Modulatory effect of Cytomegalovirus latency and sex on peripheral biomarkers and cognitive performance in old people

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Content

1 Abbreviations

HSC	Hematopoietic stem cells
DNA	Deoxyribonucleic acid
NK cells	Natural killer cells
DC	Dendritic cells
PAMP	Pathogen-associated molecular patterns
MHC	Major histocompatibility complex
CMV	Cytomegalovirus
IFN	Interferon
IL	Interleukin
IL-1RA	Interleukin 1 receptor antagonist
TNF	Tumor Necrosis Factor
sTNF-R	Soluble Tumor Necrosis Factor receptor
TCR	T-cell receptor
E	Effector
EM	Effector memory
CM	Central memory
EM3	Late differentiated effector memory
TEMRA	Effector Memory T cells re-expressing CD45RA
CD	Cluster of differentiation
TSCM	T-stem cell-like memory
PD	Programmed cell death protein
CNS	Central nervous system
CRP	C-reactive protein
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
IgG	Immunoglobulin G
CI	Confidence interval
EM	Episodic memory
WM	Working memory
Gf	Fluid intelligence
IGF-1	Insulin-like growth factor 1
SOEP	German Socio-Economic Panel
ELISA	Enzyme-linked Immunosorbent Assay
CBA	Cytometric bead array
CC	Clustering coefficient
CPL	Characteristic path length
Elocal	Local efficiency
Eglobal	Global efficiency
SWNs	Small-world networks
DHEA	Dehydroepiandrosterone
IGF-1	Insulin-like growth factor-1
IGFBP-3	IGF-binding protein
BDNF	Brain-derived neurotrophic factor
HPA	Hypothalamic-pituitary-adrenal axis

2 Summary

"Successful aging" - defined as long life in a good mental and physical health, requires appropriate immune function. However, dysregulated immunity in later life, known as "immunosenescence," results in compromised immune functions and disturbed pro- and anti-inflammatory balance. The impact of these age-related immune alterations on physical and mental health, and hence successful aging, is currently not well understood. With few exceptions, the studies on aging have not attempted to integrate psycho-neurological, metabolic, and endocrine parameters with immune function in order to dissect out which influences which. Few have taken CMV-infection into account and even gender has not always been considered. The present dissertation contributes to this developing research field both conceptually and empirically. It is publication-oriented and consists of four publications.

Publication I presents data obtained from a subgroup of the Berliner Aging Study participants, which show significant age-associated differences of T-cell subset distribution. The modulatory impact of both sex and CMV-infection on T-cell naïve and memory phenotypes, but unaffected frequencies of T-stem cell-like memory cells, were found. For the first time, the frequency of the TSCM phenotype and of PD-1^+ T-cells in peripheral circulation has been investigated, and the effects of age, CMV-serostatus, and sex on their frequency have been examined.

Publication II reviews the relevant literature on the dynamic neuroimmune interactions between the immune and the nervous systems. Bringing these two strands of research together, we propose that immunosenescence and peripheral low-grade inflammation at least partly contribute to neuroinflammation inducing neurodegeneration and age-related cognitive impairments. We review and discuss possible interventions that can prevent or at least postpone these age-related changes.

Publication III characterizes the baseline inflammatory status of aged individuals, recruited to undergo the cognitive, physical, and combined training interventions. By analyzing multiple circulating peripheral biomarkers and measures of objective cognitive function, we show that both CMV-serostatus and sex may modulate inflammatory immune factors, cognitive performance, and the relationship between the two domains, and should therefore be considered in comparative and interventional studies with elderly people.

In study IV, we propose a new strategy that allows the quantitative investigation of multiple interactions between different cytokines, receptor molecules, metabolic and neurotrophic factors, hormones, immune cells, and measures of cognitive performance. Using a graph-theoretical approach enables us not only to visualize biologically meaningful interconnections between different variables but also to compare the network topology dynamics between different groups of CMV-seronegative and -seropositive men and women in a statistically sound manner. In summary, results obtained from the studies III and IV suggest that highly integrated and segregated networks have optimal neuroimmune interactions.

Taken together, this dissertation contributes to the study of age-related functional alterations of immune and related physiological functions in two major ways. First, it expands the analysis methods that have been used to investigate markers of immunosenescence. Second, it has generated new findings on the modulatory effects of CMV-latency and sex on multiple peripheral biomarkers and cognitive function in older men and women.

3 Zusammenfassung

"Erfolgreiches Altern" - definiert als langes Leben bei guter geistiger und körperlicher Gesundheit - erfordert eine adäquate Immunfunktion. Allerdings im späteren Leben führt dysregulierte Immunität zu einer Beeinträchtigung der Immunfunktionen und zu einer Störung des pro- und anti-inflammatorischen Gleichgewichts. Die Auswirkungen dieser altersbedingten Veränderungen auf die Gesundheit und die kognitiven Fähigkeiten, und damit auf das erfolgreiche Altern, sind derzeit nur unzureichend verstanden. Bis auf wenige Ausnahmen wird in Altersstudien kaum versucht, psycho-neurologische, metabolische und endokrine Parameter mit der Immunfunktion integrativ zu untersuchen, um die gegenseitigen Einflüsse herauszufinden. Nur wenige Studien haben die Einflussnahme der CMV-Infektion in die Analyse einbezogen und auch das Geschlecht wurde kaum berücksichtigt. Diese Dissertation leistet somit konzeptuelle, methodologische und empirische Beiträge zu diesem wachsenden Forschungsfeld. Sie ist publikationsorientiert und basiert auf vier Veröffentlichungen.

Publikation I präsentiert Daten aus einer Stichprobe von Teilnehmern der Berliner Altersstudie, die signifikante altersbedingte Unterschiede in der Verteilung der T-Zell-Subpopulationen zeigen. Es wurden modulierende Auswirkungen von Geschlecht und CMV-Infektion auf naive T-Zell- und Gedächtnis-Phänotypen, aber unveränderte Frequenzen von T-Stammzell-ähnlichen Gedächtniszellen (TSCM) gefunden. Zum ersten Mal wurde die Häufigkeit des TSCM-Phänotyps und der PD-1^+ T-Zellen im peripheren Kreislauf erforscht und die Auswirkungen von Alter, CMV-Serostatus und Geschlecht auf ihre Frequenz untersucht.

In der Publikation II wird die relevante Literatur über dynamische neuroimmune Wechselwirkungen zwischen Immun- und Nervensystem behandelt. Es wird festgehalten, dass Immunseneszenz und periphere Inflammation zumindest teilweise zur Neuroinflammation beitragen, die für altersbedingte kognitive Beeinträchtigungen verantwortlich sein könnte. Es werden mögliche Interventionen erörtert und diskutiert, die diese altersbedingten Veränderungen verhindern oder zumindest verschieben können.

Publikation III charakterisiert den basalen Inflammationsstatus von älteren Menschen, die für die Teilnahme an kognitiven, physischen und kombinierten Trainingsmaßnahmen rekrutiert wurden. Die Analyse umfangreicher zirkulierender peripherer Biomarker und der Kognitionseigenschaften zeigen, dass sowohl der CMV-Serostatus als auch das Geschlecht die inflammatorischen Immunfaktoren, die kognitive Leistung, und die Beziehung zwischen den beiden Domänen modulieren können und daher in vergleichenden und interventionsbezogenen Studien mit älteren Menschen berücksichtigt werden sollen.

In der Studie IV schlagen wir eine neue Strategie vor, die quantitative Untersuchung von multiplen Wechselwirkungen zwischen verschiedenen zirkulierenden Biomarkern und kognitiver Leistungsfähigkeit ermöglicht. Die Verwendung eines graphen-theoretischen Ansatzes ermöglicht es uns, nicht nur biologisch sinnvolle Zusammenhänge zwischen verschiedenen Variablen zu visualisieren und darzustellen, sondern auch die Dynamik der Netzwerktopologien zwischen verschiedenen Gruppen von CMV-seronegativen und -seropositiven Männern und Frauen auf statistisch fundierte Weise zu vergleichen. Die Ergebnisse der Studien III und IV deuten darauf hin, dass hochintegrierte und segregierte Netzwerke optimale neuroimmune Interaktionen aufweisen.

Zusammenfassend, leistet diese Dissertation einen Beitrag zur Erforschung altersbedingter funktioneller Veränderungen auf zwei wesentliche Arten. Erstens, durch Erweiterung der

Analysemethoden, die verwendet werden, um Marker der Immunseneszenz zu untersuchen. Zweitens, durch neue Erkenntnisse über modulierende Wirkung von CMV-Infektion und Sex auf periphere Biomarker und die kognitive Leistungsfähigkeit bei älteren Männern und Frauen.

4 Publications embedded in this thesis

Di Benedetto, S., Derhovanessian, E., Steinhagen-Thiessen, E., Goldeck, D., Müller, L., & Pawelec, G. (2015). Impact of age, sex and CMV-infection on peripheral T cell phenotypes: Results from the Berlin BASE-II Study. Biogerontology. doi:10.1007/s10522-015-9563-2

Di Benedetto, S., Müller, L., Wenger, E., Düzel, S. & Pawelec, G. (2017). Contribution of neuroinflammation and immunity to brain aging and the mitigating effects of physical and cognitive interventions. Neuroscience & Biobehavioral Reviews. *75*, 114-128. doi:10.1016/j.neubiorev.2017.01.044

Di Benedetto, S., Gaetjen, M. & Müller, L. (2019). The Modulatory Effect of Gender and Cytomegalovirus-Seropositivity on Circulating Inflammatory Factors and Cognitive Performance in Elderly Individuals. Int. J. Mol. Sci. 2019, 20(4), 990. doi.org/10.3390/ijms20040990

Di Benedetto, S., Müller, L., Rauskolb, S., Sendtner, M., Deutschbein, T., Pawelec, G., & Müller, V. (2019). Network topology dynamics of circulating biomarkers and cognitive performance in older Cytomegalovirus-seropositive or -seronegative men and women. Immunity & Ageing. doi:10.1186/s12979-019-0171-x

5 Personal contribution

Publication 1: I was involved in the experiments and phenotypic analysis of peripheral blood lymphocytes. All flow cytometry measures, analyses of the data, and preparation of the manuscript were performed by me.

Experimental performance
Collection and assembly of data
Data analysis and interpretation
Manuscript writing

Publication 2: I was involved in the recherché of the current literature, discussion of the paper design, and in the writing of the manuscript.

Outline performance
Literature recherché and assembly
Analysis of published data and interpretation
Manuscript writing

Publication 3: I was involved in the experiments and evaluation of the pro- and anti-inflammatory markers in the peripheral blood of old people. All measures of cytokines, soluble receptor, cytokine receptor antagonist, hormones, CMV-serostatus were performed by me. I conducted the analysis of the results and was involved in interpretation of the data and in writing of the manuscript.

Experimental performance
Collection and assembly of data
Data analysis and interpretation
Manuscript writing

Publication 4: I was involved in the experiments and evaluation of the pro- and anti-inflammatory markers in the peripheral blood of old people. I was involved in the analysis and interpretation of the data and in the preparation of the manuscript.

Experimental performance
Collection and assembly of data
Data analysis and interpretation
Manuscript writing

6 Publications not embedded in this thesis

Di Benedetto, S. and Müller, L. (2019). Aging, Immunity, and Neuroinflammation: The Modulatory Potential of Nutrition. In M. Mahmoudi & N. Rezaei (Eds.), Nutrition and Immunity. *Springer Nature*.

Müller, L., Di Benedetto, S., & Pawelec, G. (2019). Human immune system in aging. In D. Gu & M. E. Dupre (Eds.), Encyclopedia of gerontology and population aging. Cham: *Springer Nature*. Advance online publication. doi:10.1007/978-3-319-69892-2_68-1

Müller, L., Di Benedetto, S., & Pawelec, G. (2019). The immune system and its dysregulation with aging. In J. R. Harris & V. I. Korolchuk (Eds.), Biochemistry and cell biology of ageing: Part II clinical science (Subcellular Biochemistry No. 91) (pp. 21-43). Singapore: *Springer Nature*. doi:10.1007/978-981-13-3681-2_2

Wistuba-Hamprecht K., Di Benedetto S., Schilling B., Sucker A., Schadendorf D., Garbe C., Weide B., Pawelec G. (2016). Phenotypic characterization and prognostic impact of circulating γδ and αβ T-cells in metastatic malignant melanoma. *Int J Cancer.* 138(3):698-704.

Di Benedetto, S., Wistuba-Hamprecht, K., Goldeck, D., Öttinger, L., Demuth, I., Pawelec, G. & Müller, L. (2017). The modulatory effect of age, gender and Cytomegalovirus (CMV) persistence on peripheral blood immune cell subsets in participants of the Berlin Aging Study II. *Psychophysiology* 54:86 (Abstract).

7 Introduction

Changes in the human immune system accompanying aging represent a universal, multidimensional process, the spectrum of which is generally referred to as “immunosenescence.” Many factors and mechanisms are attributed to immunosenescence, including defects in haematopoiesis, thymus involution and changes in formation, maturation, migration, and homeostasis of peripheral lymphocytes [1,2]. Aging affects both the innate and adaptive arms of the immune system and is commonly accompanied by an increased tendency to low-grade inflammation thought to be the contributory factor to neuroinflammation and to impairments in cognitive functioning of elderly people. The following sections of this introduction [2-5] briefly describe the modulatory effects of aging on different components of the immune system, beginning at the origin of all hematopoietic cells—in the bone marrow (Fig. 1, left).

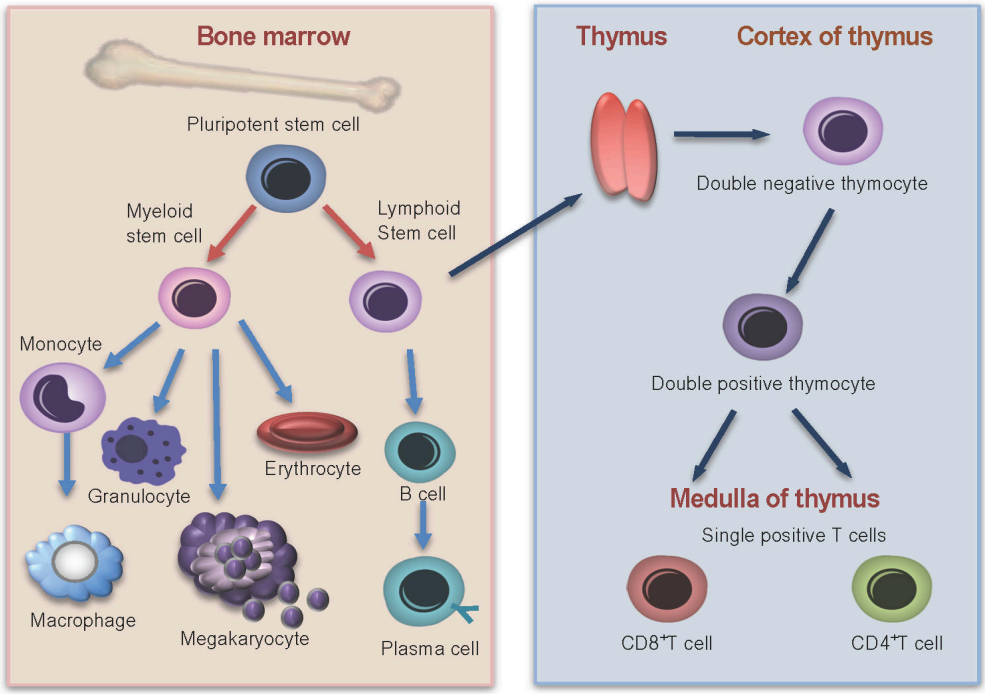


Figure 1. A simplified schematic illustration of hematopoiesis in bone marrow and thymopoiesis in thymus. CD: cluster of differentiation. Modified from [6].

7.1 Aging of hematopoietic stem cells

Age-related changes in hematopoiesis that plays a decisive role in the continuous processes of the renewal of blood and immune cells, are at least partly responsible for the functional impairments of the immune system with aging [5,7]. Throughout life, adult hematopoietic stem cells (HSCs), which consist of a heterogeneous subgroup of multipotent and unipotent progenitors (Fig. 1, left), produce the entire spectrum of cells of lymphoid and myeloid lineages of the immune system [8,9]. The microenvironment of the HSCs (so-called "niche") supports

these processes and plays an important role in the self-renewal potential of the HSCs, controlling their number and functions (Fig. 2, left). The HSC niches are populated with endothelial and mesenchymal stromal cells, adipocytes, macrophages, neutrophils, osteoclasts, and regulatory T cells, which interact with each other directly - through cell-to-cell contact, and/or indirectly - via soluble factors and molecules [7]. These reciprocal communications appear to be disturbed in the aging bone marrow - the hematopoietic compartment gradually shrinks and is progressively replaced by fatty tissue [10-12].

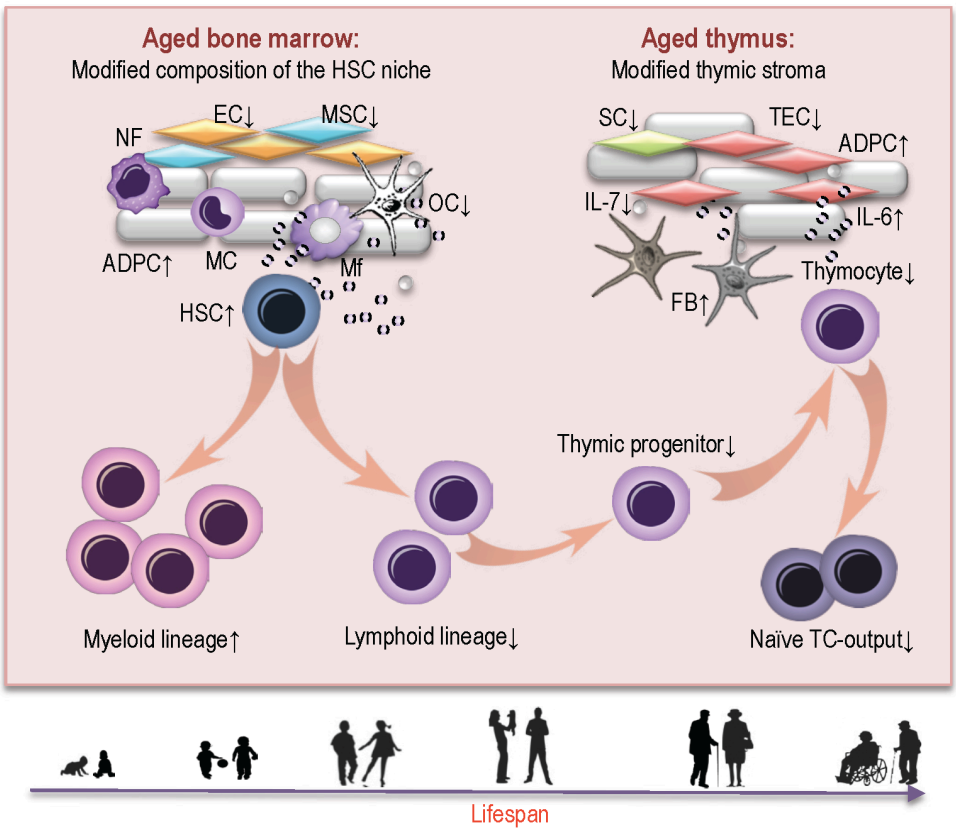


Figure 2. A schematic illustration of age-related alterations in the HSC compartment of bone marrow (left) and in the thymic stroma (right). EC: endothelial cells; MSC: mesenchymal stromal cells; NF: neutrophil; Mf: macrophage; OC: osteoclast; MC: monocyte; ADPC: adipocytes; HSC: hematopoietic stem cell; SC: stromal cell; TEC: thymic epithelial cell; FB: fibroblast; IL: interleukin. Modified from [6].

The consequences of these age-related changes in the HSC niche may include: insufficient DNA-repair capacity and epigenetic changes; increases in HSC numbers with a simultaneous decrease in their homing efficiency; a myeloid skewing of their differentiation potential, leading to downregulation of lymphoid and upregulation of myeloid differentiation genes. Whether these changes are predominantly caused by gradual intrinsic changes within the individual HSC itself or by changes in the cellular composition and supportive activity of the HSC niche is not yet fully understood. In all probability, both processes play a decisive role. Aging HSC is characterized by an accumulation of DNA damage and telomere shortening combined with an increase in intracellular reactive oxygen species - the events that eventual-

ly lead to its reduced functionality and genomic instability [7,13-16]. This may contribute to a malignant transformation of HSCs, especially in the myeloid lineage, and to an increased incidence of myeloproliferative disorders. Age-associated alterations in the lymphoid and myeloid lineage resulting in the skewing towards myeloid differentiation are thought to be one of the central mechanisms contributing to the deterioration of the immune competence in aged individuals [5,7,17].

7.2 Age–related changes to the thymus

Another aging-related major event that is thought to have pronounced influence on the aging immune system is the process of thymic involution beginning at puberty and continuing over the lifespan. This is a conserved developmental event, but contributes to immunosenescence in later life by decreasing the capacity to generate new naïve T cells, which are essential for development of adaptive immunity against new challenges (Fig. 1, right). The role of the thymus in establishing and providing a suitable microenvironment is crucial for supporting the proliferative expansion of progenitors and the maturation and selection of T lymphocytes [18].

In general, thymopoiesis is dependent on (i) a continuous supply of T-lymphoid progenitors, (ii) the existence of a suitable thymic microenvironment for progenitor engraftment, and (iii) the maintenance of thymocyte migration, as well as (iv) their dynamic expansion in the cortical stroma. These processes are controlled by mutual inter-communications between the thymic and bone marrow components as well as by developing lymphocytes [19]. Age-related alterations at this level of regulation may have crucial consequences for global immune functions. The progressive reduction of thymic epithelial areas of active thymopoiesis [20] with a concomitant increase of the perivascular areas comprising connective, adipose and fibrous tissue [11] are accompanied by changed immunometabolism in the thymic microenvironment [21], reduced production of thymo-supportive interleukin (IL)-7 [22] and enhanced secretion of thymo-suppressive mediators. These modifications adversely impact the functions and numbers of thymic epithelial and stromal cells (Fig. 2, right), what has negative consequences for thymopoiesis. The age-related decline in thymic function leads to a contraction of the T-cell repertoire and contributes significantly to the age-related increase in the incidence of infectious disease [23]. Furthermore, a decline of thymic function may also be additionally induced by environmental factors – prenatally and postnatally, as well as by genetic predisposition, and sexual dimorphism – with men presenting different patterns and dynamics of thymic involution than women [24]. The thymectomy in early childhood was found to be associated with an early onset of immune changes reminiscent of immunosenescence [25,26]. Thus, the reduced thymic output and consequently diminished capacity for replacement of the naïve T

lymphocytes after their activation and differentiation in the peripheral circulation is considered to be a decisive factor in the development and progression of immunosenescence [20,27].

7.3 Effects of aging on innate and adaptive immune system

Age-related changes in haematopoiesis combined with thymic involution contribute at least partly to the diminished immune functions of the cells both innate and adaptive immune system. In this section, we start with the short overview of the age-associated changes in the cells of the innate immunity and further focus on the adaptive immunity, where the most age-related alterations can be found.

The innate immune cells possess an exclusive capacity to respond immediately to pathogens in a generic way by activating such defence mechanisms as phagocytosis, inflammatory reactions, activation of the complement system, and recruitment of essential cells such as eosinophils, neutrophils, macrophages, natural killer cells (NKs), and dendritic cells (DCs) to sites of detected infection (Fig. 3). Various age-associated functional impairments have been reported in phagocytic mechanisms, in chemotaxis and the generation of toxic free radicals, as well as in the susceptibility to apoptosis [28]. Some of the diminished functions in neutrophils with advanced age were found to be associated with the altered production of chemokines and cytokines, with the reduced expression of receptors recognising Pathogen-Associated Molecular Patterns (PAMPs; such as Toll-like receptors), and with the lower expression of the Major Histocompatibility Complex class II (MHC-II) molecules[29].

Monocytes and macrophages also play an important role in terms of phagocytosis and antigen presentation (Figs. 3 and 4). Monocytes act very efficiently in controlling invading bacteria - the feature that is most important for the aged population, characterized by a high prevalence of infectious diseases. Macrophages are involved in the initiation of inflammatory responses, in the direct destruction of pathogens and elimination of malignant cells, as well as in interactions with, and activation of, the adaptive immune response by means of antigen presentation [30]. They are then able to eliminate their target antigens directly or indirectly - through production and secretion of immune molecules and immune factors, such as interleukin (IL)-1, Tumor Necrosis Factor (TNF), and Interferon (IFN)-γ, which in turn, are capable of activating and recruiting additional immune cells [31,32].

The proportion of circulating monocytes in the peripheral blood of aged individuals does not seem to be significantly changed with age, but decreased numbers of macrophages were reported in the bone marrow of 80 to 100-year-old people. The phagocytic and functional features of macrophages are also impaired with age, accompanied by a decreased produc-

tion of reactive oxygen species, such as NO_2 and H_2O_2, and pro-inflammatory cytokines, TNF and IL-1 [27,33,34].

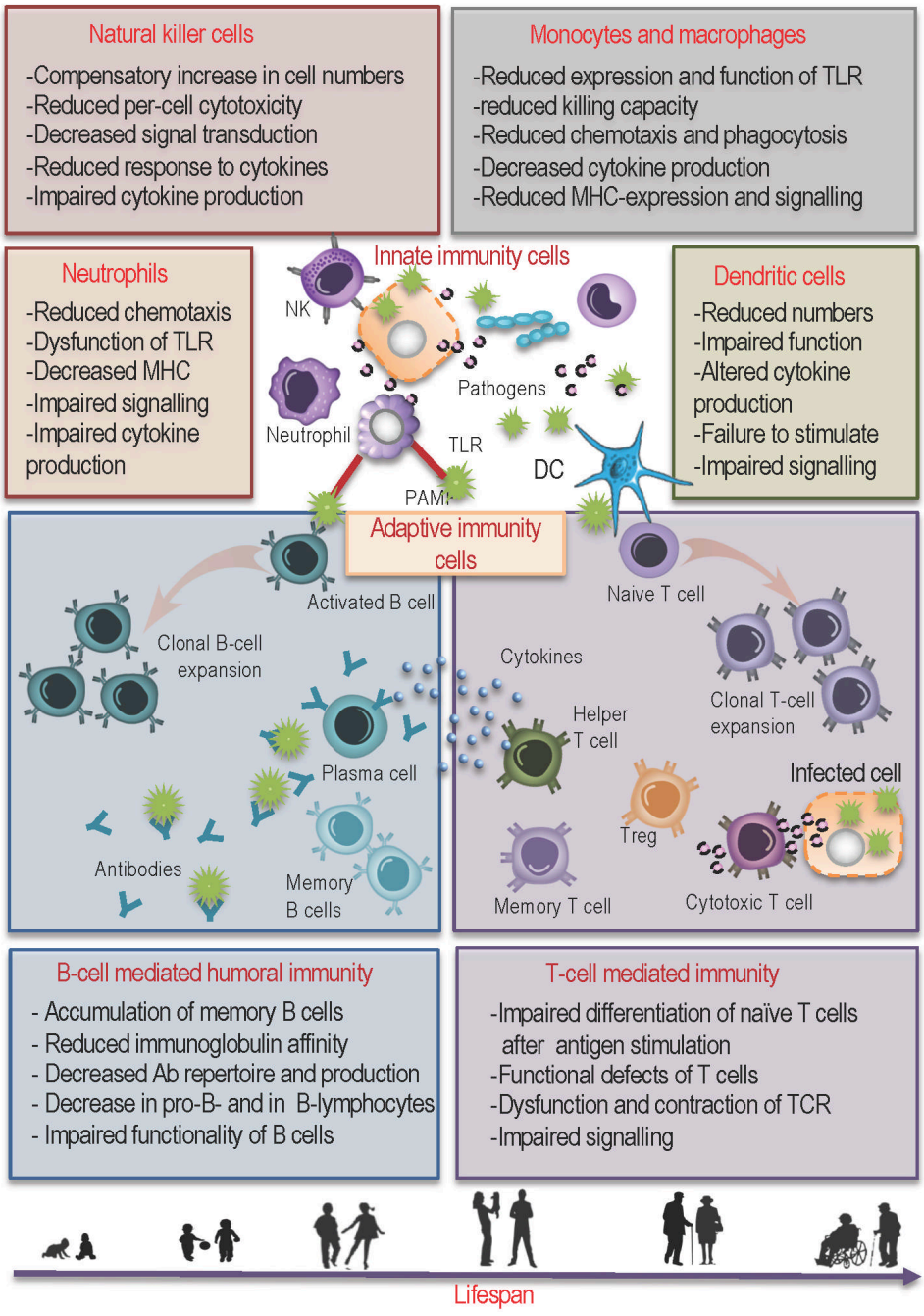


Figure 3. Modulatory effect of aging on functions in innate and adaptive immunity. EC: endothelial cells; MSC: mesenchymal stromal cells; TLR: toll-like receptor; PAMP: pathogen-associated molecular pattern; MHC: major histocompatibility complex; TCR: T-cell receptor; Treg: regulatory T cell; NK: natural killer cell; DC: dendritic cell; Ab: antibody. Adopted from [6].

Dendritic cells (Fig. 3) are important players in the immune response and are said to bridge the innate and adaptive immune systems. They are capable of recognising PAMPs and take up and process pathogens to present MHC-bound antigens to T lymphocytes. Thus, it is un-

derstandable that even small age-related alterations in the function of dendritic cells could greatly affect T-cell functionality.

DCs do appear to be impaired by aging in terms of their migratory capacity and distribution, in their ability to process antigen, in their expression of costimulatory signals, and in their ability to secrete cytokines (Fig. 4). Despite the fact that the antigen presentation by DCs appears to be relatively unimpaired in the elderly, slightly decreased numbers of DC found in peripheral blood and follicles could probably explain reduced immune supportive capacity of this cell fraction in immune defence against pathogens and tumours [31,34,35].

NK cells are cytotoxic lymphocytes that are responsible for and are involved in early defence, with their specific role in the detection and elimination of virus-infected cells in an MHC-unrestricted manner (Fig. 3). Due to this exceptional immune competence of NK cells, they are considered as the most important players in cancer immune surveillance during aging. It was shown that in elderly individuals the numbers of NK cells may be increased, but they have mostly a more mature phenotype compared to NK cells in young individuals as well as an impaired cytotoxic function [36,37].

Adaptive immunity is involved in, and contributes to, a highly regulated multidirectional interplay between innate cells and adaptive cells - such and T and B lymphocytes (Figs. 3 and 4). These specific interactions activate, induce, and support pathogen-specific immunologic effector pathways and the generation of immunological memory mediated by subpopulations of long-lived memory T and B cells [27,38].

Concerning age-related alterations occurring in B cells and their subpopulations is relatively less known than in T cells. It has been consistently reported that there are decreased numbers of functional immunoglobulin-secreting B cells in aged people, correlating with decreased titres of antigen-specific antibodies. Not only the B-cell subsets themselves but also the repertoire of immunoglobulins produced by them are altered in both specificity and isotype. The consequence of this may well be the reduced duration of humoral response with reduced B-cell capacity for providing specific primary and secondary responses seen in aged individuals [39,40]. Age-associated deficiencies in T cells can have modulatory effects on their cognate interactions with B cells and reduce the quality of antibodies in terms of their avidities, and their titres [41]. Age-related alterations in the epigenetic status are likely to be playing an important role here as well, and might be responsible for altered B-cell function. Such epigenetic changes may induce important modifications in the B-cell differentiation pathways [27,39,42]. During the course of aging, after long-term residence in the aging host, naïve T cells begin to suffer impaired differentiation into effector cells after antigen stimulation, as well as further functional impairments, such as an altered production of cytokines, and a more re-

stricted T-cell receptor (TCR) repertoire [43,44] . Epigenetic age-related changes, such as methylation of cytokine gene promoters can also lead to impaired immune function [34,45]. The altered DNA methylation profiles were found to be present over the course of aging for both immune-specific genes and for the genes involved in common pathways normally related to cell homeostasis [33,42] (Fig. 4).

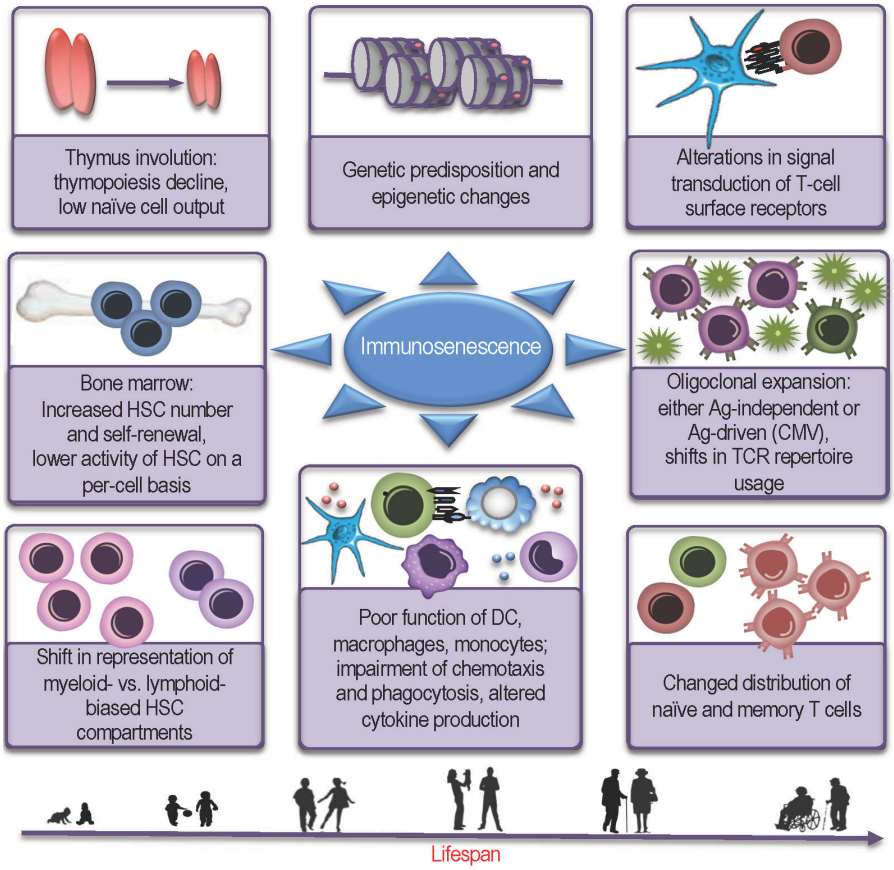


Figure 4. Immunosenescence and related processes. CMV: cytomegalovirus; Ag: antigen; HSC: hematopoietic stem cell; DC: dendritic cell; TCR: T-cell receptor. Modified from [6].

Basically, the functional potency of T lymphocytes from aged individuals was found to be reduced relative to cells of the same phenotype from the young individuals [46]. For an appropriate activation and differentiation of T cells, a necessary requirement is the integrity of the genes coding for the main key factors. This in turn, may be dependent on different environmental factors, such as exposure to hormones or chemicals, infection with different pathogens etc., which influence this process. Such factors can change the epigenetic profile of T lymphocytes, affecting their gene expression and modifying these processes. The consequence of such immune remodelling that usually accompanies aging is a significantly increased morbidity and mortality commonly observed in the elderly population [33,41,47,48]. Another important driver of the remodelling of the peripheral T-cell compartment over the lifespan is chronic immune activation by different pathogens and particularly by persistent cytomegalovirus (CMV).

7.4 Modulatory effect of CMV on immunosenescence and loss of T-cell diversity

CMV resides in a latent form in cells, which are broadly distributed throughout the body, including HSC, monocytes, macrophages, dendritic cells and endothelial cells [49-51]. A lifelong coexistence with CMV has significant implications for the immune system, leading to functional alterations and eventually driving immunosenescence [52]. It is expected that latent CMV undergo sporadical reactivations, but it is not definitely confirmed that such reactivations happen more often in the elderly than the young [53]. It is anticipated that virus reactivation produces immunogenic viral transcripts, which then induce the expansion of CMV-specific cytotoxic memory lymphocytes. Thus, CMV-persistence represents a dynamic process of continuous balancing over the lifetime between virus immune evasion and host immune recognition [54,55]. As result of such persistent CMV-infection, an expansion of the $CD8^+$ T cells occurs, with an accumulation of late-stage differentiated effector memory T cells in the peripheral circulation [52]. In people infected with CMV and especially in the elderly, a large proportion of circulating $CD8^+$ T cells is specific for CMV. This phenomenon of sequential increases of CMV-specific $CD8^+$ T cells over the lifespan is known as “memory inflation” [56,57]. Therefore, due to memory inflation, the absolute number of functional CMV-specific T cells may be even higher in the old than in the young individuals – the fact highlighting the crucial importance of immunosurveillance against this virus [58].

In a systematic review, Weltevrede and colleagues analyzed existing evidence regarding the link between immunosenescence and CMV [59]. They found that, in the majority of studies, CMV-seropositivity appears to induce the accumulation of T-cell phenotypes known to be generally associated with immunosenescence (i.e., increased proportions of Effector Memory (EM) and TEMRA (Effector Memory T cells re-expressing CD45RA) cells in both $CD4^+$ and the $CD8^+$ T-cell subsets) in the peripheral circulation of CMV^+ versus to CMV^- old individuals. No strong evidence, however, was found for a decrease in proportions of naïve T lymphocytes in CMV^+ related to CMV^- elderly [59]. It was suggested that CMV might contribute to immunosenescence in a clinically-relevant sense - by being associated with mortality in the elderly [50,52,58,60,61] - as well as with frailty and with impaired survival [62-64].

An intact TCR repertoire is generally considered to be important for supporting adequate immune competence, in particular, against new pathogens. In this concern, the age-associated limitations regarding diversity are supposed to have negative implications. The TCR-repertoire shrinkage may lead or at least contribute to the lowered responses to new pathogens and to poor vaccination response in this age group [52], but surprisingly an insufficient amount of data are available on this issue.

Thus, as we have seen above, age-related changes in peripheral T-cell dynamics are associated with altered haematopoiesis, with thymic involution and with lifelong immune stimulation by multitudinous antigens, and particularly by CMV. Such alterations may contribute to lowered proportions of naïve T cells due to the lower HSC output and to the minimal remaining thymic function and, possibly, to a reduced diversity of the T-cell repertoire [23,65,66]. In the peripheral circulation, a shift occurs towards accumulations of T-cell populations with memory and effector phenotypes, as well as to an accumulation of putatively senescent immune cells.

7.5 Inflammaging and its contribution to neuroinflammation

The aging-related accumulation of the functionally exhausted memory T lymphocytes, commonly secreting pro-inflammatory cytokines, together with mediators and factors of the innate immune system, is considered to be one of the sources contributing to the low-grade inflammation (inflammaging) often observed in old people [67-69]. The senescent immune cells not only secrete inflammatory mediators but are also able to modulate their microenvironment. It was hypothesized that inflammaging appears to be a decisive contributor of, and is associated with, different age-related diseases, and may play an essential role in their aetiology and pathology [68,70-73].

Even in overtly healthy individuals, chronic low-grade inflammation has repeatedly been identified during aging, as reflected by increased levels of circulating pro-inflammatory cytokines such as IL-6, IL-1β, TNF, and IFN-γ [69]. These cytokines influence nearly all physiological systems and affect their functional ability, including neurological functions, and, accordingly, have an impact on behavioral and cognitive parameters [4,74]. It is assumed that peripheral immunosenescence may contribute at the systemic level to the age-related alterations in the proportions and functions of cells in the circulation (Fig. 5) and that cytokines released from such aged and functionally deteriorated cells in the periphery can approach the brain via several routes and disturb neurological functions. The phenotype and function of microglia can be changed due to low-grade brain inflammation; the astrocytes and neurons as well as peripheral immune cells such as T cells, monocytes, and macrophages may participate in the neuroinflammation [4,75]. Such altered conditions may lead to a loss of neuroprotective functions (normally provided by microglia) and consequently to neuronal dysfunction [76-78] and contribute to the risk of developing age-related cognitive impairment, neurodegenerative changes and neurological disorders [4,79-81,82]. A more pronounced degree of cognitive impairment after immune challenge is often seen in the elderly relative to the young individuals. Neuroinflammation is often accompanied by an elevated and more sustained release of pro-inflammatory cytokines in the otherwise healthy brain of aged people [83].

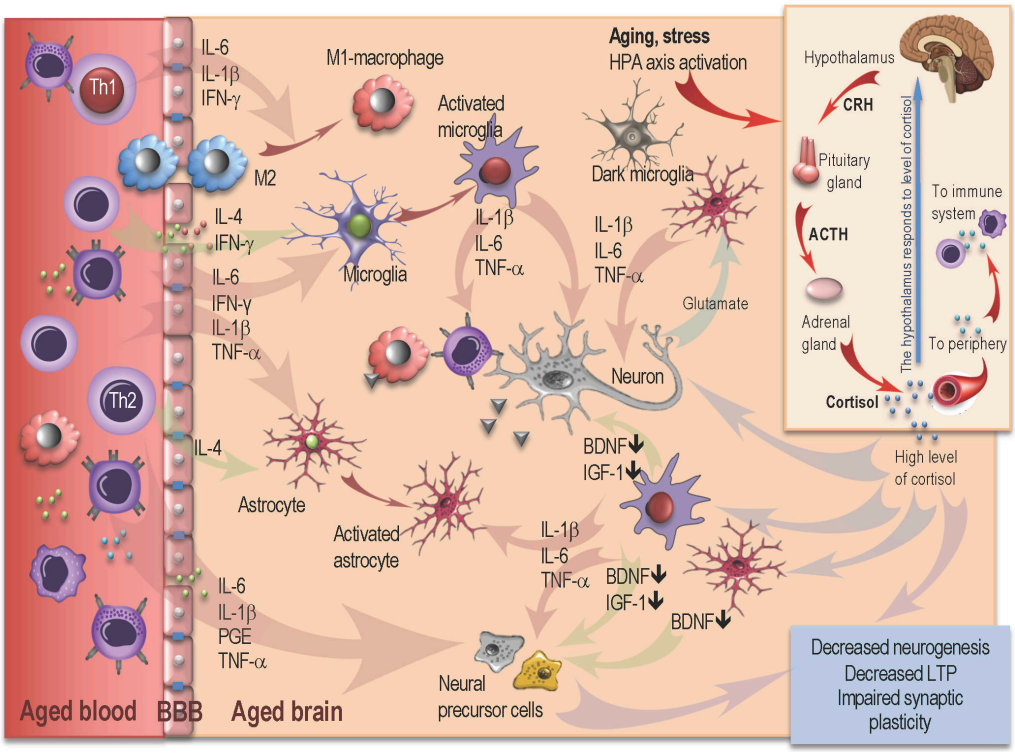


Figure 5. Immunosenescence, inflammaging, and neuroinflammation. HPA: Hypothalamic-pituitary-adrenal axis; CRH: corticotropin releasing hormone; ACTH: adrenocorticotropin; IL: interleukin; IFN: interferon; TNF: tumor necrosis factor; LTP: long term memory potentiation; BDNF: brain-derived neurotrophic factor; IGF: insulin-like growth factor. Modified from [4].

It is also known that aging together with chronic stress affects the neuroendocrine system, activating the hypothalamic-pituitary-adrenal axis and stimulating the production of glucocorticoids and releasing them into the peripheral circulation [84]. The main glucocorticoid, cortisol, can modulate immune functions in a bidirectional way: by controlling the production and release of cytokines, chemokines, and adhesion molecules, and by modifying maturation, differentiation, and migration of immune cells [83,85]. Raised levels of cortisol can have negative consequences for hippocampal neurogenesis [4]. The produced modulatory effects result in the induction of an inflammatory milieu that contains resident and peripheral immune cells, which participate in complex mutual interactions between secreted inflammatory mediators and cell surface receptors [86]. Activated microglia and astrocytes change under the influence of the inflammatory milieu their morphological and functional features and start, in turn, to release pro-inflammatory cytokines IL-1β, IL-6, and TNF (Fig. 5). It was reported that microglia in an aged brain experience a process of senescence, comparable to the immune cells in the peripheral circulation. Recent studies report a detection of senescent and hyperactive microglia, which was found in the diseased and aged brain [87]. It is supposed that also an aging brain can, through different mechanisms, be able to modulate and to regulate the immune functions and to support the recruitment of immune cells from the periphery, contributing further to immunosenescence and to neuroinflammation [4,88].

7.6 Short description of BASE-II and AKTIV studies

Longitudinal Berliner Aging Study II (BASE-II)

The aging trajectories of different individuals are distinctive – whereas some people preserve their physical and cognitive health into advanced ages, others experience age-related impairments and early decline. With the aim of identifying and characterizing mechanisms responsible for such different outcomes, researchers from Berlin and Tübingen launched the Berlin Aging Study II (BASE-II). BASE-II is a multidisciplinary and multi-institutional longitudinal study ascertaining a wide range of aging-related variables from different functional domains. The project consists of a baseline sample of 1,600 older adults aged 60 to 80 years and 600 younger adults aged 20 to 35 years. The research teams that are involved in the BASE-II study as well as various phenotypic domains, which are examined in each participant (for more details see [89,90]) are briefly described below:

- Internal medicine and geriatrics (Charité Research Group of Geriatrics, Berlin): performs in-depth medical assessment accompanied by providing a history of previous and current physical conditions as well as an extensive array of functional and laboratory tests.
- Immunology (University of Tübingen): performs assessments of immunological parameters with the aim of determining specific “immune risk profiles” for each participant and identifying potential biomarkers of immunosenescence.
- Psychology (Max Planck Institute for Human Development): performs a comprehensive array of cognitive tests characterizing a large number of cognitive and behavioural abilities, exploring self-rated health and well-being, as well as coping and general attitudes towards the aging process.
- Socio-economics and survey methodology (German Socio-Economic Panel Study): this assessment is carried out by TNS Infratest on behalf of the German Institute for Economic Research. The BASE-II participants complete two questionnaires at home - one individual and one on their household, which consist of over 120 socio-economic and behavioural variables and are comparable to a representative longitudinal survey of 20,000 adults in the whole Germany.
- Genetics (Max Planck Institute for Molecular Genetics, Berlin): each study participant is subjected to genome-wide microarray-based genotyping of single nucleotide polymorphisms to reveal the impact of genetic polymorphisms on the phenotypes assessed by the other research groups.

Thus, applying a multidisciplinary approach, BASE-II investigates the physical, cognitive, immunological, genetic and social conditions that may lead to successful aging.

Interventional study "Aktives Altern für Körper und Geist" (AKTIV)

Some modifiable lifestyle factors, such as poor diet as well as physical and cognitive inactivity have been identified in animal and in human studies to negatively influence individual aging trajectories. It was demonstrated that physical activity in advanced age and also cognitive training programs may elicit positive benefits in elderly people. Furthermore, interventional studies using a training design that combines cognitive and physical training programs demonstrated a synergistic effect. It was postulated that physical activity might create a balanced and neuroprotective environment (associated with reducing or abrogating neuroinflammation) that is more receptive to cognitive stimulation. Cognitive training in combination with physical activity were hypothesized to promote essential plastic brain changes that might significantly increase the potential of neurogenesis and synaptogenesis.

Based on this hypothesis, the aim of the interventional AKTIV study was to identify the kind of motivating and meaningful training intervention that older adults can easily integrate into their daily lives, with the aim of promoting healthy ageing. For this purpose, the intervention study was designed to investigate the effects of physical activity (using bicycle ergometers) and cognitive stimulation (by learning a foreign language) as well as their combined effects.

The healthy elderly participants (aged from 64 to 75 years) were partly drawn from the Berlin Aging Study II, where a longitudinal dataset had already been collected. Participants were randomly assigned to one of the four different groups:

- a language training group,
- a physical exercise training group,
- a combined language and exercise training group, and
- an active control group.

All participants underwent a sports medicine type of assessment of their fitness levels including blood collection, cognitive assessment, and magnetic resonance imaging before training. In the peripheral circulation of the study participants examination of the main inflammatory and anti-inflammatory biomarkers, such as cytokines, receptor antagonists, soluble receptors, immune cells, hormones, neurotrophins and their regulators, as well as relevant metabolic factors was accomplished.

The cognitive assessment was performed immediately before beginning the training period and included a broad range of measures of learning and memory performance, processing speed, working memory, and executive functioning. After 6 months of training all participants were re-invited for post-testing consisting of the same assessments.

7.7 Objectives

The aim of the research presented within this dissertation was to increase our understanding of the mechanisms and multiple interactions contributing to the age-related functional alterations in immune, cognitive, and related physiological functions. Using datasets and biobanked materials from both the BASE-II and AKTIV studies we intended to investigate the influence of age, sex, and CMV-serostatus on the peripheral biomarkers and functional abilities of aged people. For this purpose, the present work posited four main goals:

First, we aimed to examine phenotypic changes and the distribution of different T-cell subpopulations influenced by age, sex, and CMV-serostatus in the circulation in a subgroup of 157 participants of the Berlin Aging Study II. Applying polychromatic flow cytometry allowed us to define and to characterize changes in the following T-cell subpopulations: naïve, central memory, effector memory, terminally-differentiated T-effector memory cells, “exhausted” T cells, potentially “senescent” T cells and T-stem cell-like memory T cells.

Second, we reviewed the current literature on normal aging bringing together the findings of immunological research with information from neuroscience, in order to provide the most comprehensive picture of the complex interactions between the two most complex physiological systems of the organism and to convince the neuroscientists that immunological investigations should be an indispensable part of aging-related and interventional studies.

In the next step, we measured and characterized the baseline inflammatory status of 161 aged individuals recruited for an AKTIV intervention study before starting the cognitive, physical, and combined training. To this end, we evaluated major inflammatory and anti-inflammatory biomarkers, such as circulating cytokines, receptor antagonists, soluble receptors, immune cells, and relevant metabolic markers. By analyzing multiple circulating peripheral biomarkers and measures of objective cognitive function, we aimed to examine the modulatory effect of CMV-serostatus and sex on inflammatory immune factors, cognitive performance, and the relationship between the two domains.

Finally, we aimed to develop a new strategy that allows the quantitative investigation of multiple interactions between different cytokines, receptor molecules, metabolic and neurotrophic factors, hormones, immune cells, and measures of cognitive performance. With help of a graph-theoretical approach we intended not only to visualize biologically meaningful interconnections between different variables but also to compare the network topology dynamics between different groups of CMV-seronegative and -seropositive men and women in a statistically sound manner.

8 Overview of papers

8.1 *Paper I:* Impact of age, sex and CMV-infection on peripheral T-cell phenotypes: Results from the Berlin BASE-II study.

Although it is widely known that with advancing age the phenotypic characteristics of immune cells are altered, the influence of gender, age, and CMV-infection on these alterations are not completely understood. The present study examines phenotypic changes and the distribution of different T-cell subpopulations under the influence of age, sex, and CMV-serostatus in peripheral circulation of 157 (98 old and 59 young) participants of the Berlin Aging Study II.

Graphical abstract

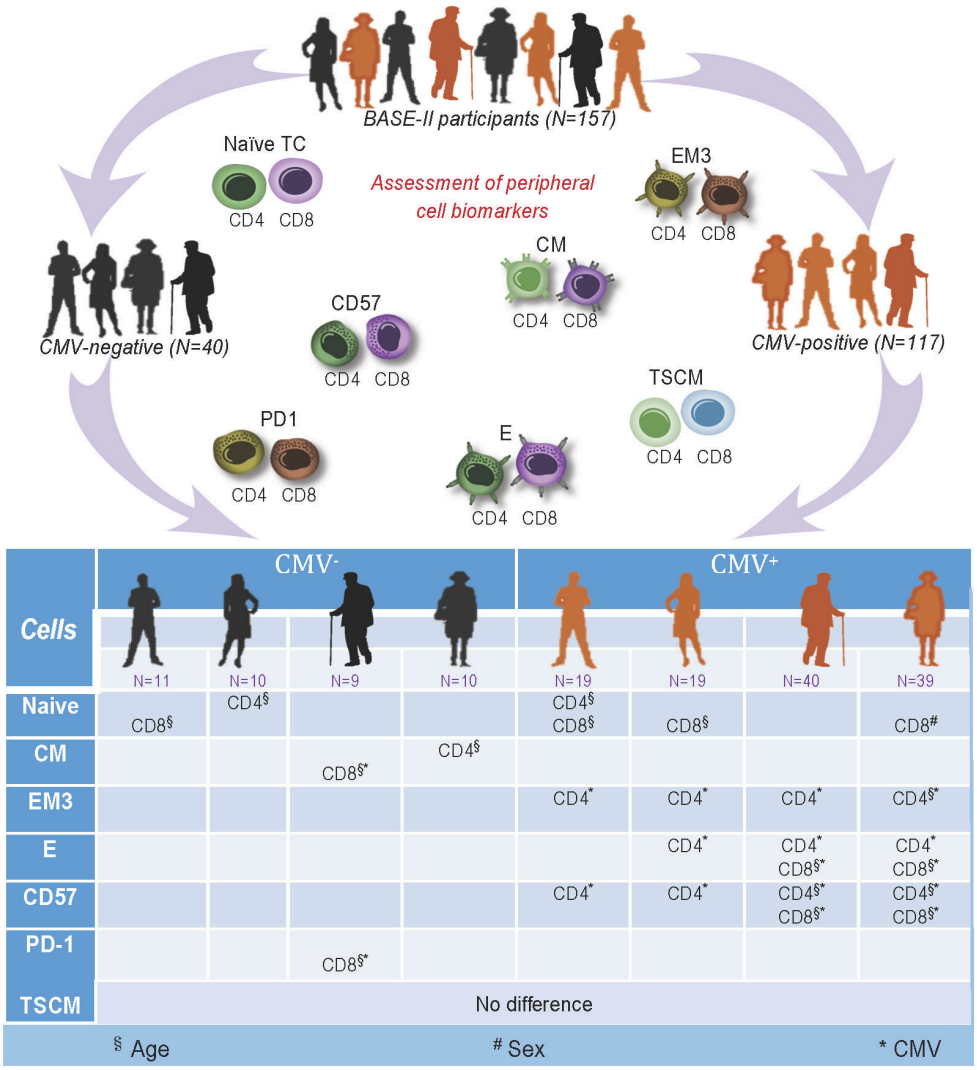


Figure 6. A schematic illustration of summarized results on the influence of age, gender and CMV-serostatus on peripheral T-cell phenotypes. The names of the analyzed parameters with significantly higher values are placed in the corresponding column of the CMV-negative or CMV-positive old or young men and women accordingly, whereby the notation (#) implies: a gender-related-higher value,

the notation (*) implies: a CMV-related-higher value, and the notation (§) implies: an age-related-higher value.

Results and discussion

For the simultaneous assessment of multiple surface marker phenotypes, we applied polychromatic flow cytometry that allows to define the following T-cell subpopulations: (N) naïve ($CD45RA^+CCR7^+CD27^+CD28^+$); (CM) Central Memory ($CD45RA^-CCR7^+CD27^+CD28^+$); (EM3) effector memory ($CD45RA^-CCR7^-CD27^-CD28^-$); (E) terminally-differentiated T-effector memory cells as an extended TEMRA phenotype ($CD45RA^+CCR7^-CD27^-CD28^-$); "exhausted" T cells [$PD\text{-}1^+$ ($CD279^+$)]; potentially "senescent" T cells ($CD57^+$), and T-stem cell-like memory T cells (TSCM, defined as $CD45RA^+CCR7^+CD27^+CD28^+CD95^+$).

If we look at the frequencies of the naïve T cells, we can see that the main differences are age-related. Within the $CD4^+$ population, both young CMV-negative females and young CMV-positive males show a significantly higher percentage of naïve T cells compared to older subjects (publication 1, Fig. 1c). These results confirm the, already known from literature, general tendency of naïve T cells to decrease with age even independently of CMV-infection [91].

Within the $CD8^+$ T-cell population, we also found that the young generation has an advantage in the distribution of naïve T cells. Their frequency is significantly higher in young CMV-negative and -positive men, and also in young CMV-positive women, than in the older participants from the corresponding groups (publication 1, Figure 1d). Most previous studies have reported that the age-related decrease in the frequency of naïve T cells is more pronounced in the $CD8^+$ compared to $CD4^+$ T lymphocytes. Our results, however, show that both $CD8^+$ and $CD4^+$ populations of naïve T cells decrease under the influence of age. Among other known factors, age-related thymic involution could play an enormous role in such reduction, as progressive thymic involution is associated with the reduced efficiency of T-cell development and reduced migratory capacity of naïve T cells to the periphery [23,92].

We also found a gender-specific effect, where the CMV-positive group of older female subjects showed a significantly higher proportion of naïve T cells compared to older men (publication 1, Fig. 1d). In general, surprisingly little is known about gender differences in the ageing immune system and age-related distribution of T-cell subpopulations in men and women [93]. It has been reported that the total number of lymphocytes in the peripheral blood of both sexes does not differ, although men had a lower proportion of T cells in their lymphocyte population than women [94,95]. However, the gender-specific distribution of the naïve T cells was not considered in these studies.

Age also has a decisive influence on the CM T cells. In the CMV-seronegative group, older men have a significantly higher frequency of CM T cells within the $CD8^+$ fraction, and older women within the $CD4^+$ population compared to young subjects. In addition, the CMV influence is evident in older males, since, within the $CD8^+$ population, the CMV-negative group has a higher proportion of CM T cells compared to CMV-positive individuals (publication 1, Fig. 1e-f).

The late-differentiated Effector Memory (EM3) T cells within the $CD4^+$ fraction show not only an age-related influence, but also a much more pronounced modulatory effect of CMV infection. Here, the CMV-positive older females have a higher proportion of EM3 T cells compared to the young women, and the same applies to both young and older men as well as young and older women in the CMV-positive group compared to the CMV-negative subjects (Publication 1, Fig. 2a).

If we look at the E T cells within the $CD4^+$ population, we can find an influence of CMV-seropositivity on the distribution of these cells. The frequency of E T cells in women of both age groups and in old men is significantly higher in CMV-positive subjects compared to individuals without CMV-infection (publication 1, Figure 2c). Within the $CD8^+$ population, the same phenomenon is observed, but limited to older men and women. Furthermore, the older men and women show a significantly higher percentage of E T cells compared to the young individuals (publication 1, Fig. 2d).

In summary, we found a higher frequency of CM $CD4^+$ and $CD8^+$ T cells exclusively in CMV-negative older subjects, while CMV-positive individuals exhibited a phenotype of late-differentiated EM3 and E in $CD4^+$ T cells. This is in line with previous findings, in which it was demonstrated that CMV predominantly drives the accumulation of late-differentiated $CD4^+$ and $CD8^+$ memory T cells [49,96,97]. Thus, the CMV-seropositivity seems to have a decisive influence on the distribution of these subpopulations. The prolonged coexistence with the latent CMV over the course of life and recurrent immune stimulation during virus reactivation, requires continuous immune surveillance and may explain the accumulation of "late differentiated" subpopulations of T cells in the peripheral circulation of elderly people.

The higher frequency of E T cells of the $CD8^+$ fraction observed exclusively in older participants is consistent with our earlier results from other cohorts as well as with results already described in the literature. Presumably, the CMV reactivation may occur more frequently in elderly people, leading to an age-related increase in memory T-cell pools in this age group [53,91,98]. However, it is also understandable that this increase can also be additionally influenced by other factors such as genetic background or general health. The importance of considering CMV-infection as one of the central parameters in our study was also previously

illustrated by results of the Swedish longitudinal studies OCTO and NONA, which defined an immune risk profile (IRP) predicting 2, 4, and 6 years survival [60,99]. They showed that CMV-induced accumulation of late-differentiated T cells can correlate with elevated mortality with increasing age.

The data of our experiments summarized here suggest that in the course of aging the differentiation status of $CD4^+$ and $CD8^+$ T cells progresses towards “late-differentiated” phenotype and that CMV-associated "senescence of T cells" may be more pronounced in older men than in older women. The results of the other studies also reported a significant increase in EM cells in older men but not in older women, although their CMV-serostatus was not investigated [100]. On the contrary, another study from Cuba reported an increased frequency of highly differentiated T cells in women compared to men [101]. Therefore, the influence of sex and CMV-persistency on the distribution of different T-cell subpopulations in peripheral blood still remains incompletely investigated and understood.

In our study, we found that the gender-related alterations in the differentiation status of T cells do not seem to play a decisive role in the group of CMV-negative individuals. This fact might be considered as another hint towards the notion that the differentiation status of T cells is primarily under the immunomodulatory effect of long-term immune surveillance to control CMV-infection. In general, it can also be assumed that the frequency and magnitude of CMV reactivations may also be different in men and women, but this should be the subject of future investigations, as no serological detection methods are currently available to determine the duration of virus persistence or/and the number of reactivations that occur over the lifespan.

Broadly speaking, the gender-specific divergence in the influence on immunity in men and women may be due to the different secretion patterns of sex hormones and their changes over the lifespan [102,103]. It is known that estrogens enhance humoral immunity, while androgens and progesterone tend to suppress it [104,105]. In particular, thymic involution is more pronounced in men compared to women, due to their higher androgen levels [106]. On the contrary, women appear to have a stronger humoral and cell-mediated immune response and generally, higher antibody levels, and increased concentrations of circulating IL-1, IL-4 and IFN-γ [100,107]. It is also known that the incidence of infectious and autoimmune diseases varies between men and women and may also be explained by gender-specific differences in the immune system [108,109].

The study of the expression of CD57 on T lymphocytes, which also acts as a biomarker for the late-differentiated T cells, revealed a CMV-induced increase of these cells within the $CD4^+$ fraction in both young and older men and women. Among $CD8^+$ T cells, only older men

and women showed a significantly higher CMV-related population of CD57-expressing cells. In addition, an age-related influence was also observed in CMV-positive individuals in both $CD4^+$ and $CD8^+$ populations (publication 1, Figs. 3a-b). Since CD57 is considered to be a potential "senescence marker" for late-differentiated T cells [91,110-112], our results are consistent with the results known from literature, where also the age- and CMV-related differences within the $CD4^+$ and $CD8^+$ subpopulations were clearly visible - with more pronounced differences in the $CD4^+$ fraction.

PD-1 (programmed cell death protein 1) is a protein that is regarded as an inhibitory immune regulator and is mostly expressed on $CD8^+$ T cells. Our results show a significantly higher frequency of PD-1-expressing $CD8^+$ T cells in older males relative to younger males in the CMV-negative group (publication 1, Fig. 3c). This is consistent with the results known from literature, which suggest that an increased expression of PD-1 on T cells is associated with exhausted T-cell phenotype and replicative senescence [113,114]. In addition, the CMV influence is evident in the old men – whereby the group with the negative CMV-serostatus unexpectedly shows a significantly higher percentage of PD-1-expressing T cells than the CMV-positive male group. We can only speculate that possibly CMV-induced chronic activation of T cells might suppress the expression of these inhibitory molecules. However, further investigations are necessary to confirm and to investigate this phenomenon more deeply.

We have assessed a novel T-cell subpopulation of TSCM (T-stem-cell-like memory) cells known for its stem cell-like properties. This rare subpopulation seems to have an increased capacity for self-renewal and for the generation of multipotent CM-, EM-, and E- cells. In the context of age-related reduced precursor-cell production from bone marrow and impaired thymus function [14], this population may represent an important additional source of peripheral T-cell regeneration in old age. This novel population, however, has not been studied for the influence of age or CMV-serostatus yet. The results of our study demonstrate that neither CMV-serostatus nor age or sex had a significant influence on the frequency of TSCM cells (publication 1, Fig. 3d-f). Since the maintenance of the constant TSCM population might possibly represent evolutionary beneficial adaptation later in life by retaining functionality of the aging immune, this finding seems to be very important – but needs, however, to be confirmed in further studies. Another important question that remains to be unanswered and needs further investigations is, whether these cells are still functionally intact in the elderly people.

8.2 *Paper II:* Contribution of neuroinflammation and immunity to brain aging and the mitigating effects of physical and cognitive interventions.

This review played an important role in the further work included in this dissertation. It was supposed to represent a kind of bridge, to bring together the findings of immunological research with the facts from neuroscience, in order to provide the most comprehensive picture of the complex interactions between two major physiological systems of the organism. It should provide a basis for convincing the neuroscientists that immunological investigations should be an indispensable part of the aging-related and interventional studies. The publication of this review article and positive feedback on it, have played a decisive role for, and contributed to, the inclusion of immunological assessment in the design of the AKTIV study. The aim of the AKTIV study was to identify the kind of motivating and meaningful training intervention that older adults can easily integrate into their daily lives, with the aim of promoting healthy ageing.

While the most of the review articles are dealing rather with pathological conditions, the publications that are dedicated to the so-called “normal aging” are much scarcer. Therefore, the aim of this review was to describe the neuroimmune processes and the age-related functional changes that are important in “non-pathological” ageing. Ageing of the immune system usually has negative consequences for the entire ageing organism and *vice versa*. Unfortunately, very often age-related changes are investigated and described rather isolated in one or another physiological system. Especially the multiple reciprocal connections and complex interactions between the immune and nervous systems were ignored for a long time. The prevailing opinion of the scientific community was, that the existence of the blood-brain barrier makes it impossible for the immune system to participate in, and to influence, processes of the brain. Consequently, the appearance of immune cells in the CNS was mostly attributed exclusively to the pathological states associated with neurodegenerative processes. It has also long been thought that the brain has no lymphatic system and therefore no anatomical pathways exist to allow immune cells to enter this sensitive area. For a long time, the opinion prevailed that neurogenesis is exclusively limited to the embryonic phase of development, and the important role of microglia was not properly understood.

New technological developments have made it possible to reconsider many of these dogmatic notions. With the discovery of the meningeal lymphatic system involved in the transport of macromolecules and immune cells, as well as with the new findings in the field of neuroscience, it has become clear that the two largest and most important physiological systems of the organism are involved in a constant and very intense cross-talk (Fig. 7). Even more, it is now widely accepted that these continuous intercommunications play an immense role in maintaining the functionality and integrity of the individual.

The main focus of our review on the current literature was on the dynamic neuroimmune interactions that are becoming modulated or disrupted with advancing age as well as on the possible interventions that can prevent or at least postpone these age-related changes.

Graphical abstract

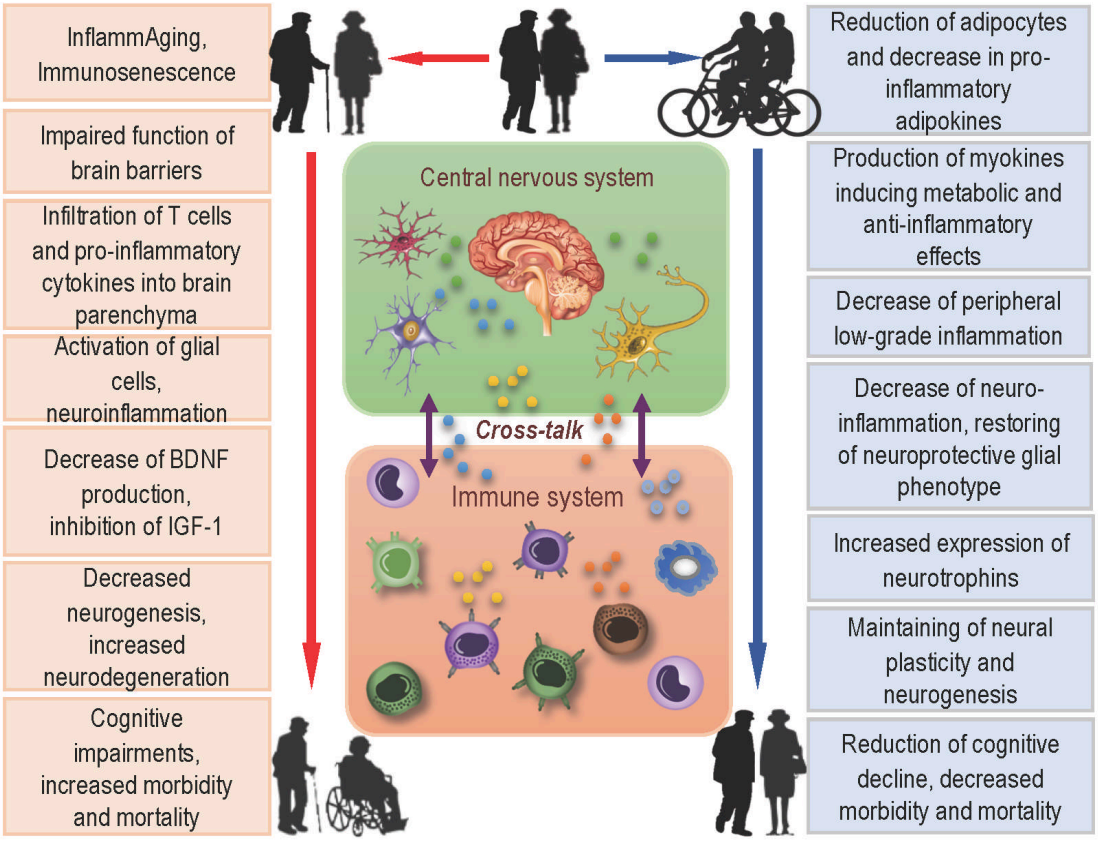


Figure 7. Overview of the mechanisms contributing to brain aging and the mitigating effects of physical activity in elderly people. BDNF: brain-derived neurotrophic factor; IGF: insulin-like growth factor.

In a first step, we have summarized the current findings describing the involvement of the immune system in supporting such important brain processes as neuroprotection, neurogenesis, learning, and memory. We have shown that in a homeostatic brain environment the CNS-specific $CD4^+$ T cells produce cytokines and neurotrophic factors that are able to exert a direct neuroprotective function on the neurons or indirectly stimulate the microglia cells to produce neurotrophins and cytokines that promote neurogenesis, learning, and memory processes (publication 2, Fig. 1).

In the next step, we described the function of three major brain barriers, their role in neuroimmune interactions, and their specific and complex mechanisms that enable these dynamic interactions (publication 2, Fig. 2). In the area of *Pia Arachnoid,* we have also schematically depicted the recently discovered lymphatic vascular system that is thought to be responsible for the transport of immune cells and macromolecules.

In the next section of our review, we describe the processes of immunosenescence and inflammaging and discuss the potential mechanisms responsible for this (publication 2, Fig. 3). Although immune senescence seems to affect both arms of the immune system (adaptive and innate), most of the described age-related changes are manifested in the adaptive immunity. The relatively low numbers of naïve T cells in peripheral circulation, with concurrently increased numbers of late-differentiated memory cells, which exhibit a reduced antigen receptor repertoire, belong to the hallmark of immunosenescence. The central mechanisms involved in these changes include: (i) age-related impairment in the functions of the hematopoietic stem cell compartment, (ii) progressive thymus involution, and (iii) lifelong stimulation with various antigens, whereby a latent infection with CMV plays the most important role in these alterations.

Immunosenescence is often accompanied by inflammatory aging, which is characterized by increased secretion of pro-inflammatory cytokines and is generally considered to be the major contributor to the age-related mortality and morbidity. Immunosenescence, together with inflammatory processes in the peripheral circulation, can also contribute to neuroinflammation in the brain - by modulating microglia cells towards the more pro-inflammatory phenotype. Such activated microglia are characterized by a changed morphological appearance and by losing their neuroprotective function, which can further lead to neuronal dysfunction, increased damage of the brain cells, and neurodegeneration.

The most important immuno- and neuromodulators, which play a decisive role in these processes (publication 2, Fig. 4), are described and characterized in detail in the next review section. These include glial cells, which represent macrophage-like immune cells of the central nervous system. The glial cells can appear in different stages of activation and are accordingly involved in performing of different functions. Their role ranges from the active support of the brain plasticity through the release of various cytokines, neurotransmitters, and neurotrophins, to the pruning of synapses and the removal of cellular waste.

T cells stimulated either in the periphery or in the *choroid plexus* can differentiate into different types of effector cells. Depending on the function and type of cytokines they produce, $CD4^+$ T cells can be separated into Th1 T cells secreting pro-inflammatory cytokines and Th2 T cells producing predominantly anti-inflammatory cytokines. Monocytes, which are able to differentiate either to inflammatory macrophages of the M1- or to anti-inflammatory macrophages of the M2-phenotype (depending on the cytokine environment surrounding them), varying also in their functions.

Cytokines represent, in general, a type of signalling molecules that enable vitally important communications between different cells of the organism. They are not only involved in a con-

stant cross-talk between these cells but also possess immense modulatory abilities that allow them to influence almost all systemic processes in the body. Cytokines, act within complex networks and modulate metabolic and neuro-endocrine interactions, neurotransmitters' and neurotrophins' production, processes of neurogenesis and brain plasticity – all of which can ultimately lead to the modulation of cognitive function and are particularly vulnerable in the elderly.

Peripheral immunosenescence and inflammaging may also lead to functional changes in the immune cells, which produce more inflammatory cytokines and less anti-inflammatory mediators. The consequence of the chronic influence of inflammatory factors may lead to a disruption of the brain barrier functions and permit the unhindered entry of the immune cells and inflammatory mediators into the brain parenchyma (publication 2, Fig. 5). These disorders may induce functional and morphological changes in the glial cells, which develop an activated phenotype. The microglia and astrocytes start to produce more inflammatory cytokines, and also macrophages change their homeostatic M2-phenotype into the inflammatory M1-phenotype. All these alterations can induce a neuroinflammatory environment in the brain, disturb production and release of neurotrophic factors, and eventually impair the neuronal functions.

In the last section of our review, we focus on the overview of the recent literature that describes the impact of the different types of intervention (in both humans and animals) with the aim of maintaining and, in the best case, improving physical and cognitive health in old age. In Figure 6 (publication 2), we schematically summarize in a simplified way the potential effects of physical intervention and its contribution to mitigating neuroimmune senescence and inflammaging. Physical training appears to exert its effects in a multidirectional manner - with most effects being on the function of immune and CNS cells, on muscle and fat tissues, and on cardiovascular and metabolic systems. All these changes seem to induce anti-inflammatory and neuroprotective effects, which together may lead to the maintenance or even enhancement of neuronal plasticity and neurogenesis and to the improvement of cognitive processes in elderly people.

Some intervention studies have also demonstrated positive effects by applying cognitive training programs. Even more, a training design combining physical and cognitive programs showed significantly better results than the separate variants of both training options, suggesting a synergy effect. This hypothesis was confirmed by some cross-sectional, longitudinal, and controlled intervention trials. It could therefore be postulated that physical training could create a balanced and neuroprotective environment (partly by neutralizing neuroinflammation) that is more susceptible to cognitive stimulation. Cognitive training in coopera-

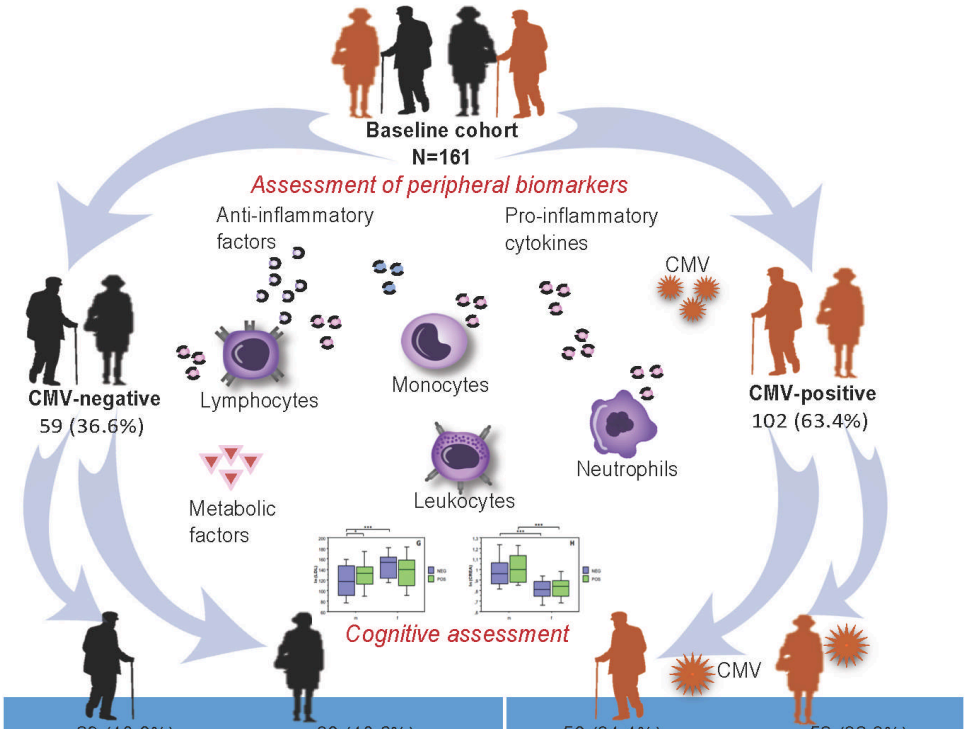
tion with physical activity seems to induce important plastic brain changes that significantly increase the potential of neurogenesis and synaptogenesis.

Based on this hypothesis, the intervention study with healthy elderly participants (AKTIV) was designed to investigate the effects of physical activity (using bicycle ergometers) and cognitive stimulation (by learning a foreign language) as well as its combined effect. Originally, it was planned to limit the investigation of different training options to the assessment of changes in brain structure and behavior. However, as it has been recognized how important it would be, to assess the inflammatory status in participants of different training groups, the study design was supplemented by the assessment of the pro- and anti-inflammatory parameters.

8.3 *Paper III:* The modulatory effect of gender and Cytomegalovirus-seropositivity on circulating inflammatory factors and cognitive performance in elderly Individuals.

Aging is characterized by a chronic increase in the systemic levels of circulating inflammatory cytokines even in seemingly healthy people. The disbalance between pro- and anti-inflammatory equilibrium can be linked to the increase in age-related functional alterations. Cytomegalovirus, operating as a persistent pro-inflammatory driver, may contribute to chronic inflammation, and together with metabolic and other risk factors might represent a causal factor for age-related cognitive impairments – with sexual dimorphism additionally influencing these underlying processes. Very few studies, however, focused on the investigation of the influence of CMV and sex on the cognitive abilities of elderly people and their findings are inconsistent. Therefore, in the present study we pursued four main aims. First, we intended to measure and characterize the baseline inflammatory status of aged individuals recruited for an intervention study of “active aging” before starting the cognitive, physical, and combine training. For this reason, we evaluated the main inflammatory and anti-inflammatory biomarkers, such as circulating cytokines (IL-1β, TNF, IL-6, IL-10, IL-18), receptor antagonist IL-1RA, soluble receptor (sTNF-R), immune cells (lymphocytes, leukocytes, monocytes, neutrophils), and relevant metabolic markers: high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides. Second, we investigated the impact of sex and CMV-seropositivity on the immune and metabolic markers assessed at baseline. Third, we studied the associations among pro- and anti-inflammatory mediators and metabolic factors, and evaluated whether CMV-seropositivity modifies these interactions. Fourth, we investigated the influence of the assessed pro- and anti-inflammatory factors on the measures of cognitive performance, such as fluid intelligence, episodic memory, speed, and working memory, in the context of CMV-serostatus and gender.

Graphical abstract



	TNF#	TNF*	
sTNF-R#			sTNF-R*
			IL-1RA* IL-1RA#
IL-10#		IL-10#	
		IL-18* IL-18#	
	LDL* LDL#	LDL*	
HDL*	HDL#		HDL#
		Trigl* Trigl#	
		Lymphocytes*	Lymphocytes*
Monocytes#		Monocytes#	
Neutrophils*	Neutrophils*		
Fluid intelligence#			
Working memory#			

Figure 8. A schematic illustration of summarized results on the influence of gender and CMV-serostatus on circulating pro- and anti-inflammatory cytokines, receptor antagonist, metabolic factors, immune cells, and cognitive abilities in the baseline cohort of elderly participants. The names of the analyzed parameters with significantly higher values are placed in the corresponding column of the CMV-negative or CMV-positive men and women accordingly, whereby the notation (#) implies: a gender-related-higher value, and the notation (*) implies: a CMV-related-higher value. The pro-inflammatory mediators are written in red; the anti-inflammatory are green; the cognitive latent factors are blue; and immune cells are left in black. TNF: tumor necrosis factor; sTNF-R: soluble tumor necrosis factor receptor; IL: interleukin; IL-1β: interleukin 1 beta; IL-1RA: interleukin 1 receptor antagonist; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

Results and discussion

In a first step, we investigated the influence of sex and CMV-serostatus on circulating pro- and anti-inflammatory mediators, immune cells and metabolic factors using MANOVA (multivariate ANOVAs), and, in the next step, we additionally applied bootstrapping analysis (publication 3, Figs. 2-3). The results obtained by these different statistical methods appears to be mostly consistent and complement each other.

In short, the analyses showed that both sex and CMV-seropositivity played a pronounced modulatory role by influencing in individual or/and combine action circulating peripheral pro- and anti-inflammatory biomarkers in older participants. As for inflammatory TNF, we were able to determine both a gender-specific influence and an effect of the CMV-infection. In the CMV-seronegative group, female participants showed a significantly elevated level of TNF compared to men. Furthermore, the concentration of TNF in the CMV-seropositive group of male participants was significantly increased compared to CMV-seronegative males (publication 3, Fig. 3).

In contrast, the anti-inflammatory soluble receptor sTNF-R showed elevated levels in CMV-seropositive women compared to non-infected female participants. In addition, the concentration of sTNF-R in men was significantly higher compared to women in the CMV-seronegative group. While the analysis of pro-inflammatory IL-1β showed no significant differences between the groups, its receptor antagonist, IL-1RA, experienced both a modulating effect of CMV and an influence of gender. The IL-1RA levels were significantly higher in CMV-seropositive women compared to CMV-seronegative female participants. In addition, women had a higher concentration of this inhibitory receptor than men in the CMV-seropositive group (publication 3, Fig. 3).

The analysis of the pro-inflammatory IL-18 revealed both gender-specific and CMV-related differences. Cytokine levels were significantly higher in men compared to women in the CMV-seropositive group. In addition, the IL-18 concentration of CMV-seropositive males was increased compared to CMV-seronegative males. The study of anti-inflammatory IL-10 showed the influence of gender (male > female) in both CMV-seronegative and CMV-seropositive groups.

We can speculate that the gender-specific difference, where CMV-positive men demonstrated a significantly lower level of anti-inflammatory IL-1RA compared to their female counterparts, was probably responsible for the increased levels of pro-inflammatory IL-18 in their circulation, as IL-1RA is known for its ability to attenuate the effects of inflammatory cytokines [28,115-117].

In general, there are only few and rather controversial reports on the investigations of gender-specific differences in the cytokine profile of older individuals [118,119]. One study provided *in vitro* evidence that testosterone was able to suppress the production of the pro-inflammatory cytokines TNF, IL-1β, and IL-6 and to amplify the release of the anti-inflammatory cytokine IL-10 [120]. Our results are partly in line with these finding. We observed in the CMV-negative male group lower levels of TNF and detected higher levels of IL-10 in both CMV-positive and -negative groups compared to their female counterparts. But interestingly, infection with CMV however, appears to compromise this positive effect of testosterone – the CMV-positive male subjects displayed less of anti-inflammatory sTNF-R and IL-1RA in their blood, but significantly higher levels of pro-inflammatory TNF and IL-18 compared to non-infected males.

In our earlier publication, we found that both the chronic presence of CMV and the stress of the necessity for a continuous monitoring of latent CMV-infection can modulate the differentiation status of immune cells [2]. In this case and with regard to the cytokines, a similar explanation can be applied, because it is precisely these CMV-exhausted, late-differentiated immune cells that may secrete pro-inflammatory cytokines, which, in turn, contribute to immunosenescence and may promote age-related functional changes through complementary chronic inflammations [121]. The CMV-modulating influence on ageing immune cells (e.g., increased levels of lymphocytes and reduced numbers of neutrophils in CMV-infected participants) and also gender-specific differences (e.g., men showing a higher proportion of monocytes than women) once again emphasized in our cohort the fact known from publications that these cells can trigger inflammation and thus may promote "unhealthy" ageing [115].

The fact that, on the one hand, CMV-positive women exhibit elevated anti-inflammatory factors compared to non-infected subjects and, on the other hand, women in the CMV-negative group showed a higher pro-inflammatory status compared to men (publication 3, Fig. 6) is really remarkable. These results can also be attributed to both the historical coexistence of the individuals with CMV [122,123] and to the gender-specific differences in immune responses in previous years [97,124]. It is a well-known fact that the immune responses of men are different and less strong than those of women. For this reason, it may be assumed that the primary immune response to CMV-infection in both sexes at a young age may have a quite different course of actions. These differences, together with other factors, may later contribute to the induction of a pro-inflammatory environment in older male subjects, whereas in the postmenopausal women it may lead to the production of anti-inflammatory factors arising in response to pro-inflammatory cytokines.

Also, lipoproteins can be negatively influenced by inflammatory cytokines, which in turn can modulate the production of pro-inflammatory cytokines. We found elevated levels of HDL in

CMV-seronegative compared to CMV-seropositive males. In addition, women showed a significantly higher concentration of HDL than men, regardless of their CMV-status. Interestingly, CMV-seropositive males displayed significantly higher LDL levels compared to CMV-seronegative males, while women showed an inverse picture. In addition, there were gender-specific differences in the CMV-seronegative group of subjects, with women having significantly higher LDL values compared to men.

In general, HDL possesses strong anti-inflammatory properties and the remarkable ability to modulate the inflammatory response in different cell types. In a chronic inflammatory environment, however, HDL may itself become dysfunctional [125] and thus not relieve cells of excessive and oxidized LDL cholesterol. The most frequent consequences of chronic inflammatory conditions are declines in serum HDL and elevations of triglycerides, total cholesterol, and LDL [126]. We found similar effects of increased LDL and triglyceride concentrations, but reduced HDL levels in the inflammatory environments of CMV-negative women and CMV-positive men (publication 3, Fig. 6). In addition, it also appears that both CMV-seropositivity and sex in combination (an increase in LDL in CMV-seronegative women and an increase in triglycerides in CMV-positive men) and separately (a decrease in HDL in CMV-seropositive men) contributed to these effects.

Findings from animal experiments showed that inflammatory cytokines, such as TNF, IL-1β, and IL-6, significantly increased serum concentrations of triglyceride fatty acid [127,128]. We have also observed a positive correlation between the proinflammatory IL-6 levels and triglyceride concentrations, but only among CMV-positive participants (publication 3, Fig. 4). Moreover, our results also demonstrate several significant associations of HDL- and LDL-cholesterols with pro- and anti-inflammatory cytokines and their receptors (publication 3, Fig. 4). Furthermore, the magnitude of these associations appears to be altered by CMV-infection. Moreover, the inflammatory environment in the groups with higher concentrations of serum HDL seemed to be less pronounced (publication 3, Fig. 6). But it is obvious that more research with different pro- and anti-inflammatory biomarkers and their modulators is needed, to better elucidate these complex and dynamic interactions and their implications for inflammaging and immunosenescence.

We investigated the influence of CMV-infection and the impact of sex on the cognitive performance of older people and found pronounced gender-specific effects on fluid intelligence and on working memory. Males had significantly higher performance values in these domains compared to females, but only in the CMV-seronegative participant group (publication 3, Fig. 5). No influence of CMV or sex was found for processing speed and episodic memory in any of the tested groups. Although some literature describes the modest influence of sex on cognitive performance [3,129-131] and also attributes this impact with different mechanisms

(e.g., to levels of sexual hormones, or/and sexual dimorphism in the brain structure), it does not take into consideration the modulating effect of CMV-infection.

In our investigations, however, the group of CMV-positive males showed no advantageous differences in cognitive performance compared to CMV-positive women. Both these groups were found to have a relative adverse inflammatory environment in their peripheral circulation (publication 3, Fig. 6). Therefore, our results suggest that an environment with increased inflammatory factors (high TNF and IL-18 concentrations), a comparatively unfavorable metabolic status (elevated LDL cholesterol and triglycerides), and high percentages of monocytes and lymphocytes in the peripheral circulation (e.g., of CMV-positive males vs. CMV-negative males) may possibly induce a state of persistent low-grade peripheral inflammation, that may contribute to inflammatory conditions in the central nervous system and thus, to impairments in cognitive functioning [3,132,133].

Our study has shown negative correlations of fluid intelligence as well as episodic and working memory with pro-inflammatory TNF in CMV-negative individuals (publication 3, Table 1). It is known that TNF may elicit in the nervous system both physiological neuroprotective as well as pathological neurodegenerative effects [134]. On the one hand, the cognitive deficits have been found in transgenic mice overexpressing TNF [135]. On the other hand, TNF and IL-1β revealed that they were able to physiologically modulate synaptic plasticity and synaptic scaling in several brain regions such as the hippocampus, striatum and cortex [136,137].

In the CMV-negative group we have detected a positive association of episodic memory with anti-inflammatory IL-10, which is generally known to play an inhibitory role on the production of inflammatory cytokines by microglia and for its neuroprotective function on neurons and astrocytes [138].

Remarkably, in the CMV-seropositive group, fluid intelligence, and episodic- and working-memory scores correlated negatively with the anti-inflammatory IL-1RA (publication 3, Table 1), whose values were seemingly concomitantly raised as a reaction to the elevations of pro-inflammatory cytokines in their periphery. Similar findings were reported by other groups [139,140], who observed that persons with high levels of inflammatory markers also tend to show increased levels of anti-inflammatory factors. We have also found in the CMV-positive group a negative correlation of pro-inflammatory IL-6 with episodic memory and fluid intelligence. A number of pro-inflammatory mediators, such as cytokines IL-6, IL-1β and TNF, were identified as being associated with cognitive impairments [139,141,142], therefore our findings on the negative association of cognitive functioning with inflammatory cytokines are congruent with these results.

In our study of the interrelations between different inflammatory markers, we also observed that CMV-latency affected the relationships between various inflammatory mediators, potentially promoting the induction of these CMV-related inflammatory environments. In other words, CMV infection appears not only to shift the levels of individual cytokines but also to alter the relationships between different immune mediators and molecules. Considering the exploratory nature of the study on these associations, further investigations are needed to elucidate these associations and the changed interactions among multiple biomarkers under the modulatory influence of CMV-infection.

Thus, we can conclude that CMV-latency can trigger a variety of modulatory effects on the inflammatory and immune factors and their relationships in the peripheral circulation of elderly people. These modulatory effects may have different outcomes for older men and women and may therefore have different functional and cognitive consequences. For this reason, comparative and interventional studies with older people should always consider both CMV-serostatus and sex dimorphism together with other factors.

8.4 *Paper IV:* Network topology dynamics of circulating biomarkers and cognitive performance in older Cytomegalovirus-seropositive or -negative men and women.

It is known that our immune system is not functionally isolated but depends on interactions with other physiological systems in the organism. These systems communicate with each other within complex networks through cytokines, receptor molecules, hormones, neuropeptides, and metabolic and neurotrophic factors [143,144]. With advancing age, however, impairments in this communication can facilitate inflammatory environments, which may range from a disturbance of homeostasis towards to pathological conditions [4,17,27]. The importance of pro-inflammatory and anti-inflammatory homeostasis for cognitive health in old age and the decisive role of various inflammatory cytokines in neuroimmune communications have been repeatedly described [134,138,145-147]. At the same time, it is also known that a latent CMV-infection and the related chronic immune activity can modify the mode of action and function of inflammatory factors [4,148].

The aim of our present work was to quantitatively describe multiple interactions between different cytokines, receptor molecules, metabolic and neurotrophic factors, hormones, immune cells and measurements of cognitive performance using a graph-theoretical approach. This enabled us not only to visualize biologically meaningful interconnections between the nodes of different variables, but also, for the first time, to statistically compare the network topology metrics between different groups of CMV-seronegative and -positive men and women.

Graphical abstract

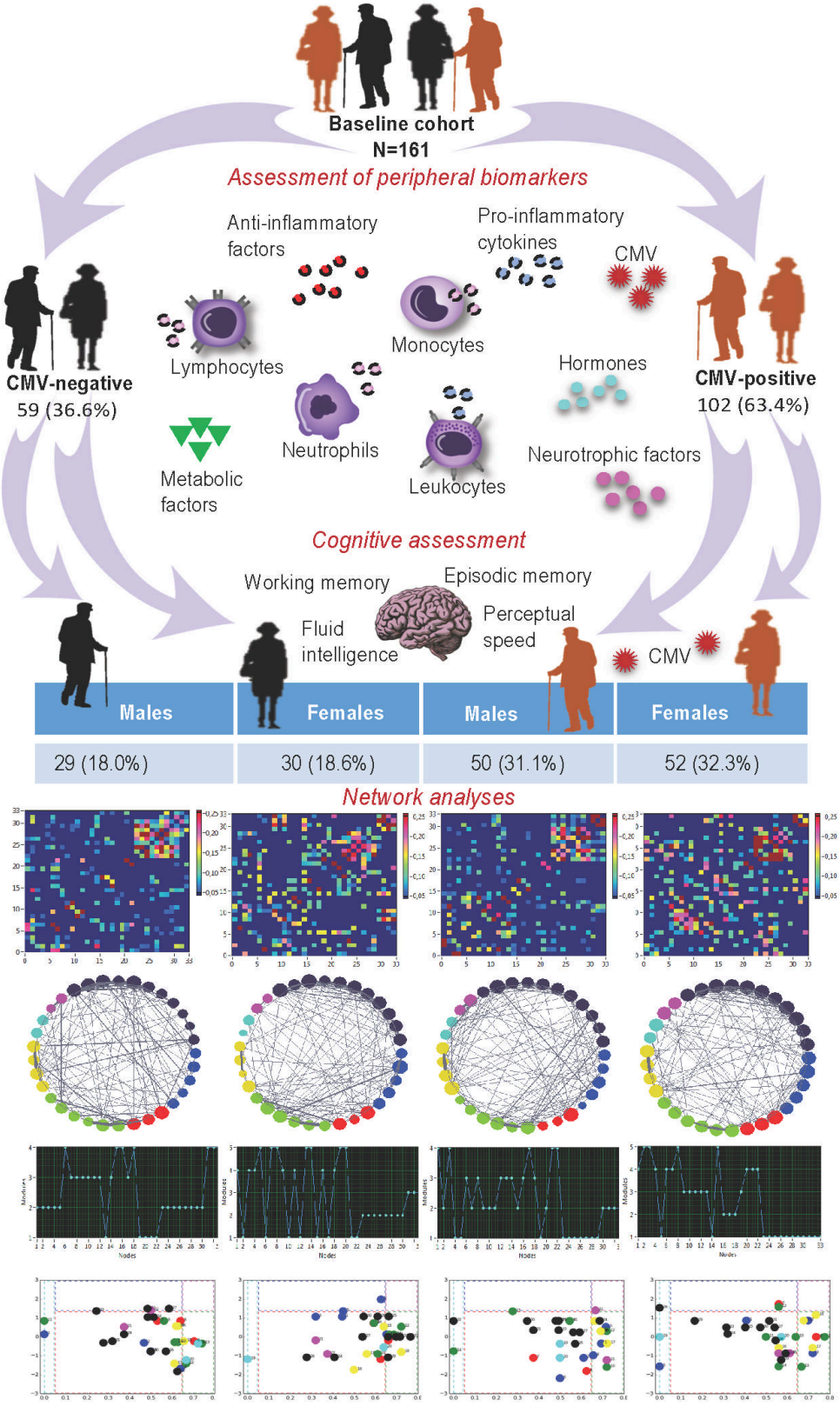


Figure 9. Graphical abstract for study on network topology dynamics of circulating inflammatory markers and cognitive performance in CMV-seropositive and –negative men and women at baseline prior to six-months training intervention. CMV: Cytomegalovirus.

Results and discussion

The examination of the network patterns revealed that the connectivity matrices had a group-specific structure in all four participant groups (manuscript 4, Fig. 3A). The calculation of the network strengths as the sum of the connections of each individual node showed that cognitive nodes had high strengths, which appear to be mainly due to the strong connections between the cognitive nodes themselves, especially in the female groups (manuscript 4, Fig. 3B). In the male groups, cognitive nodes are also strongly linked to other variables such as cytokines (CMV-negative group), metabolic factors (CMV-positive group), and immune cells (manuscript 4, Fig. 4). The CMV-seronegative male group showed several strong connections between nodes of pro-inflammatory cytokines (IL-1β, TNF, IL-18) and cognitive nodes (episodic memory and fluid intelligence). Currently available evidence suggests that pro-inflammatory cytokines can have a dose-dependent physiological neuroprotective effect, but, under certain circumstances, for example, at elevated concentrations, can also mediate pathological neurodegenerative effects [134]. Both IL-1β and TNF have been shown to have such a dual function, in which they can act both as pro-inflammatory factors on the one hand, leading to neuroinflammation, and as neuromodulators on the other, influencing cognitive processes in a positive way [145,146].

Less strong, but still numerous, connections were found in the CMV-seronegative male group between nodes of anti-inflammatory cytokines and cognition (manuscript 4, Fig. 4). This is partly consistent with our previous findings on the positive association of episodic memory with anti-inflammatory cytokine IL-10 in older men and women in the CMV-seronegative group [3]. It is known that IL-10 can play a neuroprotective role due to its inhibitory effect on inflamed microglia [138]. The same CMV-negative male group also showed significantly increased levels of anti-inflammatory IL-10 and sTNF-R and reduced levels of pro-inflammatory cytokines in their peripheral circulation, as reported in our recent study [3]. In the light of this information, we can speculate that strong connections between cognitive nodes and the nodes of (low concentration) pro-inflammatory cytokines, on the one hand, and numerous connections of cognition to the nodes of (high concentration) anti-inflammatory cytokines, on the other hand, might explain the cognitive advantage in fluid intelligence and working memory found for this group of participants in our previous work [3]. Interestingly, this was the only group in which pro- and anti-inflammatory cytokines showed no direct connections to each other (manuscript 4, Fig. 4). The other three groups (two of which were CMV-seronegative women and CMV-seropositive men, characterized in our previous study by heterogeneous unbalanced pro-inflammatory and anti-inflammatory mediators and an adverse metabolic environment), showed various more or less strong connections between pro-

inflammatory and anti-inflammatory cytokines. These numerous interactions were probably important and necessary homeostatic responses to these imbalanced peripheral conditions.

In our previous study, the group of CMV-seropositive women (showing multiple connections between nodes of pro- and anti-inflammatory cytokines in the present study) demonstrated significantly higher values of the anti-inflammatory factors sTNF-R and IL-1RA. We also previously found that, in the CMV-seropositive group, fluid intelligence and episodic and working memory were negatively associated with the anti-inflammatory factor IL-1RA, which was thought to be simultaneously elevated in response to the increase in pro-inflammatory cytokines in the periphery [3] - the phenomenon that has also been reported by other researchers. They supposed [140,149] that individuals with high levels of pro-inflammatory cytokines also apparently tend to show an increased proportion of anti-inflammatory factors. The network analyses in the present study made it possible, to visualize these diverse and mutual connections between pro- and anti-inflammatory biomarkers, which were only assumed in our previous work [3].

It is known that pro-inflammatory cytokines are involved in dynamic interactions with the major neurotrophic factor IGF (insulin-like growth factor)-1 and its regulator IGFBP (IGF-binding protein)-3 by reducing IGF-1 signaling and increasing IGFBP-3 production. Conversely, IGF-1 is able to inhibit pro-inflammatory cytokine production by increasing anti-inflammatory IL-10 secretion [150-152]. Our results show that both IGF-1 and IGFBP-3 have relatively strong links to metabolic nodes in CMV-seronegative males, but only a weak link to CRP. In contrast, all three other networks showed multiple connections to pro- and anti-inflammatory cytokines, possibly due to their involvement in dynamic interactions aimed at balancing the pro- and anti-inflammatory equilibrium (manuscript 4, Fig. 4).

Regarding the connections between neurotrophins and cognitive nodes, there were few connections in CMV-seronegative and -positive men and only one connection in CMV-seronegative and -positive women. There is substantial evidence that IGF-1 deficiency is often seen in older people and might represent a contributory factor to reduced cognitive ability [153,154]. Thus, we can speculate that the relatively low number of connections between neurotrophins and cognitive nodes, as seen in all four networks, may be due to the general age-related decrease of these neurotrophic factors in the peripheral circulation of older participants.

Interestingly, the network of CMV-seronegative men showed some direct connections between Dehydroepiandrosterone (DHEA) and cognitive nodes but also to the nodes of anti-inflammatory and metabolic factors. In general, it is known that inflammatory reactions are influenced by various mechanisms, including neuroendocrine interactions. Both DHEA and

cortisol represent multifunctional adrenocortical hormones with such immunomodulatory properties. They exert strong and broad influences on the body and brain and together influence a multitude of processes associated with metabolic, immune and cognitive functions [155]. Interestingly, nodes of both cortisol and DHEA in the CMV-seronegative males represent non-hub connectors exhibiting numerous links to diverse modules in the modular organization of the network. This indicates that these nodes play a crucial role in the communication between different subsystems. Inverse correlations between DHEA concentrations and neuroinflammatory-related diseases have repeatedly been found in the elderly [132,133,154,155]. Similar to DHEA, the cortisol nodes in our study also demonstrated a very heterogeneous and group-specific picture regarding their connections (manuscript 4, Fig. 4). This may be partly due to the fact that being typically immunosuppressive, cortisol may induce (under certain concentrations and in a delayed-response fashion) a delayed systemic augmentation of inflammation [156], contributing to a further complexity in interpreting of these already complex interactions.

Using graph-theoretical approach, we analyzed and compared the network topology metrics between different groups of participants (manuscript 4, Fig. 5). Modularity analyses revealed that all four networks of the CMV-seropositive and -negative men and women demonstrated a highly differentiated modular organization (manuscript 4, Fig. 6). All four networks represented so-called “small-world networks” (SWNs) at all levels of wiring costs and were identified as SWNs with more random characteristics (manuscript 4, Fig. 7). Interestingly, the network of CMV-seronegative men contains more hub nodes but fewer connector nodes, compared to the other three groups. This indicates that modules in this group are more autonomous and the information flow between the modules may be realized through a small number of connector nodes. Interestingly, three of the four hubs are cognitive variables and the fourth one is IGFBP-3. Thus, cognitive nodes, such as fluid intelligence, working memory, and perceptual speed play a central role in the network of CMV-negative male participants, driving or controlling the connections within the corresponding modules.

In this work, we also investigated the integration and segregation properties of the individual networks of the CMV-seropositive and -negative men and women by applying such network topology measures as a clustering coefficient, a characteristic path length, and local and global efficiency (manuscript 4, Fig. 5). We compared network topology dynamics and found that a mean clustering coefficient was the highest and CPL shortest in the network of the CMV-seronegative males. Additionally, this network is also characterized through the highest local and global efficiency, allowing it to be identified as the network with optimal features of segregation and integration (manuscript 4, Fig. 5A). In our previous study, the same group of participants displayed the most balanced inflammatory status in their peripheral circulation (with low levels of pro-inflammatory cytokines and high levels of anti-inflammatory bi-

omarkers) as well as significantly higher cognitive performance in working memory and fluid intelligence [3], suggesting that highly integrated and segregated networks may have optimal neuroimmune interactions. Further studies, however, are required to confirm these findings and to better understand such complex relationships and network topology changes in older CMV-seropositive and -negative men and women.

9 Concluding remarks, studies' limitations, and future directions

Data obtained in both BASE-II and AKTIV studies have clearly suggested that age, sex, and CMV-serostatus influence the peripheral circulating biomarkers and functional abilities of aged people. In summary, the research presented in this dissertation contributes to the investigation of age-related functional alterations of immune and related physiological functions in two major ways. First, it has extended the design and introduced novel analysis methods that have been used to investigate markers of immunosenescence. Second, it has generated new findings on the modulatory effects of CMV-latency and sex on multiple peripheral biomarkers and cognitive function in older men and women.

Results obtained from a subgroup of BASE-II participants have revealed significant cross-sectional age-associated differences of T-cell subset distribution in a representative German urban population and demonstrated the impact of both sex and CMV-infection on T-cell naïve and memory phenotypes, but unaffected frequencies of T-stem cell-like memory cells. To the best of our knowledge, this was the first study, in which the frequency of the TSCM phenotype and of PD-1$^+$ T cells in peripheral circulation has been investigated, and the effects of age, CMV-serostatus, and sex on their frequency have been examined. Certainly, further analyses on the whole BASE-II cohort of 2200 participants are needed to confirm and to generalize these results. Although the subgroup of participants was selected on the basis of a distribution similar to the whole cohort, the sample size may have been too small, particularly after a further subdivision into subgroups, based on the differences in age, sex and CMV-seropositivity. Additionally, differences in the number of CMV-seronegative young subjects compared to CMV-seropositive participants may potentially limit the power to identify some significant associations and might affect our results. Due to the multidisciplinary nature of the BASE-II study, future investigations will allow to search for associations of immunologic parameters with the health and socio-economic status of the subjects, their genetic background, and behavioral, and psychological characteristics. This will permit the investigation of the influence of these parameters on the immune function, and *vice versa*, to better understand their complex relationships and the age-related alterations occurring in the process of aging.

Data obtained from a baseline cohort of the interventional AKTIV study with healthy elderly participants have revealed that CMV-latency may induce various modulatory effects on the inflammatory and immune factors in the circulation of aged men and women. This was the first study to extensively characterize inflammatory and functional status in elderly participants by assessing various pro- and anti-inflammatory cytokines, receptor antagonists, soluble receptors, metabolic factors, immune cells, and multiple measures of objective cognitive function at baseline prior to physical, cognitive, and combine interventions. We found that not only both sex and CMV-seropositivity influence levels of circulating peripheral biomarkers, but also that CMV-infection modifies the observed associations among the latter. Moreover, we detected an interaction between CMV-serostatus and sex associations with cognitive abilities: Sex differences in fluid intelligence and working memory were found only in the CMV-negative participants. It was revealed that in CMV-seronegative individuals fluid intelligence, episodic memory, and working memory correlated negatively with pro-inflammatory TNF levels; and episodic memory correlated positively with anti-inflammatory IL-10 levels. In CMV-seropositive participants, episodic memory and fluid intelligence correlated negatively with pro-inflammatory IL-6; and episodic memory, fluid intelligence, and working memory correlated negatively with anti-inflammatory IL-1RA. We conclude that both CMV-serostatus and sex may modulate neuroimmune factors, cognitive performance and the relationship between the two domains and should therefore be considered in comparative and interventional studies with elderly people.

There are also several other limitations for this study that should be acknowledged. The first is related to the fact that our pre-training cohort contained comparatively healthy, non-obese, and well-educated Berlin citizens with a relatively low CMV seroprevalence. Therefore, the generalizability of some of our findings may be restricted to the Berlin healthy aging population or to similar European populations in urban areas. Another limitation is one that is also frequently reported in studies by others, namely that cytokines such as IL-1β, TNF, and IL-6 are not very abundant in the circulation of relatively healthy non-obese people, and thus the quantities of these cytokines may be tending towards the lower end or below the levels of detection for these assays. Hence, a technical limitation may be the sensitivity of the assays used for cytokine detection. The commonly applied quantification of cytokine concentrations using the ELISA technique may occasionally not be sensitive enough, also due to potential inhibition by some biological agents naturally present in blood. Also, multiplex techniques (and even the CBA Enhanced Sensitivity Flex Set used in our study) are primarily designed for a simultaneous assessment of numerous analytes, and, therefore, compromises made for the individual analytes may be unavoidable. Despite these limitations, however, results obtained in the present study for most of the pro- and anti-inflammatory cytokines and other factors related to low-grade inflammation are quite consistent.

In the next study included in this dissertation, a new strategy was designed by means of a graph-theoretical approach that allowed the quantitative investigation of multiple interactions between different cytokines, receptor molecules, metabolic and neurotrophic factors, hormones, immune cells, and measures of cognitive performance. To the best of our knowledge, simultaneous network analyses of multiple inflammation-related mediators and cognitive performance in older CMV-seropositive and CMV-seronegative men and women were not previously accomplished. We were able not only to visualize biologically meaningful interconnections between various nodes, but also to compare the network topology dynamics between different groups in a statistically sound manner. The examination of separate nodes in the networks showed that these network topology differences were particularly evident for cytokines and cognitive nodes. We investigated the segregation and integration properties of the individual networks of CMV-seropositive and -negative older men and women by applying such network topology measures as a clustering coefficient, characteristic path length, local and global efficiency. Using the rewiring procedure, we found that the mean clustering coefficient was highest and characteristic path length shortest in the network of the CMV-seronegative males. The same network also manifested the highest local and global efficiency, allowing it to be identified as the network with optimal features of segregation and integration. We conclude that analyses of network topology dynamics provide decisive information about interactions between various circulating pro- and anti-inflammatory biomarkers, immune cells, and measures of cognitive performance and can be in general applied for analyzing interactions between different physiological systems and subsystems.

Due to the exploratory character of our study of the network patterns and their relationships, we are well aware that our choice of variables in the present study, selected on the basis on their involvement in the known age-associated functional alterations in the immune, nervous, and other central physiological systems, does not necessarily cover all potential actors. Therefore, we need further and more extended network analyses to obtain a comprehensive picture on their dynamic interactions. Thus, further studies are required to confirm these findings and to better understand such complex relationships and network topology differences between various groups of older CMV-seropositive and -negative men and women.

Moreover, it is anticipated that the graph-theoretical approach, that has been successfully established for the investigation of the multifunctional networks in the AKTIV-study participants at baseline, will be useful for future analyses of the results obtained after six months of training intervention. By using the advantages of this tool for investigating complex physiological interactions, it should be possible to both analyze the outcomes of the individual training program within each interventional group and to compare training effects between different interventional groups under consideration of CMV-latency and sexual dimorphism.

10 References

1. Müller L, Fülöp T, Pawelec G. Immunosenescence in vertebrates and invertebrates. *Immun Ageing* 2013; **10**(1): 12.

2. Di Benedetto S, Derhovanessian E, Steinhagen-Thiessen E, Goldeck D, Muller L, Pawelec G. Impact of age, sex and CMV-infection on peripheral T cell phenotypes: results from the Berlin BASE-II Study. *Biogerontology* 2015; **16**(5): 631-43.

3. Di Benedetto S, Gaetjen M, Muller L. The Modulatory Effect of Gender and Cytomegalovirus-Seropositivity on Circulating Inflammatory Factors and Cognitive Performance in Elderly Individuals. *International journal of molecular sciences* 2019; **20**(4).

4. Di Benedetto S, Müller L, Wenger E, Duzel S, Pawelec G. Contribution of neuroinflammation and immunity to brain aging and the mitigating effects of physical and cognitive interventions. *Neuroscience and biobehavioral reviews* 2017; **75**: 114-28.

5. Müller L, Di Benedetto S, Pawelec G. The Immune System and Its Dysregulation with Aging. *Subcell Biochem* 2019; **91**: 21-43.

6. Müller L, Di Benedetto S, Pawelec G. Human Immune System in Aging. In: Gu D, Dupre ME, eds. Encyclopedia of Gerontology and Population Aging. Cham: Springer International Publishing; 2019: 1-12.

7. Konieczny J, Arranz L. Updates on Old and Weary Haematopoiesis. *International journal of molecular sciences* 2018; **19**(9).

8. Notta F, Zandi S, Takayama N, et al. Distinct routes of lineage development reshape the human blood hierarchy across ontogeny. *Science* 2016; **351**(6269): aab2116.

9. Pietras EM, Reynaud D, Kang YA, et al. Functionally Distinct Subsets of Lineage-Biased Multipotent Progenitors Control Blood Production in Normal and Regenerative Conditions. *Cell Stem Cell* 2015; **17**(1): 35-46.

10. Compston JE. Bone marrow and bone: a functional unit. *J Endocrinol* 2002; **173**(3): 387-94.

11. Gruver AL, Hudson LL, Sempowski GD. Immunosenescence of ageing. *J Pathol* 2007; **211**(2): 144-56.

12. Crane GM, Jeffery E, Morrison SJ. Adult haematopoietic stem cell niches. *Nature reviews Immunology* 2017; **17**(9): 573-90.

13. Dykstra B, de Haan G. Hematopoietic stem cell aging and self-renewal. *Cell Tissue Res* 2008; **331**(1): 91-101.

14. Warren LA, Rossi DJ. Stem cells and aging in the hematopoietic system. *Mechanisms of ageing and development* 2009; **130**(1-2): 46-53.

15. Geiger H, de Haan G, Florian MC. The ageing haematopoietic stem cell compartment. *Nature reviews Immunology* 2013; **13**(5): 376-89.

16. Jasiulionis MG. Abnormal Epigenetic Regulation of Immune System during Aging. *Frontiers in immunology* 2018; **9**: 197.

17. Müller L, Pawelec G. As we age: Does slippage of quality control in the immune system lead to collateral damage? *Ageing research reviews* 2015; **23**(Pt A): 116-23.

18. Yan F, Mo X, Liu J, Ye S, Zeng X, Chen D. Thymic function in the regulation of T cells, and molecular mechanisms underlying the modulation of cytokines and stress signaling (Review). *Mol Med Rep* 2017; **16**(5): 7175-84.

19. Hakim FT, Gress RE. Reconstitution of the lymphocyte compartment after lymphocyte depletion: a key issue in clinical immunology. *Eur J Immunol* 2005; **35**(11): 3099-102.

20. Aspinall R, Pitts D, Lapenna A, Mitchell W. Immunity in the elderly: the role of the thymus. *Journal of comparative pathology* 2010; **142 Suppl 1**: S111-5.

21. Kugelberg E. Immunometabolism: Unravelling the puzzle to longevity and immunity. *Nature reviews Immunology* 2016; **16**(2): 74-5.

22. Sempowski GD, Hale LP, Sundy JS, et al. Leukemia inhibitory factor, oncostatin M, IL-6, and stem cell factor mRNA expression in human thymus increases with age and is associated with thymic atrophy. *Journal of immunology* 2000; **164**(4): 2180-7.

23. Lynch HE, Goldberg GL, Chidgey A, Van den Brink MR, Boyd R, Sempowski GD. Thymic involution and immune reconstitution. *Trends in immunology* 2009; **30**(7): 366-73.

24. Gui J, Mustachio LM, Su DM, Craig RW. Thymus Size and Age-related Thymic Involution: Early Programming, Sexual Dimorphism, Progenitors and Stroma. *Aging and disease* 2012; **3**(3): 280-90.

25. Appay V, Sauce D, Prelog M. The role of the thymus in immunosenescence: lessons from the study of thymectomized individuals. *Aging* 2010; **2**(2): 78-81.

26. Sauce D, Appay V. Altered thymic activity in early life: how does it affect the immune system in young adults? *Current opinion in immunology* 2011; **23**(4): 543-8.

27. Müller L, Pawelec G. Aging and immunity - impact of behavioral intervention. *Brain, behavior, and immunity* 2014; **39**: 8-22.

28. Shaw AC, Joshi S, Greenwood H, Panda A, Lord JM. Aging of the innate immune system. *Current opinion in immunology* 2010; **22**(4): 507-13.

29. Fulop T, Larbi A, Douziech N, et al. Signal transduction and functional changes in neutrophils with aging. *Aging cell* 2004; **3**(4): 217-26.

30. Oishi Y, Manabe I. Macrophages in age-related chronic inflammatory diseases. *NPJ Aging Mech Dis* 2016; **2**: 16018.

31. Derhovanessian E, Solana R, Larbi A, Pawelec G. Immunity, ageing and cancer. *Immunity & ageing : I & A* 2008; **5**: 11.

32. Keller R. The macrophage response to infectious agents: mechanisms of macrophage activation and tumour cell killing. *Research in immunology* 1993; **144**(4): 271-3; discussion 94-8.

33. Fernandez-Morera JL, Calvanese V, Rodriguez-Rodero S, Menendez-Torre E, Fraga MF. Epigenetic regulation of the immune system in health and disease. *Tissue antigens* 2010; **76**(6): 431-9.

34. Gonzalo S. Epigenetic alterations in aging. *Journal of applied physiology* 2010; **109**(2): 586-97.

35. Della Bella S, Bierti L, Presicce P, et al. Peripheral blood dendritic cells and monocytes are differently regulated in the elderly. *Clinical immunology* 2007; **122**(2): 220-8.

36. Manser AR, Uhrberg M. Age-related changes in natural killer cell repertoires: impact on NK cell function and immune surveillance. *Cancer Immunol Immunother* 2016; **65**(4): 417-26.

37. Solana C, Tarazona R, Solana R. Immunosenescence of Natural Killer Cells, Inflammation, and Alzheimer's Disease. *Int J Alzheimers Dis* 2018; **2018**: 3128758.

38. Cooper MD. 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: evolution of adaptive immunity in vertebrates. *Clinical and experimental immunology* 2010; **160**(1): 58-61.

39. Colonna-Romano G, Bulati M, Aquino A, et al. B cell immunosenescence in the elderly and in centenarians. *Rejuvenation research* 2008; **11**(2): 433-9.

40. Ademokun A, Wu YC, Dunn-Walters D. The ageing B cell population: composition and function. *Biogerontology* 2010; **11**(2): 125-37.

41. Dewan SK, Zheng SB, Xia SJ, Bill K. Senescent remodeling of the immune system and its contribution to the predisposition of the elderly to infections. *Chinese medical journal* 2012; **125**(18): 3325-31.

42. Shanley DP, Aw D, Manley NR, Palmer DB. An evolutionary perspective on the mechanisms of immunosenescence. *Trends in immunology* 2009; **30**(7): 374-81.

43. Ferrando-Martinez S, Ruiz-Mateos E, Hernandez A, et al. Age-related deregulation of naive T cell homeostasis in elderly humans. *Age* 2011; **33**(2): 197-207.

44. Weiskopf D, Weinberger B, Grubeck-Loebenstein B. The aging of the immune system. *Transplant international : official journal of the European Society for Organ Transplantation* 2009; **22**(11): 1041-50.

45. Calvanese V, Lara E, Kahn A, Fraga MF. The role of epigenetics in aging and age-related diseases. *Ageing research reviews* 2009; **8**(4): 268-76.

46. Liu K, Catalfamo M, Li Y, Henkart PA, Weng NP. IL-15 mimics T cell receptor crosslinking in the induction of cellular proliferation, gene expression, and cytotoxicity in CD8+ memory T cells. *Proceedings of the National Academy of Sciences of the United States of America* 2002; **99**(9): 6192-7.

47. Larbi A, Franceschi C, Mazzatti D, Solana R, Wikby A, Pawelec G. Aging of the immune system as a prognostic factor for human longevity. *Physiology* 2008; **23**: 64-74.

48. Solana R, Pawelec G, Tarazona R. Aging and innate immunity. *Immunity* 2006; **24**(5): 491-4.

49. Fulop T, Larbi A, Pawelec G. Human T Cell Aging and the Impact of Persistent Viral Infections. *Frontiers in immunology* 2013; **4**: 271.

50. Pawelec G, Derhovanessian E. Role of CMV in immune senescence. *Virus research* 2011; **157**(2): 175-9.

51. Solana R, Tarazona R, Aiello AE, et al. CMV and Immunosenescence: from basics to clinics. *Immunity & ageing : I & A* 2012; **9**(1): 23.

52. Müller L, Hamprecht K, Pawelec G. The role of CMV in "immunosenescence". In: Bueno V, Lord JM, Jackson TA, eds. The ageing immune system and health: Springer; 2017: 53-68.

53. Stowe RP, Kozlova EV, Yetman DL, Walling DM, Goodwin JS, Glaser R. Chronic herpesvirus reactivation occurs in aging. *Experimental gerontology* 2007; **42**(6): 563-70.

54. Jackson SE, Redeker A, Arens R, et al. CMV immune evasion and manipulation of the immune system with aging. *Geroscience* 2017; **39**(3): 273-91.

55. Nikolich-Zugich J, Goodrum F, Knox K, Smithey MJ. Known unknowns: how might the persistent herpesvirome shape immunity and aging? *Curr Opin Immunol* 2017; **48**: 23-30.

56. Karrer U, Sierro S, Wagner M, et al. Memory inflation: continuous accumulation of antiviral CD8+ T cells over time. *J Immunol* 2003; **170**(4): 2022-9.

57. Kim J, Kim AR, Shin EC. Cytomegalovirus Infection and Memory T Cell Inflation. *Immune Netw* 2015; **15**(4): 186-90.

58. Ouyang Q, Wagner WM, Voehringer D, et al. Age-associated accumulation of CMV-specific CD8+ T cells expressing the inhibitory killer cell lectin-like receptor G1 (KLRG1). *Exp Gerontol* 2003; **38**(8): 911-20.

59. Weltevrede M, Eilers R, de Melker HE, van Baarle D. Cytomegalovirus persistence and T-cell immunosenescence in people aged fifty and older: A systematic review. *Exp Gerontol* 2016; **77**: 87-95.

60. Pawelec G, Derhovanessian E, Larbi A, Strindhall J, Wikby A. Cytomegalovirus and human immunosenescence. *Reviews in medical virology* 2009; **19**(1): 47-56.

61. Pawelec G, McElhaney JE, Aiello AE, Derhovanessian E. The impact of CMV infection on survival in older humans. *Current opinion in immunology* 2012; **24**(4): 507-11.

62. Savva GM, Pachnio A, Kaul B, et al. Cytomegalovirus infection is associated with increased mortality in the older population. *Aging Cell* 2013; **12**(3): 381-7.

63. Wikby A, Ferguson F, Forsey R, et al. An immune risk phenotype, cognitive impairment, and survival in very late life: impact of allostatic load in Swedish octogenarian and nonagenarian humans. *J Gerontol A Biol Sci Med Sci* 2005; **60**(5): 556-65.

64. Spyridopoulos I, Martin-Ruiz C, Hilkens C, et al. CMV seropositivity and T-cell senescence predict increased cardiovascular mortality in octogenarians: results from the Newcastle 85+ study. *Aging Cell* 2015.

65. Naylor K, Li G, Vallejo AN, et al. The influence of age on T cell generation and TCR diversity. *J Immunol* 2005; **174**(11): 7446-52.

66. Holder A, Mella S, Palmer DB, Aspinall R, Catchpole B. An Age-Associated Decline in Thymic Output Differs in Dog Breeds According to Their Longevity. *PLoS One* 2016; **11**(11): e0165968.

67. Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *The journals of gerontology Series A, Biological sciences and medical sciences* 2014; **69 Suppl 1**: S4-9.

68. Franceschi C, Capri M, Monti D, et al. Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. *Mechanisms of ageing and development* 2007; **128**(1): 92-105.

69. Franceschi C, Garagnani P, Vitale G, Capri M, Salvioli S. Inflammaging and 'Garb-aging'. *Trends Endocrinol Metab* 2017; **28**(3): 199-212.

70. Franceschi C, Bonafe M, Valensin S, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. *Annals of the New York Academy of Sciences* 2000; **908**: 244-54.

71. Jenny NS. Inflammation in aging: cause, effect, or both? *Discovery medicine* 2012; **13**(73): 451-60.

72. Soysal P, Stubbs B, Lucato P, et al. Inflammation and frailty in the elderly: A systematic review and meta-analysis. *Ageing Res Rev* 2016; **31**: 1-8.

73. Vallejo AN. Immune remodeling: lessons from repertoire alterations during chronological aging and in immune-mediated disease. *Trends Mol Med* 2007; **13**(3): 94-102.

74. Alboni S, Maggi L. Editorial: Cytokines as Players of Neuronal Plasticity and Sensitivity to Environment in Healthy and Pathological Brain. *Front Cell Neurosci* 2015; **9**: 508.

75. Liang Z, Zhao Y, Ruan L, et al. Impact of aging immune system on neurodegeneration and potential immunotherapies. *Prog Neurobiol* 2017.

76. Giunta B, Fernandez F, Nikolic WV, et al. Inflammaging as a prodrome to Alzheimer's disease. *J Neuroinflammation* 2008; **5**: 51.

77. von Bernhardi R, Tichauer JE, Eugenin J. Aging-dependent changes of microglial cells and their relevance for neurodegenerative disorders. *J Neurochem* 2010; **112**(5): 1099-114.

78. Smith LK, White CW, 3rd, Villeda SA. The systemic environment: at the interface of aging and adult neurogenesis. *Cell Tissue Res* 2018; **371**(1): 105-13.

79. Pizza V, Agresta A, D'Acunto CW, Festa M, Capasso A. Neuroinflammation and ageing: current theories and an overview of the data. *Rev Recent Clin Trials* 2011; **6**(3): 189-203.

80. Harrison NA. Brain Structures Implicated in Inflammation-Associated Depression. *Curr Top Behav Neurosci* 2016.

81. Goldeck D, Witkowski JM, Fulop T, Pawelec G. Peripheral Immune Signatures in Alzheimer Disease. *Curr Alzheimer Res* 2016; **13**(7): 739-49.

82. Ownby RL. Neuroinflammation and cognitive aging. *Curr Psychiatry Rep* 2010; **12**(1): 39-45.

83. Barrientos RM, Kitt MM, Watkins LR, Maier SF. Neuroinflammation in the normal aging hippocampus. *Neuroscience* 2015; **309**: 84-99.

84. Barrientos RM, Frank MG, Watkins LR, Maier SF. Aging-related changes in neuroimmune-endocrine function: implications for hippocampal-dependent cognition. *Horm Behav* 2012; **62**(3): 219-27.

85. Hansel A, Hong S, Camara RJ, von Kanel R. Inflammation as a psychophysiological biomarker in chronic psychosocial stress. *Neurosci Biobehav Rev* 2010; **35**(1): 115-21.

86. Doty KR, Guillot-Sestier MV, Town T. The role of the immune system in neurodegenerative disorders: Adaptive or maladaptive? *Brain Res* 2015; **1617**: 155-73.

87. Deleidi M, Jaggle M, Rubino G. Immune aging, dysmetabolism, and inflammation in neurological diseases. *Front Neurosci* 2015; **9**: 172.

88. Gemechu JM, Bentivoglio M. T Cell Recruitment in the Brain during Normal Aging. *Front Cell Neurosci* 2012; **6**: 38.

89. Bertram L, Bockenhoff A, Demuth I, et al. Cohort profile: The Berlin Aging Study II (BASE-II). *Int J Epidemiol* 2014; **43**(3): 703-12.

90. Gerstorf D, Bertram L, Lindenberger U, et al. Editorial. *Gerontology* 2016; **62**(3): 311-5.

91. Koch S, Larbi A, Derhovanessian E, Ozcelik D, Naumova E, Pawelec G. Multiparameter flow cytometric analysis of CD4 and CD8 T cell subsets in young and old people. *Immunity & ageing : I & A* 2008; **5**: 6.

92. Qi Q, Zhang DW, Weyand CM, Goronzy JJ. Mechanisms shaping the naive T cell repertoire in the elderly - thymic involution or peripheral homeostatic proliferation? *Experimental gerontology* 2014; **54**: 71-4.

93. Caruso C, Accardi G, Virruso C, Candore G. Sex, gender and immunosenescence: a key to understand the different lifespan between men and women? *Immunity & ageing : I & A* 2013; **10**(1): 20.

94. Bouman A, Schipper M, Heineman MJ, Faas MM. Gender difference in the non-specific and specific immune response in humans. *Am J Reprod Immunol* 2004; **52**(1): 19-26.

95. Hirokawa K, Utsuyama M, Hayashi Y, Kitagawa M, Makinodan T, Fulop T. Slower immune system aging in women versus men in the Japanese population. *Immunity & ageing : I & A* 2013; **10**(1): 19.

96. Chidrawar S, Khan N, Wei W, et al. Cytomegalovirus-seropositivity has a profound influence on the magnitude of major lymphoid subsets within healthy individuals. *Clinical and experimental immunology* 2009; **155**(3): 423-32.

97. Villacres MC, Longmate J, Auge C, Diamond DJ. Predominant type 1 CMV-specific memory T-helper response in humans: evidence for gender differences in cytokine secretion. *Hum Immunol* 2004; **65**(5): 476-85.

98. Derhovanessian E, Maier AB, Hahnel K, et al. Lower proportion of naive peripheral CD8+ T cells and an unopposed pro-inflammatory response to human Cytomegalovirus proteins in vitro are associated with longer survival in very elderly people. *Age* 2013; **35**(4): 1387-99.

99. Wikby A, Nilsson BO, Forsey R, et al. The immune risk phenotype is associated with IL-6 in the terminal decline stage: findings from the Swedish NONA immune longitudinal study of very late life functioning. *Mechanisms of ageing and development* 2006; **127**(8): 695-704.

100. Yan J, Greer JM, Hull R, et al. The effect of ageing on human lymphocyte subsets: comparison of males and females. *Immunity & ageing : I & A* 2010; **7**: 4.

101. Garcia Verdecia B, Saavedra Hernandez D, Lorenzo-Luaces P, et al. Immunosenescence and gender: a study in healthy Cubans. *Immunity & ageing : I & A* 2013; **10**(1): 16.

102. Nussinovitch U, Shoenfeld Y. The role of gender and organ specific autoimmunity. *Autoimmunity reviews* 2012; **11**(6-7): A377-85.

103. Tower J, Arbeitman M. The genetics of gender and life span. *J Biol* 2009; **8**(4): 38.

104. Gameiro C, Romao F. Changes in the immune system during menopause and aging. *Frontiers in bioscience* 2010; **2**: 1299-303.

105. Sakiani S, Olsen NJ, Kovacs WJ. Gonadal steroids and humoral immunity. *Nat Rev Endocrinol* 2013; **9**(1): 56-62.

106. Ongradi J, Kovesdi V. Factors that may impact on immunosenescence: an appraisal. *Immunity & ageing : I & A* 2010; **7**: 7.

107. Ansar Ahmed S, Penhale WJ, Talal N. Sex hormones, immune responses, and autoimmune diseases. Mechanisms of sex hormone action. *Am J Pathol* 1985; **121**(3): 531-51.

108. McCombe PA, Greer JM, Mackay IR. Sexual dimorphism in autoimmune disease. *Curr Mol Med* 2009; **9**(9): 1058-79.

109. Qi Q, Liu Y, Cheng Y, et al. Diversity and clonal selection in the human T-cell repertoire. *Proceedings of the National Academy of Sciences of the United States of America* 2014; **111**(36): 13139-44.

110. Pera A, Campos C, Corona A, et al. CMV latent infection improves CD8+ T response to SEB due to expansion of polyfunctional CD57+ cells in young individuals. *PloS one* 2014; **9**(2): e88538.

111. Strioga M, Pasukoniene V, Characiejus D. CD8+ CD28- and CD8+ CD57+ T cells and their role in health and disease. *Immunology* 2011; **134**(1): 17-32.

112. Tarazona R, DelaRosa O, Alonso C, et al. Increased expression of NK cell markers on T lymphocytes in aging and chronic activation of the immune system reflects the accumulation of effector/senescent T cells. *Mechanisms of ageing and development* 2000; **121**(1-3): 77-88.

113. Catakovic K, Klieser E, Neureiter D, Geisberger R. T cell exhaustion: from pathophysiological basics to tumor immunotherapy. *Cell Commun Signal* 2017; **15**(1): 1.

114. Lages CS, Lewkowich I, Sproles A, Wills-Karp M, Chougnet C. Partial restoration of T-cell function in aged mice by in vitro blockade of the PD-1/ PD-L1 pathway. *Aging cell* 2010; **9**(5): 785-98.

115. Shaw DM, Merien F, Braakhuis A, Dulson D. T-cells and their cytokine production: The anti-inflammatory and immunosuppressive effects of strenuous exercise. *Cytokine* 2018; **104**: 136-42.

116. Arend WP, Malyak M, Guthridge CJ, Gabay C. Interleukin-1 receptor antagonist: role in biology. *Annu Rev Immunol* 1998; **16**: 27-55.

117. Dinarello CA. Interleukin-1 and interleukin-1 receptor antagonist. *Nutrition* 1995; **11**(5 Suppl): 492-4.

118. Alvarez-Rodriguez L, Lopez-Hoyos M, Munoz-Cacho P, Martinez-Taboada VM. Aging is associated with circulating cytokine dysregulation. *Cellular immunology* 2012; **273**(2): 124-32.

119. Goetzl EJ, Huang MC, Kon J, et al. Gender specificity of altered human immune cytokine profiles in aging. *FASEB J* 2010; **24**(9): 3580-9.

120. Malkin CJ, Pugh PJ, Jones RD, Kapoor D, Channer KS, Jones TH. The effect of testosterone replacement on endogenous inflammatory cytokines and lipid profiles in hypogonadal men. *J Clin Endocrinol Metab* 2004; **89**(7): 3313-8.

121. Accardi G, Caruso C. Immune-inflammatory responses in the elderly: an update. *Immunity & ageing : I & A* 2018; **15**: 11.

122. Gubbels Bupp MR. Sex, the aging immune system, and chronic disease. *Cellular immunology* 2015; **294**(2): 102-10.

123. Gubbels Bupp MR, Potluri T, Fink AL, Klein SL. The Confluence of Sex Hormones and Aging on Immunity. *Frontiers in immunology* 2018; **9**: 1269.

124. Ostan R, Monti D, Gueresi P, Bussolotto M, Franceschi C, Baggio G. Gender, aging and longevity in humans: an update of an intriguing/neglected scenario paving the way to a gender-specific medicine. *Clin Sci (Lond)* 2016; **130**(19): 1711-25.

125. Odegaard JI, Chawla A. Old HDL learns a new (anti-inflammatory) trick. *Nature immunology* 2014; **15**(2): 138-9.

126. Catapano AL, Pirillo A, Norata GD. Vascular inflammation and low-density lipoproteins: is cholesterol the link? A lesson from the clinical trials. *Br J Pharmacol* 2017; **174**(22): 3973-85.

127. Feingold KR, Grunfeld C. Role of cytokines in inducing hyperlipidemia. *Diabetes* 1992; **41 Suppl 2**: 97-101.

128. Nakagomi A, Seino Y, Noma S, et al. Relationships between the serum cholesterol levels, production of monocyte proinflammatory cytokines and long-term prognosis in patients with chronic heart failure. *Intern Med* 2014; **53**(21): 2415-24.

129. Orsini A, Chiacchio L, Cinque M, Cocchiaro C, Schiappa O, Grossi D. Effects of age, education and sex on two tests of immediate memory: a study of normal subjects from 20 to 99 years of age. *Percept Mot Skills* 1986; **63**(2 Pt 2): 727-32.

130. Zhang J, Zhou W, Wang L, Zhang X, Harvard Aging Brain S. Gender differences of neuropsychological profiles in cognitively normal older people without amyloid pathology. *Compr Psychiatry* 2017; **75**: 22-6.

131. Munro CA, Winicki JM, Schretlen DJ, et al. Sex differences in cognition in healthy elderly individuals. *Neuropsychol Dev Cogn B Aging Neuropsychol Cogn* 2012; **19**(6): 759-68.

132. Wilson CJ, Finch CE, Cohen HJ. Cytokines and cognition--the case for a head-to-toe inflammatory paradigm. *Journal of the American Geriatrics Society* 2002; **50**(12): 2041-56.

133. Shields GS, Moons WG, Slavich GM. Inflammation, Self-Regulation, and Health: An Immunologic Model of Self-Regulatory Failure. *Perspect Psychol Sci* 2017; **12**(4): 588-612.

134. Perry RT, Collins JS, Wiener H, Acton R, Go RC. The role of TNF and its receptors in Alzheimer's disease. *Neurobiol Aging* 2001; **22**(6): 873-83.

135. Fiore M, Angelucci F, Alleva E, Branchi I, Probert L, Aloe L. Learning performances, brain NGF distribution and NPY levels in transgenic mice expressing TNF-alpha. *Behav Brain Res* 2000; **112**(1-2): 165-75.

136. Rizzo FR, Musella A, De Vito F, et al. Tumor Necrosis Factor and Interleukin-1beta Modulate Synaptic Plasticity during Neuroinflammation. *Neural Plast* 2018; **2018**: 8430123.

137. Yirmiya R, Goshen I. Immune modulation of learning, memory, neural plasticity and neurogenesis. *Brain, behavior, and immunity* 2011; **25**(2): 181-213.

138. Lobo-Silva D, Carriche GM, Castro AG, Roque S, Saraiva M. Balancing the immune response in the brain: IL-10 and its regulation. *J Neuroinflammation* 2016; **13**(1): 297.

139. Marsland AL, Gianaros PJ, Abramowitch SM, Manuck SB, Hariri AR. Interleukin-6 covaries inversely with hippocampal grey matter volume in middle-aged adults. *Biol Psychiatry* 2008; **64**(6): 484-90.

140. Tegeler C, O'Sullivan JL, Bucholtz N, et al. The inflammatory markers CRP, IL-6, and IL-10 are associated with cognitive function--data from the Berlin Aging Study II. *Neurobiol Aging* 2016; **38**: 112-7.

141. Jefferson AL, Massaro JM, Beiser AS, et al. Inflammatory markers and neuropsychological functioning: the Framingham Heart Study. *Neuroepidemiology* 2011; **37**(1): 21-30.

142. Simpson EE, Hodkinson CF, Maylor EA, et al. Intracellular cytokine production and cognition in healthy older adults. *Psychoneuroendocrinology* 2013; **38**(10): 2196-208.

143. Talbot S, Foster SL, Woolf CJ. Neuroimmunity: Physiology and Pathology. *Annu Rev Immunol* 2016; **34**: 421-47.

144. Morel PA, Lee REC, Faeder JR. Demystifying the cytokine network: Mathematical models point the way. *Cytokine* 2017; **98**: 115-23.

145. McAfoose J, Baune BT. Evidence for a cytokine model of cognitive function. *Neuroscience and biobehavioral reviews* 2009; **33**(3): 355-66.

146. Vitkovic L, Bockaert J, Jacque C. "Inflammatory" cytokines: neuromodulators in normal brain? *J Neurochem* 2000; **74**(2): 457-71.

147. Tangestani Fard M, Stough C. A Review and Hypothesized Model of the Mechanisms That Underpin the Relationship Between Inflammation and Cognition in the Elderly. *Front Aging Neurosci* 2019; **11**: 56.

148. Bennett JM, Glaser R, Malarkey WB, Beversdorf DQ, Peng J, Kiecolt-Glaser JK. Inflammation and reactivation of latent herpesviruses in older adults. *Brain, behavior, and immunity* 2012; **26**(5): 739-46.

149. Morrisette-Thomas V, Cohen AA, Fulop T, et al. Inflamm-aging does not simply reflect increases in pro-inflammatory markers. *Mechanisms of ageing and development* 2014; **139**: 49-57.

150. Ashpole NM, Sanders JE, Hodges EL, Yan H, Sonntag WE. Growth hormone, insulin-like growth factor-1 and the aging brain. *Exp Gerontol* 2015; **68**: 76-81.

151. Junnila RK, List EO, Berryman DE, Murrey JW, Kopchick JJ. The GH/IGF-1 axis in ageing and longevity. *Nat Rev Endocrinol* 2013; **9**(6): 366-76.

152. O'Connor JC, McCusker RH, Strle K, Johnson RW, Dantzer R, Kelley KW. Regulation of IGF-I function by proinflammatory cytokines: at the interface of immunology and endocrinology. *Cell Immunol* 2008; **252**(1-2): 91-110.

153. Wennberg AMV, Hagen CE, Machulda MM, et al. The association between peripheral total IGF-1, IGFBP-3, and IGF-1/IGFBP-3 and functional and cognitive outcomes in the Mayo Clinic Study of Aging. *Neurobiol Aging* 2018; **66**: 68-74.

154. Willis EL, Wolf RF, White GL, McFarlane D. Age- and gender-associated changes in the concentrations of serum TGF-1beta, DHEA-S and IGF-1 in healthy captive baboons (Papio hamadryas anubis). *Gen Comp Endocrinol* 2014; **195**: 21-7.

155. Kamin HS, Kertes DA. Cortisol and DHEA in development and psychopathology. *Horm Behav* 2017; **89**: 69-85.

156. Elenkov IJ. Neurohormonal-cytokine interactions: implications for inflammation, common human diseases and well-being. *Neurochem Int* 2008; **52**(1-2): 40-51.

157. Schmiedek F, Lovden M, Lindenberger U. Hundred Days of Cognitive Training Enhance Broad Cognitive Abilities in Adulthood: Findings from the COGITO Study. *Front Aging Neurosci* 2010; **2**.

158. Lindenberger U, Mayr U, Kliegl R. Speed and intelligence in old age. *Psychol Aging* 1993; **8**(2): 207-20.

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12 Publication I

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RESEARCH ARTICLE

Impact of age, sex and CMV-infection on peripheral T cell phenotypes: results from the Berlin BASE-II Study

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Abstract Advancing age is characterized by functional and phenotypic alterations in the distribution of circulating T-cell subsets, some of which are exacerbated by a latent infection with the persistent herpesvirus, cytomegalovirus (CMV). The influence of age, sex and CMV-infection on T-cell subpopulations in the peripheral blood remains incompletely understood. Here, T cells from 157 participants of the Berlin Aging Study II (BASE-II) were characterized at 21–34 (n = 59) and 62–85 (n = 98) years of age. We found that the frequency of naïve $CD8^+$ T cells was significantly lower in the older group than in the young, and was different in men and women. Elderly men had a significantly lower proportion of naïve $CD8^+$ T cells than younger men, regardless of their CMV-status, but in older women, this was seen only in the CMV-seropositive group. Reciprocally, older men had a higher proportion of late-differentiated, potentially "senescent" $CD57^+$ T cells. Thus, T-cell senescence may be more pronounced in older men than women. Within the $CD4^+$ population, in the elderly of both sexes there was a significantly higher proportion of late-differentiated TEMRA cells (T effector memory cells re-expressing CD45RA), but these were present exclusively in CMV-positive subjects. Finally, for the first time, we examined the so-called TSCM cell (T-stem cell-like memory) subpopulations in both $CD4^+$ and $CD8^+$ subsets and found that neither CMV-seropositivity nor age or sex affected their frequencies. This study confirms significant cross-sectional age-associated differences of T-cell subset distribution in a representative German urban population and emphasizes the impact of both sex and CMV-infection on T-cell naïve and memory phenotypes, but unaffected frequencies of T-stem cell-like memory cells.

Keywords Immunosenescence · Aging T-cell phenotype · CMV · Differentiation status · Sex difference · BASE-II study

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Introduction

Changes in the human immune system accompanying aging are generally referred to as indicating "immunosenescence". Many factors and mechanisms are attributed to immunosenescence including defects in hematopoiesis, thymus involution and defects in formation, maturation, migration and homeostasis of peripheral lymphocytes (Müller et al. 2013). Age-related modulation of the immune system can be assessed as differences in the distribution of peripheral T cells at different stages of differentiation. The frequencies of memory T cells depend on lifetime exposure of the individual to pathogens, above all to cytomegalovirus (CMV). As many studies now confirm, infection with this herpesvirus drives specific T cells to a late stage of differentiation, which may be confused with the process of aging itself if not properly controlled for (Chidrawar et al. 2009; Derhovanessian et al. 2010; Fülöp et al. 2013; Looney et al. 1999; Pawelec 2014a, b; Smithey et al. 2012). These late-stage differentiated CMV-specific $CD8^+$ T cells have a reduced or absent proliferative capacity, increased ability for activation of senescence pathways and a significantly increased resistance to apoptosis in vitro (Akbar and Fletcher 2005). In aged people, oligoclonally expanded T cells show increased expression of "late-stage" differentiation markers. The accumulation of these highly differentiated T-cell pools in combination with a reduced frequency of naïve T cells could possibly contribute to causing age-related mortality (Almanzar et al. 2005; Chidrawar et al. 2009; Pawelec 2014b; Qi et al. 2014a, b; Wertheimer et al. 2014). Nonetheless, some recent findings indicate that this late-differentiated T-cell constellation is not universally to be viewed as detrimental to survival, but depends on the circumstances. Thus, in an extremely elderly Dutch population, lower frequencies of naïve $CD8^+$ T cells and higher frequencies of late-differentiated $CD8^+$ T cells were associated with a survival benefit on 7 year follow-up (Derhovanessian et al. 2012). Thus, the immunological remodeling of the memory cell pool, which takes place with advancing age, is likely to represent an adaptation of the aged immune system conferring survival advantages (Pawelec 2012). Hence, it remains important to establish the impact of age and CMV-infection in different human populations experiencing different current and earlier exposures and environments, and to establish whether this is different in men and women. For this reason, the Berlin BASE II study was established to examine the impact of multiple health, socioeconomic, psychological and other parameters on healthy ageing, enabling a large-scale study of the contribution of immune ageing to this process. Here we present a subset analysis of the cross-sectional base-line of this study, for which longitudinal follow-up will be available later.

We have used polychromatic flow cytometry to simultaneously measure multiple surface marker phenotypes, defining the following subpopulations: (N) naïve ($CD45RA^+CCR7^+CD27^+CD28^+$); (CM) central memory ($CD45RA^-CCR7^+CD27^+CD28^+$); (EM3) effector memory ($CD45RA^-CCR7^-CD27^-CD28^-$); (E) terminally-differentiated T-effector memory cells as an extended TEMRA phenotype ($CD45RA^+CCR7^-CD27^-CD28^-$); "exhausted" T cells [$PD\text{-}1^+$ ($CD279^+$)]; potentially "senescent" T cells ($CD57^+$) and T stem cell-like memory T cells (TSCM, defined as $CD45RA^+CCR7^+CD27^+CD28^+CD95^+$). We have analyzed the frequency of these subpopulations from the viewpoint of age, influence of sex and effect of a latent CMV-infection. For this reason we first examined the effect of age on the differentiation status of the T cells in both CMV-positive and CMV-negative men and women. The influence of sex in different age groups with different CMV-status and the influence of the CMV-status in men and women in different age groups have been explored. In addition, to the best of our knowledge for the first time, the frequency of the TSCM phenotype (Lugli et al. 2013) and of $PD\text{-}1^+$ T-cells has been included, and the effects of age, CMV-serostatus and sex on their frequency have been examined.

Materials and methods

Subjects

A subgroup of 157 participants of the BASE-II study selected on the basis of a distribution of age, sex and CMV-infection similar to the whole cohort has been analyzed here (Table 1). BASE-II is a multidisciplinary and multi-institutional project that ascertains a large number of ageing-related variables from a wide

Table 1 Participants were subdivided into eight different groups according to the following characteristics

Group	CMV-status	Sex	Age group	Mean age (age range)	n
1	Negative	Female	Young	28 (24–32)	10
2	Negative	Female	Old	74 (67–80)	10
3	Negative	Male	Young	30 (26–34)	11
4	Negative	Male	Old	69 (65–73)	9
5	Positive	Female	Young	28 (23–33)	19
6	Positive	Female	Old	74 (63–85)	39
7	Positive	Male	Young	27 (21–32)	19
8	Positive	Male	Old	72 (62–82)	40

range of different functional domains (Bertram et al. 2014). Phenotypic assessments include factors related to geriatrics and internal medicine, immunology, genetics, psychology, sociology and economics. Baseline recruitment of the BASE-II cohort was recently completed and has led to the sampling of 1600 older adults (age range 60–85 years), as well as 600 younger adults (20–35 years) serving as the basic population for in-depth analyses. The study was approved by the local ethics committees and written informed consent was obtained from all participants.

Samples

Venous blood was taken from the subjects of the BASE-II study during medical examinations by the Geriatric Research Group at the Charité in Berlin and sent to Tübingen in three EDTA tubes (7 ml) packed in iso-containers, to minimize temperature variations. The PBMC were further isolated under sterile conditions and frozen at −196 °C in the gas phase of liquid nitrogen until further processing.

Flow cytometry

All staining steps were performed in PFEA buffer (PBS, 2 % FCS, 2 mM EDTA, and 0.01 % azide). After thawing, PBMCs were treated with human Ig, GAMUNEX (Bayer, Leverkusen, Germany), and ethidium monoazide (EMA) bromide (MoBiTec GmbH, Göttingen, Germany) for 10 min on ice to block surface FcRs and label nonviable cells. Cells were first stained with unconjugated primary Ab CCR7 (R&D System) for 20 min at 4 °C, followed by staining with Pacific Orange-conjugated F(ab́) fragment of goat anti-mouse IgG (Invitrogen) for another 20 min on ice. Mouse serum (Millipore, Temecula California, USA) was added for 15 min to block nonspecific binding to anti-mouse secondary Ab, followed by addition of directly conjugated mAbs, CD3-Alexa Fluor700, CD4-PerCP, CD8-allophycocyanin-H7 (all from BD Biosciences, Heidelberg, Germany), CD27-allophycocyanin (BioLegend, San Diego, CA), CD45RA-V450 (BD Horizon, Heidelberg, Germany) CD28-PE (BD Pharmingen, Germany), PD1-PerCP-Cy5.5 (BioLegend, San Diego, CA), CD95-PE-Cy7 (eBioscience, San Diego, CA), and CD57-FITC (Immunotools, Freiburg, Germany). After 20 min incubation on ice, cells were washed and analyzed immediately on a LSR II cytometer (BD, Heidelberg) with FACSDiva software (BD Biosciences). The spectral overlap between all channels was calculated automatically by the BD FACSDiva software, after measuring negative and single-color controls. Data were analyzed using FlowJo 7.6.5 software (Tree Star, Portland, USA). T-cell subsets were characterized according to previously published models (Derhovanessian et al. 2010; Romero et al. 2007; Sallusto et al. 1999). The gating strategy is shown in Supplemental Material, Fig. S1, (a, b). In brief, the lymphocyte population was gated in FSC versus SSC dot plots. After exclusion of EMA^+ dead cells, viable lymphocytes were gated within $CD3^+$ gate and then selected for either $CD8^+$ (Fig. S1a) or $CD4^+$ (Fig. S1b) T-cell subsets, which have further been subdivided into main T-cell subsets (N, CM, EM and TEMRA) using CD45RA and CCR7. These subsets were also stained for CD27 and CD28 expression to better characterize their differentiation status: N ($CD45RA^+CCR7^+CD27^+CD28^+$); CM ($CD45RA^-CCR7^+CD27^+CD28^+$); EM3 ($CD45RA^-CCR7^-CD27^-CD28^-$); E (an extended TEMRA phenotype defined as $CD45RA^+CCR7^-CD27^-CD28^-$). Additionally, N cells have been gated

for CD95 expression to identify TSCM ($CD45RA^{+-}$ $CCR7^{+}CD27^{+}$ $CD28^{+}CD95^{+}$) cells and PD-1 (CD279) was determined within the $CD3^{+}$ population. Flow cytometry staining and data analysis were performed on blinded samples.

Screening of CMV-serostatus

Anti-CMV IgG titers were measured in plasma of BASE-II participants using a CMV IgG kit (Omega Diagnostic Group, Scotland, UK) based on enzyme immunoassay technology.

Statistics

Statistical analysis used GraphPad V6 (GraphPad Software, Inc., La Jolla, USA). For comparisons between two independent groups the Mann–Whitney U Test was used. For all analyses, the significance level (p-values) has been set to 0.05, adjusted for multiple comparisons using the Bonferroni correction.

Results

Frequency of $CD4^{+}$ and $CD8^{+}$ T cells within the $CD3^{+}$ T-cell population

Age-related differences in the proportion of $CD4^{+}$ T cells within the $CD3^{+}$ pool are apparent in Fig. 1a and Table S1 (Supplementary Material). Older CMV-seronegative women have significantly higher frequencies of peripheral $CD4^{+}$ T cells than younger subjects ($p = 0.0004$). However, a latent infection with CMV is associated with a lower percentage of $CD4^{+}$ T cells, regardless of sex and age of the subjects ($p = 0.0109$ for young and $p = 0.0007$ for old women; $p = 0.0026$ for young and $p = 0.0067$ for old men).

The percentage of $CD8^{+}$ T cells is significantly lower in elderly subjects of either sex ($p = 0.0021$ for women; $p = 0.0024$ for men), but only in CMV-negative people (Fig. 1b; Table S1, in Suppl). However, no significant differences were observed between sexes. An effect of CMV infection on this parameter was only seen in the elderly where both older women ($p = 0.0017$), and older men ($p = 0.0024$) had a higher percentage of $CD8^{+}$ T cells than CMV-seronegatives.

No significant differences in the percentage of $CD3^{+}$ T cells (Fig. 3f; Table S1) were observed between different subgroups, except in the group of old females, where CMV-positive individuals had a significantly higher frequency of $CD3^{+}$ T cells than CMV-negative ($p = 0.0053$).

Frequency of early-differentiated T cells within the $CD4^{+}$ and $CD8^{+}$ T-cell subsets

We found a significant difference between old and young subjects in the frequency of naïve T cells (defined as $CD45RA^{+}CCR7^{+}CD27^{+}CD28^{+}$) within the $CD4^{+}$ T-cell population. Elderly women had a lower percentage ($p = 0.008$) of naïve $CD4^{+}$ T cells compared to younger women, but only in the group of CMV-negative individuals (Fig. 1c). In contrast, although there were also lower frequencies of $CD4^{+}$ naïve T cells in older men, in this case this was only seen in the CMV-seropositive group ($p = 0.0063$). These results imply that at least some of the observed sex differences in naïve T-cell distribution in the elderly are not only dependent on the different frequency of CMV-infection in men and women, but reflect a difference in response to CMV after infection.

$CD8^{+}$ T cells are generally reported to show greater age-associated differences than $CD4^{+}$ T cells. Consistent with this, Fig. 1d shows a greater impact of age on the frequencies of $CD8^{+}$ naïve T cells than $CD4^{+}$ naïve T cells, in both CMV^{+} and CMV^{-} subjects. Elderly men had a significantly lower proportion of these naïve T cells than younger men, regardless of their CMV-status ($p = 0.0012$ for CMV^{-}; $p < 0.0001$ for CMV^{+}). However, in women, this was only the case in the CMV-positive group ($p = 0.0004$) (Fig. 1d; Table S1, in Suppl). There is an additional sex difference, in that older men had a lower proportion of naïve $CD8^{+}$ T cells ($p = 0.0081$) than elderly women, only in the CMV-positive group (Fig. 1d).

Similarly, age-related differences can be seen in the central memory (CM) $CD4^{+}$ T-cell population ($CD45RA^{-}CCR7^{+}CD27^{+}CD28^{+}$), particularly evident in the CMV-seronegative female group. Thus, elderly women had a significantly higher frequency ($p = 0.0027$) of CM T cells than younger women (Fig. 1e).

In the $CD8^{+}$ T-cell population, a significant influence of age on the distribution of CM T cells

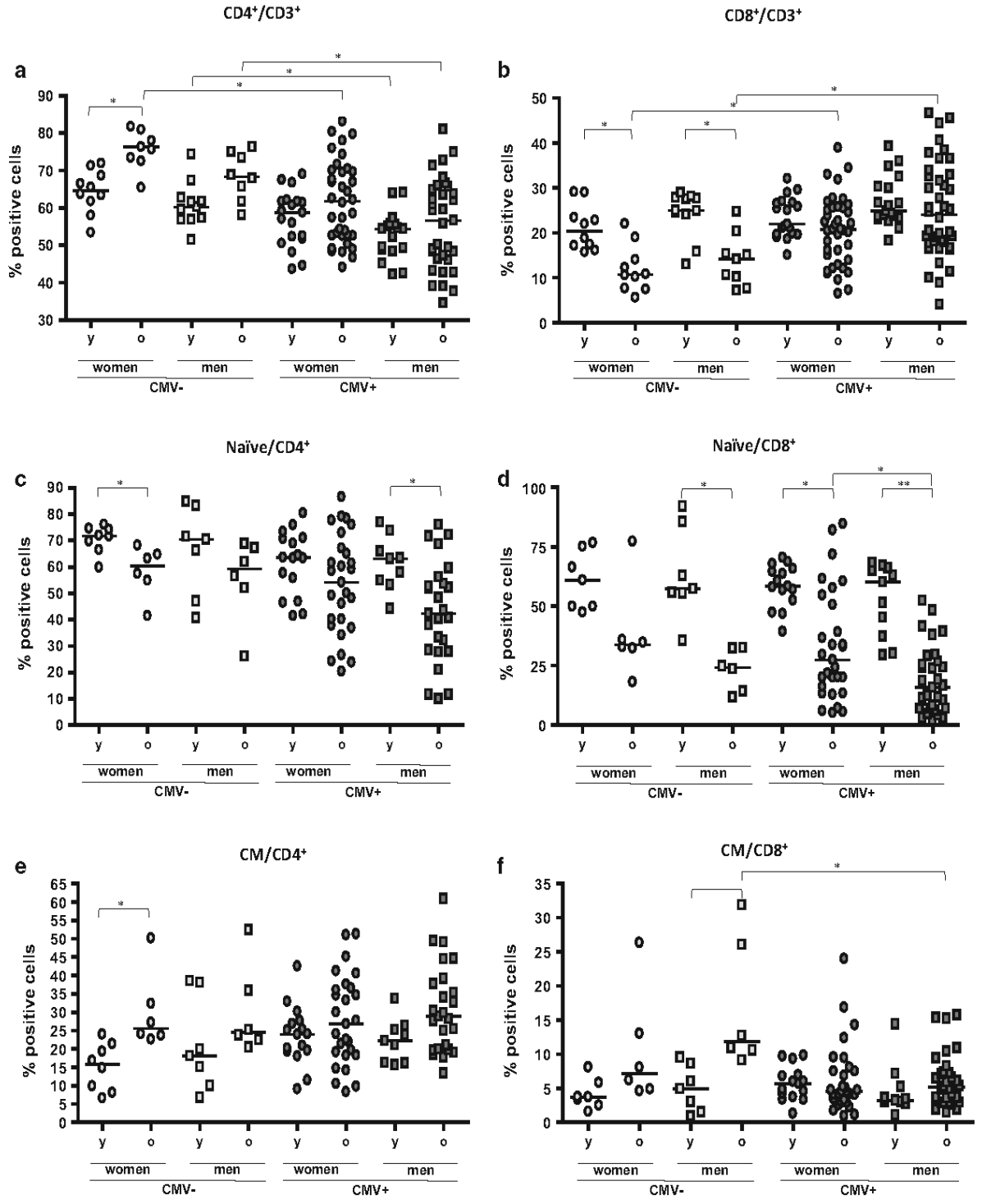

Fig. 1 Frequency of $CD4^+$ (**a**), $CD8^+$ (**b**) T cells within the $CD3^+$ population; frequency of naïve (N) T cells (**c**, **d**) and central memory (CM) T cells (**e**, **f**) within $CD4^+$ and $CD8^+$ T cell populations for every subject of the eight different groups. The *horizontal bars* represent the median values for each group. N T cells are defined as $CD45RA^+CCR7^+CD27^+CD28^+$; CM cells are defined as CD45RA $CCR7^+CD27^+CD28^+$; *y* young, *o* old. Significance levels: *$p < 0.05$ and **$p < 0.01$ Bonferroni-corrected

was seen only in CMV-seronegative men (Fig. 1f; Table S1, in Suppl). The percentage of $CD8^+$ CM T cells in elderly subjects was significantly higher ($p = 0.0023$) in comparison to younger people. In addition, CMV-seropositive older men have a significantly lower percentage ($p = 0.0016$) of CM cells than seronegative elderly men.

Frequency of late-differentiated T cells within the $CD4^+$ and $CD8^+$ T-cell subsets

Age-related differences in the frequencies of effector memory (EM3) T cells ($CD45RA^-CCR7^-CD27^-CD28^-$) can be clearly seen in CMV-positive women (Fig. 2a). Thus, the proportion of $CD4^+$ EM3 T cells in older women is significantly higher than in the younger women ($p = 0.01$). A strong effect of CMV-infection is also apparent in that CMV-negative subjects exhibit a significantly lower frequency of $CD4^+$ EM3 T cells than CMV-positive subjects, regardless of sex and age ($p = 0.0003$ for young and $p = 0.0002$ for old women; $p = 0.0115$ for young and 0.0014 for old men). Thus, sex seems to make no difference to the distribution of EM3 T cells.

In contrast to the $CD4^+$ T-cell population, no effect of age, sex or CMV-status was noted for the $CD8^+$ population of EM3 T cells (Fig. 2b; Table S1, in Suppl).

Finally, we assessed the frequencies of the potentially "terminally-differentiated" T-effector memory T cells characterized by their re-expression of CD45RA (so-called TEMRA cells). Although the presence of cells with this phenotype in the $CD8^+$ T-cell subset is well-accepted, it has remained

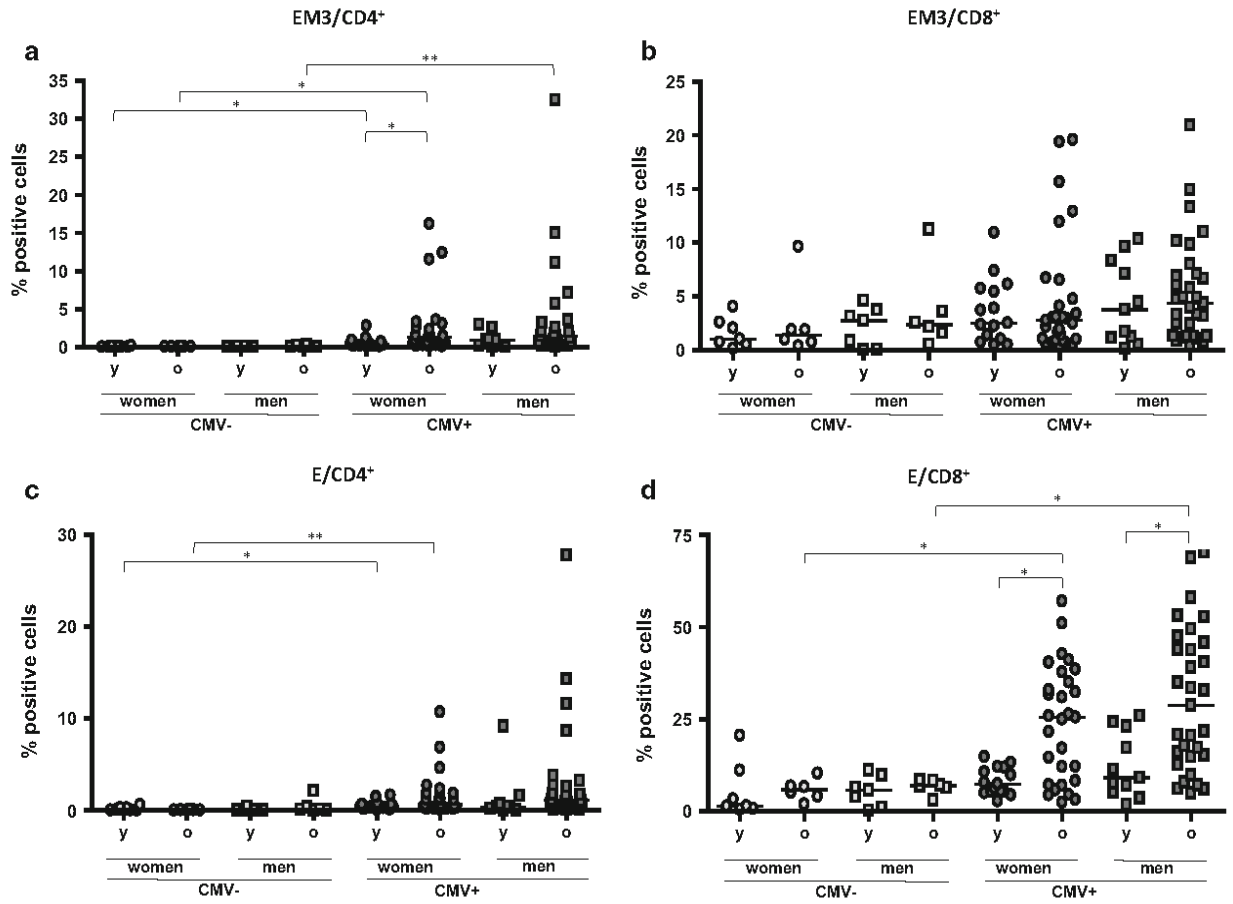

Fig. 2 Frequency of effector memory (EM3) (**a**, **b**) and TEMRA effector (E) (**c**, **d**) T cells within $CD4^+$ (**a**, **c**) and $CD8^+$ (**b**, **d**) T-cell populations for every subject of the eight different groups. The *horizontal bars* represent the median values for each group. EM3 T cells are defined as CD45RA CCR7 CD27 CD28 ; E cells are defined as $CD45RA^+CCR7$ CD27 CD28 ; *y* young, *o* old. Significance levels: *$p < 0.05$ and **$p < 0.01$ Bonferroni-corrected

controversial whether they exist in the $CD4^+$ T-cell subset. Therefore we analyzed an extended phenotype of TEMRA subset, designated effector (E) T cells and characterized by negativity for the two costimulatory receptors CD27 and CD28 ($CD45RA^{+-}$ $CCR7^-CD27^-CD28^-$). Within the $CD4^+$ population of CMV-seronegative people, it was indeed the case that this subset of TEMRA cells was essentially absent (Fig. 2c). However, in CMV-infected older subjects, some individuals did possess $CD4^+$ T cells with this phenotype (Fig. 2c). There was a significantly higher proportion of these cells in CMV-positive people ($p = 0.0044$ for young and $p = 0.0002$ for old women; and $p = 0.0116$ for old men) (Fig. 2c).

Concerning the frequency of $CD8^+$ E cells, we found a significant influence of age on this subset but again, only in subjects with latent CMV-infection ($p = 0.0021$ for women; $p = 0.0072$ for men). Furthermore, the frequency of these cells in CMV-positive elderly individuals was significantly higher ($p = 0.0062$ for women; $p = 0.0028$ for men) in comparison to CMV-negative old people, regardless of their sex. In the younger subjects no significant differences could be detected (Fig. 2d; Table S1, in Suppl).

Frequency of $CD57^+$ T cells within the $CD4^+$ and $CD8^+$ T-cell subsets

With the objective to corroborate the results described above, the expression of CD57 (as an independent marker of late-differentiated, potentially "senescent" T cells) has been investigated and compared within the $CD4^+$ and $CD8^+$ populations. CD57 is often referred to in the literature as a marker of T-cell "senescence" reflecting its presence on cells thought to be beyond the terminally differentiated state and possibly indicating true replicative senescence, at least in the $CD8^+$ subset. (Tarazona et al. 2000). We found CD57 expression also on $CD4^+$ T-cells in the elderly, relative to younger subjects, especially those who were CMV-infected ($p = 0.0039$ for women; $p = 0.0007$ for men). With respect to the influence of CMV-status, the frequency of $CD57^+$ T cells was significantly higher in CMV-positive than CMV-negative subjects, regardless of both age and sex ($p = 0.0002$ for young and $p < 0.0001$ for old women; $p = 0.0005$ for young and $p = 0.0002$ for old men).

Also in the $CD8^+$ T-cell subset (Fig. 3b; Table S1, in Suppl), we found that the percentage of $CD57^+$ T cells in elderly CMV-positive subjects was significantly higher than in younger subjects, regardless of sex ($p = 0.0098$ for women; $p = 0.0002$ for men).

As in the $CD4^+$ T-cell population, a clear influence of CMV-status can be seen on the expression of CD57 by $CD8^+$ T cells. The percentage of $CD57^+$ T cells in CMV-positive older women ($p = 0.0083$) and in CMV-positive older men ($p = 0.0017$) was significantly higher compared to CMV-negative subjects (Fig. 3b).

Frequency of PD-1^+ ($CD279^+$) T cells within the $CD8^+$ subset

We also investigated the expression of PD-1 (programmed cell death protein 1), which is mostly expressed on $CD8^+$ T cells and is known as an inhibitory immunoregulator, potentially marking "exhausted" T cells (Barber et al. 2006). We found that elderly men had a significantly higher percentage of $CD279^+$ T cells than younger subjects, but only in the CMV-negative group ($p = 0.0098$) (Fig. 3d; Table S1, in Suppl).

Considering the influence of CMV-status on the expression of PD-1, a significant difference was found only for CMV-negative-versus-positive men who had a higher proportion of PD-1^+ T cells ($p = 0.0095$). No sex-related differences were observed.

Frequency of T-stem cell-like memory (TSCM)-cells within the $CD4^+$ and $CD8^+$ T-cell subsets

A novel T-cell subpopulation with stem cell-like properties has been described (Gattinoni et al. 2011), characterized by the phenotype $CD45RA^+CCR7^{+-}$ $CD27^+$ $CD28^+CD95^+$, and designated TSCM. This long-lived memory T-cell population has an increased capacity for self-renewal and for the generation of multipotent CM-, EM-, and E-cells (Gattinoni et al. 2011). However, this rare population has not been studied in terms of the influence of age or CMV-serostatus. Thus, we examined the distribution of TSCM cells within the $CD4^+$ and $CD8^+$ populations, and summarize the results in Fig. 3d and e. It is apparent that neither CMV-status nor age has any significant associations with the frequency of TSCM-cells. We also found no effect of sex (data not shown).

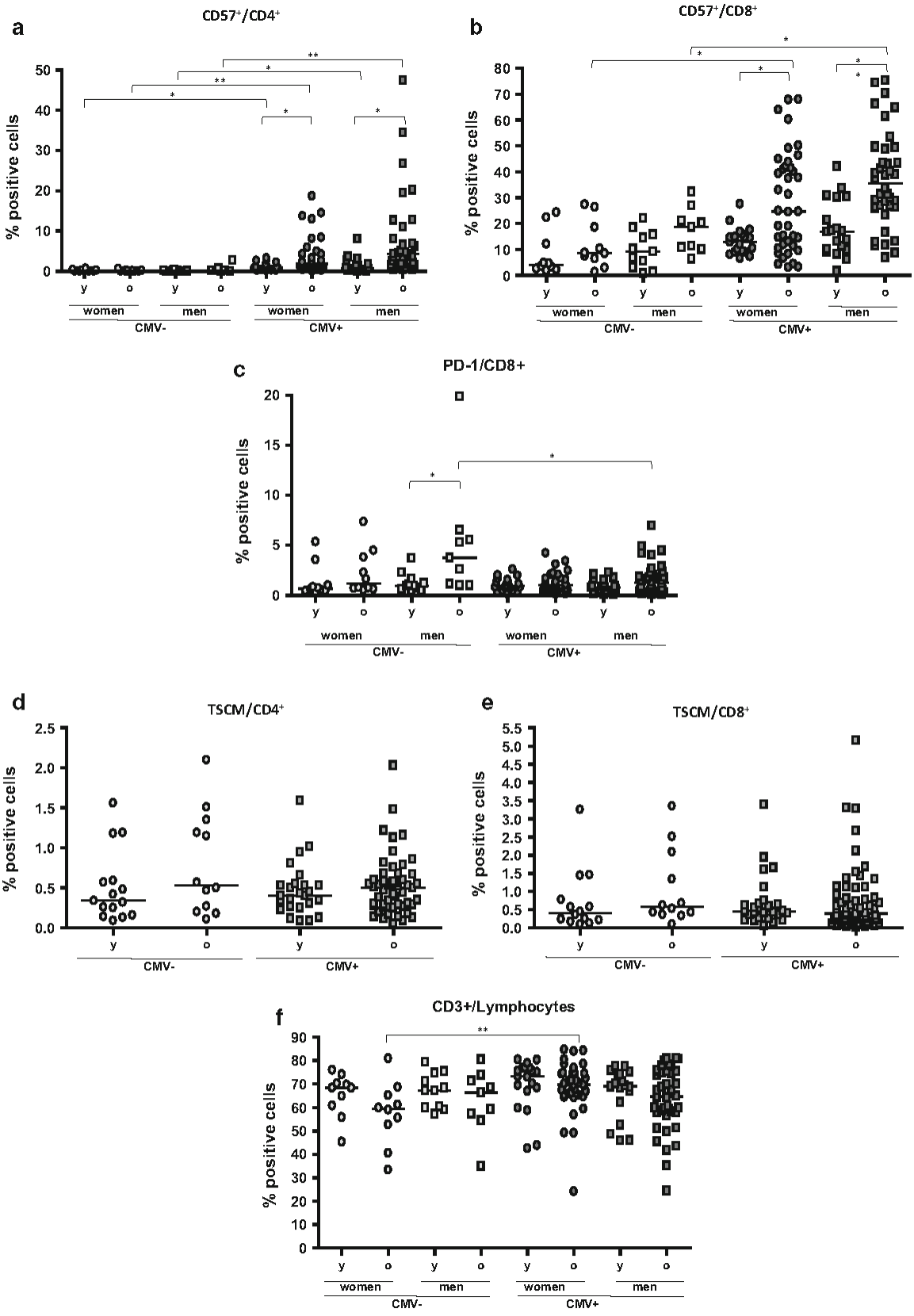
a
CD57+/CD4+
% positive cells
women
men
CMV-
CMV+
b
CD57+/CD8+
c
PD-1/CD8+
d
TSCM/CD4+
e
TSCM/CD8+
f
CD3+/Lymphocytes

Fig. 3 Frequency of $CD57^+$ T cells within $CD4^+$ (**a**) and $CD8^+$ (**b**) T cell populations as well as percentage of PD-1 ($CD279^+$) T cells within the $CD8^+$ T cell population (**c**) for every subject of the eight different groups. Frequency of TSCM cells within $CD4^+$ (**d**) and $CD8^+$ (**e**) T cell populations for every subject of the four different groups. The *horizontal bars* represent the median values for each group. TSCM cells are defined as $CD45RA^+CCR7^+CD27^+$ $CD28^+CD95^+$; *y* young, *o* old. Significance levels: $^*p < 0.05$ and $^{**}p < 0.01$ Bonferroni-corrected

Hence, this potentially important source of effector and memory T cells appears to be well-conserved in both sexes regardless of either age or CMV-infection, all of which are factors markedly affecting the distribution of other T-cell differentiation phenotypes in humans.

Discussion

Influence of age on differentiation status in CMV-positive and CMV-negative men and women

The results of our study have confirmed a general tendency for reduced frequencies of naïve T cells in the peripheral blood of subjects at advanced age, regardless of CMV-infection. This is more pronounced in the $CD8^+$ subset but is also apparent in $CD4^+$ T cells. Interestingly, this tendency is particularly pronounced in women who are CMV-negative women but in men who are CMV-positive, suggesting that there is a sex difference in the immunological impact of CMV. A recent study by another group yielded slightly different results, where aging in the absence of CMV was associated with decreased naïve $CD8^+$ but not $CD4^+$ T cells (Wertheimer et al. 2014). However, that study did not examine men and women separately. This may explain the difference, because we found that the age-associated lower frequency of naïve T cells in CMV-seronegative individuals was sex-sensitive.

We have observed a higher frequency of CM $CD4^+$ T cells exclusively in the CMV-negative elderly, while CMV-positive individuals had a more advanced differentiation phenotype of EM3 and E CD4 T cells. These results are consistent with CMV predominantly driving the accumulation of late-stage $CD4^+$ as well as $CD8^+$ memory T cells in both sexes. Thus, as in most previous studies, more marked than for $CD4^+$ T cells, we have observed lower frequencies of naïve $CD8^+$ T cells at older age for all groups. Both CMV-positive and CMV-negative old subjects of both sexes showed a lower percentage of naïve $CD8^+$ T cells. Similar to the $CD4^+$ T cells, also here a corresponding increase in the frequency of CM cells in CMV-seronegative elderly people was observed. The same trend was observed for the "late-differentiated" effector population in CMV-seropositive subjects.

Consistent with this interpretation, higher frequencies of T cells expressing CD57 in both the $CD4^+$ and $CD8^+$ subsets in the elderly were observed exclusively in CMV-seropositive subjects. As CD57 is considered to be a potential "senescence marker" for late differentiated T cells (Koch et al. 2008; Pera et al. 2014; Strioga et al. 2011; Tarazona et al. 2000), this finding was expected. Again, there were marked differences between the $CD4^+$ and $CD8^+$ subsets, especially in the young. Interestingly, and perhaps counter-intuitively, the frequencies of "exhausted" $PD\text{-}1^+$ $CD8^+$ T cells were not affected by age or CMV infection; the same was true for TSCM cells in these subjects.

Age-related thymic involution is associated with a decreased efficiency of T-cell development and with a reduced migration of naïve T cells into the periphery (Lynch et al. 2009; Qi et al. 2014b). The consequences of thymic involution in old people contribute to a reduction of naïve T cells in periphery, regardless of CMV-infection. However, the higher frequency of memory cells present exclusively in CMV-positive subjects could be explained by the coexistence of latent CMV and duration of immune system stimulation by the virus, requiring permanent immunosurveillance. According to Stowe et al. CMV-reactivation may occur more often in older people and this could provide an explanation for an age-related increase in the memory T-cell pool in this age group (Stowe et al. 2007).

Influence of sex in different age groups with different CMV-status

It is known that the impact of aging on immunity in men and women is different (Caruso et al. 2013) but details on immune status are sparse. Although the total number of lymphocytes in the peripheral blood of both sexes is similar, men have a lower percentage of T cells within their lymphocyte population (Bouman

et al. 2004; Hirokawa et al. 2013). The difference in incidence of infectious diseases and the different prevalence of autoimmune diseases between men and women could also be attributed to sex-related differences in the immune system (McCombe et al. 2009; Qi et al. 2014a). However, little is known about the effects of sex differences on the aging immune system (Nunn et al. 2009). There are few publications so far on sex differences in the age-related distribution of T-cell subpopulations. Yan et al. studied men and women of different age groups, but without taking their CMV-status into account. They reported significantly higher frequencies of EM cells in older men, but not in older women (Yan et al. 2010), but in a study of the Cuban population (with a higher prevalence of CMV-infection at all ages) the frequency of highly differentiated T cells was higher in women (Garcia Verdecia et al. 2013). Thus, the impact of sex and CMV-persistence on distinct T-cell subpopulations in the peripheral blood remains incompletely quantified and understood. In our experiments reported here, we have observed multiple sex-related differences in the effects of age and CMV-infection on the differentiation status of both $CD4^+$ and $CD8^+$ T cells. Together, the data suggest that the CMV-associated "senescence of T cells" in older men may be more pronounced than in elderly women.

Although sex-specific differences in sex hormone secretion patterns and their changes over the lifespan are clearly candidates intimately involved in controlling ageing trajectories, their impact on immunity is not well-established (Nussinovitch and Shoenfeld 2012; Tower and Arbeitman 2009). It is known that estrogens enhance humoral immunity, while androgens and progesterone tend to suppress it (Gameiro and Romao 2010; Sakiani et al. 2013). Women in general seem to have stronger humoral and cell-mediated immune responses to immune stimulation compared to men. In addition, they generally have higher antibody levels and increased levels of circulating IL-1, IL-4 and IFN-γ (Ansar Ahmed et al. 1985; Yan et al. 2010). It has been reported that men have more pronounced thymic involution, a process that could be due to higher androgens levels in men (Ongradi and Kovesdi 2010).

Although the functionality of the immune system in men and women seems to be different, these differences can only partly explain our results. Such differences for example, seem to play no crucial role in the subpopulation of CMV-negative individuals on the distribution of T-cell subpopulations. Therefore, we can only assume that the sex differences in the differentiation status of the T cells could first emerge under the immunomodulating effect of the stress of long-term immunosurveillance to control CMV-infection. It might be assumed that CMV reactivation in men and women manifests differently. However, this has not been investigated yet, and currently available methods of serological detection neither allow the determination of the duration of virus persistence nor the number of reactivations occuring over the life span.

Influence of CMV-status in men and women in different age groups

As discussed above, repetitive reactivation of CMV or re-infection could lead to exhaustion and dysfunction of CMV-specific T cells, so that larger amounts of immune cells are needed to control the CMV-infection. In our experiments we found that the CMV-serostatus had a decisive influence on the distribution of different subpopulations of both $CD4^+$ and $CD8^+$ T cells. In $CD4^+$ T cells, an accumulation of "late-differentiated" subpopulations was directly associated with the CMV-status, being seen only in infected individuals. This finding on the subgroup of BASE-II participants is in line with our earlier results in other cohorts (Derhovanessian et al. 2012; Derhovanessian and Pawelec 2012; Koch et al. 2008) and by Lachmann et al. (2012). These data are consistent with the hypothesis that persistent CMV-infection accelerates age-related increases in the proportion of memory T cells. This is likely to be influenced by many other factors, such as general health and genetic background, which still have not been taken into account at this stage of the study. Because BASE-II is collecting a large data set on health parameters, cognitive and psychosocial data, as well as genetic data, these variables will also be taken into account as the study progresses.

The importance of this type of analysis, and including the hitherto "innocuous" CMV as an important parameter is emphasized by earlier findings that the CMV-induced accumulation of late-differentiated T cells with advancing age may correlate with increased mortality. The Swedish OCTO and NONA longitudinal studies defined an immune risk profile

(IRP) predicting 2-, 4- and 6-year survival. All subjects in the IRP group were CMV-positive, as opposed to 85 % of those not in the IRP group (Pawelec et al. 2009; Wikby et al. 2006).

Frequency of the TSCM-cells among $CD4^+$ and $CD8^+$ T-cell populations

To the best of our knowledge, in the present study we have investigated for the first time the impact of age and CMV-status on the frequency of the relatively rare long-lived TSCM subpopulation with stem-cell-like characteristics. Interestingly, neither age, sex or CMV infection affected the frequency of TSCM cells within the $CD4^+$ and $CD8^+$ subsets. Retention of these potentially important cells through life could be a crucial aspect of the maintenance of immune system functionality. As there is age-related impairment of progenitor cell production from the bone marrow (Warren and Rossi 2009), as well the age-dependent decreased thymic function discussed above, the TSCM population might represent an important additional source of T-cell regeneration in the periphery. It remains to be determined whether the maintained levels of TSCM cells in the elderly are also functionally intact.

Study limitations

Some limitations of the present study need to be acknowledged. First, the study included a small subgroup of the BASE-II study containing 157 participants. Although selected on the basis of a distribution similar to the whole cohort, the sample size is small, especially after further subdivision into subgroups, based on the differences in age, sex and CMV-positivity. Also the differences in the number of CMV-seronegative young participants compared to CMV-seropositive donors may affect our results and limit the power to detect some significant associations. Further, there are several outliers mainly in the older groups that can affect results of our statistical analysis, although we used nonparametric tests that are recommended for the data being subject to outliers or extreme values. Therefore we are planning further analyses on the whole BASE-II cohort of 2200 participants to confirm and to generalize these results and to include social and other data.

Conclusions

Data obtained from a subgroup of participants of the BASE-II study have demonstrated that age, sex and CMV-status all influence the differentiation phenotypes of peripheral blood T lymphocytes. The multidisciplinary nature of BASE-II will allow the correlation of immunologic parameters with health and socio-economic status of the subjects, their genetic background and psychological characteristics. This will allow us to dissect out the influences of these parameters on immune function, and vice versa, at baseline of the planned longitudinal follow-up of BASE-II.

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References

Akbar AN, Fletcher JM (2005) Memory T cell homeostasis and senescence during aging. Curr Opin Immunol 17:480–485. doi:10.1016/j.coi.2005.07.019

Almanzar G et al (2005) Long-term cytomegalovirus infection leads to significant changes in the composition of the $CD8^+$ T-cell repertoire, which may be the basis for an imbalance in the cytokine production profile in elderly persons. J Virol 79:3675–3683. doi:10.1128/JVI.79.6.3675-3683.2005

Ansar Ahmed S, Penhale WJ, Talal N (1985) Sex hormones, immune responses, and autoimmune diseases. Mechanisms of sex hormone action. Am J Pathol 121:531–551

Barber DL et al (2006) Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 439:682–687. doi:10.1038/nature04444

Bertram L et al (2014) Cohort profile: the Berlin Aging Study II (BASE-II). Int J Epidemiol 43:703–712. doi:10.1093/ije/dyt018

Bouman A, Schipper M, Heineman MJ, Faas MM (2004) Gender difference in the non-specific and specific immune response in humans. Am J Reprod Immunol 52:19–26. doi:10.1111/j.1600-0897.2004.00177.x

Caruso C, Accardi G, Virruso C, Candore G (2013) Sex, gender and immunosenescence: a key to understand the different

lifespan between men and women? Immun Ageing 10:20. doi:10.1186/1742-4933-10-20
Chidrawar S, Khan N, Wei W, McLarnon A, Smith N, Nayak L, Moss P (2009) Cytomegalovirus-seropositivity has a profound influence on the magnitude of major lymphoid subsets within healthy individuals. Clin Exp Immunol 155:423–432. doi:10.1111/j.1365-2249.2008.03785.x
Derhovanessian E, Pawelec G (2012) Vaccination in the elderly. Microb Biotechnol 5:226–232. doi:10.1111/j.1751-7915.2011.00283.x
Derhovanessian E et al (2010) Hallmark features of immunosenescence are absent in familial longevity. J Immunol 185:4618–4624. doi:10.4049/jimmunol.1001629
Derhovanessian E et al (2012) Lower proportion of naive peripheral CD8$^+$ T cells and an unopposed pro-inflammatory response to human Cytomegalovirus proteins in vitro are associated with longer survival in very elderly people. Age (Dordr) 35:1387–1399. doi:10.1007/s11357-012-9425-7
Fülöp T, Larbi A, Pawelec G (2013) Human T cell aging and the impact of persistent viral infections. Front immunol 4:271. doi:10.3389/fimmu.2013.00271
Gameiro C, Romao F (2010) Changes in the immune system during menopause and aging. Front Biosci (Elite Ed) 2:1299–1303
Garcia Verdecia B et al (2013) Immunosenescence and gender: a study in healthy Cubans. Immun Ageing 10:16. doi:10.1186/1742-4933-10-16
Gattinoni L et al (2011) A human memory T cell subset with stem cell-like properties. Nat Med 17:1290–1297. doi:10.1038/nm.2446
Hirokawa K, Utsuyama M, Hayashi Y, Kitagawa M, Makinodan T, Fulop T (2013) Slower immune system aging in women versus men in the Japanese population. Immun Ageing 10:19. doi:10.1186/1742-4933-10-19
Koch S, Larbi A, Derhovanessian E, Ozcelik D, Naumova E, Pawelec G (2008) Multiparameter flow cytometric analysis of CD4 and CD8 T cell subsets in young and old people. Immun Ageing 5:6. doi:10.1186/1742-4933-5-6
Lachmann R, Bajwa M, Vita S, Smith H, Cheek E, Akbar A, Kern F (2012) Polyfunctional T cells accumulate in large human cytomegalovirus-specific T cell responses. J Virol 86(2)1001–1009. doi:10.1128/JVI.00873-11
Looney RJ et al (1999) Role of cytomegalovirus in the T cell changes seen in elderly individuals. Clin Immunol 90:213–219. doi:10.1006/clim.1998.4638
Lugli E et al (2013) Superior T memory stem cell persistence supports long-lived T cell memory. J Clin Invest 123:594–599. doi:10.1172/JCI66327
Lynch HE, Goldberg GL, Chidgey A, Van den Brink MR, Boyd R, Sempowski GD (2009) Thymic involution and immune reconstitution. Trends Immunol 30:366–373. doi:10.1016/j.it.2009.04.003
McCombe PA, Greer JM, Mackay IR (2009) Sexual dimorphism in autoimmune disease. Curr Mol Med 9:1058–1079
Müller L, Fülöp T, Pawelec G (2013) Immunosenescence in vertebrates and invertebrates. Immun Ageing 10:12. doi:10.1186/1742-4933-10-12
Nunn CL, Lindenfors P, Pursall ER, Rolff J (2009) On sexual dimorphism in immune function. Philos Trans R Soc London B 364:61–69. doi:10.1098/rstb.2008.0148
Nussinovitch U, Shoenfeld Y (2012) The role of gender and organ specific autoimmunity. Autoimmun Rev 11:A377–A385. doi:10.1016/j.autrev.2011.11.001
Ongradi J, Kovesdi V (2010) Factors that may impact on immunosenescence: an appraisal. Immun Ageing 7:7. doi:10.1186/1742-4933-7-7
Pawelec G (2012) Hallmarks of human "immunosenescence": adaptation or dysregulation? Immun Ageing 9:15. doi:10.1186/1742-4933-9-15
Pawelec G (2014a) Immunosenenescence: role of cytomegalovirus. Exp Gerontol 54:1–5. doi:10.1016/j.exger.2013.11.010
Pawelec G (2014b) T-cell immunity in the aging human. Haematologica 99:795–797. doi:10.3324/haematol.2013.094383
Pawelec G, Derhovanessian E, Larbi A, Strindhall J, Wikby A (2009) Cytomegalovirus and human immunosenescence. Rev Med Virol 19:47–56. doi:10.1002/rmv.598
Pera A, Campos C, Corona A, Sanchez-Correa B, Tarazona R, Larbi A, Solana R (2014) CMV latent infection improves CD8$^+$ T response to SEB due to expansion of polyfunctional CD57$^+$ cells in young individuals. PLoS One 9:e88538. doi:10.1371/journal.pone.0088538
Qi Q et al (2014a) Diversity and clonal selection in the human T-cell repertoire. Proc Natl Acad Sci USA 111:13139–13144. doi:10.1073/pnas.1409155111
Qi Q, Zhang DW, Weyand CM, Goronzy JJ (2014b) Mechanisms shaping the naive T cell repertoire in the elderly—thymic involution or peripheral homeostatic proliferation? Exp Gerontol 54:71–74. doi:10.1016/j.exger.2014.01.005
Romero P et al (2007) Four functionally distinct populations of human effector-memory CD8$^+$ T lymphocytes. J Immunol 178:4112–4119
Sakiani S, Olsen NJ, Kovacs WJ (2013) Gonadal steroids and humoral immunity. Nat Rev Endocrinol 9:56–62. doi:10.1038/nrendo.2012.206
Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A (1999) Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature 401:708–712. doi:10.1038/44385
Smithey MJ, Li G, Venturi V, Davenport MP, Nikolich-Zugich J (2012) Lifelong persistent viral infection alters the naive T cell pool, impairing CD8 T cell immunity in late life. J Immunol 189:5356–5366. doi:10.4049/jimmunol.1201867
Stowe RP, Kozlova EV, Yetman DL, Walling DM, Goodwin JS, Glaser R (2007) Chronic herpesvirus reactivation occurs in aging. Exp Gerontol 42:563–570. doi:10.1016/j.exger.2007.01.005
Strioga M, Pasukoniene V, Characiejus D (2011) CD8$^+$ CD28 and CD8$^+$ CD57$^+$ T cells and their role in health and disease. Immunology 134:17–32. doi:10.1111/j.1365-2567.2011.03470.x
Tarazona R, DelaRosa O, Alonso C, Ostos B, Espejo J, Pena J, Solana R (2000) Increased expression of NK cell markers on T lymphocytes in aging and chronic activation of the immune system reflects the accumulation of effector/senescent T cells. Mech Ageing Dev 121:77–88
Tower J, Arbeitman M (2009) The genetics of gender and life span. J Biol 8:38. doi:10.1186/jbiol141

Warren LA, Rossi DJ (2009) Stem cells and aging in the hematopoietic system. Mech Ageing Dev 130:46–53. doi:10.1016/j.mad.2008.03.010

Wertheimer AM et al (2014) Aging and cytomegalovirus infection differentially and jointly affect distinct circulating T cell subsets in humans. J Immunol 192:2143–2155. doi:10.4049/jimmunol.1301721

Wikby A et al (2006) The immune risk phenotype is associated with IL-6 in the terminal decline stage: findings from the Swedish NONA immune longitudinal study of very late life functioning. Mech Ageing Dev 127:695–704. doi:10.1016/j.mad.2006.04.003

Yan J, Greer JM, Hull R, O'Sullivan JD, Henderson RD, Read SJ, McCombe PA (2010) The effect of ageing on human lymphocyte subsets: comparison of males and females. Immun Ageing 7:4. doi:10.1186/1742-4933-7-4

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Contribution of neuroinflammation and immunity to brain aging and the mitigating effects of physical and cognitive interventions

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ABSTRACT

It is widely accepted that the brain and the immune system continuously interact during normal as well as pathological functioning. Human aging is commonly accompanied by low-grade inflammation in both the immune and central nervous systems, thought to contribute to many age-related diseases. This review of the current literature focuses first on the normal neuroimmune interactions occurring in the brain, which promote learning, memory and neuroplasticity. Further, we discuss the protective and dynamic role of barriers to neuroimmune interactions, which have become clearer with the recent discovery of the meningeal lymphatic system. Next, we consider age-related changes of the immune system and possible deleterious influences of immunosenescence and low-grade inflammation (inflammaging) on neurodegenerative processes in the normally aging brain. We survey the major immunomodulators and neuroregulators in the aging brain and their highly tuned dynamic and reciprocal interactions. Finally, we consider our current understanding of how physical activity, as well as a combination of physical and cognitive interventions, may mediate anti-inflammatory effects and thus positively impact brain aging.

Contents

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1. Introduction

For many years the brain was considered as an immune-privileged space, functioning fully autonomically in isolation from the immune system, separated by a relatively impenetrable blood-brain barrier. However, recent findings are resulting in a radical shift in this view. First, it used to be believed that the brain has no lymphatic system, reflecting lack of entry of lymphocytes into this sensitive area. The appearance of immune cells in the brain was considered an exceptionally harmful pathological incident leading to neurodegeneration. Second, for many years, neurogenesis was thought to be restricted to embryonic and developmental stages, but this view is now also being revised following the discovery of adult neurogenesis. It is now well-accepted that the brain is plastic and actually capable of change throughout the lifespan, adapting its function to different external and internal demands by altering its structure (Lövdén et al., 2013). The term "neuroplasticity" encompasses the potential for a number of functional and structural mechanisms, regulated by diverse extrinsic and intrinsic cues, all of which allow neuronal remodeling, formation of novel synapses and birth of new neurons (Calabrese et al., 2014). The immune system actively participates in this process, and immune cells and their secreted mediators can modulate adult neurogenesis under both homeostatic conditions and in phases of remodeling (Aimone et al., 2014; Kempermann et al., 2002; Leiter et al., 2016; Singhal et al., 2014; Villeda et al., 2011; Yau et al., 2015; Yirmiya and Goshen, 2011; Ziv et al., 2006).

The central nervous system (CNS) is no longer considered as being restricted to limited interactions with the peripheral immune system. We now know that these two major physiological systems communicate with each other constantly and extensively through multiple pathways (Ellwardt et al., 2016; Quan and Banks, 2007). Recent technological advances allow us to address this crosstalk using such techniques as brain imaging, cell-specific targeting and sequencing. Animal models have additionally helped to shed light on the complex mechanisms of neuroimmune regulation (Berry et al., 2010; Capoccia et al., 2013; Veiga-Fernandes and Mucida, 2016). Scientific interest in these interactions has markedly increased since the discovery of a meningeal lymphatic system capable of carrying fluid, immune cells, and macromolecules from the CNS to the draining lymph nodes (Louveau et al., 2015; Raper et al., 2016).

It could be postulated that the immune system and CNS represent the two major adaptive systems of the body. In this context, chronic inflammation can be regarded as a result of the maladjustment of these two major adaptive systems to resolve acute inflammation, which in turn may affect the course of the aging process (Elenkov et al., 2005). The interplay between aging, genetic predisposition, and environmental exposures initiates systemic and local metabolic changes as well as inflammatory reactions that predispose an individual to neuropsychiatric and neurodegenerative diseases (Deleidi et al., 2015). Even conditions of the prenatal environment (such as maternal chronic stress) may have long-term consequences influencing postnatal development (Berry et al., 2015). Maternal obesity may already prove detrimental by providing an intrauterine environment with elevated glucocorticoids, insulin resistance and increased inflammation that influences fetal developmental pathways associated with unhealthy aging in later life (Hanson and Gluckman, 2014; Holvoet, 2012; Iozzo et al., 2014).

The focus of the present review is on neuroimmune interactions in "normal" aging, which have received relatively little attention, rather than neurodegenerative pathologies, which have been extensively reviewed recently (Da Mesquita et al., 2016; Feigenson et al., 2014; Goldeck et al., 2016; Hansel et al., 2010; Leza et al., 2015; Litteljohn et al., 2014; Na et al., 2014; Norden et al., 2015; Nunes et al., 2013; Swardfager et al., 2016; Tansey, 2010; Tansey and Goldberg, 2010; von Bernhardi et al., 2010). Thus, we summarize representative studies and reviews concerning the multitude of reciprocal and dynamic communications between the nervous and immune systems during normal aging, the systemic consequences of age-related dysfunction of these communications, and possible interventions to mitigate this process. First, we will introduce the neuroimmunomodulatory mechanisms involved in the process of learning and memory under normal conditions, and then discuss their dysregulation in aging.

2. Immune modulation of neuroplasticity

The immune system communicates constantly with the CNS and is involved in modulating behavior and in many other critical neurological functions throughout the lifespan (Wilson et al., 2002). Normal learning and memory processes are dependent on hippocampal neurogenesis and deficits in such processes may lead to impairments in both spatial and non-spatial learning tasks (Yau et al., 2015). It has been well established that hippocampal neurogenesis in the adult brain is regulated by various intrinsic and extrinsic mechanisms (Kempermann et al., 2002). One of the mechanisms for optimal hippocampal neurogenesis is dependent on the immune system, an unexpected finding first demonstrated in mice with severe combined immune deficiency (SCID mice) and in mice lacking certain immune cell populations (Brynskikh et al., 2008; Kipnis et al., 2004; Wolf et al., 2009; Ziv et al., 2006). The role of systemic immune cells in supporting brain function and plasticity has been demonstrated for hippocampus-dependent functions such as spatial memory and sensorimotor gating (Kipnis et al., 2004; Ron-Harel et al., 2011; Wolf et al., 2009). Remarkably, it was found that systemic depletion of $CD4^+$ T lymphocytes led to significantly reduced hippocampal neurogenesis, impaired reversal learning in the Morris water maze, and decreased brain-derived neurotrophic factor (BDNF) expression in the brain (Wolf et al., 2009). Repopulation with $CD4^+$ T cells restored the deficits observed in immune-deficient mice, highlighting the role of this T-cell population as being pro-neurogenic under physiological conditions (Leiter et al., 2016). Apparently, hippocampus-dependent cognitive ability is supported by CNS-specific T cells, which accumulate within the brain meningeal spaces and produce interleukin (IL)-4, inducing BDNF production (Fig. 1) during the performance of cognitive tasks (Derecki et al., 2010). $CD4^+$ T cells were shown to promote and maintain neurogenesis by positively influencing microglia and regulating insulin-growth factor (IGF)-1 transport into the brain, thereby also regulating BDNF levels (Wekerle, 2006; Ziv et al., 2006). CNS-specific $CD4^+$ T cells are thought to be stimulated by macrophages, which circulate through the brain parenchyma, phagocytizing and processing CNS-derived self-antigens, such as myelin and/or neural debris. They are able to present these processed antigens and to stimulate naïve T cells in the periphery, resulting in the development of CNS-specific memory T cells (Fig. 1, bottom left), which later appear in the meningeal cerebrospinal fluid (CSF). Here they can be re-stimulated by brain-surveying macrophages (Fig. 1, top left) to produce neuroprotective cytokines and neurotrophic factors, supporting normal cognitive performance, learning and memory (Ron-Harel et al., 2011). T cells found in the CSF are mostly of central memory phenotype, expressing CCR7, CD27 and the activation marker CD69 (Ellwardt et al., 2016), in contrast to those within the choroid plexus (CP), which appear to be of effector-memory type (Baruch and Schwartz, 2013). Cytokines secreted by T cells, such as IL-4 and transforming growth factor β (TGF-β), have a protective effect on neurons and neural precursor cells (Fig. 1, central). Additionally, IL-4 stimulates microglia to produce BDNF, IGF-1, TGF-β, which all influence neuronal functioning (Burch, 2014; Ellwardt et al., 2016). IL-4 also

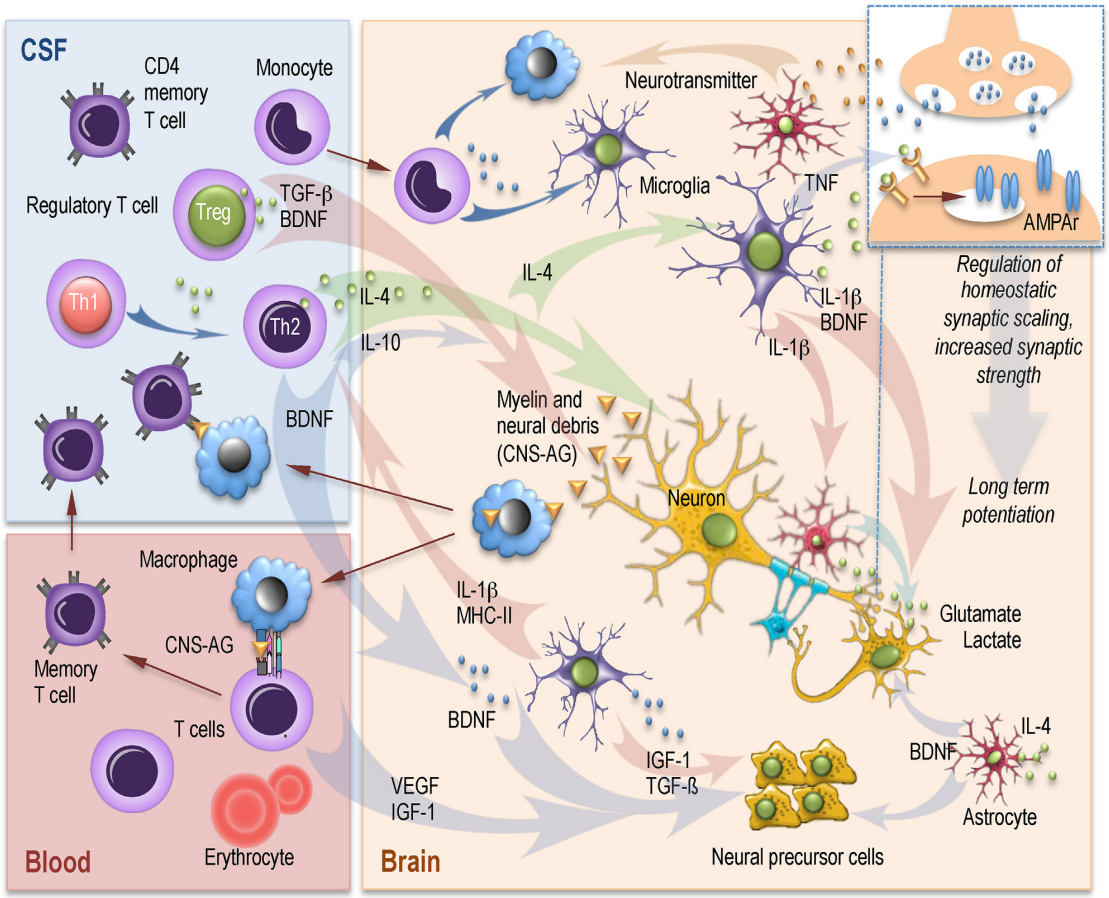

Fig. 1. Immune maintenance of brain homeostasis.
Adult hippocampal neurogenesis occurs in a homeostatic microenvironment that is partially dependent on T-cell surveillance of the CNS. Tregs can induce shift from Th1- to Th2-cells, which are neuroprotective. CNS-specific CD4-positive T cells (stimulated by myelin and/or neural debris-derived self antigens) support normal cognitive performance, learning and memory by production of IL-4 and BDNF. IL-4 has a direct neuroprotective function on neurons and on neural precursor cells. Furthermore, IL-4 stimulates microglia to produce BDNF, IGF-1 and TGF-β, which influence neuronal functioning. Hippocampal microglia also secrete TNF that regulates synaptic scaling, increases synaptic strength and supports LTP. IL-1β released at low concentration activates astrocytes to produce BDNF and TNF, as well as additional glutamate and lactate, all of which are important for long-term memory consolidation. Endothelial cells of the cerebrovasculature can also be stimulated (by low concentrations of IL-1β) to produce the neurotrophic factors IGF-1 and VEGF. All these molecular and cellular interactions (here depicted in a highly simplified scheme) allow supportive immunomodulation of brain function, neurogenesis and plasticity.
Abbreviations: CNS: central nervous system; Tregs: regulatory T cells; Th: T-helper cells; CD: cluster of differentiation; IL: interleukin; LTP: long-term memory potentiation; MHC: major histocompatibility complex; BDNF: brain-derived neurotrophic factor; IGF: insulin-like growth factor; TGF: transforming growth factor; TNF: tumor necrosis factor; VEGF: vascular endothelial growth factor.

promotes astrocyte expression of BDNF that is crucial for learning and cognition (Ron-Harel et al., 2011). Infiltrating macrophages also play an important role in maintaining homeostatic conditions in the brain (Fig. 1, central). Together with glial cells they regulate the physiological milieu of the brain by removing dead cells and cell debris, buffering toxic compounds and producing growth factors needed for cell survival and renewal, and also by down-regulating pro-inflammatory factors such as IL-1β and tumor necrosis factor (TNF) (Shechter et al., 2009). Microglial expression of major histocompatibility complex (MHC) class II molecules and of IL-1β (at low levels) can induce T cells to communicate with glia and meningeal myeloid cells via production of IL-4. Endothelial cells of the cerebrovasculature can also be stimulated by low concentrations of IL-1β to produce neurotrophic factors IGF-1 and vascular endothelial growth factor (VEGF) (Goshen and Yirmiya, 2009; Wohleb and Delpech, 2016; Yirmiya and Goshen, 2011). VEGF stimulates precursor cell proliferation in the subgranular zone and has neurogenic and angiogenic properties (Leiter et al., 2016).

Regulatory T cells (Treg) were also shown to modulate neuroinflammation by inducing apoptosis in pro-inflammatory M1-type glia, thereby promoting the neuroprotective M2 phenotype. They also decrease neurotoxicity by inhibition of glutamate secretion and generation of reactive oxygen species (ROS). Additionally, regulatory T cells (Fig. 1, top left) can induce a Th1-to-Th2 shift by down-regulating Th1 cytokine-producing T cells, and up-regulating Th2 cytokine-producing T cells, which are neuroprotective (Ellwardt et al., 2016; Kipnis et al., 2012; Xie et al., 2015).

Although most studies have reported deleterious effects of TNF on synaptic plasticity, it was also shown that physiologically low levels of TNF might be important in brain development (Stellwagen and Malenka, 2006). At glutamatergic synapses, plasticity usually requires changes in the number of postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (Bains and Oliet, 2007). The TNF secreted by hippocampal microglia is crucial for synaptic plasticity (Fig. 1, top right) underlying higher brain functions. It regulates synaptic scaling, increases synaptic strength and supports long-term potentiation (LTP, which

is known to reflect a long-lasting increase in synaptic efficacy). This is thought to be an important underlying mechanism of learning and memory formation (Baune et al., 2008; Beattie et al., 2002; Stellwagen and Malenka, 2006). The TNF released from microglia in response to decreased neuronal activity up-regulates the number of synaptic AMPA receptors, and thus synaptic strength, allowing the homeostatic adjustment of neuronal excitability (Bains and Oliet, 2007; Stellwagen and Malenka, 2006).

Like TNF, also IL-1β has a dual role: when released at low concentrations and under homeostatic conditions, IL-1β activates astrocytes to produce BDNF and TNF, as well as additional glutamate and lactate, all of which are important for long-term memory consolidation (Goshen et al., 2008; Goshen and Yirmiya, 2009). Goshen and colleagues demonstrated that physiological levels of IL-1β support memory formation, such that a slight increase in its level can even improve memory (Goshen and Yirmiya, 2009). However, any deviation from this physiological range results in impaired memory formation.

In the next section we will consider the important role of brain barriers in the dynamic regulation and maintenance of brain homeostasis and pursue the question of which mechanisms allow this vital cross communication between the CNS and immune system.

3. Role of brain barriers in neuroimmunology

Under homeostatic conditions the brain parenchyma is physically separated from the peripheral milieu by sophisticated cellular structures that are not only responsible for maintaining brain homeostasis but also for specific communication with other systems outside the CNS (Banks, 2015; Da Mesquita et al., 2016; Stolp et al., 2013). In fact, the question of how peripheral immune cells, cytokines and other immune mediators can affect a central behavioral response arose a long time ago. The first possible explanation was provided by invoking the activity of existing circumventricular regions of the brain, where the blood-brain barrier (BBB) is less impenetrable (such as the organum vasculosum lateralis terminalis, OVLT) and allows for direct entry (Maier et al., 1998). Additionally, for immune mediators like TNF, IL-1 and soluble IL-1-receptors, specific active transport mechanisms have been described (Dunn, 1992; Gutierrez et al., 1993, 1994). Moreover, binding of cytokines to endothelial receptors of the cerebrovasculature with subsequent release of other mediators (such as chemokines, endothelial cell adhesion molecules, prostaglandins and nitric oxide) leads to impairment of BBB integrity (Anthony et al., 1997) and allows entry into the brain.

Other mechanisms that may allow activated T cells to cross the BBB could be dependent on molecules such as P-selectin (Hickey, 2000). Peripheral cytokines crossing the BBB can also act indirectly by inducing release of more cytokine from brain stores (Banks, 2015). An alternative route by which the immune system might communicate with the CNS is via direct cytokine stimulation of vagal sensory nerve activity or via the sympathetic nervous system (Hansen et al., 2000; Hansen et al., 1998). Apparently, cytokine stimulation of peripheral sensory neural afferents induces central cytokine production or central alterations in neurotransmission (Hasegawa-Ishii et al., 2016).

In the following, we briefly describe the structure and function of the three main barriers of the brain depicted in Fig. 2:

(i) The BBB is formed by highly specialized, low pinocytotic endothelial cells, which line brain capillaries and are connected by tight junctions (Fig. 2, bottom right). At the interface between blood and brain, these tight junctions restrict the passage of solutes. Endothelial cells utilize different mechanisms to transduce signals from the vascular system and from the brain, described in more detail in the previous paragraph. The endothelial layer is surrounded by pericytes, astrocytic endfeet, and branches of circulating microglia. Together with the neural and dendritic processes they constitute the neurovascular unit that allows responses to external alterations in a very rapid and dynamic manner (Da Mesquita et al., 2016). The complex interplay between endothelial cells, astrocytes and brain cells as well as between endothelial cells and blood-derived immune cells is necessary to create a dynamic and protective blood–brain barrier. The BBB allows the controlled, regulated exchange of chemokines, cytokines, and immune cells between the CNS and the blood, as well as the secretion of immunomodulatory molecules by the BBB itself (Banks, 2015; Engelhardt et al., 2016; Quan and Banks, 2007; Shechter et al., 2013).

(ii) The blood-CSF barrier (BCSFB) is formed by tight junctions between epithelial cells of the choroid plexus (Fig. 2, bottom left). The CP contains a single layer of epithelium (with numerous microvilli) surrounding the inner stroma and blood vessels, with the endothelial layer of the latter being fenestrated. The CP is responsible for the production of CSF, providing a nutrient-rich milieu, and participating in brain homeostasis. CP regulates transport of serum-derived substances and immune cells across its epithelial layer. In contrast to the BBB, the BCSF barrier is permeable to immune cells also under normal conditions, allowing immunosurveillance (Plata-Salaman, 1991; Ron-Harel et al., 2011; Shechter et al., 2013).

Recent studies have recognized the CP as an active immunological interface and an important neuroimmune compartment in maintaining and restoring brain homeostasis. The CP is enriched with CNS-specific T cells and is strategically positioned for signal exchange between the CNS (through CSF) and circulation (through epithelium-immune cell interactions) participating in modulating brain functions (Baruch and Schwartz, 2013).

(iii) The CSF-brain barrier (CSFBB) at the level of the pia arachnoid is formed by tight junctions between endothelial cells of the arachnoid vessels (Fig. 2, top left). The recent discovery of functional classic lymphatic vessels (LV) in this area has opened a new window for understanding neuroimmune interactions within the CNS (Engelhardt et al., 2016; Raper et al., 2016; Shechter et al., 2013). The meningeal lymphatic system emerged as a new player in these interactions. It is hypothesized that the main role of the meningeal lymphatic system is not the drainage of the water content of the CSF, but rather a more pronounced immunological function, such as drainage of macromolecules and immune cells into cervical lymph nodes to maintain brain immune surveillance (Raper et al., 2016).

4. Immunosenescence and “inflammaging”

As we age, our immune system undergoes an imprecisely-defined process of “immunosenescence” that affects both adaptive and innate immune systems. Monocytes, which are innate immune cells, are known to be mediators of the inflammatory response and comprise at least three different subsets, based on their expression of CD14 (a pattern recognition receptor binding microorganism-derived lipopolysaccharide) and CD16 (a low affinity receptor for the Fc portion of immunoglobulins), namely classical, intermediate and non-classical monocytes. An age-related increase in frequencies of intermediate and non-classical monocytes has been reported (de Pablo-Bernal et al., 2016; Hearps et al., 2012). Our results from the Berlin Aging Study II confirmed these findings, where we also found an age-related increase in frequencies of intermediate and non-classical monocytes (unpublished results). It has been suggested that aging is associated with chronic innate immune activation and significant changes in monocyte functions, which may have impli-

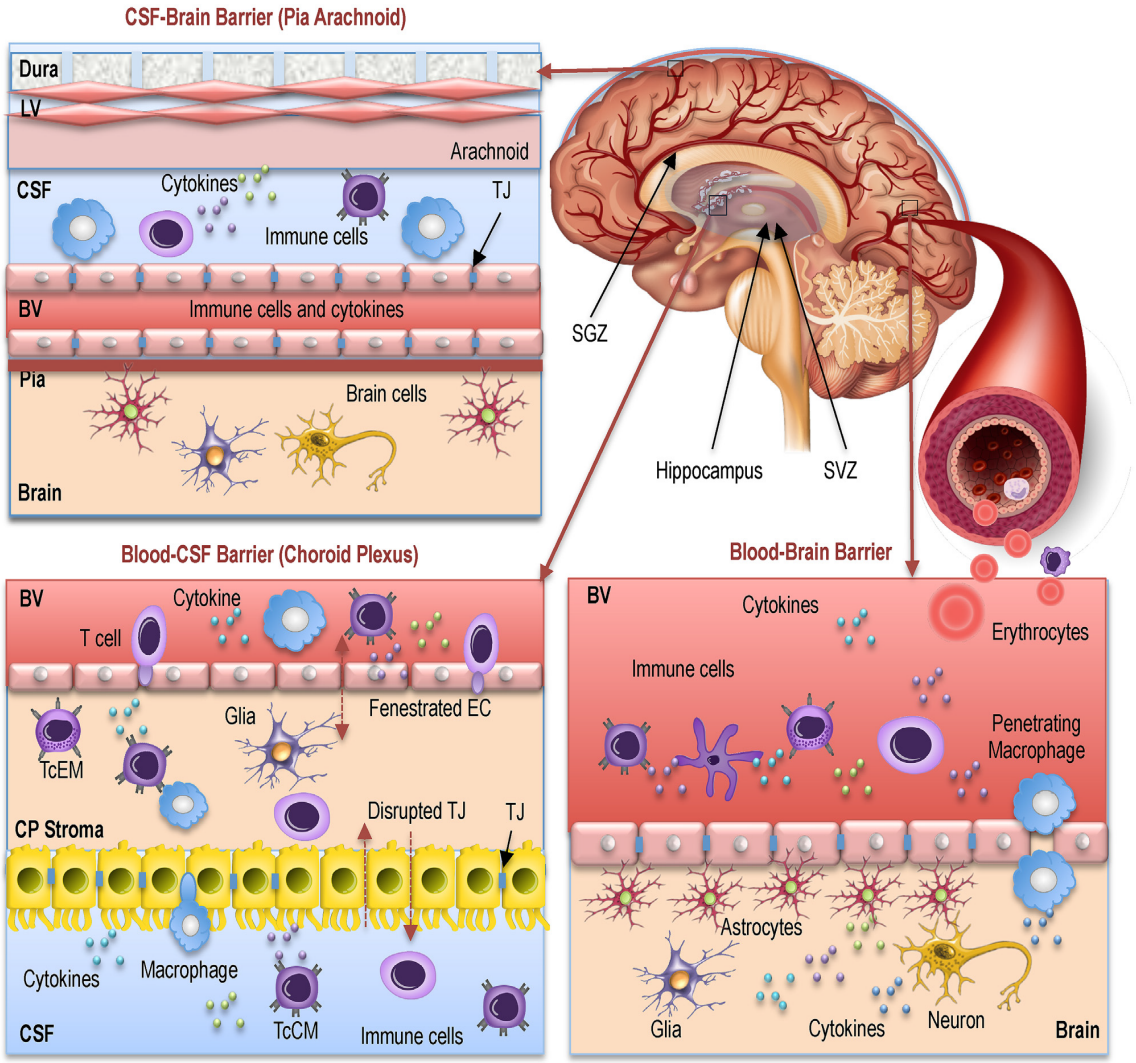

Fig. 2. Protective and dynamic barriers of the brain.
Schematic depiction of the three main barriers of the brain:
BBB—the blood-brain barrier (bottom right) is formed by highly specialized, low pinocytotic endothelial cells, which are connected by tight junctions. At the interface between blood and the brain these tight junctions restrict the passage of solutes. The BBB allows the controlled, regulated exchange of chemokines, cytokines, and immune cells between the CNS and the blood as well as the secretion of immunomodulatory molecules by the BBB itself.
BCSFB—the blood-CSF barrier (bottom left) is formed by tight junctions between epithelial cells of the CP. The CP contains a single layer of epithelium (with numerous microvilli) surrounding the inner stroma and blood vessels, with the endothelial layer of the latter being fenestrated. The CP is responsible for the production of CSF and regulates transport of serum-derived substances and immune cells across its epithelial layer. In contrast to the BBB, the BCSF is permeable to immune cells also under normal conditions, allowing immunosurveillance.
CSFBB—the CSF-brain barrier (top left) at the level of the pia arachnoid is formed by tight junctions between endothelial cells of the arachnoid vessels. Recently discovered lymphatic vessels (LV) drain macromolecules and immune cells from the meninges and the CSF.
Abbreviations: TJ: tight junctions; BV: blood vessel; CP: choroid plexus; EC: epithelial cell; CSF cerebrospinal fluid; LV: lymphatic vessel; TcEM: Effector Memory T cells; TcCM: Central Memory T cells; SVZ: subventricular zone; SGZ: subgranular zone.

cations for increased low-grade chronic inflammation and for the development of age-related diseases (Hearps et al., 2012). Thus, macrophage activation, together with inflammatory monocytes contribute to the subclinical chronic inflammatory process dubbed "inflammaging" (Franceschi et al., 2000; Franceschi et al., 2007).

The most marked changes in adaptive immunity are decreased numbers of peripheral naïve T cells and concomitant accumulation of late-stage differentiated memory T cells (Ben-Smith et al., 2008; Di Benedetto et al., 2015; Malaguarnera et al., 2010; Müller et al., 2013; Müller and Pawelec, 2015; Qi et al., 2014a,b; Vescovini et al., 2014; Wistuba-Hamprecht et al., 2015) with reduced antigen receptor repertoire diversity (Johnson et al., 2014; Naylor et al., 2005; Qi et al., 2014b; Salam et al., 2013) (Fig. 3). This phenomenon results from poorly-understood age-related impairments in the hematopoietic stem cell compartment which generates fewer T-cell precursors in adult and later life on the one hand, and thymic involution at puberty which markedly reduces the production of mature T cells from their precursors on the other hand (Arnold et al., 2011).

Life-long exposure to different pathogens is regarded as a major driving factor of the phenotypic changes in the distribution of T-cell subsets over the life course. For unclear reasons, especially a latent infection with Cytomegalovirus (CMV), but not with any other herpesviruses, with recurrent episodes of reactivation has been found to promote memory T-cell "inflation" and drive T cells to a late stage of differentiation. In aged individuals, oligoclonally-expanded $CD8^+$ T cells show increased expression of late-stage differentiation

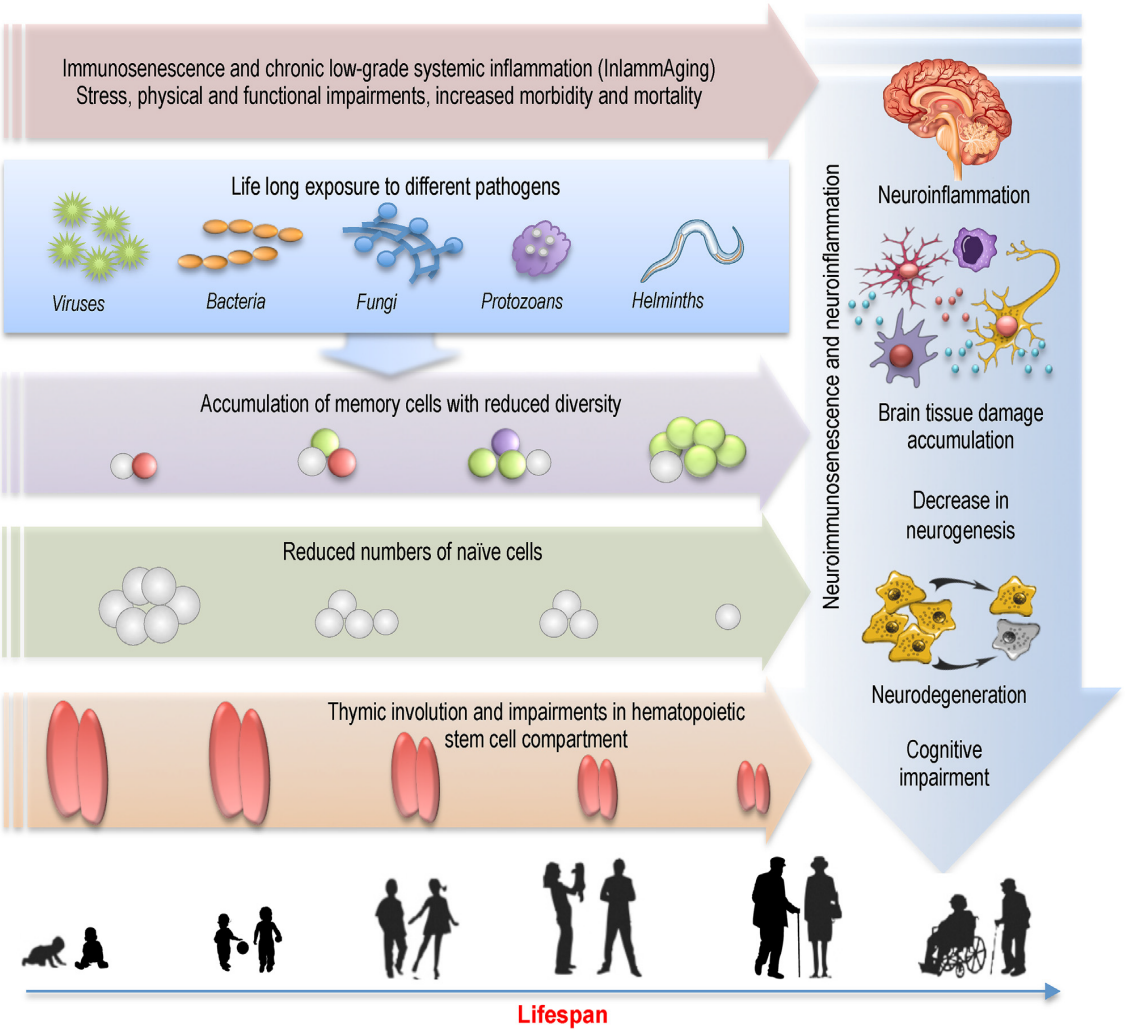

Fig. 3. Schematic representation of potential mechanisms of immunosenescence and neurosenescence.
Immunosenescence affects both adaptive and innate immune systems. The most relevant changes to adaptive immunity are decreased peripheral naïve T cells and concomitant accumulation of late-stage differentiated memory T cells with reduced antigen receptor repertoire diversity. This phenomenon results from age-related impairments in the hematopoietic stem cell compartment (which generates few T-cell precursors) in adult and later life, and thymic involution at puberty (which markedly reduces the production of mature T cells from their precursors). Life-long exposure to different pathogens is the major driver of the phenotypic changes in the distribution of T cell subsets over the life course. Aging is characterized by a chronic, low-grade inflammation (termed "inflammaging") that is a highly significant contributor for morbidity and mortality in the elderly people. Peripheral immunosenescence and inflammaging may promote neuroinflammation by modulating glial cells towards a more active pro-inflammatory state, leading to loss of neuroprotective function, to neuronal dysfunction and accumulation of brain tissue damage. Systemic inflammation may increase the risk for developing cognitive impairments and neurodegeneration.

markers. The accumulation of these highly differentiated T-cells, at least some of which may be truly senescent, contributes to the age-associated increased production of pro-inflammatory cytokines and could thus possibly also contribute to age-related morbidity and mortality (Bennett et al., 2012; Müller et al., 2013; Pawelec and Derhovanessian, 2011; Pawelec et al., 2010).

As mentioned above, aging is characterized by a chronic, low-grade inflammation that is probably a significant contributor to morbidity and mortality in the elderly (Franceschi et al., 2000; Franceschi and Campisi, 2014). Thus, peripheral immunosenescence and inflammaging may promote neuroinflammation by modulating glial cells towards a more active pro-inflammatory state, leading to loss of neuroprotective function, to neuronal dysfunction and accumulation of brain tissue damage (Giunta et al., 2008; von Bernhardi et al., 2010). Systemic inflammation may therefore increase the risk for developing cognitive impairment, neurological disorders and neurodegeneration (Goldeck et al., 2016; Harrison, 2016; Pizza et al., 2011). In the next section we will have a closer look at the main players contributing to the process of neuroinflammation during aging.

5. Main immunomodulators and neuroregulators in the aging brain

5.1. Cytokines

Cytokines, together with neurotransmitters and hormones, are signaling molecules possessing unique immunomodulatory functions. They can influence virtually every physiological system including neuroendocrine interactions, neurotransmitter metabolism and neuroplasticity, thereby affecting behavioral and cognitive functioning. The cytokine network, composed of the cytokines themselves, their receptors and their regulators, is present throughout the brain and other physiological systems and is highly regulated during the lifespan. Cytokines can operate within complex cascade patterns and act synergistically or antago-

nistically. These molecules enable cross-communication between different cell types, translating environmental signals into molecular signals (Alboni and Maggi, 2015). Cytokines are normally grouped according to their effects on inflammation, as some are considered likely to increase inflammation, especially IL-1, IL-6 and TNF, whereas others (IL-4, IL-10, TGF-β) tend to decrease it (Ownby, 2010). It is well-established that peripherally-produced cytokines can access the brain and thus affect CNS functions via several routes. Bidirectional transport of different cytokines, including IL-1, has been demonstrated (Khairova et al., 2009). Thus, the presence of IL-1 in CSF is not only a result of local synthesis by brain cells, but is also dependent on the concentration of IL-1 in the peripheral blood. Recent work by Scheinert and colleagues demonstrated that cytokine levels change across the lifespan and vary by cognitive status, at least in rats, suggesting that serum inflammatory biomarkers are predictive of cognitive decline (Scheinert et al., 2015).

5.2. Immune cells

T cells (Fig. 4B) are activated by antigen-presenting cells, which are specialized to capture, process and present antigens for recognition by these cells. This can occur either in the periphery or in the CSF of the choroid plexus. Depending on the pattern of cytokine secretion, the functions of T cells, and the molecules that drive their differentiation, different T-helper cell phenotypes are identified. Th1 cells secrete pro-inflammatory cytokines such as IFN-γ or TNF. Th2 cells have an anti-inflammatory function by producing IL-4 and IL-10 (Aloisi et al., 2000; Gemechu and Bentivoglio, 2012).

In addition to CNS-specific $CD4^+$ T cells, which appear to be neuroprotective (Derecki et al., 2010; Kipnis et al., 2012), a novel population of $CD8^+$ T cells has been reported in several regions of the brain, including the meninges and choroid plexus. These aging-related T cells had effector memory and tissue-resident phenotypes and were shown to modulate microglia (Ritzel et al., 2016). The principal function of regulatory T cells in this context is to prevent or to counteract excessive CNS inflammation by release of anti-inflammatory cytokines such as IL-10 or TGF-β (Xie et al., 2015).

Bone marrow-derived monocytes (Fig. 4C) reside in the perivascular space, choroid plexus and meninges of the brain, and are also able to differentiate into macrophages or dendritic cells (Auffray et al., 2007; Auffray et al., 2009). Macrophages scan the brain for pathogens and present antigens to T cells (Prinz et al., 2011). They exhibit different states of activation depending on the cytokine milieu to which they are exposed. Increased levels of IL-6 drive their differentiation into inflammatory M1-phenotype, while IL-4 exposure induces differentiation to anti-inflammatory M2-macrophages.

In addition to cytokine production, immune cells (predominantly T cells) can produce and respond to neurotransmitters such as acetylcholine, glutamate, dopamine, and serotonin. Dopamine and acetylcholine are immunostimulating neurotransmitters, whereas epinephrine and serotonin induce immunosuppression (Plata-Salaman, 1991). Moreover, these neurotransmitters are essential for modulating learning, memory and LTP. They affect not only neurons but also regulate the production and secretion of inflammatory factors from astrocytes and microglia (Leiter et al., 2016).

5.3. Microglia and astrocytes

Microglia are macrophage-like immune cells of the CNS involved in numerous physiological and pathological brain functions. They actively support brain development and plasticity through release of neurotransmitters and neurotrophic factors or through the pruning of synaptic elements (Deleidi et al., 2015). Given the functional significance of glial cells in the brain, it was suggested that they might represent a third element of the synapse (in addition to pre- and post-synaptic neurons), with the potential to influence synaptic transmission and in particular synaptic plasticity processes (Di Filippo et al., 2013). Therefore, the function of microglia and astrocytes is different in different states of their activation (Fig. 4A). Under baseline conditions, they are relatively quiescent and can be found in a resting state characterized by a ramified morphology with many processes, which constantly survey the surrounding area (Nimmerjahn et al., 2005). Under such resting conditions microglia carry out their essential functions of phagocytosis and clearance of debris, neuronal support as well as synaptic monitoring and remodeling (Da Mesquita et al., 2016). Following activation, both microglia and astrocytes change their shape, become amoeboid and undergo phenotypic polarization. Their activation phenotype is largely dependent on the levels of specific cytokines (Hefendehl et al., 2014; Jones and Lynch, 2015; Norden et al., 2015; Patterson, 2015).

In addition to these main phenotypes, a new "dark" microglial phenotype has been described (Bisht et al., 2016; Tay et al., 2016) that exhibits condensed cytoplasm, giving those microglia a dark appearance. Dark microglia could represent a subset of stressed, hyperactive microglia, which might be implicated in the loss of synapses (Bisht et al., 2016).

Astrocytes are closely associated with neuronal synapses and have similar neuroprotective functions as microglia in the normal brain but can also display neurotoxic effects, which depend on the cytokine milieu causing neuroinflammation (Singhal et al., 2014). It has also been demonstrated that astrocytes and the environment in the CNS have the capacity to regulate T-cell characteristics (Beurel et al., 2014), mediating inflammation and immune reactivity in the brain.

5.4. BDNF and IGF-1

It is now widely accepted that neurotrophic factors, such as BDNF and IGF-1, are crucial mediators of neuronal plasticity, because they are abundant in the regions particularly relevant for plasticity, where they participate in the axonal and dendritic growth and their remodeling. They are responsible for the production of neurotransmitters, for proper formation of synapses and for their functioning (Bramham and Messaoudi, 2005; Lu et al., 2005).

It could be suggested that an exaggerated brain inflammatory response, occurring during aging and/or a secondary immune challenge, may degrade the ability to provide the BDNF needed for memory-related plasticity processes at hippocampal synapses (Patterson, 2015). Many studies have shown that inflammation detrimentally affects the expression of BDNF within the brain (Guan and Fang, 2006; Schnydrig et al., 2007). The effect of a peripheral immune challenge on different BDNF transcripts has also been demonstrated (Aid et al., 2007; Chapman et al., 2012), indicating that inflammation may affect specific isoforms of this neurotrophin. It was hypothesized that one of the mechanisms by which inflammation may affect brain function could involve BDNF modulation (Calabrese et al., 2014).

IGF-1 is another important neurotrophic factor. Deficiency of this mediator has been reported to influence the development of cognitive impairment and dementia in older adults. A growing body of evidence indicates that synaptic function decreases with age and that IGF-1 contributes to this diminished information processing in the brain (Deak and Sonntag, 2012). Therefore, low levels of IGF-1 are associated with worse cognitive function in elderly persons. Interestingly, results of a recent study found an optimal level of IGF-1 for optimal cognitive functioning suggesting that both high and low levels of IGF-1 may be associated with poor cognition (Tumati et al., 2016).

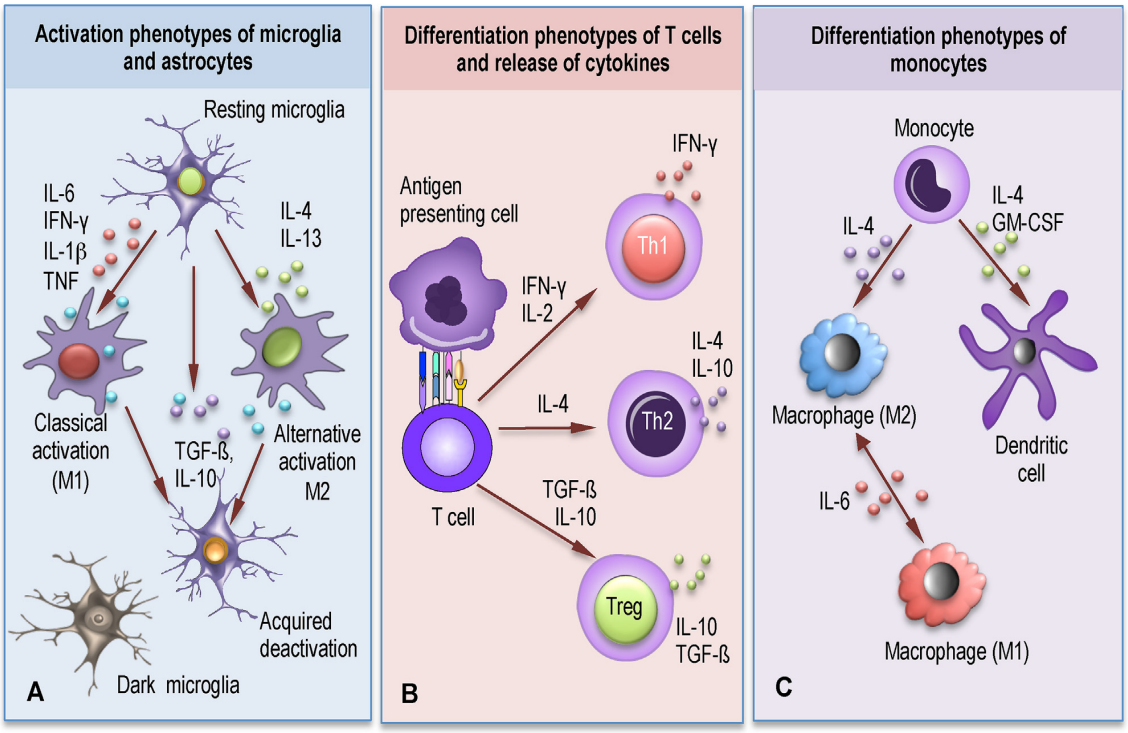

Fig. 4. Overview of the main players in brain homeostasis and neuroinflammation.
Abbreviations: IL: interleukin; IFN: interferon; TNF: tumor necrosis factor; TGF: transforming growth factor; CP: choroid plexus; Th: T-helper cells; CNS: central nervous system; DC: dendritic cell.

6. The aging brain and neuroinflammation

Cognitive aging is characterized by an impairment of cognitive abilities. Although no agreement exists on the basic underlying mechanisms involved in this process, neuroinflammation appears to be the main contributor that links many factors associated with cognitive aging together (Ownby, 2010). As we age, we experience greater susceptibility to memory impairments following an immune challenge that is characterized by increased and prolonged production of pro-inflammatory cytokines in the otherwise healthy aged brain (Barrientos et al., 2015). It is widely established that both aging and stress can affect the neuroendocrine system, activate the hypothalamic-pituitary-adrenal (HPA) axis to release corticotropin-releasing hormone (CRH) from the paraventricular nucleus of the hypothalamus and cause the anterior pituitary gland to secrete adrenocorticotropin (ACTH) (Fig. 5, top right). This, in turn, induces the release of glucocorticoids from the adrenal gland into the circulation (Barrientos et al., 2012). Cortisol affects the immune system in different ways: by regulating the expression of cytokines (Capoccia et al., 2013), chemokines and adhesion molecules, and by affecting immune cell migration, maturation and differentiation (Barrientos et al., 2015; Hansel et al., 2010). High levels of cortisol can negatively influence hippocampal neurogenesis directly or indirectly through regulation of expression of cytokines and their receptors on brain and immune cells. It appears that CNS levels of certain cytokines and their modulators increase as a function of age, at least in rodents (Barrientos et al., 2012; Scheinert et al., 2015; Ye and Johnson, 1999) and that aging microglia develop an altered profile characterized by an increased inflammatory state (Norden et al., 2015). Norden and colleagues referred to this as a primed profile and defined it by (i) increased baseline expression of inflammatory markers and mediators; (ii) a decreased threshold "to be activated and to switch" to a pro-inflammatory state; and (iii) exaggerated inflammatory response following immune activation (Norden et al., 2015).

Recent ultrastructural analyses have uncovered a new player in the age-related remodeling of neuronal circuits, especially at the synapse level, that is rare under homeostatic conditions, but becomes abundant during aging, neurodegeneration and chronic stress (Bisht et al., 2016). These hyperactive so-called "dark microglia" frequently reach into synaptic clefts with their highly ramified and thin processes, extensively encircle axon terminals and dendritic spines and engulf them (Bisht et al., 2016; Tay et al., 2016). Aging and neurodegeneration are exactly characterized by dysregulated interactions with synapses, resulting in neuronal loss, which in turn represents the best pathological correlate of cognitive decline (Tay et al., 2016). These conditions can sensitize the aged brain to produce an exaggerated response following exposure to a stressor or to the presence of an immune stimulus in the periphery (Sparkman and Johnson, 2008). Altered microglia profiles together with impairments in key regulatory systems can lead to prolonged neuroinflammation and age-related neurobehavioral complications (Norden et al., 2015).

On the systemic level, peripheral immunosenescence and inflammaging lead to age-related changes in the blood (Fig. 5, left). Chronic exposure to inflammatory mediators may disrupt the endothelial barrier and allow the transfer of immune cells and numerous pro-inflammatory cytokines into the brain parenchyma that, in turn, can modulate microglial phenotype and reactivity and drive low-grade brain inflammation. Brain cells, such as microglia, astrocytes, and neurons but also peripheral immune cells, such as T cells, monocytes, and macrophages participate in inflammation (Fig. 5, central). This induces an inflammatory milieu that is populated by all these resident and additional infiltrating immune cells, which participate in a complex interplay between secreted inflammatory modulators and activated cell surface receptors, such

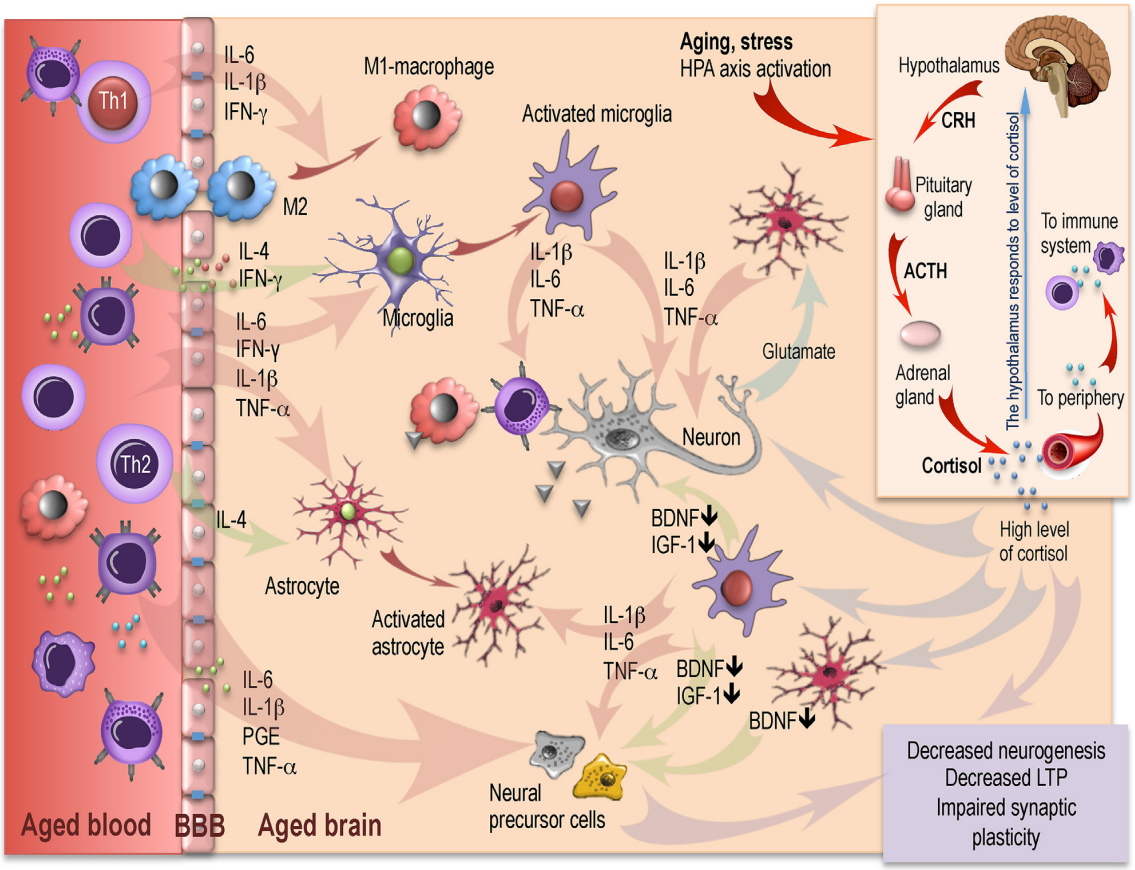

Fig. 5. Aging brain and neuroinflammation.
Aging and stress activate the HPA axis to release CRH from the paraventricular nucleus of the hypothalamus and cause the anterior pituitary gland to secrete ACTH. This, in turn, induces the release of glucocorticoids from the adrenal gland into the circulation. High levels of cortisol can negatively influence hippocampal neurogenesis directly, or indirectly through regulation of expression of cytokines and their receptors on brain and immune cells. Peripheral immunosenescence and inflammaging lead to age-related changes in the blood. Chronic exposure to inflammatory mediators may disrupt the endothelial barrier and allow the transfer of immune cells and numerous pro-inflammatory cytokines into the brain parenchyma that, in turn, can modulate microglial phenotype and reactivity and drive low-grade brain inflammation. Activated microglia and astrocytes change their morphology and function, and produce pro-inflammatory cytokines such as IL-1 β, IL-6 and TNF. Macrophages change their protective M2-phenotype to the proinflammatory M1-phenotype, thus contributing to further neuroinflammation. This over-production of pro-inflammatory mediators disrupts the delicate balance needed for LTP-induction, impairs synaptic plasticity and reduces production of BDNF and IGF-1. This has detrimental consequences for neural precursor cells (decrease of neurogenesis), as well as for the normal neuronal functioning.
Abbreviations: HPA: Hypothalamic-pituitary-adrenal axis; CRH: corticotropin releasing hormone; ACTH: adrenocorticotropin; IL: interleukin; IFN: interferon; TNF: tumor necrosis factor; LTP: long term memory potentiation; BDNF: brain-derived neurotrophic factor; IGF: insulin-like growth factor.

as toll-like receptors. These receptors are primarily expressed on cells that play central roles in the inflammatory response, including macrophages and microglia (Doty et al., 2015).

Activated microglia and astrocytes change their morphology and function, and produce pro-inflammatory cytokines such as IL-1β, IL-6 and TNF. Recent work suggests that microglia undergo a process of senescence, similar to the one in peripheral immune cells. Senescent and hyperactive microglia have been detected in the aged and diseased brain (Deleidi et al., 2015). The aging brain in turn is apparently able to modulate the immune system and to support recruitment of immune cells from the periphery, thereby contributing further to immunosenescence and neuroinflammation (Gemechu and Bentivoglio, 2012). In addition, macrophages change their protective M2-phenotype to the pro-inflammatory M1-phenotype, thus contributing to further neuroinflammation. As the aged brain is already "primed" to respond to inflammatory stimuli, additional stress or infection may induce more detrimental changes in cognitive functioning of aged individuals (Campbell, 2004; Sparkman and Johnson, 2008). Furthermore, the age-related concurrent reduction of anti-inflammatory molecules also contributes to a sensitization to extrinsic and intrinsic stressors. T cells produce less IL-4 and IL-10, and more IFN-γ, thereby promoting microglial activation. This has detrimental consequences for neural precursor cells leading to a decrease of neurogenesis, as well as increased decrements in learning and memory (Chen et al., 2016; Leza et al., 2015).

Taken together, even neurologically-intact aged individuals show a progressive increase in neuroinflammation characterized by increased glial activation, elevated steady-state levels of inflammatory cytokines and decreased production of anti-inflammatory molecules. This over-production of pro-inflammatory mediators disrupts the delicate balance needed for LTP-induction, reduces production of brain plasticity-related molecules, such as BDNF and IGF-1 and impairs synaptic plasticity.

7. Impact of physical and cognitive interventions

With increasing age, even healthy and able individuals experience some decline of cognitive performance. Investigating the nature of cognitive changes in normal aging, and the possibilities of how an enriched and stimulating lifestyle in aging could impede this decline, attracts wide scientific and social interest. Physical activity is one of the most promising and relatively simple behavioral interventions that may prevent or at least delay cognitive

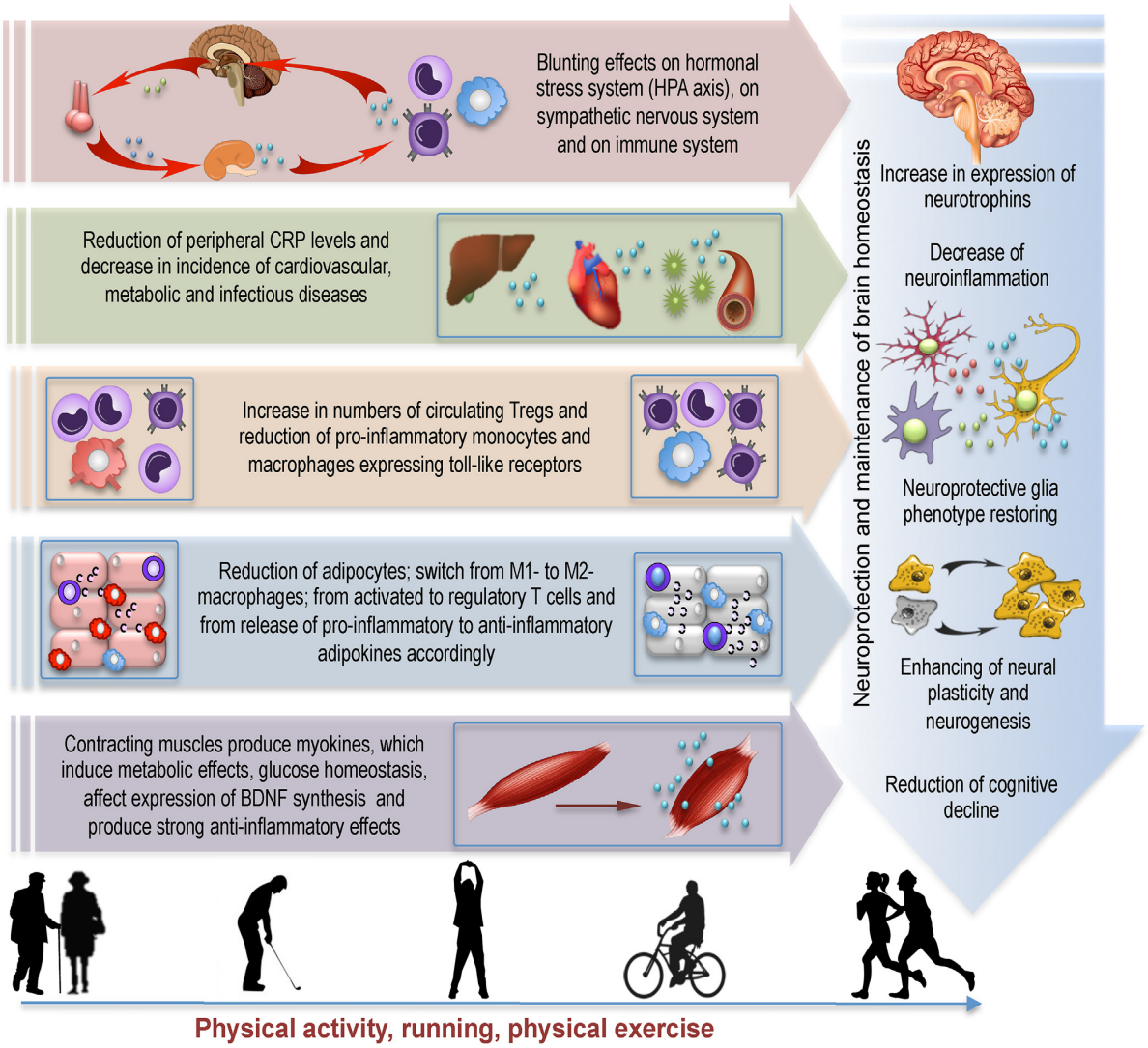

Fig. 6. Overview of the potential effects of physical activity on neuroimmunosenescence. Abbreviations: HPA: Hypothalamic-pituitary-adrenal axis.

decline. It has been reported that physical exercises slow down cognitive impairment and maintain the volume of the hippocampus (Ahlskog et al., 2011; Colcombe and Kramer, 2003; Cotman and Berchtold, 2002; Erickson et al., 2011; Maass et al., 2015). Results from both animal (Speisman et al., 2013a; Speisman et al., 2013b) and human studies demonstrate that physical activity plays an important role in altering metabolic, structural, and functional networks of the brain and possibly in protecting from cognitive impairments in aged adults (Kim et al., 2010; Kirk-Sanchez and McGough, 2014; Snigdha et al., 2014). Promising evidence from recent animal models indicates that physical activity and the exposure to enriched environments show beneficial effects on physiological markers of brain aging, such as LTP, long-term depression (LTD), and cell excitability and can improve cognitive performance (Kumar et al., 2012). Beneficial effects of physical exercise on structural and neurochemical measures within brain regions has been shown in human too, both in younger and older adulthood (Düzel et al., 2016). Observational studies continue to suggest that adults who engage in physical activity reduce their risk of cognitive decline and dementia (Dik et al., 2003; Ferencz et al., 2014; Podewils et al., 2005; Yaffe et al., 2001). Physical inactivity is a significant risk factor for cognitive decline in aging and for Alzheimer's Disease (Norton et al., 2014), and exercise can exert a protective effect (Ahlskog et al., 2011; Geda et al., 2012; Ngandu et al., 2015; Prakash et al., 2015; Wirth et al., 2014), even if initiated in later life (Tolppanen et al., 2015). Physical exercise and physical activity in later life might target modifiable risk factors of aging and induce neuroprotective mechanisms (Fig. 6) (Carvalho et al., 2014; Kirk-Sanchez and McGough, 2014).

Although the exact mechanisms through which physical exercise affects cognition are not yet fully understood, there is growing evidence that selected aspects of cognition are responsive to increases in physical exercise (Colcombe and Kramer, 2003; Cotman and Berchtold, 2002; Erickson et al., 2011; Maass et al., 2015). Numerous studies have revealed anti-inflammatory and anti-oxidative effects following physical activity, which potentially exert beneficial effects on neuroplasticity, affect the expression of neurotrophins and therefore normal neuronal functions (Moylan et al., 2013). Barrientos and colleagues observed an inhibition of neuroinflammation (caused by infection) in rats performing exercises, and increased induction of BDNF mRNA in the brain of otherwise sedentary animals (Barrientos et al., 2011). Further studies with aged mice revealed that wheel running inhibits the pro-inflammatory state of microglia and their ability to proliferate,

thus inducing a pro-neurogenic phenotype (Kohman et al., 2012). The findings by Speisman and colleagues demonstrated that daily exercises may have protective effects on cognition by modulating immune and neuroimmune cytokines and neurogenesis (Speisman et al., 2013a) in aged rats. In another recent study it was shown that an intensive training program inhibited pro-inflammatory responses in hippocampus (such as gene expression level and protein content of IL-1β cytokine), thereby inducing neuroprotection (Chennaoui et al., 2015).

Certainly, the anti-inflammatory effects of exercise are mediated through multidirectional pathways. Some of them are exerted on adipose tissue, skeletal muscles, immune system cells, and the cardiovascular system (Fig. 6). These effects include modulation of anti-inflammatory and pro-inflammatory cytokine profiles, redox-sensitive transcription factors, anti-oxidant and pro-oxidant enzymes, and repair proteins (Majka et al., 2009). Regular physical activity can reduce the size of adipocytes and attenuate inflammation in adipose tissue by phenotype switching from pro-inflammatory M1- to anti-inflammatory M2-type macrophages (Fig. 6). In addition, a reduction in the numbers of circulating pro-inflammatory type monocytes and an increase in numbers of regulatory T cells were found in the peripheral blood of individuals following physical activity (Pedersen, 2011; Timmerman et al., 2008).

Recent evidence suggests that contracting muscles release myokines (Dishman et al., 2006; Minter et al., 2016; Müller and Pawelec, 2014; Pedersen and Hoffman-Goetz, 2000; Petersen and Pedersen, 2005; Phillips et al., 2014), which affect the synthesis of BDNF in the dentate gyrus of the hippocampus (Phillips et al., 2014). A recent *meta*-analysis based on 29 studies estimated the strength of association between exercise and increased BDNF levels in humans across multiple exercise paradigms. Effect size analysis supported the role of exercise as a successful strategy to enhance BDNF activity in humans. Interestingly, the magnitude of these effects was lower in females than males (Szuhany et al., 2015). In general, differences between the sexes over the course of aging have been repeatedly reported in both animal and human studies (Beery and Zucker, 2011; Laws et al., 2016; Zagni et al., 2016). Estrogens, which are known to have inhibitory effects on neuroinflammation (in particular on microglia) appear to contribute to such sexual dimorphism (Au et al., 2016; Vegeto et al., 2008). Fluctuations in estrogen levels might alter the anti-inflammatory activity of this hormone and impair the neuronal protection.

Accumulating knowledge from clinical and pre-clinical data about the effects of physical exercise on brain function suggests that exercise may reduce inflammation and oxidative stress through a number of cellular and humoral neuroimmune changes. Astrocytes, microglia, endothelial cells of cerebrovasculature and T cells are known to have anti-inflammatory and neuroprotective functions via a variety of mechanisms. It is still a matter of investigation whether exercise has effects on specific neuroimmune markers, such as markers of immunosenescence (Beurel et al., 2014; Eyre and Baune, 2012; Moylan et al., 2013). Numerous studies do nevertheless suggest that regular physical activity is likely to be associated with maintenance of the more "youthful" phenotype of the immune system (Simpson, 2016; Turner, 2016).

The beneficial effects of physical fitness on mental and physical health involve protection against potentially adverse behavioral and metabolic consequences of stressful events and aging. Physical activity may have a buffering role on the hormonal stress responsive systems (Fig. 6), such as the HPA axis and the sympathetic nervous system (Simpson, 2016). Regular physical activity may also lead to a reduction of excessive inflammation and may therefore contribute to positive psycho-physiological effects. Furthermore, regular exercise may be beneficial for the brain by enriching growth factor expression and neural plasticity, thereby contributing to improved mood and cognition (Silverman and Deuster, 2014). Papenberg and colleagues investigated the impact of an inactive lifestyle and high levels of peripheral inflammatory cytokines on gray-matter volumes in a population-based study of older adults. Results showed that inflammation exacerbated negative effects on brain and cognition, and this was particularly pronounced in inactive older adults (Papenberg et al., 2016). Exercise and physical activity have been shown to improve overall health and well-being in aged individuals and their beneficial effects appear to impact all physiological systems (Simpson, 2016). Nonetheless, the majority of aged individuals does not implement exercise programs on a regular, extended basis. Varma and colleagues have recently demonstrated that even low-intensity but regular exercise such as daily walking was associated with greater hippocampal volume in older women (Varma et al., 2015). Epidemiological evidence and other results suggest that physical activity may be associated with improved cerebral blood flow and neuronal connectivity (Burdette et al., 2010), favorable changes in brain volume (Colcombe et al., 2006; Erickson et al., 2014), neurotrophic factors (Leckie et al., 2014), neurogenesis (Yau et al., 2011), and lower rates of cognitive decline. But some results on beneficial effects of exercise training remain still controversial. The effects of exercise may vary greatly depending on the individual's characteristics, as well as on type, intensity, frequency, and duration of exercise. Thus, it appears to be essential to develop personalized exercise programs in interventional designs (Majka et al., 2009).

Results from cognitive training programs have also been shown to result in improvements in cognitive abilities in older adults compared to control groups (Engvig et al., 2010; Schmiedek et al., 2010) and the effects were partly maintained up to five years after the intervention (Willis et al., 2006). Thus, increased mental activity in older people through directed cognitive training may represent an effective intervention to counteract cognitive decline and potentially even postpone the rise of dementia (Cheng et al., 2012).

It has been proposed that physical and cognitive exercise might interact synergistically, thereby inducing larger beneficial effects (Bamidis et al., 2014; Hotting and Roder, 2013; Kempermann et al., 2010; Kraft, 2012; Lustig et al., 2009). Several intervention studies are in line with this hypothesis demonstrating larger benefits on cognitive test performance for combined physical and cognitive activities than for either activity alone. Those studies comprise observational cross-sectional (Eskes et al., 2010), longitudinal (Karp et al., 2006), as well as controlled interventional designs (Fabre et al., 2002; Oswald et al., 2006). Results of studies with patients at risk of cognitive decline suggest that cognitive and physical exercise training represent promising non-pharmaceutical tools for improving cognition in elderly at-risk individuals (Bherer, 2015; Fiatarone Singh et al., 2014).

What might be the advantage of combined physical and cognitive exercise? Physical activity could be boosting the potential for adult neurogenesis while cognitive stimulation is increasing the recruitment of cells in an enriched environment (Kempermann et al., 2010). From an evolutionary perspective, physical and cognitive challenges have always appeared in a highly interlinked fashion (Kempermann et al., 2010). It might be assumed that the neurobiological mechanisms triggered by physical and cognitive exercise cooperate to induce plastic changes and to increase the potential for neurogenesis and synaptogenesis (Fissler et al., 2013). In other words, physical exercise may (through mitigating neuroinflammation) induce an appropriate neuroprotective environment and prepare the brain to respond to cognitive stimulation. Cognitive training then induces neuronal changes in specific networks associated with the trained skill.

Taken together, accumulating and promising evidence suggests that plastic changes are less pronounced but possible in old age,

and might be boosted by combining both physical and cognitive activities.

8. Concluding remarks

Around the world, and especially in many European countries, the older segment of the adult population is growing in size and proportion (Vaupel et al., 2003). Given recent major advances in prophylaxis and medical care for cardiovascular disease and cancer, the most important societal, public health and personal challenges in dealing with demographic change will relate to cognitive function and finally, neurodegeneration. What people make out of the added years of their lives will be the most important aspect, but will only be advantageous to the individual and to society at large if people can remain active participants in daily life and work. Maintaining cognitive function will be paramount, but cognitive performance is known to decrease with increasing age, even in overtly healthy individuals. Preventing, attenuating and delaying cognitive decline is probably the most effective measure for postponing the point of time at which individuals are no longer able to lead an independent life. Effective pharmacological treatments for cognitive decline remain unavailable. Some modifiable lifestyle factors, such as poor diet and physical and cognitive inactivity have been identified as associated with increased risk of cognitive decline over the normal aging process, as opposed to overt pathological conditions (Lindenberger, 2014). Encouragingly, exercise can have a protective effect, even if initiated in advanced old age (Tolppanen et al., 2015). In this review we have described potential basic underlying processes of age-related decline, namely a progressive increase in neuroinflammation characterized by increased glial activation, elevated steady-state levels of inflammatory cytokines and decreased production of neurotrophic molecules, as well as possible positive effects of physical exercise and cognitive interventions. It is our hypothesis, as partly summarized here, that a judicious combination of exercise and dietary interventions, cognitive training, pharmacological manipulation of immunosenescence and inflammatory processes will eventually deliver an optimal individualized regime for the maintenance of the best possible cognitive function over the lifespan of every individual.

Conflict of interest

The authors have no conflicting financial interests to declare.

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References

Ahlskog, J.E., Geda, Y.E., Graff-Radford, N.R., Petersen, R.C., 2011. Physical exercise as a preventive or disease-modifying treatment of dementia and brain aging. Mayo Clin. Proc. 86, 876–884.
Aid, T., Kazantseva, A., Piirsoo, M., Palm, K., Timmusk, T., 2007. Mouse and rat BDNF gene structure and expression revisited. J. Neurosci. Res. 85, 525–535.
Aimone, J.B., Li, Y., Lee, S.W., Clemenson, G.D., Deng, W., Gage, F.H., 2014. Regulation and function of adult neurogenesis: from genes to cognition. Physiol. Rev. 94, 991–1026.
Alboni, S., Maggi, L., 2015. Editorial: cytokines as players of neuronal plasticity and sensitivity to environment in healthy and pathological brain. Front. Cell. Neurosci. 9, 508.
Aloisi, F., Ria, F., Adorini, L., 2000. Regulation of T-cell responses by CNS antigen-presenting cells: different roles for microglia and astrocytes. Immunol. Today 21, 141–147.
Anthony, D.C., Bolton, S.J., Fearn, S., Perry, V.H., 1997. Age-related effects of interleukin-1 beta on polymorphonuclear neutrophil-dependent increases in blood-brain barrier permeability in rats. Brain 120 (Pt 3), 435–444.
Arnold, C.R., Wolf, J., Brunner, S., Herndler-Brandstetter, D., Grubeck-Loebenstein, B., 2011. Gain and loss of T cell subsets in old age–age-related reshaping of the T cell repertoire. J. Clin. Immunol. 31, 137–146.
Au, A., Feher, A., McPhee, L., Jessa, A., Oh, S., Einstein, G., 2016. Estrogens, inflammation and cognition. Front. Neuroendocrinol. 40, 87–100.
Auffray, C., Fogg, D., Garfa, M., Elain, G., Join-Lambert, O., Kayal, S., Sarnacki, S., Cumano, A., Lauvau, G., Geissmann, F., 2007. Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. Science 317, 666–670.
Auffray, C., Fogg, D.K., Narni-Mancinelli, E., Senechal, B., Trouillet, C., Saederup, N., Leemput, J., Bigot, K., Campisi, L., Abitbol, M., Molina, T., Charo, I., Hume, D.A., Cumano, A., Lauvau, G., Geissmann, F., 2009. CX3CR1+ CD115+ CD135+ common macrophage/DC precursors and the role of CX3CR1 in their response to inflammation. J. Exp. Med. 206, 595–606.
Bains, J.S., Oliet, S.H., 2007. Glia: they make your memories stick! Trends Neurosci. 30, 417–424.
Bamidis, P.D., Vivas, A.B., Styliadis, C., Frantzidis, C., Klados, M., Schlee, W., Siountas, A., Papageorgiou, S.G., 2014. A review of physical and cognitive interventions in aging. Neurosci. Biobehav. Rev. 44, 206–220.
Banks, W.A., 2015. The blood-brain barrier in neuroimmunology: tales of separation and assimilation. Brain Behav. Immun. 44, 1–8.
Barrientos, R.M., Frank, M.G., Crysdale, N.Y., Chapman, T.R., Ahrendsen, J.T., Day, H.E., Campeau, S., Watkins, L.R., Patterson, S.L., Maier, S.F., 2011. Little exercise, big effects: reversing aging and infection-induced memory deficits, and underlying processes. J. Neurosci. 31, 11578–11586.
Barrientos, R.M., Frank, M.G., Watkins, L.R., Maier, S.F., 2012. Aging-related changes in neuroimmune-endocrine function: implications for hippocampal-dependent cognition. Horm. Behav. 62, 219–227.
Barrientos, R.M., Kitt, M.M., Watkins, L.R., Maier, S.F., 2015. Neuroinflammation in the normal aging hippocampus. Neuroscience 309, 84–99.
Baruch, K., Schwartz, M., 2013. CNS-specific T cells shape brain function via the choroid plexus. Brain Behav. Immun. 34, 11–16.
Baune, B.T., Ponath, G., Rothermundt, M., Riess, O., Funke, H., Berger, K., 2008. Association between genetic variants of IL-1beta, IL-6 and TNF-alpha cytokines and cognitive performance in the elderly general population of the MEMO-study. Psychoneuroendocrinology 33, 68–76.
Beattie, E.C., Stellwagen, D., Morishita, W., Bresnahan, J.C., Ha, B.K., Von Zastrow, M., Beattie, M.S., Malenka, R.C., 2002. Control of synaptic strength by glial TNFalpha. Science 295, 2282–2285.
Beery, A.K., Zucker, I., 2011. Sex bias in neuroscience and biomedical research. Neurosci. Biobehav. Rev. 35, 565–572.
Ben-Smith, A., Gorak-Stolinska, P., Floyd, S., Weir, R.E., Lalor, M.K., Mvula, H., Crampin, A.C., Wallace, D., Beverley, P.C., Fine, P.E., Dockrell, H.M., 2008. Differences between naive and memory T cell phenotype in Malawian and UK adolescents: a role for Cytomegalovirus? BMC Infect. Dis. 8, 139.
Bennett, J.M., Glaser, R., Malarkey, W.B., Beversdorf, D.Q., Peng, J., Kiecolt-Glaser, J.K., 2012. Inflammation and reactivation of latent herpesviruses in older adults. Brain Behav. Immun. 26, 739–746.
Berry, A., Carnevale, D., Giorgio, M., Pelicci, P.G., de Kloet, E.R., Alleva, E., Minghetti, L., Cirulli, F., 2010. Greater resistance to inflammation at adulthood could contribute to extended life span of p66(Shc-/-) mice. Exp. Gerontol. 45, 343–350.
Berry, A., Panetta, P., Luoni, A., Bellisario, V., Capoccia, S., Riva, M.A., Cirulli, F., 2015. Decreased Bdnf expression and reduced social behavior in periadolescent rats following prenatal stress. Dev. Psychobiol. 57, 365–373.
Beurel, E., Harrington, L.E., Buchser, W., Lemmon, V., Jope, R.S., 2014. Astrocytes modulate the polarization of CD4+ T cells to Th1 cells. PLoS One 9, e86257.
Bherer, L., 2015. Cognitive plasticity in older adults: effects of cognitive training and physical exercise. Ann. N. Y. Acad. Sci. 1337, 1–6.
Bisht, K., Sharma, K.P., Lecours, C., Sanchez, M.G., El Hajj, H., Milior, G., Olmos-Alonso, A., Gomez-Nicola, D., Luheshi, G., Vallieres, L., Branchi, I., Maggi, L., Limatola, C., Butovsky, O., Tremblay, M.E., 2016. Dark microglia: a new phenotype predominantly associated with pathological states. Glia 64, 826–839.
Bramham, C.R., Messaoudi, E., 2005. BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis. Prog. Neurobiol. 76, 99–125.
Brynskikh, A., Warren, T., Zhu, J., Kipnis, J., 2008. Adaptive immunity affects learning behavior in mice. Brain Behav. Immun. 22, 861–869.
Burch, D., 2014. What could computerized brain training learn from evidence-based medicine? PLoS Med. 11, e1001758.
Burdette, J.H., Laurienti, P.J., Espeland, M.A., Morgan, A., Telesford, Q., Vechlekar, C.D., Hayasaka, S., Jennings, J.M., Katula, J.A., Kraft, R.A., Rejeski, W.J., 2010. Using network science to evaluate exercise-associated brain changes in older adults. Front Aging Neurosci. 2, 23.
Calabrese, F., Rossetti, A.C., Racagni, G., Gass, P., Riva, M.A., Molteni, R., 2014. Brain-derived neurotrophic factor: a bridge between inflammation and neuroplasticity. Front. Cell. Neurosci. 8, 430.
Campbell, A., 2004. Inflammation, neurodegenerative diseases, and environmental exposures. Ann. N. Y. Acad. Sci. 1035, 117–132.
Capoccia, S., Berry, A., Bellisario, V., Vacirca, D., Ortona, E., Alleva, E., Cirulli, F., 2013. Quality and timing of stressors differentially impact on brain plasticity and neuroendocrine-immune function in mice. Neural Plast. 2013, 971817.

Carvalho, A., Rea, I.M., Parimon, T., Cusack, B.J., 2014. Physical activity and cognitive function in individuals over 60 years of age: a systematic review. Clin. Interv. Aging 9, 661–682.

Chapman, T.R., Barrientos, R.M., Ahrendsen, J.T., Hoover, J.M., Maier, S.F., Patterson, S.L., 2012. Aging and infection reduce expression of specific brain-derived neurotrophic factor mRNAs in hippocampus. Neurobiol. Aging 33 (83), e831–814.

Chen, W.W., Zhang, X., Huang, W.J., 2016. Role of neuroinflammation in neurodegenerative diseases (Review). Mol. Med. Rep. 13, 3391–3396.

Cheng, Y., Wu, W., Feng, W., Wang, J., Chen, Y., Shen, Y., Li, Q., Zhang, X., Li, C., 2012. The effects of multi-domain versus single-domain cognitive training in non-demented older people: a randomized controlled trial. BMC Med. 10, 30.

Chennaoui, M., Gomez-Merino, D., Drogou, C., Geoffroy, H., Dispersyn, G., Langrume, C., Ciret, S., Gallopin, T., Sauvet, F., 2015. Effects of exercise on brain and peripheral inflammatory biomarkers induced by total sleep deprivation in rats. J. Inflamm. (Lond.) 12, 56.

Colcombe, S., Kramer, A.F., 2003. Fitness effects on the cognitive function of older adults: a meta-analytic study. Psychol. Sci. 14, 125–130.

Colcombe, S.J., Erickson, K.I., Scalf, P.E., Kim, J.S., Prakash, R., McAuley, E., Elavsky, S., Marquez, D.X., Hu, L., Kramer, A.F., 2006. Aerobic exercise training increases brain volume in aging humans. J. Gerontol. A: Biol. Sci. Med. Sci. 61, 1166–1170.

Cotman, C.W., Berchtold, N.C., 2002. Exercise: a behavioral intervention to enhance brain health and plasticity. Trends Neurosci. 25, 295–301.

Düzel, E., van Praag, H., Sendtner, M., 2016. Can physical exercise in old age improve memory and hippocampal function? Brain 139, 662–673.

Da Mesquita, S., Ferreira, A.C., Sousa, J.C., Correia-Neves, M., Sousa, N., Marques, F., 2016. Insights on the pathophysiology of Alzheimer's disease: the crosstalk between amyloid pathology, neuroinflammation and the peripheral immune system. Neurosci. Biobehav. Rev. 68, 547–562.

Deak, F., Sonntag, W.E., 2012. Aging, synaptic dysfunction, and insulin-like growth factor (IGF)-1. J. Gerontol. A. Biol. Sci. Med. Sci. 67, 611–625.

Deleidi, M., Jaggle, M., Rubino, G., 2015. Immune aging, dysmetabolism, and inflammation in neurological diseases. Front. Neurosci. 9, 172.

Derecki, N.C., Cardani, A.N., Yang, C.H., Quinnies, K.M., Crihfield, A., Lynch, K.R., Kipnis, J., 2010. Regulation of learning and memory by meningeal immunity: a key role for IL-4. J. Exp. Med. 207, 1067–1080.

Di Benedetto, S., Derhovanessian, E., Steinhagen-Thiessen, E., Goldeck, D., Muller, L., Pawelec, G., 2015. Impact of age, sex and CMV-infection on peripheral T cell phenotypes: results from the Berlin BASE-II Study. Biogerontology 16, 631–643.

Di Filippo, M., Chiasserini, D., Gardoni, F., Viviani, B., Tozzi, A., Giampa, C., Costa, C., Tantucci, M., Zianni, E., Boraso, M., Siliquini, S., de Iure, A., Ghiglieri, V., Colcelli, E., Baker, D., Sarchielli, P., Fusco, F.R., Di Luca, M., Calabresi, P., 2013. Effects of central and peripheral inflammation on hippocampal synaptic plasticity. Neurobiol. Dis. 52, 229–236.

Dik, M., Deeg, D.J., Visser, M., Jonker, C., 2003. Early life physical activity and cognition at old age. J. Clin. Exp. Neuropsychol. 25, 643–653.

Dishman, R.K., Berthoud, H.R., Booth, F.W., Cotman, C.W., Edgerton, V.R., Fleshner, M.R., Gandevia, S.C., Gomez-Pinilla, F., Greenwood, B.N., Hillman, C.H., Kramer, A.F., Levin, B.E., Moran, T.H., Russo-Neustadt, A.A., Salamone, J.D., Van Hoomissen, J.D., Wade, C.E., York, D.A., Zigmond, M.J., 2006. Neurobiology of exercise. Obesity 14, 345–356.

Doty, K.R., Guillot-Sestier, M.V., Town, T., 2015. The role of the immune system in neurodegenerative disorders: adaptive or maladaptive? Brain Res. 1617, 155–173.

Dunn, A.J., 1992. Endotoxin-induced activation of cerebral catecholamine and serotonin metabolism: comparison with interleukin-1. J. Pharmacol. Exp. Ther. 261, 964–969.

Elenkov, I.J., Iezzoni, D.G., Daly, A., Harris, A.G., Chrousos, G.P., 2005. Cytokine dysregulation, inflammation and well-being. Neuroimmunomodulation 12, 255–269.

Ellwardt, E., Walsh, J.T., Kipnis, J., Zipp, F., 2016. Understanding the role of T cells in CNS homeostasis. Trends Immunol. 37, 154–165.

Engelhardt, B., Carare, R.O., Bechmann, I., Flugel, A., Laman, J.D., Weller, R.O., 2016. Vascular, glial, and lymphatic immune gateways of the central nervous system. Acta Neuropathol. 132, 317–338.

Engvig, A., Fjell, A.M., Westlye, L.T., Moberget, T., Sundseth, O., Larsen, V.A., Walhovd, K.B., 2010. Effects of memory training on cortical thickness in the elderly. Neuroimage 52, 1667–1676.

Erickson, K.I., Voss, M.W., Prakash, R.S., Basak, C., Szabo, A., Chaddock, L., Kim, J.S., Heo, S., Alves, H., White, S.M., Wojcicki, T.R., Mailey, E., Vieira, V.J., Martin, S.A., Pence, B.D., Woods, J.A., McAuley, E., Kramer, A.F., 2011. Exercise training increases size of hippocampus and improves memory. Proc. Natl. Acad. Sci. U. S. A. 108, 3017–3022.

Erickson, K.I., Leckie, R.L., Weinstein, A.M., 2014. Physical activity, fitness, and gray matter volume. Neurobiol. Aging 35 (Suppl. (2)), S20–28.

Eskes, G.A., Longman, S., Brown, A.D., McMorris, C.A., Langdon, K.D., Hogan, D.B., Poulin, M., 2010. Contribution of physical fitness, cerebrovascular reserve and cognitive stimulation to cognitive function in post-menopausal women. Front Aging Neurosci. 2, 137.

Eyre, H., Baune, B.T., 2012. Neuroimmunological effects of physical exercise in depression. Brain Behav. Immun. 26, 251–266.

Fabre, C., Chamari, K., Mucci, P., Masse-Biron, J., Prefaut, C., 2002. Improvement of cognitive function by mental and/or individualized aerobic training in healthy elderly subjects. Int. J. Sports Med. 23, 415–421.

Feigenson, K.A., Kusnecov, A.W., Silverstein, S.M., 2014. Inflammation and the two-hit hypothesis of schizophrenia. Neurosci. Biobehav. Rev. 38, 72–93.

Ferencz, B., Laukka, E.J., Welmer, A.K., Kalpouzos, G., Angleman, S., Keller, L., Graff, C., Lovden, M., Backman, L., 2014. The benefits of staying active in old age: physical activity counteracts the negative influence of PICALM, BIN1, and CLU risk alleles on episodic memory functioning. Psychol. Aging 29, 440–449.

Fiatarone Singh, M.A., Gates, N., Saigal, N., Wilson, G.C., Meiklejohn, J., Brodaty, H., Wen, W., Singh, N., Baune, B.T., Suo, C., Baker, M.K., Foroughi, N., Wang, Y., Sachdev, P.S., Valenzuela, M., 2014. The Study of Mental and Resistance Training (SMART) study-resistance training and/or cognitive training in mild cognitive impairment: a randomized, double-blind, double-sham controlled trial. J. Am. Med. Dir. Assoc. 15, 873–880.

Fissler, P., Kuster, O., Schlee, W., Kolassa, I.T., 2013. Novelty interventions to enhance broad cognitive abilities and prevent dementia: synergistic approaches for the facilitation of positive plastic change. Prog. Brain Res. 207, 403–434.

Franceschi, C., Campisi, J., 2014. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J. Gerontol. A. Biol. Sci. Med. Sci. 69 (Suppl. (1)), S4–S9.

Franceschi, C., Bonafe, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., De Benedictis, G., 2000. Inflamm-aging: an evolutionary perspective on immunosenescence. Ann. N. Y. Acad. Sci. 908, 244–254.

Franceschi, C., Capri, M., Monti, D., Giunta, S., Olivieri, F., Sevini, F., Panourgia, M.P., Invidia, L., Celani, L., Scurti, M., Cevenini, E., Castellani, G.C., Salvioli, S., 2007. Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mech. Ageing Dev. 128, 92–105.

Geda, Y.E., Silber, T.C., Roberts, R.O., Knopman, D.S., Christianson, T.J., Pankratz, V.S., Boeve, B.F., Tangalos, E.G., Petersen, R.C., 2012. Computer activities physical exercise, aging, and mild cognitive impairment: a population-based study. Mayo Clin. Proc. 87, 437–442.

Gemechu, J.M., Bentivoglio, M., 2012. T cell recruitment in the brain during normal aging. Front. Cell. Neurosci. 6, 38.

Giunta, B., Fernandez, F., Nikolic, W.V., Obregon, D., Rrapo, E., Town, T., Tan, J., 2008. Inflammaging as a prodrome to Alzheimer's disease. J. Neuroinflammation 5, 51.

Goldeck, D., Witkowski, J.M., Fulop, T., Pawelec, G., 2016. Peripheral immune signatures in alzheimer disease. Curr. Alzheimer Res. 13, 739–749.

Goshen, I., Yirmiya, R., 2009. Interleukin-1 (IL-1): a central regulator of stress responses. Front. Neuroendocrinol. 30, 30–45.

Goshen, I., Kreisel, T., Ben-Menachem-Zidon, O., Licht, T., Weidenfeld, J., Ben-Hur, T., Yirmiya, R., 2008. Brain interleukin-1 mediates chronic stress-induced depression in mice via adrenocortical activation and hippocampal neurogenesis suppression. Mol. Psychiatry 13, 717–728.

Guan, Z., Fang, J., 2006. Peripheral immune activation by lipopolysaccharide decreases neurotrophins in the cortex and hippocampus in rats. Brain Behav. Immun. 20, 64–71.

Gutierrez, E.G., Banks, W.A., Kastin, A.J., 1993. Murine tumor necrosis factor alpha is transported from blood to brain in the mouse. J. Neuroimmunol. 47, 169–176.

Gutierrez, E.G., Banks, W.A., Kastin, A.J., 1994. Blood-borne interleukin-1 receptor antagonist crosses the blood-brain barrier. J. Neuroimmunol. 55, 153–160.

Hansel, A., Hong, S., Camara, R.J., von Kanel, R., 2010. Inflammation as a psychophysiological biomarker in chronic psychosocial stress. Neurosci. Biobehav. Rev. 35, 115–121.

Hansen, M.K., Taishi, P., Chen, Z., Krueger, J.M., 1998. Vagotomy blocks the induction of interleukin-1beta (IL-1beta) mRNA in the brain of rats in response to systemic IL-1beta. J. Neurosci. 18, 2247–2253.

Hansen, M.K., Nguyen, K.T., Goehler, L.E., Gaykema, R.P., Fleshner, M., Maier, S.F., Watkins, L.R., 2000. Effects of vagotomy on lipopolysaccharide-induced brain interleukin-1beta protein in rats. Auton. Neurosci. 85, 119–126.

Hanson, M.A., Gluckman, P.D., 2014. Early developmental conditioning of later health and disease: physiology or pathophysiology? Physiol. Rev. 94, 1027–1076.

Harrison, N.A., 2016. Brain structures implicated in inflammation-associated depression. Curr. Top. Behav. Neurosci.

Hasegawa-Ishii, S., Inaba, M., Umegaki, H., Unno, K., Wakabayashi, K., Shimada, A., 2016. Endotoxemia-induced cytokine-mediated responses of hippocampal astrocytes transmitted by cells of the brain-immune interface. Sci. Rep. 6, 25457.

Hearps, A.C., Martin, G.E., Angelovich, T.A., Cheng, W.J., Maisa, A., Landay, A.L., Jaworowski, A., Crowe, S.M., 2012. Aging is associated with chronic innate immune activation and dysregulation of monocyte phenotype and function. Aging Cell 11, 867–875.

Hefendehl, J.K., Neher, J.J., Suhs, R.B., Kohsaka, S., Skodras, A., Jucker, M., 2014. Homeostatic and injury-induced microglia behavior in the aging brain. Aging Cell 13, 60–69.

Hickey, W.F., 2000. P selectin, pioneer cells and the path to inflammation. Brain 123 (Pt 6), 1073–1074.

Holvoet, P., 2012. Stress in obesity and associated metabolic and cardiovascular disorders. Scientifica (Cairo) 2012, 205027.

Hotting, K., Roder, B., 2013. Beneficial effects of physical exercise on neuroplasticity and cognition. Neurosci. Biobehav. Rev. 37, 2243–2257.

Iozzo, P., Holmes, M., Schmidt, M.V., Cirulli, F., Guzzardi, M.A., Berry, A., Balsevich, G., Andreassi, M.G., Wesselink, J.J., Liistro, T., Gomez-Puertas, P., Eriksson, J.G., Seckl, J., 2014. Developmental ORIgins of Healthy and Unhealthy AgeiNg: the role of maternal obesity—introduction to DORIAN. Obes Facts 7, 130–151.

Johnson, P.L., Goronzy, J.J., Antia, R., 2014. A population biological approach to understanding the maintenance and loss of the T-cell repertoire during aging. Immunology 142, 167–175.

Jones, R.S., Lynch, M.A., 2015. How dependent is synaptic plasticity on microglial phenotype? Neuropharmacology 96, 3–10.

Kempermann, G., Gast, D., Gage, F.H., 2002. Neuroplasticity in old age: sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. Ann. Neurol. 52, 135–143.

Kempermann, G., Fabel, K., Ehninger, D., Babu, H., Leal-Galicia, P., Garthe, A., Wolf, S.A., 2010. Why and how physical activity promotes experience-induced brain plasticity. Front. Neurosci. 4, 189.

Khairova, R.A., Machado-Vieira, R., Du, J., Manji, H.K., 2009. A potential role for pro-inflammatory cytokines in regulating synaptic plasticity in major depressive disorder. Int. J. Neuropsychopharmacol. 12, 561–578.

Kim, S.E., Ko, I.G., Kim, B.K., Shin, M.S., Cho, S., Kim, C.J., Kim, S.H., Baek, S.S., Lee, E.K., Jee, Y.S., 2010. Treadmill exercise prevents aging-induced failure of memory through an increase in neurogenesis and suppression of apoptosis in rat hippocampus. Exp. Gerontol. 45, 357–365.

Kipnis, J., Cohen, H., Cardon, M., Ziv, Y., Schwartz, M., 2004. T cell deficiency leads to cognitive dysfunction: implications for therapeutic vaccination for schizophrenia and other psychiatric conditions. Proc. Natl. Acad. Sci. U. S. A. 101, 8180–8185.

Kipnis, J., Gadani, S., Derecki, N.C., 2012. Pro-cognitive properties of T cells. Nat. Rev. Immunol. 12, 663–669.

Kirk-Sanchez, N.J., McGough, E.L., 2014. Physical exercise and cognitive performance in the elderly: current perspectives. Clin. Interv. Aging 9, 51–62.

Kohman, R.A., DeYoung, E.K., Bhattacharya, T.K., Peterson, L.N., Rhodes, J.S., 2012. Wheel running attenuates microglia proliferation and increases expression of a proneurogenic phenotype in the hippocampus of aged mice. Brain Behav. Immun. 26, 803–810.

Kraft, E., 2012. Cognitive function, physical activity, and aging: possible biological links and implications for multimodal interventions. Neuropsychol. Dev. Cogn. Sect. B: Aging Neuropsychol. Cogn. 19, 248–263.

Kumar, A., Rani, A., Tchigranova, O., Lee, W.H., Foster, T.C., 2012. Influence of late-life exposure to environmental enrichment or exercise on hippocampal function and CA1 senescent physiology. Neurobiol. Aging 33, e1–17.

Lövdén, M., Wenger, E., Martensson, J., Lindenberger, U., Backman, L., 2013. Structural brain plasticity in adult learning and development. Neurosci. Biobehav. Rev. 37, 2296–2310.

Laws, K.R., Irvine, K., Gale, T.M., 2016. Sex differences in cognitive impairment in Alzheimer's disease. World J. Psychiatry 6, 54–65.

Leckie, R.L., Oberlin, L.E., Voss, M.W., Prakash, R.S., Szabo-Reed, A., Chaddock-Heyman, L., Phillips, S.M., Gothe, N.P., Mailey, E., Vieira-Potter, V.J., Martin, S.A., Pence, B.D., Lin, M., Parasuraman, R., Greenwood, P.M., Fryxell, K.J., Woods, J.A., McAuley, E., Kramer, A.F., Erickson, K.I., 2014. BDNF mediates improvements in executive function following a 1-year exercise intervention. Front. Hum. Neurosci. 8, 985.

Leiter, O., Kempermann, G., Walker, T.L., 2016. A common language: how neuroimmunological cross talk regulates adult hippocampal neurogenesis. Stem Cells Int. 2016, 1681590.

Leza, J.C., Garcia-Bueno, B., Bioque, M., Arango, C., Parellada, M., Do, K., O'Donnell, P., Bernardo, M., 2015. Inflammation in schizophrenia: a question of balance. Neurosci. Biobehav. Rev. 55, 612–626.

Lindenberger, U., 2014. Human cognitive aging: corriger la fortune? Science 346, 572–578.

Litteljohn, D., Nelson, E., Hayley, S., 2014. IFN-gamma differentially modulates memory-related processes under basal and chronic stressor conditions. Front. Cell. Neurosci. 8, 391.

Louveau, A., Harris, T.H., Kipnis, J., 2015. Revisiting the mechanisms of CNS immune privilege. Trends Immunol. 36, 569–577.

Lu, B., Pang, P.T., Woo, N.H., 2005. The yin and yang of neurotrophin action. Nat. Rev. Neurosci. 6, 603–614.

Lustig, C., Shah, P., Seidler, R., Reuter-Lorenz, P.A., 2009. Aging, training, and the brain: a review and future directions. Neuropsychol. Rev. 19, 504–522.

Müller, L., Pawelec, G., 2014. Aging and immunity—impact of behavioral intervention. Brain Behav. Immun. 39, 8–22.

Müller, L., Pawelec, G., 2015. As we age: does slippage of quality control in the immune system lead to collateral damage? Ageing Res. Rev. 23, 116–123.

Müller, L., Fulop, T., Pawelec, G., 2013. Immunosenescence in vertebrates and invertebrates. Immun. Ageing: I & A 10, 12.

Maass, A., Duzel, S., Goerke, M., Becke, A., Sobieray, U., Neumann, K., Lovden, M., Lindenberger, U., Backman, L., Braun-Dullaeus, R., Ahrens, D., Heinze, H.J., Muller, N.G., Duzel, E., 2015. Vascular hippocampal plasticity after aerobic exercise in older adults. Mol. Psychiatry 20, 585–593.

Maier, S.F., Goehler, L.E., Fleshner, M., Watkins, L.R., 1998. The role of the vagus nerve in cytokine-to-brain communication. Ann. N. Y. Acad. Sci. 840, 289–300.

Majka, D.S., Chang, R.W., Vu, T.H., Palmas, W., Geffken, D.F., Ouyang, P., Ni, H., Liu, K., 2009. Physical activity and high-sensitivity C-reactive protein: the multi-ethnic study of atherosclerosis. Am. J. Prev. Med. 36, 56–62.

Malaguarnera, L., Cristaldi, E., Malaguarnera, M., 2010. The role of immunity in elderly cancer. Crit. Rev. Oncol. Hematol. 74, 40–60.

Minter, M.R., Moore, Z., Zhang, M., Brody, K.M., Jones, N.C., Shultz, S.R., Taylor, J.M., Crack, P.J., 2016. Deletion of the type-1 interferon receptor in APPSWE/PS1DeltaE9 mice preserves cognitive function and alters glial phenotype. Acta Neuropathol. Commun. 4, 72.

Moylan, S., Eyre, H.A., Maes, M., Baune, B.T., Jacka, F.N., Berk, M., 2013. Exercising the worry away: how inflammation, oxidative and nitrogen stress mediates the beneficial effect of physical activity on anxiety disorder symptoms and behaviours. Neurosci. Biobehav. Rev. 37, 573–584.

Na, K.S., Jung, H.Y., Kim, Y.K., 2014. The role of pro-inflammatory cytokines in the neuroinflammation and neurogenesis of schizophrenia. Prog. Neuropsychopharmacol. Biol. Psychiatry. 48, 277–286.

Naylor, K., Li, G., Vallejo, A.N., Lee, W.W., Koetz, K., Bryl, E., Witkowski, J., Fulbright, J., Weyand, C.M., Goronzy, J.J., 2005. The influence of age on T cell generation and TCR diversity. J. Immunol. 174, 7446–7452.

Ngandu, T., Lehtisalo, J., Solomon, A., Levalahti, E., Ahtiluoto, S., Antikainen, R., Backman, L., Hanninen, T., Jula, A., Laatikainen, T., Lindstrom, J., Mangialasche, F., Paajanen, T., Pajala, S., Peltonen, M., Rauramaa, R., Stigsdotter-Neely, A., Strandberg, T., Tuomilehto, J., Soininen, H., Kivipelto, M., 2015. A 2 year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): a randomised controlled trial. Lancet 385, 2255–2263.

Nimmerjahn, A., Kirchhoff, F., Helmchen, F., 2005. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308, 1314–1318.

Norden, D.M., Muccigrosso, M.M., Godbout, J.P., 2015. Microglial priming and enhanced reactivity to secondary insult in aging, and traumatic CNS injury, and neurodegenerative disease. Neuropharmacology 96, 29–41.

Norton, S., Matthews, F.E., Barnes, D.E., Yaffe, K., Brayne, C., 2014. Potential for primary prevention of Alzheimer's disease: an analysis of population-based data. Lancet Neurol. 13, 788–794.

Nunes, S.O., Vargas, H.O., Prado, E., Barbosa, D.S., de Melo, L.P., Moylan, S., Dodd, S., Berk, M., 2013. The shared role of oxidative stress and inflammation in major depressive disorder and nicotine dependence. Neurosci. Biobehav. Rev. 37, 1336–1345.

Oswald, W.D.G., Rupprecht, T., Hagen, R., 2006. Differential effects of single versus combined cognitive and physical training with older adults: the SimA study in a 5-year perspective. Eur. J. Ageing 3.

Ownby, R.L., 2010. Neuroinflammation and cognitive aging. Curr. Psychiatry Rep. 12, 39–45.

Papenberg, G., Ferencz, B., Mangialasche, F., Mecocci, P., Cecchetti, R., Kalpouzos, G., Fratiglioni, L., Backman, L., 2016. Physical activity and inflammation: effects on gray-matter volume and cognitive decline in aging. Hum. Brain Mapp.

Patterson, S.L., 2015. Immune dysregulation and cognitive vulnerability in the aging brain: interactions of microglia, IL-1beta, BDNF and synaptic plasticity. Neuropharmacology 96, 11–18.

Pawelec, G., Derhovanessian, E., 2011. Role of CMV in immune senescence. Virus Res. 157, 175–179.

Pawelec, G., Larbi, A., Derhovanessian, E., 2010. Senescence of the human immune system. J. Comp. Pathol. 142 (Suppl. (1)), S39–44.

Pedersen, B.K., Hoffman-Goetz, L., 2000. Exercise and the immune system: regulation, integration, and adaptation. Physiol. Rev. 80, 1055–1081.

Pedersen, B.K., 2011. Exercise-induced myokines and their role in chronic diseases. Brain Behav. Immun. 25, 811–816.

Petersen, A.M., Pedersen, B.K., 2005. The anti-inflammatory effect of exercise. J. Appl. Physiol. 98, 1154–1162, 1985.

Phillips, C., Baktir, M.A., Srivatsan, M., Salehi, A., 2014. Neuroprotective effects of physical activity on the brain: a closer look at trophic factor signaling. Front. Cell. Neurosci. 8, 170.

Pizza, V., Agresta, A., D'Acunto, C.W., Festa, M., Capasso, A., 2011. Neuroinflammation and ageing: current theories and an overview of the data. Rev. Recent Clin. Trials 6, 189–203.

Plata-Salaman, C.R., 1991. Immunoregulators in the nervous system. Neurosci. Biobehav. Rev. 15, 185–215.

Podewils, L.J., Guallar, E., Kuller, L.H., Fried, L.P., Lopez, O.L., Carlson, M., Lyketsos, C.G., 2005. Physical activity, APOE genotype, and dementia risk: findings from the Cardiovascular Health Cognition Study. Am. J. Epidemiol. 161, 639–651.

Prakash, R.S., Voss, M.W., Erickson, K.I., Kramer, A.F., 2015. Physical activity and cognitive vitality. Annu. Rev. Psychol. 66, 769–797.

Prinz, M., Priller, J., Sisodia, S.S., Ransohoff, R.M., 2011. Heterogeneity of CNS myeloid cells and their roles in neurodegeneration. Nat. Neurosci. 14, 1227–1235.

Qi, Q., Liu, Y., Cheng, Y., Glanville, J., Zhang, D., Lee, J.Y., Olshen, R.A., Weyand, C.M., Boyd, S.D., Goronzy, J.J., 2014a. Diversity and clonal selection in the human T-cell repertoire. Proc. Natl. Acad. Sci. U. S. A. 111, 13139–13144.

Qi, Q., Zhang, D.W., Weyand, C.M., Goronzy, J.J., 2014b. Mechanisms shaping the naive T cell repertoire in the elderly—thymic involution or peripheral homeostatic proliferation? Exp. Gerontol. 54, 71–74.

Quan, N., Banks, W.A., 2007. Brain-immune communication pathways. Brain Behav. Immun. 21, 727–735.

Raper, D., Louveau, A., Kipnis, J., 2016. How do meningeal lymphatic vessels drain the CNS? Trends Neurosci. 39, 581–586.

Ritzel, R.M., Crapser, J., Patel, A.R., Verma, R., Grenier, J.M., Chauhan, A., Jellison, E.R., McCullough, L.D., 2016. Age-associated resident memory CD8 T cells in the central nervous system are primed to potentiate inflammation after ischemic brain injury. J. Immunol. 196, 3318–3330.

Ron-Harel, N., Cardon, M., Schwartz, M., 2011. Brain homeostasis is maintained by danger signals stimulating a supportive immune response within the brain's borders. Brain Behav. Immun. 25, 1036–1043.

Salam, N., Rane, S., Das, R., Faulkner, M., Gund, R., Kandpal, U., Lewis, V., Mattoo, H., Prabhu, S., Ranganathan, V., Durdik, J., George, A., Rath, S., Bal, V., 2013. T cell

ageing: effects of age on development, survival & function. Indian J. Med. Res. 138, 595–608.
Scheinert, R.B., Asokan, A., Rani, A., Kumar, A., Foster, T.C., Ormerod, B.K., 2015. Some hormone, cytokine and chemokine levels that change across lifespan vary by cognitive status in male Fischer 344 rats. Brain Behav. Immun. 49, 216–232.
Schmiedek, F., Lövdén, M., Lindenberger, U., 2010. Hundred days of cognitive training enhance broad cognitive abilities in adulthood: findings from the COGITO study. Front. Aging Neurosci. 2.
Schnydrig, S., Korner, L., Landweer, S., Ernst, B., Walker, G., Otten, U., Kunz, D., 2007. Peripheral lipopolysaccharide administration transiently affects expression of brain-derived neurotrophic factor, corticotropin and proopiomelanocortin in mouse brain. Neurosci. Lett. 429, 69–73.
Shechter, R., London, A., Varol, C., Raposo, C., Cusimano, M., Yovel, G., Rolls, A., Mack, M., Pluchino, S., Martino, G., Jung, S., Schwartz, M., 2009. Infiltrating blood-derived macrophages are vital cells playing an anti-inflammatory role in recovery from spinal cord injury in mice. PLoS Med. 6, e1000113.
Shechter, R., London, A., Schwartz, M., 2013. Orchestrated leukocyte recruitment to immune-privileged sites: absolute barriers versus educational gates. Nat. Rev. Immunol. 13, 206–218.
Silverman, M.N., Deuster, P.A., 2014. Biological mechanisms underlying the role of physical fitness in health and resilience. Interface Focus 4, 20140040.
Simpson, R.J., 2016. Aging and inflammation: directing traffic through physical activity. Brain Behav. Immun. 56, 10–11.
Singhal, G., Jaehne, E.J., Corrigan, F., Baune, B.T., 2014. Cellular and molecular mechanisms of immunomodulation in the brain through environmental enrichment. Front. Cell. Neurosci. 8, 97.
Snigdha, S., de Rivera, C., Milgram, N.W., Cotman, C.W., 2014. Exercise enhances memory consolidation in the aging brain. Front. Aging Neurosci. 6, 3.
Sparkman, N.L., Johnson, R.W., 2008. Neuroinflammation associated with aging sensitizes the brain to the effects of infection or stress. Neuroimmunomodulation 15, 323–330.
Speisman, R.B., Kumar, A., Rani, A., Foster, T.C., Ormerod, B.K., 2013a. Daily exercise improves memory, stimulates hippocampal neurogenesis and modulates immune and neuroimmune cytokines in aging rats. Brain Behav. Immun. 28, 25–43.
Speisman, R.B., Kumar, A., Rani, A., Pastoriza, J.M., Severance, J.E., Foster, T.C., Ormerod, B.K., 2013b. Environmental enrichment restores neurogenesis and rapid acquisition in aged rats. Neurobiol. Aging 34, 263–274.
Stellwagen, D., Malenka, R.C., 2006. Synaptic scaling mediated by glial TNF-alpha. Nature 440, 1054–1059.
Stolp, H.B., Liddelow, S.A., Sa-Pereira, I., Dziegielewska, K.M., Saunders, N.R., 2013. Immune responses at brain barriers and implications for brain development and neurological function in later life. Front. Integr. Neurosci. 7, 61.
Swardfager, W., Rosenblat, J.D., Benlamri, M., McIntyre, R.S., 2016. Mapping inflammation onto mood: inflammatory mediators of anhedonia. Neurosci. Biobehav. Rev. 64, 148–166.
Szuhany, K.L., Bugatti, M., Otto, M.W., 2015. A meta-analytic review of the effects of exercise on brain-derived neurotrophic factor. J. Psychiatr. Res. 60, 56–64.
Tansey, M.G., Goldberg, M.S., 2010. Neuroinflammation in Parkinson's disease: its role in neuronal death and implications for therapeutic intervention. Neurobiol. Dis. 37, 510–518.
Tansey, M.G., 2010. Inflammation in neuropsychiatric disease. Neurobiol. Dis. 37, 491–492.
Tay, T.L., Savage, J., Hui, C.W., Bisht, K., Tremblay, M.E., 2016. Microglia across the lifespan: from origin to function in brain development, plasticity and cognition. J. Physiol.
Timmerman, K.L., Flynn, M.G., Coen, P.M., Markofski, M.M., Pence, B.D., 2008. Exercise training-induced lowering of inflammatory (CD14+CD16+) monocytes: a role in the anti-inflammatory influence of exercise. J. Leukoc. Biol. 84, 1271–1278.
Tolppanen, A.M., Solomon, A., Kulmala, J., Kareholt, I., Ngandu, T., Rusanen, M., Laatikainen, T., Soininen, H., Kivipelto, M., 2015. Leisure-time physical activity from mid- to late life, body mass index, and risk of dementia. Alzheimer's Demen. 11, 434–443, e436.
Tumati, S., Burger, H., Martens, S., van der Schouw, Y.T., Aleman, A., 2016. Association between cognition and serum insulin-like growth factor-1 in middle-aged & older men: an 8 year follow-up study. PLoS One 11, e0154450.
Turner, J.E., 2016. Is immunosenescence influenced by our lifetime dose of exercise? Biogerontology 17, 581–602.
Varma, V.R., Chuang, Y.F., Harris, G.C., Tan, E.J., Carlson, M.C., 2015. Low-intensity daily walking activity is associated with hippocampal volume in older adults. Hippocampus 25, 605–615.
Vaupel, J.W., Carey, J.R., Christensen, K., 2003. Aging. It's never too late Science 301, 1679–1681.
Vegeto, E., Benedusi, V., Maggi, A., 2008. Estrogen anti-inflammatory activity in brain: a therapeutic opportunity for menopause and neurodegenerative diseases. Front. Neuroendocrinol. 29, 507–519.
Veiga-Fernandes, H., Mucida, D., 2016. Neuro-immune interactions at barrier surfaces. Cell 165, 801–811.
Vescovini, R., Fagnoni, F.F., Telera, A.R., Bucci, L., Pedrazzoni, M., Magalini, F., Stella, A., Pasin, F., Medici, M.C., Calderaro, A., Volpi, R., Monti, D., Franceschi, C., Nikolich-Zugich, J., Sansoni, P., 2014. Naive and memory CD8 T cell pool homeostasis in advanced aging: impact of age and of antigen-specific responses to cytomegalovirus. Age (Dordr) 36, 625–640.
Villeda, S.A., Luo, J., Mosher, K.I., Zou, B., Britschgi, M., Bieri, G., Stan, T.M., Fainberg, N., Ding, Z., Eggel, A., Lucin, K.M., Czirr, E., Park, J.S., Couillard-Despres, S., Aigner, L., Li, G., Peskind, E.R., Kaye, J.A., Quinn, J.F., Galasko, D.R., Xie, X.S., Rando, T.A., Wyss-Coray, T., 2011. The ageing systemic milieu negatively regulates neurogenesis and cognitive function. Nature 477, 90–94.
Wekerle, H., 2006. Breaking ignorance: the case of the brain. Curr. Top. Microbiol. Immunol. 305, 25–50.
Willis, S.L., Tennstedt, S.L., Marsiske, M., Ball, K., Elias, J., Koepke, K.M., Morris, J.N., Rebok, G.W., Unverzagt, F.W., Stoddard, A.M., Wright, E., Group, A.S., 2006. Long-term effects of cognitive training on everyday functional outcomes in older adults. JAMA 296, 2805–2814.
Wilson, C.J., Finch, C.E., Cohen, H.J., 2002. Cytokines and cognition?the case for a head-to-toe inflammatory paradigm. J. Am. Geriatr. Soc. 50, 2041–2056.
Wirth, M., Haase, C.M., Villeneuve, S., Vogel, J., Jagust, W.J., 2014. Neuroprotective pathways: lifestyle activity, brain pathology, and cognition in cognitively normal older adults. Neurobiol. Aging 35, 1873–1882.
Wistuba-Hamprecht, K., Haehnel, K., Janssen, N., Demuth, I., Pawelec, G., 2015. Peripheral blood T-cell signatures from high-resolution immune phenotyping of gammadelta and alphabeta T-cells in younger and older subjects in the Berlin Aging Study II. Immun. Ageing: I & A 12, 25.
Wohleb, E.S., Delpech, J.C., 2016. Dynamic cross-talk between microglia and peripheral monocytes underlies stress-induced neuroinflammation and behavioral consequences. Prog. Neuropsychopharmacol. Biol. Psychiatry.
Wolf, S.A., Steiner, B., Akpinarli, A., Kammertoens, T., Nassenstein, C., Braun, A., Blankenstein, T., Kempermann, G., 2009. CD4-positive T lymphocytes provide a neuroimmunological link in the control of adult hippocampal neurogenesis. J. Immunol. 182, 3979–3984.
Xie, L., Choudhury, G.R., Winters, A., Yang, S.H., Jin, K., 2015. Cerebral regulatory T cells restrain microglia/macrophage-mediated inflammatory responses via IL-10. Eur. J. Immunol. 45, 180–191.
Yaffe, K., Barnes, D., Nevitt, M., Lui, L.Y., Covinsky, K., 2001. A prospective study of physical activity and cognitive decline in elderly women: women who walk. Arch. Intern. Med. 161, 1703–1708.
Yau, S.Y., Lau, B.W., So, K.F., 2011. Adult hippocampal neurogenesis: a possible way how physical exercise counteracts stress. Cell Transplant. 20, 99–111.
Yau, S.Y., Li, A., So, K.F., 2015. Involvement of adult hippocampal neurogenesis in learning and forgetting. Neural Plast. 2015, 717958.
Ye, S.M., Johnson, R.W., 1999. Increased interleukin-6 expression by microglia from brain of aged mice. J. Neuroimmunol. 93, 139–148.
Yirmiya, R., Goshen, I., 2011. Immune modulation of learning, memory, neural plasticity and neurogenesis. Brain Behav. Immun. 25, 181–213.
Zagni, E., Simoni, L., Colombo, D., 2016. Sex and gender differences in central nervous system-related disorders. Neurosci. J. 2016, 2827090.
Ziv, Y., Ron, N., Butovsky, O., Landa, G., Sudai, E., Greenberg, N., Cohen, H., Kipnis, J., Schwartz, M., 2006. Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. Nat. Neurosci. 9, 268–275.
de Pablo-Bernal, R.S., Canizares, J., Rosado, I., Galva, M.I., Alvarez-Rios, A.I., Carrillo-Vico, A., Ferrando-Martinez, S., Munoz-Fernandez, M.A., Rafii-El-Idrissi Benhnia, M., Pacheco, Y.M., Ramos, R., Leal, M., Ruiz-Mateos, E., 2016. Monocyte phenotype and polyfunctionality are associated with elevated soluble inflammatory markers, cytomegalovirus infection, and functional and cognitive decline in elderly adults. J. Gerontol. A. Biol. Sci. Med. Sci. 71, 610–618.
von Bernhardi, R., Tichauer, J.E., Eugenin, J., 2010. Aging-dependent changes of microglial cells and their relevance for neurodegenerative disorders. J. Neurochem. 112, 1099–1114.

14 Publication III

International Journal of
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MDPI

Article

The Modulatory Effect of Gender and Cytomegalovirus-Seropositivity on Circulating Inflammatory Factors and Cognitive Performance in Elderly Individuals

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Abstract: Aging is characterized by a chronic increase in the systemic levels of inflammatory cytokines even in ostensibly healthy individuals. The drivers of age-related increase in systemic inflammation are unclear but one potential contributor may be a persistent infection with Cytomegalovirus (CMV). In this study, we characterized the inflammatory status of 161 older participants recruited to undergo a six-month training intervention. We investigated the influence of gender and CMV-seropositivity on the main inflammatory and anti-inflammatory circulating biomarkers, such as cytokines, receptor antagonist, soluble receptor, immune cells, and relevant metabolic markers. We found that both gender and CMV-seropositivity modulate circulating peripheral biomarkers, and that CMV-infection modifies associations among the latter. Moreover, we observed an interaction between CMV-serostatus and gender associations with cognitive abilities: gender differences in fluid intelligence (Gf) and working memory (WM) were noted only in CMV-negative individuals. Finally, we found that in the CMV-seronegative participants Gf, episodic memory (EM), and WM correlated negatively with pro-inflammatory tumor necrosis factor (TNF); and EM correlated positively with anti-inflammatory interleukin (IL)-10. In CMV-seropositive individuals EM and Gf correlated negatively with pro-inflammatory IL-6, while EM, Gf, and WM correlated negatively with anti-inflammatory IL-1RA. We conclude that both CMV-serostatus and gender may modulate neuroimmune factors, cognitive performance and the relationship between the two domains and should therefore be considered in comparative and interventional studies with elderly people.

Keywords: aging; immunosenescence; inflammaging; pro-inflammatory cytokines; anti-inflammatory cytokines; cytomegalovirus; gender; cognition

1. Introduction

Aging has been linked to persistent low-grade systemic inflammation that is characterized by a chronic increase in the levels of circulating pro-inflammatory cytokines, whose presence is highly related to age-related metabolic, cardiovascular, and neuro-degenerative diseases [1]. The disequilibrium between pro- and anti-inflammatory cytokines may have a negative effect on cognitive abilities, inducing learning and memory deficits in Alzheimer's disease and other neurodegenerative disorders. Although it is unclear even in pathological processes, how systemic inflammation relates to disease processes occurring in the brain, peripheral inflammation and central inflammation may be closely related [2,3]. To underscore the importance of pro- and anti-inflammatory

homeostasis in aging, and the role of chronic low-grade inflammation in shaping the aging phenotype, a term "inflammaging" has been coined [4].

Cytokines are signaling molecules possessing unique modulatory functions. They may influence many physiological processes, such as neuroendocrine interactions, neurotransmitter metabolism, and neuroplasticity and affect behavior and cognition [5]. Among numerous pro- and anti-inflammatory cytokines, some stand out as influential contributors to age-related differences in health, immunity, and cognition.

The tumor necrosis factor (TNF) that plays a key role in several neuroimmune functions is associated with the increased risk for neurodegeneration [4,6–8]. Through the activation of several pathways, this cytokine contributes to the production of pro-inflammatory interleukin-6 (IL-6) [9,10]. IL-6 that is produced mostly by adipose tissue macrophages, myokines and glial cells is elevated in persons of advanced age and people suffering from obesity. IL-6 may, however, under certain conditions exhibits anti-inflammatory properties through inhibiting TNF release as well as promoting release of anti-inflammatory molecules, such as interleukin 10 (IL-10) [11]. IL-10, an anti-inflammatory cytokine, suppresses, in turn, the release of TNF and other inflammatory cytokines [10,12].

Another prominent pro-inflammatory cytokine, interleukin 1 beta (IL-1β) is primarily produced by monocytes, but could also be secreted by myocytes, adipocytes and microglia [13]. The main actions of IL-1β are stimulation of immune cells to produce pro-inflammatory cytokines, activation of microglia, and regulation of neurotrophic activity [14,15]. Alone or in synergy with TNF, IL-1β affects nearly every cell in the organism [13]. Whereas TNF is able to directly activate neurons, IL-1β that shows increased circulating levels with advanced age [12,16], appears to act via microglia [17]. Yet another pro-inflammatory cytokine, interleukin 18 (IL-18) plays an important role in local and systemic inflammation by promoting TNF release and IL-6 production [18].

Levels of cytokines can also be affected by the properties of their receptors, and the IL-1 receptor antagonist (IL1-RA) that is secreted by monocytes, neutrophils, and other cells has an important role as an IL-1β receptor blocker, while also acting as a natural inhibitor of TNF. IL-1RA limits or buffers the inflammatory effect of other cytokines [13,19–21] and modulates the immune effects of TNF [17]. With so many actors intertwined in multiple synthesis, release and modulation pathways, attributing immune and neural differences to specific cytokines may be a challenging task.

To complicate matters, the interrelated effects of all surveyed cytokines as well as their influence on immune and neuroendocrine functions can be modified by chronic activity of an infectious agent. A lifelong persistent infection influences immunosenescence and can significantly alter the course of cognitive aging when it acts in conjunction with individual differences in cytokine production and release. Currently, consensus seems to be building around the CMV as such a chronic modifier of cytokine action. CMV exerts significant influence on the aging immune system [22–25] and thus acts as a driving factor of inflammaging [26,27]. Constant immune surveillance and the sustained efforts of the immune system to control reactivations of a dormant virus induce low-level immune response and inflammation, which may have dramatic consequences for health in the elderly [26,28–30]. In older adults, CMV has been linked to increased frailty, accelerated cognitive decline, and an increased risk of cardiovascular and Alzheimer diseases [23,26,31–35].

To date, very few studies have investigated the associations between CMV and cognition in healthy older individuals and their findings are inconsistent. In a study on 1,061 participants, the CMV-seropositive elderly showed lower cognitive performance than their CMV-seronegative counterparts, and among CMV-infected individuals, higher CMV-antibody levels were associated with lower general cognitive ability [36]. On the other hand, in a cohort of 567 octogenarians, CMV-infection was not associated with functional or cognitive decline [34]. To the best of our knowledge, only one longitudinal study, reported that higher CMV IgG titers were associated with an increased cognitive decline over a 4-year period [35].

CMV infection is thought to contribute to many chronic conditions that have been established as predictors of cognitive decline [37–40], including endothelial dysfunction, dyslipidemia, hypertension, atherosclerosis and coronary heart disease [41,42]. Subclinical inflammation, together with the metabolic and vascular risk factors might be underlying factor for age-related cognitive impairments—with sexual dimorphism additionally influencing these underlying processes.

Although it is known that the impact of aging on immunity in men and women is different [43–45], studies on the influence of gender in humans are still scarce and their results are controversial [43,46–49]. In our previous study, we have observed multiple gender-related differences in the effects of age and CMV-infection on the differentiation status of T cells [50], suggesting that the CMV-associated "senescence of T cells" in older men may be more pronounced than in older women. Others have later confirmed this effect, which was even found in the middle-aged CMV-positive males and females between 50 and 65 years old [51].

The present study posited four major goals. First, we aimed to measure and characterize the baseline inflammatory status of aged individuals recruited for an intervention study of active aging before starting the cognitive and physical training. Specifically, we assessed main inflammatory and anti-inflammatory biomarkers, such as circulating cytokines (IL-1β, TNF, IL-6, IL-10, IL-18), receptor antagonist IL-1RA, and soluble receptor (sTNF-R), immune cells (lymphocytes, leukocytes, monocytes, neutrophils), and relevant metabolic markers: high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides. Second, we aimed to explore the influence of gender and CMV-seropositivity on the immune and metabolic markers measured at baseline. Third, we examined the associations among inflammatory and metabolic factors, and assessed whether CMV-seropositivity modifies these relationships. Fourth, we explored the influence of the measured inflammatory factors on the cognitive abilities, such as fluid intelligence, episodic memory, speed, and working memory, in the context of CMV-serostatus and gender.

2. Results

2.1. CMV-Serostatus of Study Participants

Results of the CMV-serostatus in both male and female participants are presented in Figure 1. Among 161 participants 102 (63.4%) were CMV-seropositive and 59 (36.6%) CMV-seronegative, whereby CMV-positive group consisted of 50 (31.0%) male and 52 (32.3%) female persons, whereas CMV-negative group contained 29 (18.1%) men and 30 (18.6) women.

2.2. Influence of Gender and CMV-Serostatus on Circulating Levels of Pro- and Anti-Inflammatory Mediators, Immune Cells, and Metabolic Blood Values Analysed by MANOVA

For the Multivariate ANOVAs (MANOVA), the logarithmically transformed variables were grouped into pro-inflammatory (IL-1β, IL-6, IL-18, and TNF), anti-inflammatory (IL-10, IL1RA, and sTNF-R), metabolic (HDL, LDL, and triglycerides), and immune cells (lymphocytes, monocytes, and neutrophils) groups of variables. Results of MANOVA, the follow-up univariate ANOVAs, and Scheffé's post hoc test are described in the following sub-sections accordingly.

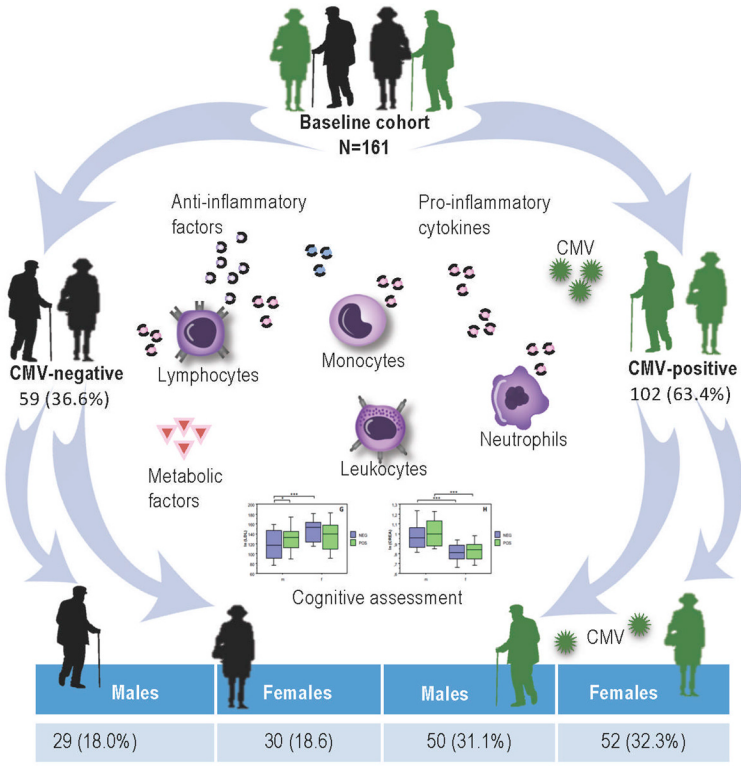

Figure 1. A schematic illustration of the study setup and Cytomegalovirus (CMV)-serostatus of study participants. CMV: Cytomegalovirus.

2.2.1. Pro- and Anti-Inflammatory Groups of Variables

MANOVA for pro-inflammatory group of variables showed no significant effects for any of the factors or for interaction between them. However, a separate univariate ANOVA on the outcome variable IL-1β revealed a significant effect of CMV-serostatus, $F(1,157) = 4.52$, $p < 0.05$, whereby according to the Scheffé's post hoc test, only male subjects showed significant differences: NEG (negative) > POS (positive), mean diff. = 0.77, crit. diff. = 0.72, $p < 0.05$ (Figure 2A).

MANOVA for anti-inflammatory variables showed a significant effect of gender, $F(3,155) = 4.16$, $p < 0.01$, whereby separate univariate ANOVAs revealed a significant effect of gender for sTNF-R only, $F(1,157) = 6.97$, $p < 0.01$. As indicated by the Scheffé's post hoc test, sex differences were significant only in CMV-negative group, mean diff. = 0.17, crit. diff. = 0.13, $p < 0.05$ (Figure 2G).

2.2.2. Group of Metabolic Risk Variables

In the case of the metabolic blood values, MANOVA showed a significant effect of the factor Gender, $F(3,155) = 14.85$, $p < 0.0001$, and a significant interaction Gender by CMV, $F(3,155) = 3.84$, $p < 0.05$. Separate univariate ANOVAs revealed a significant effect of the factor Gender for HDL, $F(1,157) = 26.39$, $p < 0.0001$, and LDL, $F(1,157) = 11.63$, $p < 0.001$, and a significant interaction Gender by CMV for LDL, $F(1,157) = 9.93$, $p < 0.01$. As shown by the Scheffè post hoc test, HDL demonstrated significant sex differences in both CMV-negative, mean diff. = 0.23, crit. diff. = 0.13, $p < 0.01$, and CMV-positive participants, mean diff. = 0.22, crit. diff. = 0.11, $p < 0.0001$ (Figure 2H), whereas LDL was higher in female as compared with male subjects only for the CMV-negative group, mean diff. = 0.29, crit. diff. = 0.14, $p < 0.0001$ (Figure 2I). In addition, there was also a significant effect of the factor CMV for HDL, $F(1,157) = 4.0$, $p < 0.05$, and for Triglycerides, $F(1,157) = 6.55$, $p < 0.05$. Interestingly, when performing the Scheffé's post hoc test, the CMV effect for HDL did not reach a significance level either in males or in females, and triglycerides revealed significant differences only in males, mean diff. = 0.24, crit. diff. = 0.22, $p < 0.05$ (Figure 2J).

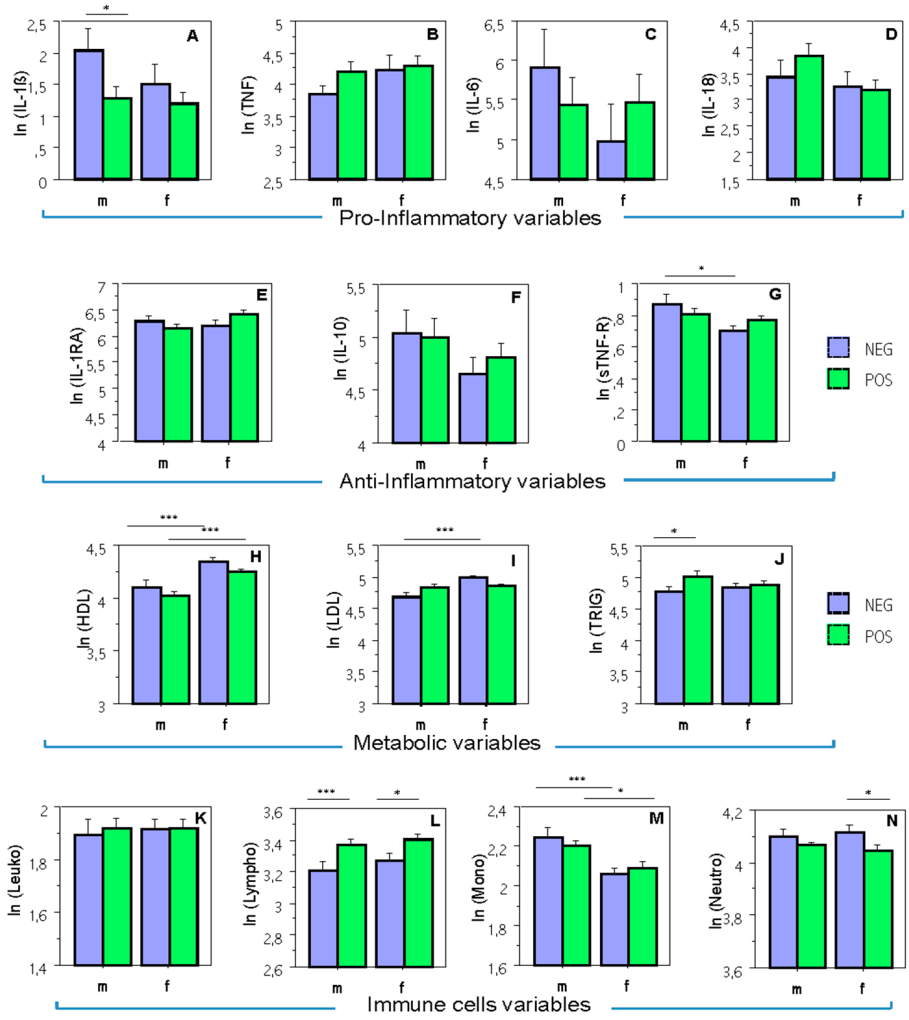

Figure 2. Means and standard errors for all dependent variables by Sex and CMV. Diagrams of concentrations (logarithmically transformed) for: (**A**–**D**) pro-inflammatory variables; (**E**–**G**) anti-inflammatory variables; (**H**–**J**) metabolic variables; (**K**–**N**) immune cells. IL: interleukin; IL-1β: interleukin 1 beta; TNF: tumor necrosis factor; IL-1RA: interleukin 1 receptor antagonist; sTNF-R: soluble tumor necrosis factor receptor; HDL: high-density lipoprotein; LDL: low-density lipoprotein; m: male; f: female; POS: NEG: CMV-seronegative CMV-seropositive; ln: natural logarithm. *, $p < 0.05$; ***, $p < 0.001$.

2.2.3. Group of Immune Cells Variables

As for the immune cells' group, there were significant effects of the factors Gender, $F(4,154) = 4.39$, $p < 0.01$, and CMV, $F(4,154) = 3.75$, $p < 0.01$ found by MANOVA. Separate univariate ANOVAs revealed a significant effect of the factor Gender for monocytes, $F(1,157) = 16.07$, $p < 0.0001$, and a significant effect of the factor CMV for lymphocytes, $F(1,157) = 13.21$, $p < 0.001$, and neutrophils, $F(1,157) = 6.55$, $p < 0.05$. Sex differences for monocytes were significant in both CMV-negative, mean diff. = 0.18, crit. diff. = 0.10, $p < 0.001$ and CMV-positive participants, mean diff. = 0.11, crit. diff. = 0.09, $p < 0.05$ (Figure 2M). CMV differences for lymphocytes were significant in both male participants, mean diff. = 0.16, crit. diff. = 0.11, $p < 0.01$ and female participants, mean diff. = 0.13, crit. diff. = 0.12, $p < 0.05$ (Figure 2L), while these differences for neutrophils were significant only in female participants, mean diff. = 0.07, crit. diff. = 0.06, $p < 0.05$ (Figure 2N).

2.3. Influence of Gender and CMV-Serostatus on Circulating Levels of Pro- and Anti-Inflammatory Mediators, Immune Cells, and Metabolic Blood Values Analysed by Bootstrapping Approach

The results of bootstrapping analyses are presented in Figure 3. As demonstrated by a confidence interval (CI = 95%) obtained from bootstrapping, the following significant group differences in mean levels were observed at $p < 0.05$.

The inflammatory TNF (Figure 3A) was significantly increased in the CMV-seropositive group of male participants compared to the CMV-seronegative males. Furthermore, there were clear sex differences in the CMV-seronegative group; namely, female participants showed an increased level of TNF compared to males.

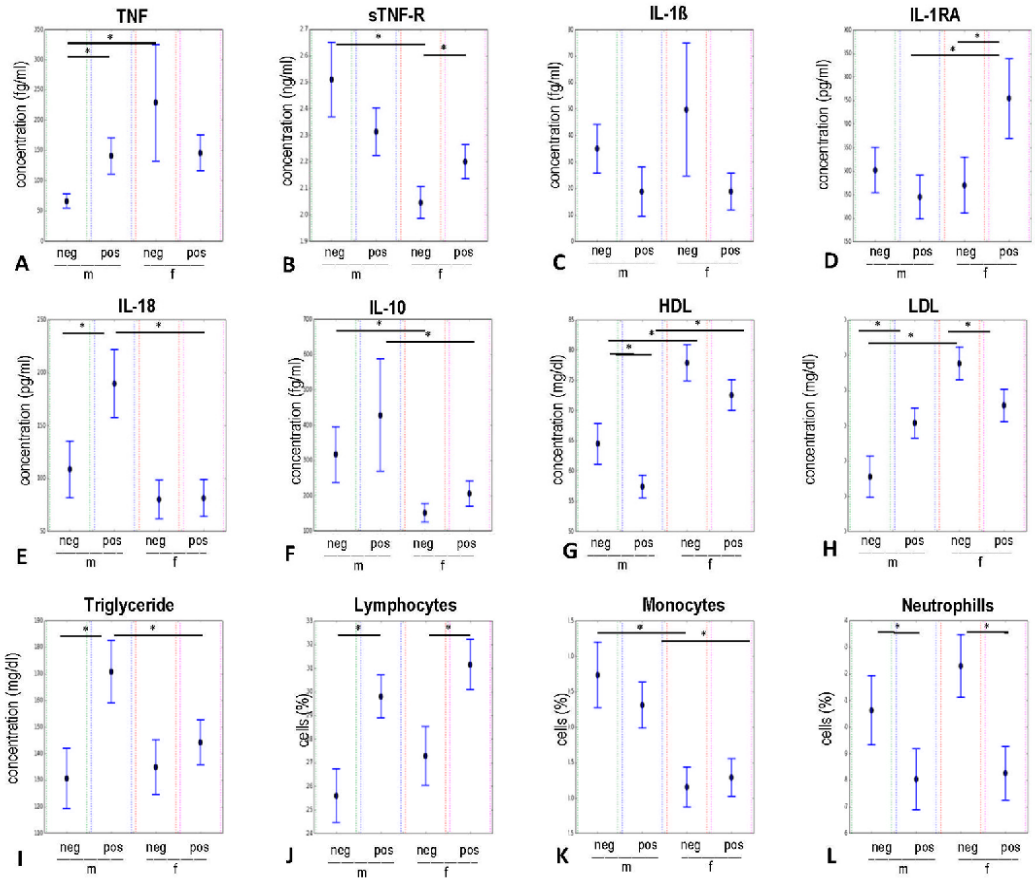

Figure 3. Bootstrapping results on the influence of CMV-serostatus and gender on circulating pro- and anti-inflammatory cytokines, receptor antagonist, immune cells, and metabolic factors. Data are presented as means with 95% confidence intervals for CMV-negative and CMV-positive males as well as for CMV-negative and CMV-positive females. * $p < 0.05$. IL-1β: interleukin 1 beta; IL-1RA: interleukin 1 receptor antagonist; TNF: tumor necrosis factor; sTNF-R: soluble tumor necrosis factor receptor; IL: interleukin; HDL: high-density lipoprotein; LDL: low-density lipoprotein; m: male; f: female; pos: CMV-seropositive; neg: CMV-seronegative.

In contrast, the anti-inflammatory sTNF-R (Figure 3B) was increased in males compared to females in the CMV-seronegative group. Furthermore, sTNF-R was significantly increased in the CMV-seropositive females compared to the CMV-negative female participants.

No significant differences between groups were found for IL-1β levels (Figure 3C). The modulating effect of CMV on IL-RA was detected in females (Figure 3D), where CMV-seropositive women produced more of this receptor antagonist than CMV-seronegative women did. Gender also influenced the IL-1RA levels, with females having higher concentrations of this inhibitor compared to males in the CMV-seropositive group.

The inflammatory IL-18 (Figure 3E) was increased in males in relation to females in the CMV-seropositive group. CMV-seropositive male participants also showed increased levels of IL-18 compared to their counterparts from the CMV-seronegative group. Levels of anti-inflammatory IL-10 (Figure 3F) showed gender differences (male > female) in both, CMV-seronegative and in CMV-seropositive groups.

CMV-seropositive males had significantly increased levels of LDL (Figure 3H) compared to CMV-seronegative male participants, whereas the CMV-seropositive females showed decreased levels of LDL compared to uninfected women. The levels of LDL in CMV-seronegative subjects were significantly increased in females compared to males.

We also looked at the differences in the concentration of HDL (Figure 3G) and found increased levels in CMV-seronegative compared to CMV-seropositive male participants. Moreover, women showed a significantly higher concentration of HDL than men, regardless of their CMV-status. Levels of triglyceride (Figure 3I) in the serum of the CMV-seropositive male group were significantly higher compared to the CMV-seronegative men. In the CMV-seropositive group men showed increased levels compared to women.

Concerning the differences in the levels of tested immune cells, we observed that CMV-seropositive men and women showed a decreased percentage of neutrophils (Figure 3L) compared to CMV-seronegative participants. In contrast, the levels of lymphocytes (Figure 3J), were increased in the groups of CMV-seropositive males and females. Additionally, we observed higher proportions of monocytes (Figure 3K) in males than in females, regardless of their CMV-serostatus.

2.4. Associations Among Various Blood Biomarkers in the CMV-Negative and CMV-Positive Groups and Modulatory Effect of CMV-Infection on the Correlation Coefficients

Figure 4 presents the Pearson's correlation coefficients for both the CMV-seropositive (red) and the CMV-seronegative (green) groups. Correlations' significance values are not adjusted for multiple comparisons [52].

CMV-seronegative

	IL-1ß	IL-6	TNF	IL-18	IL-1RA	IL-10	sTNF-R	HDL	LDL	TRIG	LEUKO	LYMPHO	MONO	NEUTRO	
IL-1ß		0,396	-0,153	-0,215	0,208	0,201	0,009	-0,185	0,052	0,076	-0,234	0,178	0,085	-0,197	IL-1ß
IL-6	0,282		-0,209	-0,218	0,345	0,350	0,355	-0,299	-0,201	0,088	0,147	-0,028	0,061	0,020	IL-6
TNF	0,132	0,118		0,053	-0,211	-0,163	0,129	0,145	-0,003	0,133	0,034	-0,016	-0,077	-0,095	TNF
IL-18	-0,046	0,143	-0,068		-0,293	-0,005	-0,047	0,035	0,054	0,118	-0,159	-0,170	-0,115	0,172	IL-18
IL-1RA	0,336	0,374	0,002	-0,038		0,256	0,271	-0,241	-0,001	0,240	0,303	-0,375	0,015	0,369	IL-1RA
IL-10	0,265	0,256	0,225	0,163	0,229		0,300	-0,360	-0,356	0,028	-0,068	0,025	0,196	-0,067	IL-10
sTNF-R	-0,008	0,258	-0,015	0,094	0,288	0,166		-0,387	-0,445	0,135	0,047	-0,036	0,296	-0,110	sTNF-R
HDL	0,012	-0,200	0,022	-0,008	-0,224	-0,066	-0,181		0,245	-0,301	0,061	-0,173	-0,291	0,212	HDL
LDL	-0,118	-0,182	-0,169	-0,119	0,005	-0,125	-0,062	-0,147		0,092	-0,066	0,110	-0,440	0,030	LDL
TRIG	-0,129	0,196	-0,155	0,029	0,241	0,062	0,064	-0,577	0,166		-0,049	0,102	-0,241	-0,040	TRIG
LEUKO	-0,130	0,220	0,017	-0,027	0,233	0,135	0,296	-0,196	0,035	0,296		-0,345	-0,088	0,369	LEUKO
LYMPHO	-0,064	-0,082	-0,116	-0,013	-0,213	-0,048	-0,021	0,049	0,024	-0,034	0,060		-0,056	-0,871	LYMPHO
MONO	0,091	0,080	0,031	0,063	-0,027	0,120	0,126	-0,033	0,063	0,108	-0,216	-0,024		-0,239	MONO
NEUTRO	-0,033	0,014	0,106	0,053	0,134	0,070	0,017	-0,045	-0,023	0,005	0,113	-0,861	-0,319		NEUTRO
	IL-1ß	IL-6	TNF	IL-18	IL-1RA	IL-10	sTNF-R	HDL	LDL	TRIG	LEUKO	LYMPHO	MONO	NEUTRO	

CMV-seropositive

p<0.05 p<0.01

Figure 4. Correlations among different blood biomarkers and the modulatory effect of CMV-seropositivity. Correlation coefficients are presented for CMV-negative in the green field and for CMV-positive in the red field. The significant values are highlighted in light blue ($p < 0.05$) and dark blue ($p < 0.01$). IL-1β: interleukin 1 beta; IL-1RA: interleukin 1 receptor antagonist; IL: interleukin; TNF: tumor necrosis factor; sTNF-R: soluble tumor necrosis factor receptor; HDL: high-density lipoprotein; LDL: low-density lipoprotein; LYMPHO: lymphocytes; LEUKO: leukocytes; MONO: monocytes; NEUTRO: neutrophils.

We found that the correlations seen in the CMV-seronegative and CMV-seropositive groups of participants were different concerning their magnitude. Below we describe results of the additionally applied Steiger's procedure showing the significant differences in the correlation coefficients between these two groups. We found that the correlation between the pro-inflammatory cytokine TNF and the anti-inflammatory cytokine IL-10 was significantly increased in the CMV-positive compared to the CMV-negative group (Zd = 0.394, $p < 0.05$). A similar significant increase in the CMV-positive compared to the CMV-negative group was found between the correlation coefficients of triglyceride and leukocytes (Zd = 0.353, $p < 0.05$), and between triglyceride and HDL (Zd = −0.347, $p < 0.05$).

In contrast, the relationship between anti-inflammatory sTNF-R and cholesterol LDL was significantly decreased (Zd = 0.416, $p < 0.05$) in the CMV-positive compared to the CMV-negative elderly participants. Furthermore, the magnitude of the correlation coefficients between monocytes and LDL (Zd = 0.535, $p < 0.01$), and between cholesterols LDL and HDL (Zd = −0.397, $p < 0.05$) were significantly lower under influence of the CMV-infection.

2.5. The Modulatory Effect of the CMV-serostatus and Gender on the Cognitive Abilities of Study Participants

The confirmatory factor analyses (CFA) of the four latent cognitive factor model (episodic memory, working memory, fluid intelligence, and perceptual speed) resulted in a good fit, $\chi^2 48 = 55.4$; CFI = 0.99; RMSEA = 0.036; SRMR = 0.047 (Figure S1 in the supplemental material).

Figure 5A–D shows bootstrapping results on the effects of gender and CMV infection on cognitive abilities of study participants. We found that both these factors had modulatory effects on fluid intelligence measurements, where male individuals showed significantly higher scores of fluid intelligence compared to females, but only in the group of CMV-seronegative participants (Figure 5C).

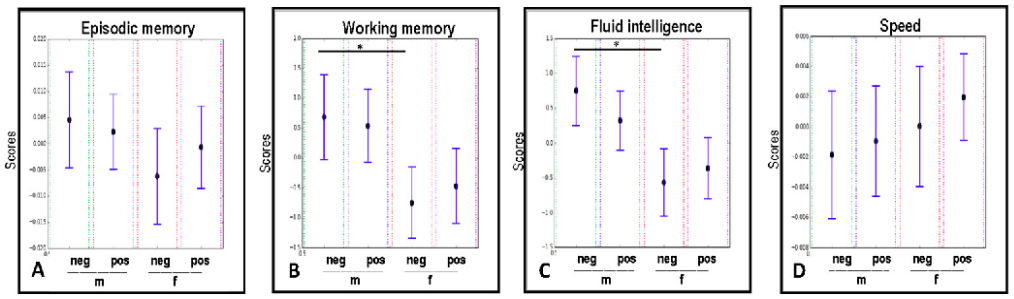

Figure 5. The influence of CMV-serostatus and gender on episodic memory, working memory, fluid intelligence, and processing speed, analyzed with the Bootstrapping approach. * $p < 0.05$. m: male; f: female; pos: CMV-seropositive; neg: CMV-seronegative.

The same phenomenon was observed also for the working memory domain (Figure 5B). Results obtained for episodic memory, and perceptual speed showed no significant differences in any of the tested groups (Figure 5A,D).

2.6. Associations Between Cognition and Circulating Inflammatory Mediators

To investigate potential associations between circulating pro- and anti-inflammatory biomarkers and cognition, we assessed correlations between scores of cognitive performance and inflammatory cytokines (TNF and IL-6), soluble receptor (sTNF-R), and receptor antagonist, IL-1RA. Table 1 summarizes the relationships between these variables. We found that in the CMV-seronegative participants, episodic and working memory as well as fluid intelligence correlated negatively with pro-inflammatory TNF levels. Episodic memory demonstrated a positive association with anti-inflammatory IL-10.

In the CMV-seropositive elderly, fluid intelligence and episodic memory correlated negatively with pro-inflammatory IL-6, but also with anti-inflammatory IL-1RA. Similarly, the working memory showed negative associations with IL-1RA in the CMV-positive individuals.

Table 1. Pearson's correlation coefficients (r) and corresponding p-values for associations between cognitive performance scores and circulating peripheral inflammatory markers.

Variables	r	p-Value
CMV-negative group ($n = 59$):		
EM [1] vs. TNF	−0.330	0.010
EM vs. IL-10	0.282	0.029
WM vs. TNF	−0.334	0.009
Gf vs. TNF	−0.415	0.001
CMV-positive group ($n = 102$):		
EM vs. IL-6	−0.201	0.042
EM vs. IL-1RA	−0.214	0.030
WM vs. IL-1RA	−0.218	0.028
Gf vs. IL-6	−0.205	0.039
Gf vs. IL-RA	−0.272	0.005

[1] EM. episodic memory; WM: working memory; Gf: fluid intelligence; TNF: tumor necrosis factor; IL: interleukin; IL-1RA: interleukin 1 receptor antagonist; CMV: Cytomegalovirus.

The correlations seen in the CMV-seronegative and CMV-seropositive groups of participants were found to be different concerning their magnitude. To test for the significant differences in the correlation coefficients between these two groups, we applied Steiger's procedure. We found that the correlation between pro-inflammatory cytokine TNF and fluid intelligence was significantly increased ($Zd = 0.387$, $p < 0.05$) in the CMV-positive group compared to the CMV-negative group. In contrast, the relationships between IL-1RA and episodic memory ($Zd = -0.445$, $p < 0.01$), and between IL-1RA and working memory ($Zd = -0.379$, $p < 0.05$) were significantly decreased in the CMV-positive compared to the CMV-negative elderly participants.

3. Discussion

In the present study, we characterized the inflammatory status of aged individuals at the baseline in a pre-intervention cohort. In a first set of analyses, we investigated the influence of gender and CMV-seropositivity on the main inflammatory and anti-inflammatory mediators and molecules assessed in this study, such as circulating cytokines, receptor antagonist, soluble receptor, immune cells, and relevant metabolic markers. We found that both gender and CMV-seropositivity jointly and separately participate in the modulation of circulating pro- and anti-inflammatory biomarkers in elderly study participants. Figure 6 illustrates the summarized results of these effects.

The influence of sexual dimorphism on the inflammatory status was demonstrated in both CMV-negative and CMV-positive participants. While in the CMV-seronegative group, males demonstrated significantly higher levels of anti-inflammatory sTNF-R, the females had elevations of its pro-inflammatory counterpart, TNF, probably, due to the missing anti-inflammatory effects of sTNF-R in their circulation. The level of the anti-inflammatory receptor antagonist, IL-1RA, was, in contrast, significantly higher in CMV-positive women than in CMV-positive men. This deficit possibly contributed to an increase in the pro-inflammatory IL-18 in men due to the property of IL-1RA to propagate and to buffer the effect of inflammatory cytokines [13,19–21].

The influence of gender on the cytokine profile in elderly humans has rarely been studied and findings are contradictory. Some studies have shown gender-related differences [53], whereas others found no differences for the majority of cytokines [12]. The in vitro evidence suggests that testosterone may suppress the production of the pro-inflammatory cytokines TNF, IL-1β, and IL-6 [54], and potentiate the release of the anti-inflammatory cytokine IL-10 [55]. Our results are mainly congruent with these findings, showing a significant gender-related increase in levels of anti-inflammatory IL-10 in men of both CMV-positive and CMV-negative groups. Pro-inflammatory TNF is also lower in men compared to women, but this concerns only the CMV-negative groups. It seems that the CMV-seropositivity might possibly diminish the positive effect of testosterone on the inflammatory status in aged males, because not only elevations of pro-inflammatory TNF and IL-18, but also

decreased levels of anti-inflammatory sTNF-R and IL-1RA have been found in CMV-infected male participants (Figure 6).

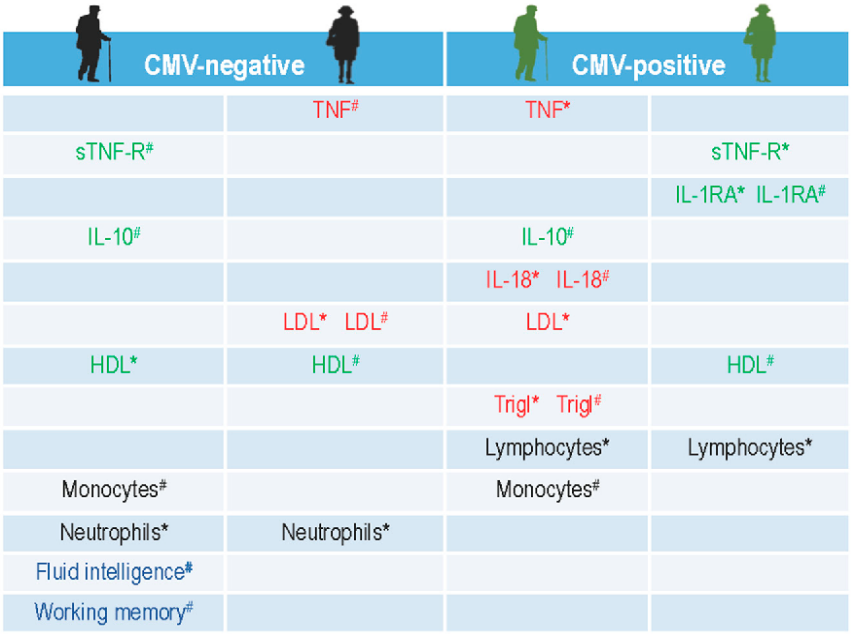

Figure 6. A schematic illustration of summarized results on the influence of gender and CMV-serostatus on circulating pro- and anti-inflammatory cytokines, receptor antagonist, metabolic factors, immune cells, and cognitive abilities in the baseline cohort of elderly participants. The names of the analyzed parameters with significantly higher values are placed in the corresponding column of the CMV-negative or CMV-positive men and women accordingly, whereby the notation ($^{\#}$) implies: a gender-related-higher value, and the notation (*) implies: a CMV-related-higher value. The pro-inflammatory mediators are written in red; the anti-inflammatory are green; the cognitive latent factors are blue; and immune cells are left in black. TNF: tumor necrosis factor; sTNF-R: soluble tumor necrosis factor receptor; IL: interleukin; IL-1β: interleukin 1 beta; IL-1RA: interleukin 1 receptor antagonist; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

In our previous publication, we assumed that "gender disparities in the differentiation status of immune cells might first emerge under the immunomodulating effect of the stress of long-term immunosurveillance to control CMV-infection" [50]. The same might also be true for circulating cytokines as well, not least because circulating inflammatory molecules are, in fact, mostly produced by the same senescent CMV-exhausted immune cells. Such senescent cells secrete various extracellular factors, including inflammatory cytokines, which can enhance and "propagate senescence with autocrine and paracrine modality, contributing to the pro-inflammatory status of ageing" [56].

The percentage of immune cells in our baseline cohort appeared to be also modulated by CMV-serostatus, with increased levels of lymphocytes and decreased proportions of neutrophils in CMV-positive old participants. At the same time, blood monocytes vary in our sample by gender, with men having higher proportion of monocytes compared to women, similar to what was reported in earlier studies [57]. These aging immune cells and particularly, the inflammatory fraction of monocytes, are thought to be responsible for inflammation-induced "unhealthy" aging [19].

Intriguing results on higher levels of anti-inflammatory mediators such as IL-1RA and sTNF-R in CMV-positive as compared to CMV-negative women on the one hand, and higher pro-inflammatory state in CMV-negative women as compared to CMV-negative men on the other hand (Figure 6) may be due to the participants' chronological history of co-existence with the CMV-antigen [58,59] and to the sex-specific differences in the immune responses [60,61]. Generally, immune responses in females are characterized by more pronounced pro-inflammatory activation that is partly regulated by estrogen

receptors (ER). ERs form complexes at gene regulatory elements and promote epigenetic changes and transcription, thereby regulating inflammatory response in a dose- and context-dependent manner. Low physiological levels of estradiol generally promote inflammatory pathways leading to production of pro-inflammatory cytokines. In some conditions, however, ER signaling inhibits these pathways even in a low estrogen environment [62]. Such a special condition may represent the latent CMV infection, which may persist on a lifelong basis.

One of the possible explanations might be that the initial immune response of young women to a primary CMV infection could be different to young men's (due to the less active immunity in the latter) and that this initial difference (together with other factors) translates to an induction of a pro-inflammatory environment in the aged CMV-positive men, while in the post-menopausal CMV-positive women, this may perhaps lead to generation of anti-inflammatory mediators instead. However, clearly more in-depth studies are required, including investigations on the impact of modulatory effects of sex hormones on immune interactions, to further define and to clearly delineate these effects.

Inflammation can also adversely affect lipoproteins, which may then, in turn, modulate the production of pro-inflammatory cytokines [63]. Results from the animal studies demonstrated that inflammatory cytokines such as TNF, IL-1β, and IL-6 increase serum triglyceride fatty acid levels [63,64]. We have also found a positive association between levels of pro-inflammatory IL-6 and triglyceride, but exclusively in the CMV-positive participants (Figure 4). Our results show also multiple significant associations of HDL- and LDL-cholesterols with pro- and anti-inflammatory cytokines and their receptors (Figure 4). Moreover, the strength of these associations seems to be modified by CMV infection. Additionally, the inflammatory environment appeared to be less pronounced in the groups with higher concentrations of serum HDL (Figure 6).

In general, HDL has potent anti-inflammatory properties and the remarkable ability to modulate the inflammatory response in various cell types. However, in a chronic inflammatory state, HDL can itself be modified to become dysfunctional [65] and therefore not able to relieve cells from excessive and oxidized LDL cholesterol. The most common effects of a chronic pro-inflammatory state are decreases in serum HDL and increases in triglycerides, total cholesterol, and LDL. Thus, in addition to affecting serum lipid levels, inflammation also adversely affects the lipoprotein function [66,67]. Our results showed similar effects of increased LDL and triglycerides concentrations, but decreased HDL levels in the inflammatory environment of CMV-negative females and CMV-positive males (Figure 6). Moreover, it appears that both CMV-seropositivity and gender jointly (increase of LDL in CMV-negative women; increase of triglycerides in CMV-positive men) and separately (decrease of HDL in CMV-positive men) contributed to these effects. However, it is clear that more studies involving different pro- and anti-inflammatory biomarkers and their modulators are required to understand these multifactorial and dynamic interrelationships and their effects on low-grade inflammation and immunosenescence.

In the present study, we assessed the cognitive performance of elderly people at baseline of a six-month intervention study. Moreover, we investigated the influence of the CMV-serostatus and gender on cognitive abilities. Here, we found that gender exerted a modulating effect on the fluid intelligence and working memory (with men showing higher scores of performance) in the CMV-negative individuals only, whereas no such influence has been detected for processing speed and episodic memory in any of the tested groups (Figure 5).

Gender differences in cognitive tests have repeatedly been reported in older adults although the magnitude of these differences seems to be modest [68] and advantage for one or another gender appears to be related to different cognitive domains [69]. Gender differences in cognitive test performance have been attributed to various factors, such as sex hormones or sexual dimorphisms in brain structure—all of which change with normal aging [70], but the modulating influence of CMV-seropositivity has not been investigated in these studies. Al-Delaimy demonstrated higher cognitive test performance among men, but not among women and these differences were related to insulin-like growth factor (IGF)-1 levels [71]. Similar results on the IGF-1 that positively influenced the

cognitive performance only in men, were also shown by another group [72]; and, again, the influence of CMV was not considered in this study.

In the current study, males from the CMV-negative group demonstrated better scores in fluid intelligence, working memory and concomitantly, a high level of peripheral anti-inflammatory factors, such as IL-10 cytokine and soluble TNF receptor (Figure 6). Furthermore, they also showed higher levels of anti-inflammatory HDL compared to males of the CMV-seropositive group. Interestingly, CMV-positive men did not show such cognitive advantage, although their levels of IL-10 were higher than in CMV-positive women. This could partly be explained by the fact that they had an elevated inflammatory status (CMV-related higher levels of TNF and IL-18), a relatively adverse metabolic environment (elevated LDL cholesterol and triglycerides), and increased levels of monocytes and lymphocytes in their peripheral circulation (Figure 6). All these factors are known to contribute to a low-grade inflammation and some of them, acting as upstream effectors might also mediate effects of peripheral inflammation on the central nervous system and have powerful effects on cognition and behavior [5,73,74]. Therefore, we can speculate that the integral effect of the above-described conditions in their peripheral circulation might influence and modulate cognitive abilities of elderly people.

In our study, we have demonstrated a negative association of fluid intelligence as well as episodic and working memory with the pro-inflammatory TNF in CMV-negative individuals. TNF is known to exert physiological neuroprotective but also pathological neurodegenerative effects [75] within the nervous system. Cognitive impairments have also been demonstrated in transgenic mice over-expressing TNF [76]. The pro-inflammatory TNF and IL-1β have been shown to physiologically modulate synaptic plasticity and synaptic scaling in different brain areas such as hippocampus, striatum and cortex [77,78].

In contrast, we found a positive association of episodic memory with anti-inflammatory IL-10 in the CMV-negative group. IL-10 is known for its inhibitory role on the production of inflammatory cytokines by microglia as well as for its neuroprotective function on neurons and astrocytes [79].

Interestingly, in the CMV-seropositive group, fluid intelligence, episodic and working memory scores were negatively associated with the anti-inflammatory IL-1RA, the levels of which were apparently simultaneously increased as a reaction to the rise of the pro-inflammatory cytokines in their periphery. This phenomenon has also been observed by other groups [80,81], who reported that individuals with elevated levels of the pro-inflammatory markers also tend to show increased levels of the anti-inflammatory markers. In the CMV-positive group we have also found a negative associations of episodic memory and fluid intelligence with pro-inflammatory IL-6. Several mediators of inflammatory activity, including such cytokines as IL-6, IL-1β, and TNF, were shown to be associated with impairments in the cognitive function [82–85]. Our results on the negative association of the cognitive performance with inflammatory cytokines are in accordance with these findings.

In our investigation of the relationships between various inflammatory biomarkers, we have also found that the CMV-latency influenced interrelations between the different mediators of inflammation, possibly contributing to the induction of such a CMV-related inflammatory environment. In other words, the CMV-infection appears not only to contribute to the shift in the levels of the particular cytokine but also to the change in the interrelationships between these immune mediators and molecules. Due to the exploratory character of the study on these associations, more in-depth investigations are required to confirm and elucidate these associations and altered interrelationships between different biomarkers under the modulatory influence of CMV-infection.

Findings from a comprehensive study that aimed to create a source of immune measurements in aging individuals including among others, clinical and functional parameters, peripheral blood mononuclear cells (PBMC) phenotypes, cytokines and gene expression in stimulated and unstimulated PBMC, as well as measures of some serum cytokines, showed that age, followed by sex and CMV status had the greatest effect on the immune system [86].

Thus, we can conclude that the CMV-latency may induce various modulatory effects on the inflammatory and immune factors in the peripheral circulation of aged individuals. This modulatory

activity may have different consequences for the aged men and women and, therefore, may also differently influence their functional and cognitive abilities. On account of this, both the CMV-serostatus and gender should always be included in the consideration together with other factors in the comparative and interventional studies with elderly people.

Our study has many strengths, including that it is one of the first studies to extensively characterize prior to physical, cognitive, and combine interventions, the inflammatory and functional status in elderly participants by accessing the multiple pro- and anti-inflammatory cytokines, receptor antagonist, soluble receptor, metabolic factors, immune cells, and multiple measures of objective cognitive function. This is also one of the first studies to assess the modulatory effect of CMV-seropositivity and gender on the inflammatory status of participants and their functional cognitive abilities at baseline.

There are several limitations in our study that should be acknowledged. The first one is related to the fact that our pre-training cohort consisted of relatively healthy, non-obese, and well-educated Berlin residents with a comparatively low seroprevalence for CMV for this age. For this reason, the generalizability of some of our findings may be limited to the Berlin healthy aging population or to a similar European population in urban areas.

Another limitation may be related to the fact that we did not evaluate the serostatus of the study participants for other chronic or latent infections, such as EBV (Epstein-Barr virus), HIV (Human Immunodeficiency virus), HBV (Hepatitis B virus), or HCV (Hepatitis C virus), to confirm that the observed results are specifically related to CMV infection.

The next limitation that has been repeatedly reported also by several other studies [12,81,87–89], may be due to the fact that such cytokines as IL-1β, TNF, and IL-6 are not highly abundant in the periphery of relatively healthy non-obese people, and the levels of such cytokines were also found in some of our participants towards the lower end or below the levels of detection for these assays. Accordingly, a further limitation may be related to the sensitivity of the techniques used to detect cytokines. The most frequently applied quantification of cytokine levels using Enzyme-linked Immunosorbent Assay (ELISA) technique may sometimes not be sensitive enough, due to the "presence of naturally occurring biological inhibitors in circulation, which might interfere with the detection of the respective cytokine" [90]. Also, multiplex techniques and even the Cytometric Bead Array (CBA) Enhanced Sensitivity Flex Set used in our study "are primarily designed to accommodate the simultaneous measurement of several analytes, and therefore compromises are inevitably made for the individual analytes" [91]. Despite these limitations, results obtained in the present study for the most of the pro- and anti-inflammatory cytokines and other factors related to low-grade inflammation are rather consistent.

While the interaction of pro- and anti-inflammatory cytokines, receptor antagonist, soluble receptor, and metabolic factors are complex and still need to be understood in the context of age-related low-grade inflammation, our results suggest that the evaluation of both gender differences and the impact of the CMV-serostatus appears to be decisive in studies dealing with age-related changes in neuroimmune factors as well as their association with cognitive and behavioral abilities in elderly people.

4. Materials and Methods

4.1. Participants

The sample consisted of 161 older adults (Figure 1) who had enrolled to participate in a training study that included physical, cognitive, and combined training interventions. Male and female subjects were recruited from volunteer participants pools at the Max Planck Institute for Human Development and by advertisements in the metropolitan area of Berlin, Germany. All the volunteers lived independently at home, leading an active life. Participants were healthy, right-handed adults, aged 64–79 years. All volunteers completed a medical assessment prior to data collection. The medical

examination was conducted at the Charité Sports Medicine, Charité Universitätsmedizin Berlin. Of the originally recruited 201 volunteers only 179 individuals met inclusion criteria for study participation after medical assessment. None of the participants had a history of head injuries, medical (e.g., heart attack), neurological (e.g., epilepsy), or psychiatric (e.g., depression) disorders. None of the volunteers had suffered from chronic inflammatory, autoimmune or cancer disease, nor had clinically evident infections. Moderately elevated and controlled blood pressure was not considered as exclusion criteria. All subjects completed the informed consent form to the study protocol which was approved by the Ethics Committee of German Society of Psychology on 27.09.2016, UL 072014.

4.2. Circulating Biomarkers Assessment

4.2.1. Cytokines TNF, IL-10, IL-6, and IL-1β

The serum levels of pro- and anti-inflammatory cytokines (TNF, IL-10, IL-6, and IL-1β) were determined using the high-sensitivity cytometric bead array (CBA) flex system (BD Biosciences, San Jose, CA, USA) that allows quantification of the serum concentration of these inflammatory markers in a single sample. All analyses were performed according to the manufacturer's instructions; to increase accuracy an additional standard dilution was added. The fluorescence produced by CBA beads was measured on a BD FACS CANTO II Flow Cytometer and analyzed using the software FCAP Array v3 (BD Biosciences).

4.2.2. sTNF-R, IL-1RA, IL-18 Levels, and CMV-Serostatus

To gauge sTNF-R (80 kDA), IL-1RA, and IL-18 levels, we used the Sandwich Enzyme-linked Immunosorbent Assay (ELISA), a sensitive method allowing for the measurement of an antigen concentration in an unknown sample. All analyses were conducted according to the manufacturer's instructions. The levels of human circulating sTNF-R (80 kDA), IL-1RA, and IL-18 were determined using the Platinum ELISA kit for the quantitative detection of the three cytokines (ThermoFisher SCIENTIFIC Invitrogen, Vienna, Austria, catalog numbers: BMS211, BMS2080 and BMS267/2).

Serum levels of the Cytomegalovirus IgG were determined using the commercial ELISA kit (IBL International GMBH, Hamburg, Germany, catalogue number: RE57061) and according to the manufacturer's instructions. Samples were considered to give a positive signal if the absorbance value exceeded 10% over the cut-off, whereas a negative signal was declared if the absorbance value was lower than 10% below the cut-off.

All samples were assessed in duplicates at 450 or 450/620 nm using Multiscan-FC Microtiter Plate Photometer. Protein concentrations were determined in relation to a four-parameter standard curve (Prism 8 GraphPad, San Diego, CA, USA) or calculated using Microsoft Excel 2011.

Levels of LDL- and HDL-cholesterols, triglyceride, lymphocytes, leukocytes, monocytes, and neutrophils were measured within the clinical diagnostics facility of Berlin, Labor28. Serum concentrations of cholesterols and triglyceride were measured using enzymatic colorimetric tests (Roche, Basel, Switzerland). The counts of the immune cells were assessed by flow cytometry (Sysmex, Norderstedt, Germany).

4.3. Cognitive Assessment

Participants were invited to one session that lasted about 3.5 h. Participants were tested in groups of four to six. The cognitive battery included a broad range of measures of learning and memory performance, processing speed, working memory, and executive functioning. The group received a standardized session protocol and started, after instructions, with a practice trial to ensure that all participants understood the task. Responses were collected via button boxes, the computer mouse, or the keyboard.

For the purpose of the present study, we focused on four latent factors representing main cognitive abilities, namely episodic memory (EM; measured by Verbal Learning and Memory Test,

Face–Profession Task, and Scene Encoding), working memory (WM; measured by Letter Updating, Number-N-Back, and Spatial Updating), fluid intelligence (Gf; measured by Figural Analogies, Letter Series, and Practical Problems), and perceptual speed (Speed; measured by Verbal Speed, Figural Speed, and Number Speed) [92–96]. The detailed description of the factors and tasks is included in the supplementary material.

4.4. Statistical Analyses

The participants were split into two groups, depending on their CMV-serostatus: CMV-seropositive and CMV-seronegative and further divided by gender. All variables distributions were examined for normality using the Kolmogorov-Smirnov and Shapiro–Wilk tests. Because variables were significantly departed from normality and exhibited variance heterogeneity, the natural logarithm transformation was applied. For cytokine levels below the detection range of the assay sensitivity, the LOD/square root of 2 (where LOD is the lowest level of detection) was used [97–99].

To investigate the influence of CMV-serostatus and gender on circulating pro- and anti-inflammatory biomarkers, immune cells, and metabolic factors, the MANOVA and bootstrapping analyses were performed. For MANOVA, the logarithmically transformed variables were grouped into pro-inflammatory (IL-1β, IL-6, IL-18, and TNF), anti-inflammatory (IL-10, IL-1RA, and sTNF-R), metabolic (HDL, LDL, and triglycerides), and immune cells (lymphocytes, monocytes, and neutrophils) groups of variables. Further, follow-up univariate ANOVAs were performed to investigate the influence of CMV-serostatus and gender on the single outcome variables. Scheffé's post hoc test was used to determine which of the paired means differed significantly.

For the bootstrap approach [100] we used untransformed data. Bootstrapping generated different samples with similar distributions and provided estimates of confidence intervals around sampling means. The procedure involved drawing 10,000 samples with replacement from a single original sample in four groups (CMV^- males; CMV^- females; CMV^+ males; CMV^+ females), calculating statistics for each sample, and inspecting the bootstrap distribution of the re-sampling means. Since the bootstrap distribution showed a normal shape and a small bias, we could obtain a 95% confidence interval (CI) for the mean by using the bootstrap standard error (SE_{boot}) and the t distribution: $CI = mean \pm t \times SE_{boot}$, using LabView software with the MatLab bootstrap function. The level of statistical significance was set at $p < 0.05$.

To investigate the relationship between the levels of inflammatory and anti-inflammatory cytokines, immune cells counts, and metabolic blood characteristics, we calculated Pearson's correlations for CMV-seropositive and CMV-seronegative groups separately. Correlation analyses were performed with logarithmically transformed data. Since it was an exploratory study, analyses were performed without adjustment for multiple comparison [52].

A test for significant differences in the correlation coefficients was performed using Steiger's method [101]. To test the null hypothesis that the correlation between two variables in one sample is the same as the correlation between these variables in another sample, we first, carried out Fisher's Z transform for each of the two correlation coefficients:

$$Z = 0.5 \times ln\left(\frac{1+r}{1-r}\right) \quad (1)$$

and then calculated the test statistic (Z_d) as follows:

$$Z_d = \frac{Z_1 - Z_2}{\sqrt{\frac{1}{n_1-3} + \frac{1}{n_2-3}}} \quad (2)$$

where n_1 and n_2 are corresponding sample numbers.

A Confirmatory Factor Analyses of four latent cognitive factors was applied to define a four-factor model of cognitive abilities in latent space by using measures of specific cognitive tasks as manifest

variables. The four intercorrelated latent factors of cognitive constructs were as follow: working memory (WM), episodic memory (EM), fluid intelligence (Gf), and processing speed (Speed). All latent factors were allowed to be correlated (Figure S1 in Supplementary Materials).

5. Conclusions

In the present study we found that both gender and CMV-seropositivity modulate circulating peripheral biomarkers, and that CMV infection modifies associations among the latter. Moreover, we observed an interaction between CMV-serostatus and gender associations with cognitive abilities: Gender differences in fluid intelligence and working memory were noted only in CMV-negative individuals. Finally, we found that in the CMV-seronegative participants fluid intelligence, episodic memory, and working memory correlated negatively with pro-inflammatory TNF. We also found that episodic memory correlated positively with anti-inflammatory IL-10. In CMV-seropositive individuals, episodic memory and fluid intelligence correlated negatively with pro-inflammatory IL-6; and episodic memory, fluid intelligence, and working memory correlated negatively with anti-inflammatory IL-1RA. We conclude that both CMV-serostatus and gender may modulate neuroimmune factors, cognitive performance and the relationship between the two domains and should therefore be considered in comparative and interventional studies with elderly people.

Supplementary Materials: Supplementary materials can be found at http://www.mdpi.com/1422-0067/20/4/990/s1.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

CMV	Cytomegalovirus
IL	Interleukin
IL-1RA	Interleukin 1 receptor antagonist
TNF	Tumor Necrosis Factor
sTNF-R	Soluble Tumor Necrosis Factor receptor
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
IgG	Immunoglobulin G
ANOVA	Analysis of variance
MANOVA	Multivariate ANOVAs
CI	Confidence interval
EM	Episodic memory
WM	Working memory
Gf	Fluid intelligence
CFA	Confirmatory Factor Analysis
IGF-1	Insulin-like growth factor 1
ELISA	Enzyme-linked Immunosorbent Assay
CBA	Cytometric bead array

References

1. Franceschi, C.; Campisi, J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* **2014**, *69* (Suppl. 1), S4–S9. [CrossRef] [PubMed]
2. Holmes, C.; Cunningham, C.; Zotova, E.; Woolford, J.; Dean, C.; Kerr, S.; Culliford, D.; Perry, V.H. Systemic inflammation and disease progression in Alzheimer disease. *Neurology* **2009**, *73*, 768–774. [CrossRef] [PubMed]
3. King, E.; O'Brien, J.T.; Donaghy, P.; Morris, C.; Barnett, N.; Olsen, K.; Martin-Ruiz, C.; Taylor, J.P.; Thomas, A.J. Peripheral inflammation in prodromal Alzheimer's and Lewy body dementias. *J. Neurol. Neurosurg. Psychiatry* **2018**, *89*, 339–345. [CrossRef] [PubMed]
4. Franceschi, C.; Garagnani, P.; Vitale, G.; Capri, M.; Salvioli, S. Inflammaging and 'Garb-aging'. *Trends Endocrinol. Metab.* **2017**, *28*, 199–212. [CrossRef] [PubMed]
5. Di Benedetto, S.; Müller, L.; Wenger, E.; Duzel, S.; Pawelec, G. Contribution of neuroinflammation and immunity to brain aging and the mitigating effects of physical and cognitive interventions. *Neurosci. Biobehav. Rev.* **2017**, *75*, 114–128. [CrossRef] [PubMed]
6. Castro, A.M.; Macedo-de la Concha, L.E.; Pantoja-Meléndez, C.A. Low-grade inflammation and its relation to obesity and chronic degenerative diseases. *Revista Médica del Hospital General de México* **2017**, *80*, 101–105. [CrossRef]
7. Chupel, M.U.; Direito, F.; Furtado, G.E.; Minuzzi, L.G.; Pedrosa, F.M.; Colado, J.C.; Ferreira, J.P.; Filaire, E.; Teixeira, A.M. Strength Training Decreases Inflammation and Increases Cognition and Physical Fitness in Older Women with Cognitive Impairment. *Front. Physiol.* **2017**, *8*, 377. [CrossRef] [PubMed]
8. Fülop, T.; Larbi, A.; Dupuis, G.; Le Page, A.; Frost, E.H.; Cohen, A.A.; Witkowski, J.M.; Franceschi, C. Immunosenescence and Inflamm-Aging As Two Sides of the Same Coin: Friends or Foes? *Front. Immunol.* **2017**, *8*, 1960. [CrossRef] [PubMed]
9. Sawada, M.; Suzumura, A.; Marunouchi, T. TNF alpha induces IL-6 production by astrocytes but not by microglia. *Brain Res.* **1992**, *583*, 296–299. [CrossRef]
10. Singh, T.; Newman, A.B. Inflammatory markers in population studies of aging. *Ageing Res. Rev.* **2011**, *10*, 319–329. [CrossRef] [PubMed]
11. Steensberg, A.; Fischer, C.P.; Keller, C.; Moller, K.; Pedersen, B.K. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. *Am. J. Physiol. Endocrinol. Metab.* **2003**, *285*, E433–E437. [CrossRef] [PubMed]
12. Alvarez-Rodriguez, L.; Lopez-Hoyos, M.; Munoz-Cacho, P.; Martinez-Taboada, V.M. Aging is associated with circulating cytokine dysregulation. *Cell. Immunol.* **2012**, *273*, 124–132. [CrossRef] [PubMed]
13. Dinarello, C.A. Interleukin-1 and interleukin-1 receptor antagonist. *Nutrition* **1995**, *11*, 492–494. [PubMed]
14. Audet, M.C.; Anisman, H. Interplay between pro-inflammatory cytokines and growth factors in depressive illnesses. *Front. Cell. Neurosci.* **2013**, *7*, 68. [CrossRef] [PubMed]
15. Calabrese, F.; Rossetti, A.C.; Racagni, G.; Gass, P.; Riva, M.A.; Molteni, R. Brain-derived neurotrophic factor: A bridge between inflammation and neuroplasticity. *Front. Cell. Neurosci.* **2014**, *8*, 430. [CrossRef] [PubMed]
16. Lutz, C.T.; Quinn, L.S. Sarcopenia, obesity, and natural killer cell immune senescence in aging: Altered cytokine levels as a common mechanism. *Aging* **2012**, *4*, 535–546. [CrossRef] [PubMed]
17. O'Connor, J.C.; McCusker, R.H.; Strle, K.; Johnson, R.W.; Dantzer, R.; Kelley, K.W. Regulation of IGF-I function by proinflammatory cytokines: At the interface of immunology and endocrinology. *Cell. Immunol.* **2008**, *252*, 91–110. [CrossRef] [PubMed]
18. Biet, F.; Locht, C.; Kremer, L. Immunoregulatory functions of interleukin 18 and its role in defense against bacterial pathogens. *J. Mol. Med.* **2002**, *80*, 147–162. [CrossRef] [PubMed]
19. Shaw, A.C.; Joshi, S.; Greenwood, H.; Panda, A.; Lord, J.M. Aging of the innate immune system. *Curr. Opin. Immunol.* **2010**, *22*, 507–513. [CrossRef] [PubMed]
20. Shaw, D.M.; Merien, F.; Braakhuis, A.; Dulson, D. T-cells and their cytokine production: The anti-inflammatory and immunosuppressive effects of strenuous exercise. *Cytokine* **2017**, *104*, 136–142. [CrossRef] [PubMed]
21. Arend, W.P.; Malyak, M.; Guthridge, C.J.; Gabay, C. Interleukin-1 receptor antagonist: Role in biology. *Annu. Rev. Immunol.* **1998**, *16*, 27–55. [CrossRef] [PubMed]

22. La Rosa, C.; Diamond, D.J. The immune response to human CMV. *Future Virol.* **2012**, *7*, 279–293. [CrossRef] [PubMed]
23. Müller, L.; Hamprecht, K.; Pawelec, G. The role of CMV in "immunosenescence". In *The Ageing Immune System and Health*; Bueno, V., Lord, J.M., Jackson, T.A., Eds.; Springer: Cham, Switzerland, 2017; pp. 53–68.
24. Pawelec, G. Immunosenenescence: Role of cytomegalovirus. *Exp. Gerontol.* **2014**, *54*, 1–5. [CrossRef] [PubMed]
25. Weltevrede, M.; Eilers, R.; de Melker, H.E.; van Baarle, D. Cytomegalovirus persistence and T-cell immunosenescence in people aged fifty and older: A systematic review. *Exp. Gerontol.* **2016**, *77*, 87–95. [CrossRef] [PubMed]
26. Nikolich-Zugich, J.; Goodrum, F.; Knox, K.; Smithey, M.J. Known unknowns: How might the persistent herpesvirome shape immunity and aging? *Curr. Opin. Immunol.* **2017**, *48*, 23–30. [CrossRef] [PubMed]
27. Jackson, S.E.; Redeker, A.; Arens, R.; van Baarle, D.; van den Berg, S.P.H.; Benedict, C.A.; Cicin-Sain, L.; Hill, A.B.; Wills, M.R. CMV immune evasion and manipulation of the immune system with aging. *Geroscience* **2017**, *39*, 273–291. [CrossRef] [PubMed]
28. Al-Attar, A.; Presnell, S.R.; Peterson, C.A.; Thomas, D.T.; Lutz, C.T. Data correlations between gender, cytomegalovirus infection and T cells, NK cells, and soluble immune mediators in elderly humans. *Data Brief* **2016**, *8*, 536–544. [CrossRef] [PubMed]
29. Bennett, J.M.; Glaser, R.; Malarkey, W.B.; Beversdorf, D.Q.; Peng, J.; Kiecolt-Glaser, J.K. Inflammation and reactivation of latent herpesviruses in older adults. *Brain Behav. Immun.* **2012**, *26*, 739–746. [CrossRef] [PubMed]
30. Simanek, A.M.; Cheng, C.; Yolken, R.; Uddin, M.; Galea, S.; Aiello, A.E. Herpesviruses, inflammatory markers and incident depression in a longitudinal study of Detroit residents. *Psychoneuroendocrinology* **2014**, *50*, 139–148. [CrossRef] [PubMed]
31. Wang, G.C.; Kao, W.H.; Murakami, P.; Xue, Q.L.; Chiou, R.B.; Detrick, B.; McDyer, J.F.; Semba, R.D.; Casolaro, V.; Walston, J.D.; et al. Cytomegalovirus infection and the risk of mortality and frailty in older women: A prospective observational cohort study. *Am. J. Epidemiol.* **2010**, *171*, 1144–1152. [CrossRef] [PubMed]
32. Kilgour, A.H.; Firth, C.; Harrison, R.; Moss, P.; Bastin, M.E.; Wardlaw, J.M.; Deary, I.J.; Starr, J.M. Seropositivity for CMV and IL-6 levels are associated with grip strength and muscle size in the elderly. *Immun. Ageing* **2013**, *10*, 33. [CrossRef] [PubMed]
33. Barnes, L.L.; Capuano, A.W.; Aiello, A.E.; Turner, A.D.; Yolken, R.H.; Torrey, E.F.; Bennett, D.A. Cytomegalovirus infection and risk of Alzheimer disease in older black and white individuals. *J. Infect. Dis.* **2015**, *211*, 230–237. [CrossRef] [PubMed]
34. Mathei, C.; Vaes, B.; Wallemacq, P.; Degryse, J. Associations between cytomegalovirus infection and functional impairment and frailty in the BELFRAIL Cohort. *J. Am. Geriatr. Soc.* **2011**, *59*, 2201–2208. [CrossRef] [PubMed]
35. Aiello, A.E.; Haan, M.; Blythe, L.; Moore, K.; Gonzalez, J.M.; Jagust, W. The influence of latent viral infection on rate of cognitive decline over 4 years. *J. Am. Geriatr. Soc.* **2006**, *54*, 1046–1054. [CrossRef] [PubMed]
36. Gow, A.J.; Firth, C.M.; Harrison, R.; Starr, J.M.; Moss, P.; Deary, I.J. Cytomegalovirus infection and cognitive abilities in old age. *Neurobiol. Aging* **2013**, *34*, 1846–1852. [CrossRef] [PubMed]
37. Ricci, G.; Pirillo, I.; Tomassoni, D.; Sirignano, A.; Grappasonni, I. Metabolic syndrome, hypertension, and nervous system injury: Epidemiological correlates. *Clin. Exp. Hypertens* **2017**, *39*, 8–16. [CrossRef] [PubMed]
38. Raz, N.; Yang, Y.; Dahle, C.L.; Land, S. Volume of white matter hyperintensities in healthy adults: Contribution of age, vascular risk factors, and inflammation-related genetic variants. *Biochim. Biophys. Acta* **2012**, *1822*, 361–369. [CrossRef] [PubMed]
39. Solfrizzi, V.; Scafato, E.; Capurso, C.; D'Introno, A.; Colacicco, A.M.; Frisardi, V.; Vendemiale, G.; Baldereschi, M.; Crepaldi, G.; Di Carlo, A.; et al. Metabolic syndrome, mild cognitive impairment, and progression to dementia. The Italian Longitudinal Study on Aging. *Neurobiol. Aging* **2011**, *32*, 1932–1941. [CrossRef] [PubMed]
40. Ng, T.P.; Feng, L.; Nyunt, M.S.; Feng, L.; Gao, Q.; Lim, M.L.; Collinson, S.L.; Chong, M.S.; Lim, W.S.; Lee, T.S.; et al. Metabolic Syndrome and the Risk of Mild Cognitive Impairment and Progression to Dementia: Follow-up of the Singapore Longitudinal Ageing Study Cohort. *JAMA Neurol.* **2016**, *73*, 456–463. [CrossRef] [PubMed]
41. Freeman, R.B., Jr. The 'indirect' effects of cytomegalovirus infection. *Am. J. Transplant.* **2009**, *9*, 2453–2458. [CrossRef] [PubMed]

42. Wang, H.; Peng, G.; Bai, J.; He, B.; Huang, K.; Hu, X.; Liu, D. Cytomegalovirus Infection and Relative Risk of Cardiovascular Disease (Ischemic Heart Disease, Stroke, and Cardiovascular Death): A Meta-Analysis of Prospective Studies Up to 2016. *J. Am. Heart Assoc.* **2017**, *6*. [CrossRef] [PubMed]
43. Caruso, C.; Accardi, G.; Virruso, C.; Candore, G. Sex, gender and immunosenescence: A key to understand the different lifespan between men and women? *Immun. Ageing* **2013**, *10*, 20. [CrossRef] [PubMed]
44. Ghosh, S.; Klein, R.S. Sex Drives Dimorphic Immune Responses to Viral Infections. *J. Immunol.* **2017**, *198*, 1782–1790. [CrossRef] [PubMed]
45. Klein, S.L.; Flanagan, K.L. Sex differences in immune responses. *Nat. Rev. Immunol.* **2016**, *16*, 626–638. [CrossRef] [PubMed]
46. Bouman, A.; Schipper, M.; Heineman, M.J.; Faas, M.M. Gender difference in the non-specific and specific immune response in humans. *Am. J. Reprod. Immunol.* **2004**, *52*, 19–26. [CrossRef] [PubMed]
47. Garcia Verdecia, B.; Saavedra Hernandez, D.; Lorenzo-Luaces, P.; de Jesus Badia Alvarez, T.; Leonard Rupale, I.; Mazorra Herrera, Z.; Crombet Ramos, T.; Lage Davila, A. Immunosenescence and gender: A study in healthy Cubans. *Immun. Ageing* **2013**, *10*, 16. [CrossRef] [PubMed]
48. Hirokawa, K.; Utsuyama, M.; Hayashi, Y.; Kitagawa, M.; Makinodan, T.; Fulop, T. Slower immune system aging in women versus men in the Japanese population. *Immun. Ageing* **2013**, *10*, 19. [CrossRef] [PubMed]
49. Yan, J.; Greer, J.M.; Hull, R.; O'Sullivan, J.D.; Henderson, R.D.; Read, S.J.; McCombe, P.A. The effect of ageing on human lymphocyte subsets: Comparison of males and females. *Immun. Ageing* **2010**, *7*, 4. [CrossRef] [PubMed]
50. Di Benedetto, S.; Derhovanessian, E.; Steinhagen-Thiessen, E.; Goldeck, D.; Muller, L.; Pawelec, G. Impact of age, sex and CMV-infection on peripheral T cell phenotypes: Results from the Berlin BASE-II Study. *Biogerontology* **2015**, *16*, 631–643. [CrossRef] [PubMed]
51. van der Heiden, M.; van Zelm, M.C.; Bartol, S.J.; de Rond, L.G.; Berbers, G.A.; Boots, A.M.; Buisman, A.M. Differential effects of Cytomegalovirus carriage on the immune phenotype of middle-aged males and females. *Sci. Rep.* **2016**, *6*, 26892. [CrossRef] [PubMed]
52. Althouse, A.D. Adjust for Multiple Comparisons? It's Not That Simple. *Ann. Thorac. Surg.* **2016**, *101*, 1644–1645. [CrossRef] [PubMed]
53. Goetzl, E.J.; Huang, M.C.; Kon, J.; Patel, K.; Schwartz, J.B.; Fast, K.; Ferrucci, L.; Madara, K.; Taub, D.D.; Longo, D.L. Gender specificity of altered human immune cytokine profiles in aging. *FASEB J.* **2010**, *24*, 3580–3589. [CrossRef] [PubMed]
54. Corcoran, M.P.; Meydani, M.; Lichtenstein, A.H.; Schaefer, E.J.; Dillard, A.; Lamon-Fava, S. Sex hormone modulation of proinflammatory cytokine and C-reactive protein expression in macrophages from older men and postmenopausal women. *J. Endocrinol.* **2010**, *206*, 217–224. [CrossRef] [PubMed]
55. Malkin, C.J.; Pugh, P.J.; Jones, R.D.; Kapoor, D.; Channer, K.S.; Jones, T.H. The effect of testosterone replacement on endogenous inflammatory cytokines and lipid profiles in hypogonadal men. *J. Clin. Endocrinol. Metab.* **2004**, *89*, 3313–3318. [CrossRef] [PubMed]
56. Accardi, G.; Caruso, C. Immune-inflammatory responses in the elderly: An update. *Immun. Ageing* **2018**, *15*, 11. [CrossRef] [PubMed]
57. Al-Attar, A.; Presnell, S.R.; Peterson, C.A.; Thomas, D.T.; Lutz, C.T. The effect of sex on immune cells in healthy aging: Elderly women have more robust natural killer lymphocytes than do elderly men. *Mech. Ageing Dev.* **2016**, *156*, 25–33. [CrossRef] [PubMed]
58. Gubbels Bupp, M.R. Sex, the aging immune system, and chronic disease. *Cell. Immunol.* **2015**, *294*, 102–110. [CrossRef] [PubMed]
59. Gubbels Bupp, M.R.; Potluri, T.; Fink, A.L.; Klein, S.L. The Confluence of Sex Hormones and Aging on Immunity. *Front. Immunol.* **2018**, *9*, 1269. [CrossRef] [PubMed]
60. Ostan, R.; Monti, D.; Gueresi, P.; Bussolotto, M.; Franceschi, C.; Baggio, G. Gender, aging and longevity in humans: An update of an intriguing/neglected scenario paving the way to a gender-specific medicine. *Clin. Sci.* **2016**, *130*, 1711–1725. [CrossRef] [PubMed]
61. Villacres, M.C.; Longmate, J.; Auge, C.; Diamond, D.J. Predominant type 1 CMV-specific memory T-helper response in humans: Evidence for gender differences in cytokine secretion. *Hum. Immunol.* **2004**, *65*, 476–485. [CrossRef] [PubMed]
62. Kovats, S. Estrogen receptors regulate innate immune cells and signaling pathways. *Cell. Immunol.* **2015**, *294*, 63–69. [CrossRef] [PubMed]

63. Nakagomi, A.; Seino, Y.; Noma, S.; Kohashi, K.; Kosugi, M.; Kato, K.; Kusama, Y.; Atarashi, H.; Shimizu, W. Relationships between the Serum Cholesterol Levels, Production of Monocyte Proinflammatory Cytokines and Long-term Prognosis in Patients with Chronic Heart Failure. *Intern. Med.* **2014**, *53*, 2415–2424. [CrossRef] [PubMed]
64. Feingold, K.R.; Grunfeld, C. Role of cytokines in inducing hyperlipidemia. *Diabetes* **1992**, *41* (Suppl. 2), 97–101. [CrossRef] [PubMed]
65. Odegaard, J.I.; Chawla, A. Old HDL learns a new (anti-inflammatory) trick. *Nat. Immunol.* **2014**, *15*, 138–139. [CrossRef] [PubMed]
66. Khovidhunkit, W.; Kim, M.S.; Memon, R.A.; Shigenaga, J.K.; Moser, A.H.; Feingold, K.R.; Grunfeld, C. Effects of infection and inflammation on lipid and lipoprotein metabolism: Mechanisms and consequences to the host. *J. Lipid Res.* **2004**, *45*, 1169–1196. [CrossRef] [PubMed]
67. Catapano, A.L.; Pirillo, A.; Norata, G.D. Vascular inflammation and low-density lipoproteins: Is cholesterol the link? A lesson from the clinical trials. *Br. J. Pharmacol.* **2017**, *174*, 3973–3985. [CrossRef] [PubMed]
68. Orsini, A.; Chiacchio, L.; Cinque, M.; Cocchiaro, C.; Schiappa, O.; Grossi, D. Effects of age, education and sex on two tests of immediate memory: A study of normal subjects from 20 to 99 years of age. *Percept. Mot. Skills* **1986**, *63*, 727–732. [CrossRef] [PubMed]
69. Zhang, J.; Zhou, W.; Wang, L.; Zhang, X. Harvard Aging Brain Study. Gender differences of neuropsychological profiles in cognitively normal older people without amyloid pathology. *Compr. Psychiatry* **2017**, *75*, 22–26. [CrossRef] [PubMed]
70. Munro, C.A.; Winicki, J.M.; Schretlen, D.J.; Gower, E.W.; Turano, K.A.; Munoz, B.; Keay, L.; Bandeen-Roche, K.; West, S.K. Sex differences in cognition in healthy elderly individuals. *Neuropsychol. Dev. Cogn. B Aging Neuropsychol. Cogn.* **2012**, *19*, 759–768. [CrossRef] [PubMed]
71. Al-Delaimy, W.K.; von Muhlen, D.; Barrett-Connor, E. Insulinlike growth factor-1, insulinlike growth factor binding protein-1, and cognitive function in older men and women. *J. Am. Geriatr. Soc.* **2009**, *57*, 1441–1446. [CrossRef] [PubMed]
72. Perice, L.; Barzilai, N.; Verghese, J.; Weiss, E.F.; Holtzer, R.; Cohen, P.; Milman, S. Lower circulating insulin-like growth factor-I is associated with better cognition in females with exceptional longevity without compromise to muscle mass and function. *Aging* **2016**, *8*, 2414–2424. [CrossRef] [PubMed]
73. Wilson, C.J.; Finch, C.E.; Cohen, H.J. Cytokines and cognition–the case for a head-to-toe inflammatory paradigm. *J. Am. Geriatr. Soc.* **2002**, *50*, 2041–2056. [CrossRef] [PubMed]
74. Shields, G.S.; Moons, W.G.; Slavich, G.M. Inflammation, Self-Regulation, and Health: An Immunologic Model of Self-Regulatory Failure. *Perspect. Psychol. Sci.* **2017**, *12*, 588–612. [CrossRef] [PubMed]
75. Perry, R.T.; Collins, J.S.; Wiener, H.; Acton, R.; Go, R.C. The role of TNF and its receptors in Alzheimer's disease. *Neurobiol. Aging* **2001**, *22*, 873–883. [CrossRef]
76. Fiore, M.; Angelucci, F.; Alleva, E.; Branchi, I.; Probert, L.; Aloe, L. Learning performances, brain NGF distribution and NPY levels in transgenic mice expressing TNF-alpha. *Behav. Brain Res.* **2000**, *112*, 165–175. [CrossRef]
77. Rizzo, F.R.; Musella, A.; De Vito, F.; Fresegna, D.; Bullitta, S.; Vanni, V.; Guadalupi, L.; Stampanoni Bassi, M.; Buttari, F.; Mandolesi, G.; et al. Tumor Necrosis Factor and Interleukin-1beta Modulate Synaptic Plasticity during Neuroinflammation. *Neural Plast.* **2018**, *2018*, 8430123. [CrossRef] [PubMed]
78. Yirmiya, R.; Goshen, I. Immune modulation of learning, memory, neural plasticity and neurogenesis. *Brain Behav. Immun.* **2011**, *25*, 181–213. [CrossRef] [PubMed]
79. Lobo-Silva, D.; Carriche, G.M.; Castro, A.G.; Roque, S.; Saraiva, M. Balancing the immune response in the brain: IL-10 and its regulation. *J. Neuroinflamm.* **2016**, *13*, 297. [CrossRef] [PubMed]
80. Morrisette-Thomas, V.; Cohen, A.A.; Fulop, T.; Riesco, E.; Legault, V.; Li, Q.; Milot, E.; Dusseault-Belanger, F.; Ferrucci, L. Inflamm-aging does not simply reflect increases in pro-inflammatory markers. *Mech. Ageing Dev.* **2014**, *139*, 49–57. [CrossRef] [PubMed]
81. Tegeler, C.; O'Sullivan, J.L.; Bucholtz, N.; Goldeck, D.; Pawelec, G.; Steinhagen-Thiessen, E.; Demuth, I. The inflammatory markers CRP, IL-6, and IL-10 are associated with cognitive function–data from the Berlin Aging Study II. *Neurobiol. Aging* **2016**, *38*, 112–117. [CrossRef] [PubMed]
82. Marsland, A.L.; Gianaros, P.J.; Abramowitch, S.M.; Manuck, S.B.; Hariri, A.R. Interleukin-6 covaries inversely with hippocampal grey matter volume in middle-aged adults. *Biol. Psychiatry* **2008**, *64*, 484–490. [CrossRef] [PubMed]

83. Jefferson, A.L.; Massaro, J.M.; Beiser, A.S.; Seshadri, S.; Larson, M.G.; Wolf, P.A.; Au, R.; Benjamin, E.J. Inflammatory markers and neuropsychological functioning: The Framingham Heart Study. *Neuroepidemiology* **2011**, *37*, 21–30. [CrossRef] [PubMed]
84. Simpson, E.E.; Hodkinson, C.F.; Maylor, E.A.; McCormack, J.M.; Rae, G.; Strain, S.; Alexander, H.D.; Wallace, J.M. Intracellular cytokine production and cognition in healthy older adults. *Psychoneuroendocrinology* **2013**, *38*, 2196–2208. [CrossRef] [PubMed]
85. Trompet, S.; de Craen, A.J.; Slagboom, P.; Shepherd, J.; Blauw, G.J.; Murphy, M.B.; Bollen, E.L.; Buckley, B.M.; Ford, I.; Gaw, A.; et al. Genetic variation in the interleukin-1 beta-converting enzyme associates with cognitive function. The PROSPER study. *Brain* **2008**, *131*, 1069–1077. [CrossRef] [PubMed]
86. Whiting, C.C.; Siebert, J.; Newman, A.M.; Du, H.W.; Alizadeh, A.A.; Goronzy, J.; Weyand, C.M.; Krishnan, E.; Fathman, C.G.; Maecker, H.T. Large-Scale and Comprehensive Immune Profiling and Functional Analysis of Normal Human Aging. *PLoS ONE* **2015**, *10*, e0133627. [CrossRef] [PubMed]
87. de Jager, W.; Bourcier, K.; Rijkers, G.T.; Prakken, B.J.; Seyfert-Margolis, V. Prerequisites for cytokine measurements in clinical trials with multiplex immunoassays. *BMC Immunol.* **2009**, *10*, 52. [CrossRef] [PubMed]
88. Goldeck, D.; Pawelec, G.; Norman, K.; Steinhagen-Thiessen, E.; Oettinger, L.; Haehnel, K.; Demuth, I. No strong correlations between serum cytokine levels, CMV serostatus and hand-grip strength in older subjects in the Berlin BASE-II cohort. *Biogerontology* **2016**, *17*, 189–198. [CrossRef] [PubMed]
89. Kern, P.A.; Ranganathan, S.; Li, C.; Wood, L.; Ranganathan, G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am. J. Physiol. Endocrinol. Metab.* **2001**, *280*, E745–E751. [CrossRef] [PubMed]
90. Tate, J.; Ward, G. Interferences in immunoassay. *Clin. Biochem. Rev.* **2004**, *25*, 105–120. [PubMed]
91. Rios-Lugo, M.J.; Martin, C.; Alarcon, J.A.; Esquifino, A.; Barbieri, G.; Solano, P.; Sanz, M. Optimization of buffer solutions to analyze inflammatory cytokines in gingival crevicular fluid by multiplex flow cytometry. *Med. Oral. Patol. Oral Cir. Bucal* **2015**, *20*, e13–e16. [CrossRef] [PubMed]
92. Chavance, M.; Escolano, S.; Romon, M.; Basdevant, A.; de Lauzon-Guillain, B.; Charles, M.A. Latent variables and structural equation models for longitudinal relationships: An illustration in nutritional epidemiology. *BMC Med. Res. Methodol.* **2010**, *10*, 37. [CrossRef] [PubMed]
93. Düzel, S.; Buchmann, N.; Drewelies, J.; Gerstorf, D.; Lindenberger, U.; Steinhagen-Thiessen, E.; Norman, K.; Demuth, I. Validation of a single factor representing the indicators of metabolic syndrome as a continuous measure of metabolic load and its association with health and cognitive function. *PLoS ONE* **2018**, *13*, e0208231. [CrossRef] [PubMed]
94. Helmstaedter, C.; Durwen, H.F. The Verbal Learning and Retention Test. A useful and differentiated tool in evaluating verbal memory performance. *Schweiz Arch. Neurol. Psychiatr. (1985)* **1990**, *141*, 21–30.
95. Lindenberger, U.; Mayr, U.; Kliegl, R. Speed and intelligence in old age. *Psychol. Aging* **1993**, *8*, 207–220. [CrossRef] [PubMed]
96. Schmiedek, F.; Lovden, M.; Lindenberger, U. Hundred Days of Cognitive Training Enhance Broad Cognitive Abilities in Adulthood: Findings from the COGITO Study. *Front. Aging Neurosci.* **2010**, *2*. [CrossRef] [PubMed]
97. Finkelstein, M.M.; Verma, D.K. Exposure estimation in the presence of nondetectable values: Another look. *AIHAJ* **2001**, *62*, 195–198. [CrossRef] [PubMed]
98. Hewett, P.; Ganser, G.H. A comparison of several methods for analyzing censored data. *Ann. Occup. Hyg.* **2007**, *51*, 611–632. [CrossRef] [PubMed]
99. Ogden, T.L. Handling results below the level of detection. *Ann. Occup. Hyg.* **2010**, *54*, 255–256. [CrossRef] [PubMed]
100. Hesterberg, T.; Monaghan, S.; Moore, D.S.; Clipson, A.; Epstein, R. *Bootstrap Methods and Permutation Tests*; W.H. Freeman and Company: New York, NY, USA, 2003.
101. Steiger, J.H. Testing Pattern Hypotheses On Correlation Matrices: Alternative Statistics And Some Empirical Results. *Multivar. Behav. Res.* **1980**, *15*, 335–352. [CrossRef] [PubMed]

15 Publication IV

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Network topology dynamics of circulating biomarkers and cognitive performance in older Cytomegalovirus-seropositive or -seronegative men and women

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Abstract

Background: Cytokines are signaling molecules operating within complex cascade patterns and having exceptional modulatory functions. They impact various physiological processes such as neuroendocrine and metabolic interactions, neurotrophins' metabolism, neuroplasticity, and may affect behavior and cognition. In our previous study, we found that sex and Cytomegalovirus (CMV)-serostatus may modulate levels of circulating pro- and anti-inflammatory cytokines, metabolic factors, immune cells, and cognitive performance, as well as associations between them.

Results: In the present study, we used a graph-theoretical approach to investigate the network topology dynamics of 22 circulating biomarkers and 11 measures of cognitive performance in 161 older participants recruited to undergo a six-months training intervention. For network construction, we applied coefficient of determination (R^2) that was calculated for all possible pairs of variables ($N = 33$) in four groups (CMV^- men and women; CMV^+ men and women). Network topology has been evaluated by clustering coefficient (*CC*) and characteristic path length (*CPL*) as well as local (E_{local}) and global (E_{global}) efficiency, showing the degree of network segregation (*CC* and E_{local}) and integration (*CPL* and E_{global}). We found that networks under consideration showed small-world networks properties with more random characteristics. Mean *CC*, as well as local and global efficiency were highest and *CPL* shortest in CMV^- males (having lowest inflammatory status and highest cognitive performance). CMV^- and CMV^+ females did not show any significant differences. Modularity analyses showed that the networks exhibit in all cases highly differentiated modular organization (with *Q*-value ranged between 0.397 and 0.453).

Conclusions: In this work, we found that segregation and integration properties of the network were notably stronger in the group with balanced inflammatory status. We were also able to confirm our previous findings that CMV-infection and sex modulate multiple circulating biomarkers and cognitive performance and that balanced inflammatory and metabolic status in elderly contributes to better cognitive functioning. Thus, network analyses provide a useful strategy for visualization and quantitative description of multiple interactions between various circulating pro- and anti-inflammatory biomarkers, hormones, neurotrophic and metabolic factors, immune cells, and measures of cognitive performance and can be in general applied for analyzing interactions between different physiological systems.

Keywords: Aging, Immunosenescence, Cytomegalovirus, Inflammatory markers, Cytokines, Neurotrophic and metabolic factors, Cognition, Network topology

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Background

Aging is accompanied by chronic low-grade inflammation that has been repeatedly identified even in overtly healthy individuals and is characterized by elevated levels of circulating pro-inflammatory cytokines [1]. Cytokines represent signaling molecules having exceptional modulatory functions. They impact virtually every physiological process such as neurotransmitter metabolism, neuroendocrine interactions, and neuroplasticity, thereby not only affecting general health but also immunity and cognitive functioning [2–4]. The cytokine network, containing cytokines, their receptors, and their regulators, is present in the brain and in various other physiological systems, and is highly controlled throughout the lifespan [5, 6]. Cytokines and their receptors operate within multifactorial networks and may act synergistically or antagonistically in a time- and concentration-dependent patterns. These interactions allow cross-communication between different cell types, at different hierarchical levels, translating environmental signals into molecular signals [2, 7]. The pro-inflammatory profile becomes strategic throughout the lifespan [8–11] - an increase of cytokine secretion, also thought to be associated with the influence of CMV-infection, may be at least partly responsible for age-associated degenerative disorders [12–16]. Previous studies usually investigated individual roles of different cytokines, inflammatory mediators or metabolic factors in the age-related physiological alterations [17–21]. With growing numbers of biomarkers, however, it may become difficult to interpret results and translate them into useful information.

In our recent work [22], we assessed inflammatory status and cognitive performance in 161 older participants recruited to undergo a six-month training intervention. We demonstrated that sex and CMV-latency have influence on levels of circulating pro- and anti-inflammatory cytokines, receptor antagonist, soluble receptor, metabolic factors, and immune cells. We also found that CMV-latency has modulatory effects on associations between individual peripheral biomarkers [22]. Furthermore, we revealed an interaction between CMV-serostatus and sex associations with cognitive abilities: sex differences in fluid intelligence and working memory were noted only in CMV-negative individuals. Even more strikingly, the same group of elderly men also exhibited a lower inflammatory status in their peripheral circulation. Therefore, a well-balanced inflammatory and anti-inflammatory equilibrium appeared apparently to be decisive for optimal physiological functions and for optimal cognitive functioning.

Pro-inflammatory cytokines often act as negative regulatory signals modulating the action of hormones and neurotrophic factors. An unbalanced cytokine state may also affect the neuroendocrine system (and vice versa) impairing interplay between them, and contributing to disrupted homeostasis [23]. Therefore, in the present study, we additionally considered such hormones as cortisol and dehydroepiandrosterone (DHEA) as well as neurotrophines and their regulators (insulin-like growth factor-1, IGF-1, and IGF-binding protein, IGFBP-3), to gain a more comprehensive image of these processes. Furthermore, we extended the number of inflammation-related metabolic factors and included measures of C-reactive protein (CRP) in our present analyses. Finally, instead of focusing on four latent factors representing the main cognitive abilities (as we did in the previous study), we included in our present analysis all 11 individual cognitive performance scores assessed within the cognitive battery of elderly individuals. Increasing complexity arose when attempting to analyze dynamic interconnections between all these factors and to investigate the modulatory impact of CMV-latency and sexual dimorphism. In an effort to better understand the relationships between the multiple circulating and functional biomarkers and to compare them regardless of their physiological hierarchical assignments, we applied a graph-theoretical approach and described constructed networks in terms of network topology and modular organization of network elements.

As stated by Bhavnani et al., network analyses offer two main advantages for studying complex physiological interactions: (i) they do not require a priori assumptions about the relationship of nodes within the data, such as the categorized assumption of hierarchical clustering; and (ii) they allow the simultaneous visualization of several raw values (such as cytokine or/and cell values, functional attributes), as well as aggregated values, and clusters in a uniform visual representation [24]. This allows not only the more rapid generation of hypotheses based on complicated multivariate interactions, but also the validation, visualization, and confirmation of the results, obtained with other methodological approaches. Moreover, this enables a more informed methodology for selecting quantitative methods to compare the patterns obtained in the different sets of data regardless of their physiological hierarchical levels [24].

The purpose of the present study was to visualize and to quantitatively describe by means of a graph-theoretical approach the complex multiple interactions among diverse pro- and anti-inflammatory mediators, immune cell populations, hormones, neurotrophic and metabolic factors as well as cognitive performance in older CMV-seropositive and -negative men and women. Moreover, we aimed to design a new strategy for quantitative investigations of the network topology dynamics in circulating biomarkers and measures of cognitive performance by applying the coefficients of determination (R^2) calculated for all possible pairs of variables in four groups of participants. In order to characterize the

segregation and integration properties of the individual networks of CMV-positive or -negative men and women, we analyzed such network topology measures as clustering coefficient, characteristic path length, local and global efficiency [25, 26]. With the aim of statistically comparing the network topology dynamics and to identifying the networks with optimal features of segregation and integration, we applied a rewiring procedure. To the best of our knowledge, simultaneous network analyses of multiple inflammation-related peripheral biomarkers and cognitive performance of older Cytomegalovirus-seropositive and -seronegative men and women have not been previously accomplished.

Results

For network analyses, the participants were separated into four groups according to their CMV-serostatus and sex (Fig. 1). For network construction, we applied coefficient of determination (R^2) that was calculated for all possible pairs of variables in four groups (CMV^- men and women; CMV^+ men and women). Network topology has been evaluated by clustering coefficient (*CC*) and characteristic path length (*CPL*) as well as local (E_{local}) and global (E_{global}) efficiency (for details see Methods section).

Network composition and network topologies in real and control networks

Before analyzing network topology changes, we compared the topology in real and control (i.e., lattice and random) networks under different cost levels (the ratio of the number of actual connections to the maximum possible number of connections in the network) in the range between 10 and 60% of wiring costs. As shown in Additional file 1: Figure 1A, *CC* is greatest in lattice networks and lowest in random networks, whereas *CC* for the real networks lies in-between. *CPL* is shortest in random and longest in lattice networks, while the real networks are between these (see Additional file 1: Figure 1B). Correspondingly, E_{local} was highest in lattice networks (at least for cost levels under 45%) and lowest in random networks (at least for cost levels under 20%), while E_{global} was highest in random and lowest in lattice networks essentially for all levels of wiring costs, with real networks always in between (see Additional file 1: Figure 2 for details).

Importantly, as shown in Fig. 2, networks under consideration are Small-Word Networks (SWNs) at all levels of wiring costs ($\sigma > 1$). As indicated by the other SW coefficient ω, which is lying at practically all levels of wiring costs in the positive range (see Fig. 2b), these networks are SWNs with more random characteristics. It can also be seen that the networks with costs lower than 25% showed rather unstable behavior that was stabilizing at the 25% level of costs and showed very similar results across all experimental groups for both SW coefficients σ and ω. Thus, for our main analyses, we decided to set the cost level to 25% that makes it possible to investigate sparse and at the same time stable network topology in all four groups of participants.

Network structure and network strengths

It can be seen that connectivity matrices (Fig. 3a) display a group-specific structure in all four participant groups. In the first step, we calculated network strengths as the sum of connections of node *i* (see also Methods section for more details). As shown in Fig. 3a, b, cognitive nodes exhibit high strengths, which are mostly due to the strong connections between the cognitive nodes themselves, especially in the female groups. In the male groups, the cognitive nodes are also strongly connected to the other systems such as cytokines (especially, in the network of CMV^- males), metabolic variables (particularly, in the network of the CMV^+ males) and immune cells.

Networks of CMV^- and CMV^+ men and women differ in their structure

Networks of the four experimental groups also display group-specific structure (Fig. 4). Individual nodes (or variables) are represented as multicolored circles coding for affinity to a particular group of variables. The size of the circle depends on the sum of connections and indicates the node's strength. The thickness of the connections corresponds to their connection strength. The nodes are numbered clock-wise beginning from the pro-inflammatory cytokine IL-1β displayed in blue. The CMV-negative male group (top, left) is characterized by multiple strong connections between pro-inflammatory cytokine nodes (IL-1β, TNF, IL-18) and cognitive nodes (episodic memory and fluid intelligence).

Less strong but numerous connections are also present for anti-inflammatory cytokines and the cognitive nodes. Interestingly, this is the only group, in which pro- and anti-inflammatory cytokines show no direct connections to each other. The nodes of perceptual speed are strongly connected with immune cell nodes (lymphocytes and neutrophils). No other groups of participants display such strong direct connections between immune biomarkers and cognition – except the network of CMV^+ men (bottom, left) with only one strong connection between CRP and fluid intelligence. The network of the CMV^+ men shows strong connections between metabolic factors and perceptual speed. The network of CMV^- women (top, right) displays strong connections between pro-inflammatory IL-6 and triglycerides as well as between anti-inflammatory sTNF-R and creatinine. The network of the CMV^+ women (bottom right) shows a strong connection between leukocytes and pro-

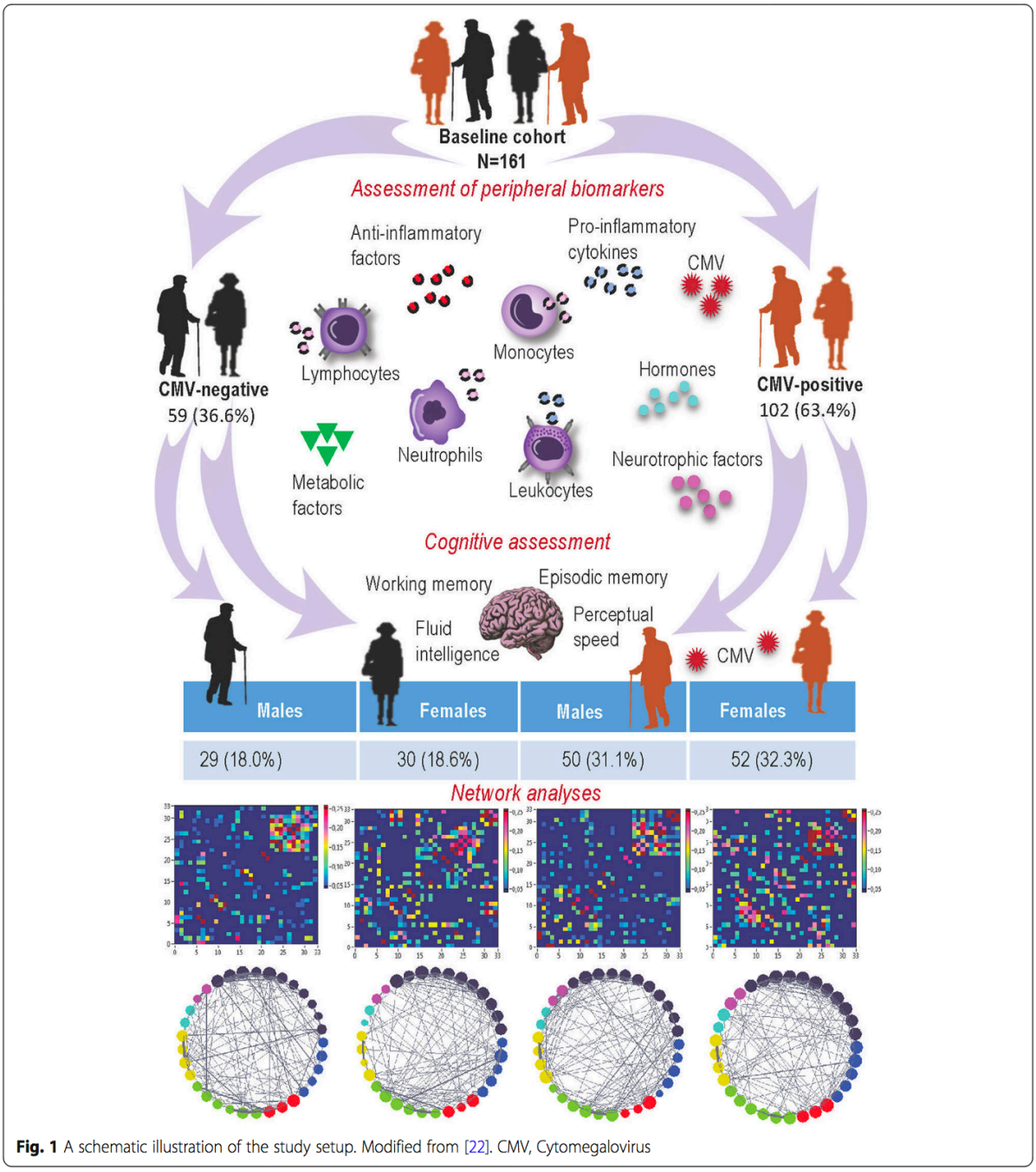

Fig. 1 A schematic illustration of the study setup. Modified from [22]. CMV, Cytomegalovirus

inflammatory IL-6. Unexpectedly, neurotrophins in the CMV$^-$ men have relatively strong connections to urea, but only one weak connection to the pro-inflammatory factor CRP. In contrast, all three of the other networks display multiple connections to both pro- and anti-inflammatory cytokines. Concerning connections between neurotrophins and cognitive nodes, we can see quite heterogeneous picture: with some connections in CMV-seronegative and -positive men, and with only one connection in the CMV-seronegative and -positive women. In general, the networks of all groups of participants show strong (but differently manifested) connections between the cognitive nodes themselves (Fig. 4).

Networks topology differences between CMV$^-$ and CMV$^+$ men and women

To be able to statistically compare the four different networks at a given cost level, we used rewiring procedure

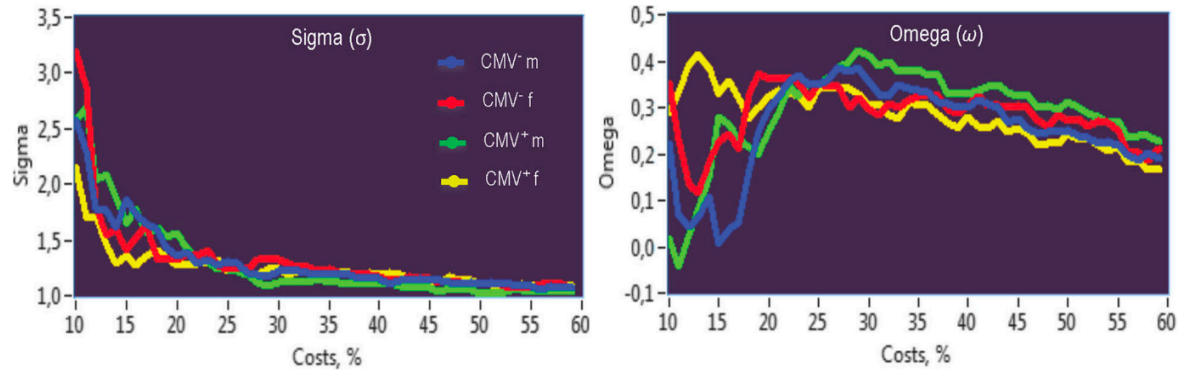

Fig. 2 Small-world coefficients sigma (σ) and omega (ω) under different levels of the wiring costs. CMV, Cytomegalovirus; CMV⁻ m, CMV-seronegative men; CMV⁺ m, CMV-seropositive men; CMV⁻ f, CMV-seronegative women; CMV⁺ f, CMV-seropositive women

with replacement of a non-existing edge through an existing one and consecutive determination of network topology metrics each time. In total, there were about 50,000 rewired networks, for which mean and standard deviation (SD) of the network topology metrics were determined. In accordance with the empirical rule, we achieved a 99.7% confidence interval (*CI*) for the mean: $CI = mean \pm 3 \times SD$. As shown in Fig. 5a, mean *CC* was highest and *CPL* shortest in CMV⁻ males and in total, higher (shorter) in males than in females. Correspondingly, local and global efficiency were both highest in CMV⁻ males and in total higher in males than in females. CMV-seronegative and -seropositive females did not show any significant differences. This indicates that segregation and integration properties of the network were notably stronger in males (especially, in CMV⁻ males) than in females. Inspection of separate nodes in the networks showed that these network topology differences were in particular stronger for cytokines and cognitive variables or nodes (Fig. 5b).

Modular organization of the networks of CMV⁻ and CMV⁺ men and women

Modularity analyses showed that the networks under consideration exhibited in all cases highly differentiated modular organization with 4 and 5 modules for males and for females, respectively. This is indicated by high modularity values or Q statistics (Fig. 6), which ranged between 0.397 and 0.453, and were considerably higher as compared with random networks (with *Q*-values close to 0). Nodes sharing the same module are displayed in Fig. 6b and d in the same color. As shown in Fig. 6a and c, cognitive nodes occupied two modules in all networks (with exception of CMV⁺ females, in which all cognitive nodes were located in one large module), whereby perceptual speed nodes occupied a separate module. Moreover, the community structure in CMV-negative males was organized in 4 modules (A-B, left), whereby all pro-inflammatory cytokines were located in the same module shared (B, blue) with cognitive variables or nodes (reflecting general intelligence and memory features). In addition, two of the three anti-inflammatory cytokines (namely, IL-10 and sTNF-R) shared the same module (B, left, red) with metabolic factors as well as with monocytes, with the exception of urea, which was located in a separate module (B, yellow) together with hormones und neurotrophins. Finally, perceptual speed nodes shared a common module (B, left, green) with IL-1RA and immune cells (namely, leukocytes, lymphocytes, and neutrophils). Interestingly, in CMV⁻ females (A-B, right), the two modules occupied by cognitive (B, right, blue) and perceptual speed nodes (B, right, cyan) were separated from all the other nodes, which were partitioned into heterogeneous modules comprising different components (e.g., cytokines, metabolic variables, immune cells, and neurotrophins). The nodes of CMV⁺ men (C-D, left) and CMV⁺ women (C-D, right) also partitioned into 4 and 5 modules, respectively, showed heterogeneous modularity structures comprising nodes of both peripheral biomarkers and cognitive features.

Z-P parameter space and nodes' specificity of the four networks

To define how the network nodes were positioned in their own module and with respect to other modules, we calculated the within-module degree (Z_{ii}) and participation coefficient (P_{ii}) of the node *i* for the given networks. The within-module degree indicates how 'well-connected' node *i* is to other nodes in the module, whereas the participation coefficient reflects how 'well-distributed' the edges of the node *i* are among the other modules. Z_i and P_i form together the so-called *Z-P* parameter space, with different regions indicating specific roles of the nodes (e.g., hubs, connectors, provincial nodes) in this parameter space [27]. As shown in Fig. 7a, the network of the CMV⁻ males contains more hub nodes but far fewer connector nodes than the other three groups. This indicates that the modules in this participants' group are more autonomous

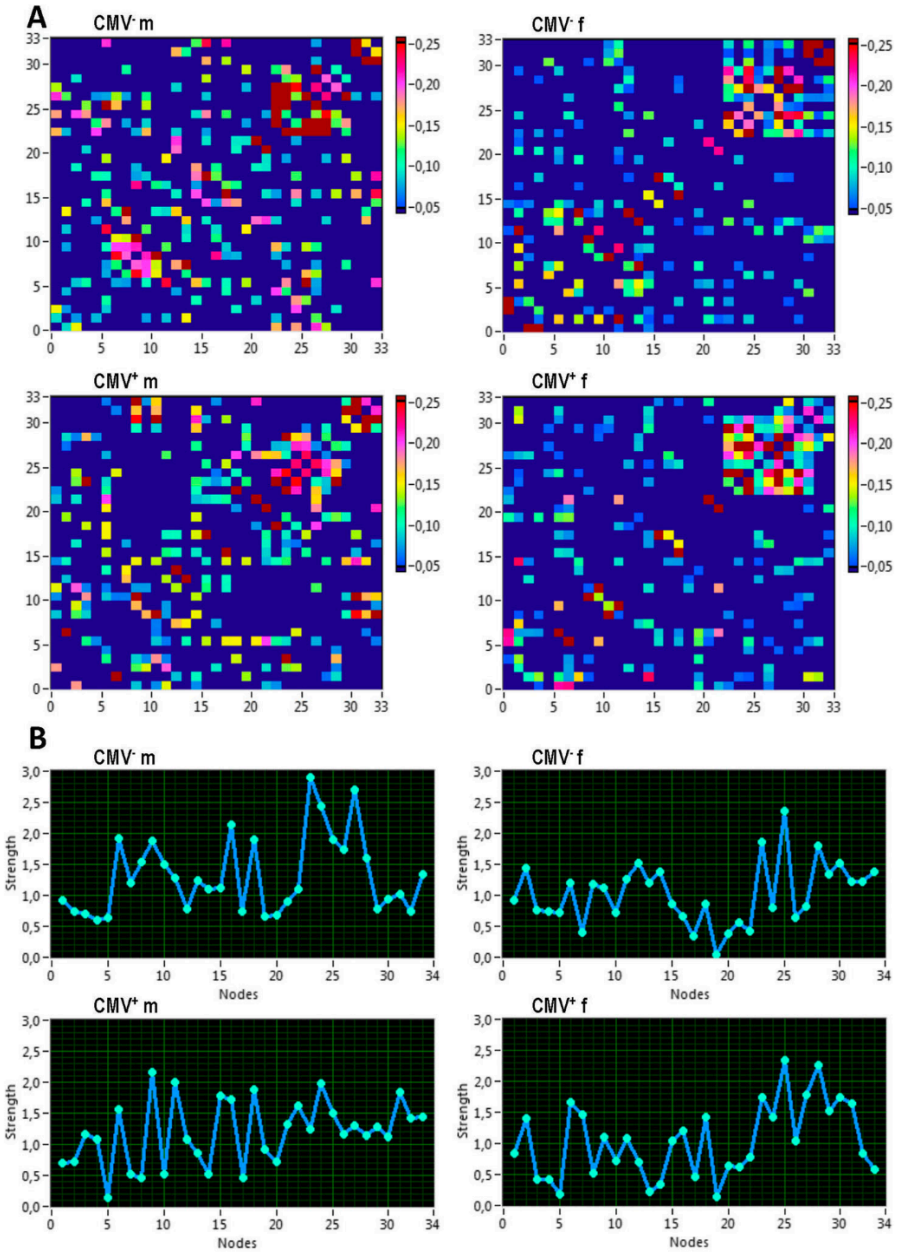

Fig. 3 (See legend on next page.)

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Fig. 3 Connectivity structure of the network and network strengths in the four groups. **a** Connectivity matrices. **b** Network strengths. CMV, Cytomegalovirus; CMV^- m, CMV-seronegative men; CMV^+ m, CMV-seropositive men; CMV^- f, CMV-seronegative women; CMV^+ f, CMV-seropositive women; IL, interleukin; IL-1β, interleukin 1 beta; TNF, tumor necrosis factor; CRP, C-reactive protein; IL-1RA, interleukin 1 receptor antagonist; sTNF-R, soluble tumor necrosis factor receptor; CHOL, cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TRIG, triglyceride; CREA, creatinine; DHEA, dehydroepiandrosterone; IGF-1, insulin-like growth factor-1; IGFBP-3, IGF-binding protein 3; Gf, fluid intelligence; EM, episodic memory; WM, working memory; Speed, perceptual speed

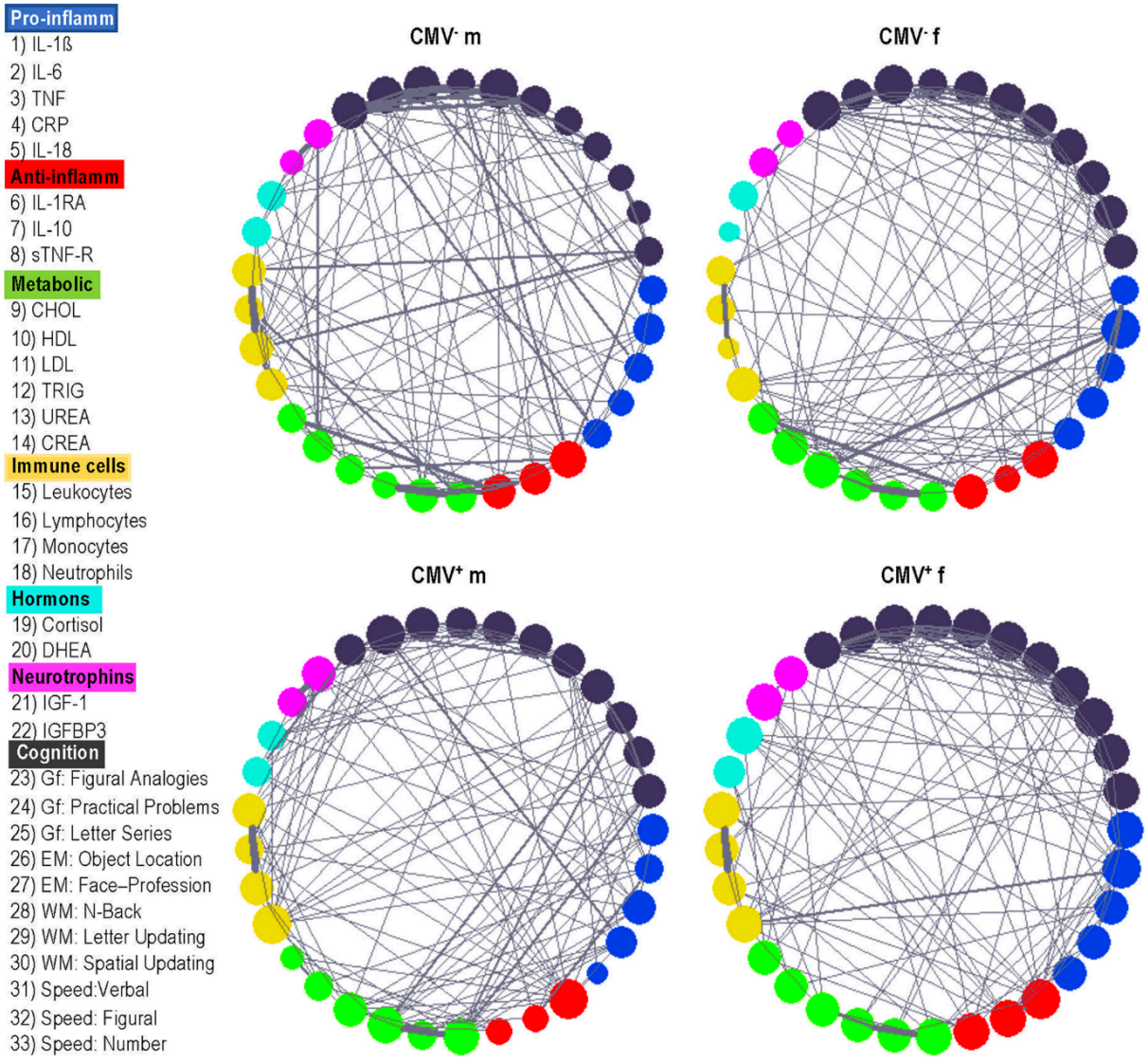

Fig. 4 Network structure differences in CMV^- and CMV^+ men and women. CMV, Cytomegalovirus; CMV^- m, CMV-seronegative men; CMV^+ m, CMV-seropositive men; CMV^- f, CMV-seronegative women; CMV^+ f, CMV-seropositive women; IL, interleukin; IL-1β, interleukin 1 beta; TNF, tumor necrosis factor; CRP, C-reactive protein; IL-1RA, interleukin 1 receptor antagonist; sTNF-R, soluble tumor necrosis factor receptor; CHOL, cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TRIG, triglyceride; CREA, creatinine; DHEA, dehydroepiandrosterone; IGF-1, insulin-like growth factor-1; IGFBP-3, IGF-binding protein 3; Gf, fluid intelligence; EM, episodic memory; WM, working memory; Speed, perceptual speed

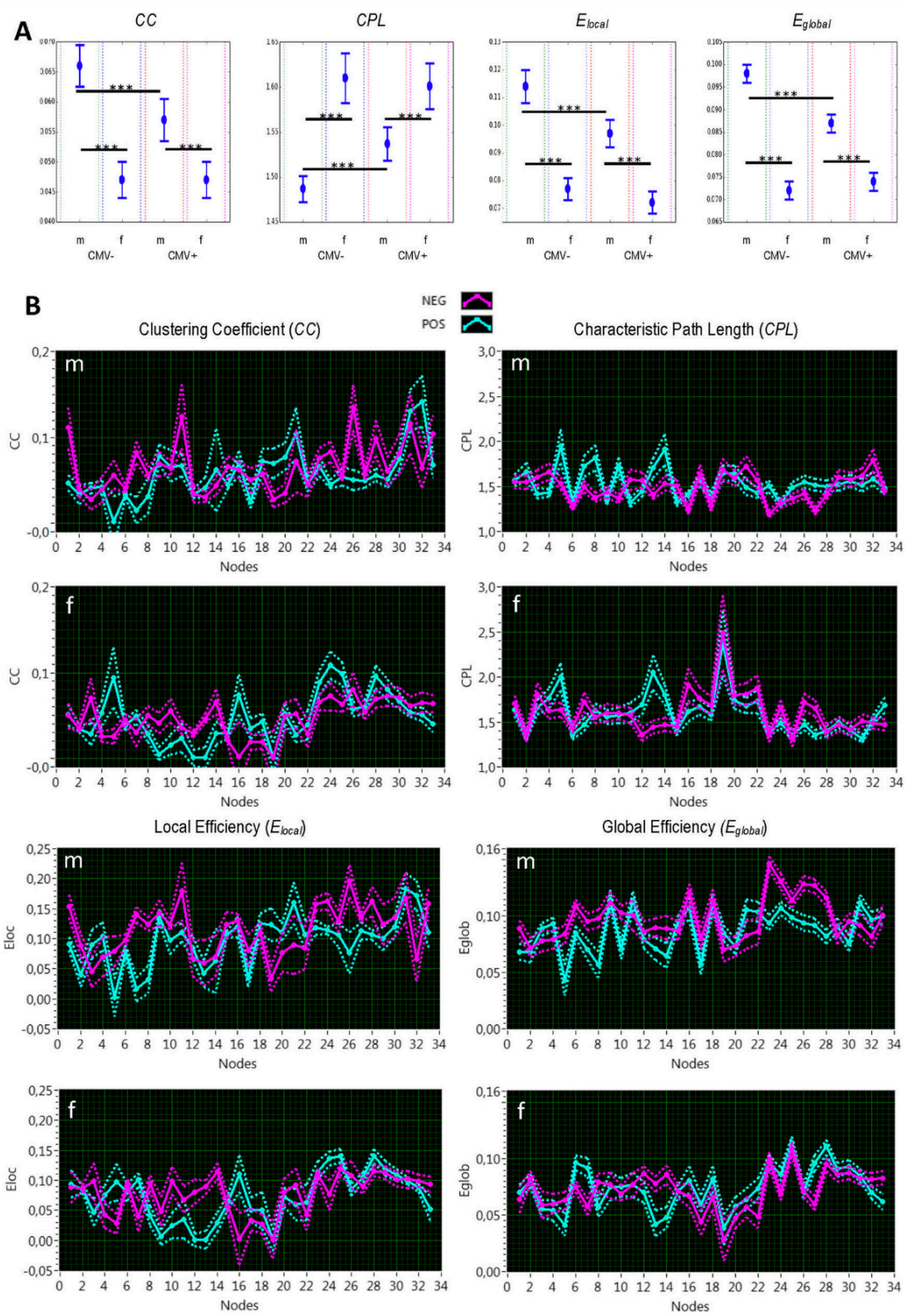

Fig. 5 Network topology differences. **a** Results of rewiring analyses for whole network. **b** Results of rewiring analyses for individual nodes. *CC*, clustering coefficient; *CPL*, characteristic path length; E_{local}, local efficiency; E_{global}, global efficiency; CMV, Cytomegalovirus; CMV-, CMV-seronegative; CMV+, CMV-seropositive; m, male; f, female; NEG, CMV-seronegative; POS, CMV-seropositive

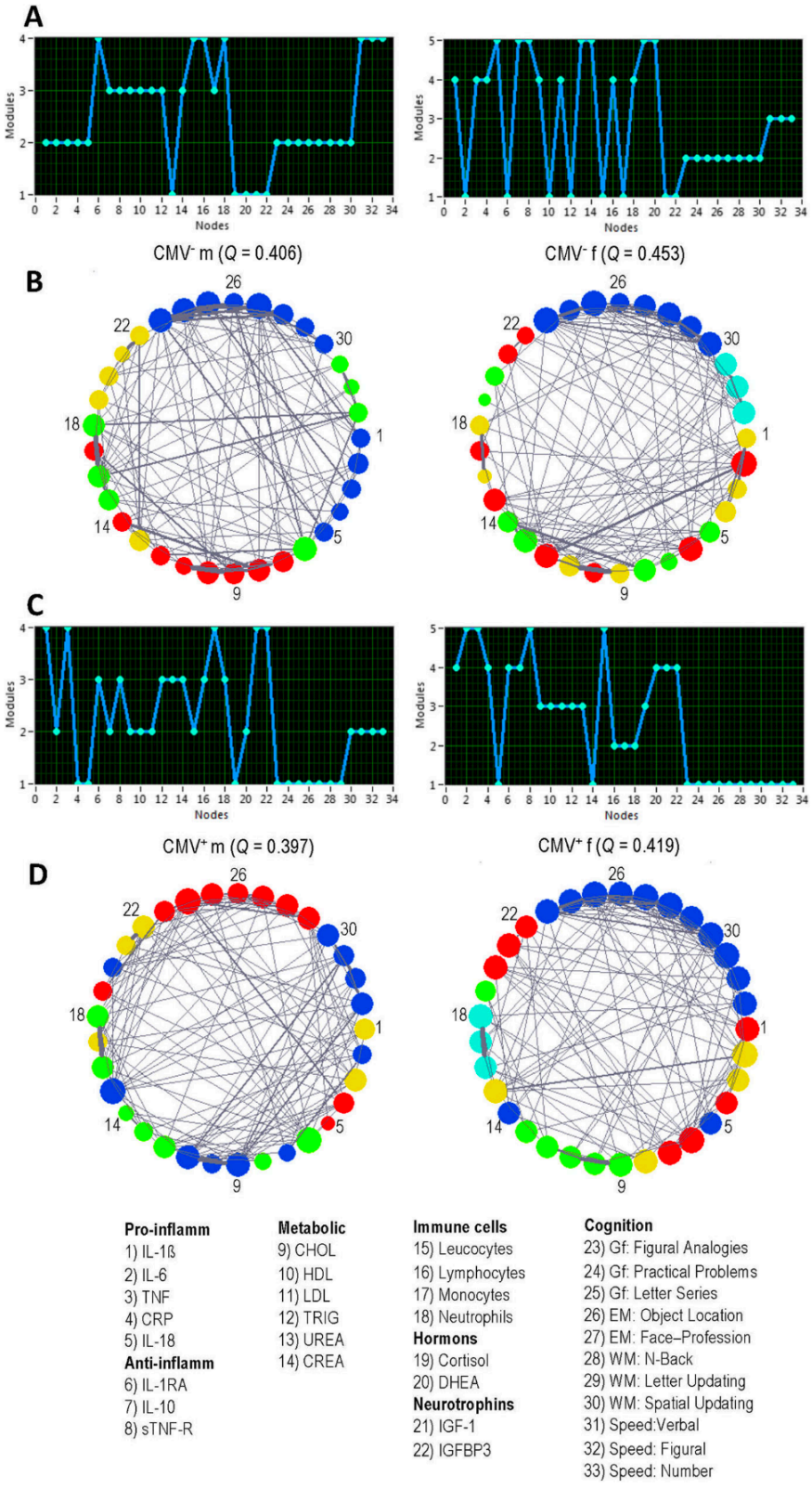

Fig. 6 (See legend on next page.)

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Fig. 6 Modular organization of the networks. **a** Modular assignment of nodes in CMV$^-$ men (left) and women (right). **b** Modular structure in CMV$^-$ men (left) and women (right). **c** Modular assignment of nodes in CMV$^+$ men (left) and women (right). **d** Modular structure in CMV$^+$ men (left) and women (right). Note that nodes sharing the same module are displayed in the same color. CMV, Cytomegalovirus; CMV$^-$ m, CMV-seronegative men; CMV$^+$ m, CMV-seropositive men; CMV$^-$ f, CMV-seronegative women; CMV$^+$ f, CMV-seropositive women; Q, modularity value

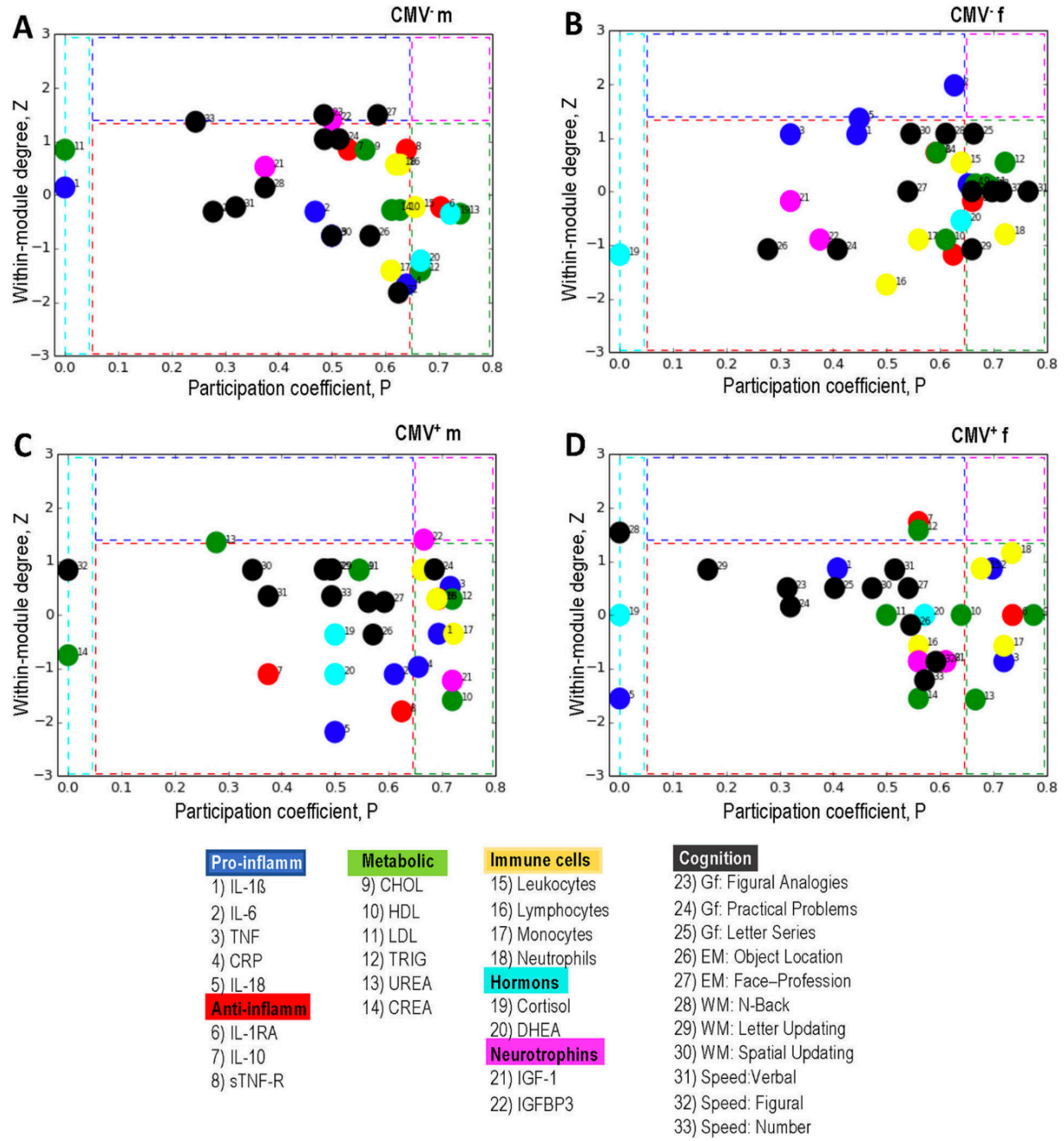

Fig. 7 *Z-P* parameter space and node' specificity for networks in four groups. **a** *Z-P* parameter space for CMV-seronegative men, (**b**) *Z-P* parameter space for CMV-seronegative women, (**c**) *Z-P* parameter space for CMV-seropositive men, and (**d**) *Z-P* parameter space for CMV-seropositive women. Different regions separated by dotted lines contain: left – ultra-peripheral nodes; central – provincial nodes; top – hubs; top right – connector hubs; right – connectors. CMV, Cytomegalovirus; CMV$^-$ m, CMV-seronegative men; CMV$^+$ m, CMV-seropositive men; CMV$^-$ f, CMV-seronegative women; CMV$^+$ f, CMV-seropositive women

and the information flow between the modules is either reduced or is realized through a small number of connector nodes. Interestingly, three of the four hubs are cognitive variables and the fourth one is IGFBP3. Thus, cognitive nodes, such as fluid intelligence, working memory, and perceptual speed, play a central role in the network of CMV^- males driving or controlling the connections within the corresponding modules. Further, the networks of CMV^- females (B) and CMV^+ males (C) are characterized by high numbers of the non-hub connectors responsible for the connectivity between the modules. Thus, the modules in these two groups are apparently worse separated from each other than, for example, in the CMV^- males. The network of the CMV^+ females (D) contains two hubs and eight non-hub connectors, and thus demonstrates a modular structure with moderate number of hubs and connectors. Note also that all cognitive nodes in this group are provincial nodes and therefore play a secondary role in the network. In summary, it can be stated that the networks under consideration exhibit a different balance between intra- and inter-modular information flow with different numbers of hub and connector nodes playing a significant role for this balance and for network functioning. Which of these types of modular organization is more effective, remains to be investigated.

Discussion

There is a growing body of evidence supporting the notion that the immune system is not hermetically self-regulated but functions in intimate interrelations with other physiological systems, including the nervous system [5, 28]. These interactions are present at the various levels of organization – at the local, as well as at the whole organism level – by sharing a common language of a wide range of cytokines, receptor molecules, hormones, neuropeptides, metabolic and neurotrophic factors allowing cross-communication [29, 30]. Particularly in the process of aging, this reciprocal cross-talk may under certain circumstances permit augmentation of maladaptive inflammatory loops, which could disturb homeostasis and contribute to the age-related functional alterations or even to pathological conditions [2, 31–33].

Several analytical techniques to investigate these interactions have been established so far, but our understanding of the interplay between different factors in such interrelated processes is still in its infancy. Despite some progress, there is a further need to place the data from different physiological and functional levels in a biological context with the aim of interpreting their multifaceted orchestration as a whole. Many studies highlight the role of different inflammatory cytokines in the low-grade inflammation, dubbed “inflammaging”, and the importance of pro-inflammatory and anti-inflammatory homeostasis for cognitive health in aging [17, 18, 34–36]. Additionally, the interrelated effects of inflammatory factors and their influence on neuroimmune and neuroendocrine functions can be modified by the chronic immune activity required to control lifelong persistent CMV infection [2, 37]. In the present work, we propose a strategy for quantitative description of multiple interactions between different cytokines, receptor molecules, metabolic and neurotrophic factors, hormones, immune cells, and measures of cognitive performance with the help of a graph-theoretical approach. To the best of our knowledge, simultaneous network analyses of multiple inflammation-related mediators and cognitive performance in older CMV-seropositive and CMV-seronegative men and women have not been previously accomplished.

Aging is associated with modulatory effects on the immune system – resulting in the universal, multifactorial changes, known as immunosenescence. This leads to functional changes in the immune cells, which produce more inflammatory cytokines and less anti-inflammatory mediators. CMV-persistence is associated with constant chronic stimulation of the immune system that could further contribute to induction and accumulation of the specific immune cell phenotypes known to be generally associated with immunosenescence. The fact that CMV has considerable influence on immunosenescence was first described 20 years ago [38] and has continuously been supported by numerous studies since then [15, 16, 39–44]. In the large-scale immune profiling and functional analysis of normal aging, it was impressively shown that the immune system alterations (determined as a number of significantly affected analytes) caused specifically by CMV were comparable to the differences seen between the sexes [45]. A lifelong persistent infection influences immune aging and can significantly modify the course of cognitive aging by acting in combination with individual differences in cytokine release [37, 46–48]. The modulatory effect of CMV-latency and sex were also demonstrated in our previous study [22]. Therefore, for the network analyses in the present study, we separated the participants into four groups according to their CMV-serostatus and sex.

We found that the modulatory impact of CMV and sex was also reflected in the specific differences of the network structure and the network topology dynamics observed between the four groups. In particular, CMV^- males were characterized through several strong connections between nodes of the pro-inflammatory cytokines IL-1β, TNF, IL-18 and cognitive nodes including variables of episodic memory and fluid intelligence. Currently available evidence shows that pro-inflammatory cytokines exert a dose-dependent physiological neuroprotective but can however also mediate pathological neurodegenerative effects under certain circumstances

[18]. IL-1β and TNF were demonstrated to have such a dual function, acting on the one hand as pro-inflammatory factors and on the other as neuromodulators, subserving memory and other cognitive processes. In other words, they not only play a role in neuroinflammation, but (at their low concentrations) also in complex processes such as synaptic plasticity, neurogenesis, long-term potentiation and memory consolidation [34, 35].

Less strong but numerous connections were found between nodes of the anti-inflammatory cytokines and cognition in the network of CMV^- males. This is partly in line with our previous findings on the positive association of episodic memory with the anti-inflammatory cytokine IL-10 in the CMV^- elderly men and women [22]. IL-10 is known to have a neuroprotective role due to its inhibitory action on inflamed microglia [17]. The same CMV^- male group also has significantly elevated levels of anti-inflammatory IL-10 and sTNF-R as well as reduced levels of pro-inflammatory cytokines in their peripheral circulation, as reported in our recent study [22]. Having this information in mind, we can speculate that strong connections between cognitive nodes and the nodes of (low-levels) pro-inflammatory cytokines on the one hand and numerous connections of cognition to the nodes of the (high-level) anti-inflammatory cytokines on the other, could possibly explain the cognitive advantage in the fluid intelligence and working memory found for this group of participants in our previous work [22]. Remarkably, this was the only group in which nodes of pro- and anti-inflammatory cytokines had no direct connections to each other. The other three groups, (two of which, CMV^- females and CMV^+ males, were characterized in our previous study by heterogeneously unbalanced levels of pro- and anti-inflammatory mediators and by an adverse metabolic environment) demonstrated, in contrast, various more or less strong connections between pro- and anti-inflammatory cytokines, which were probably important and necessary homeostatic responses to these unbalanced peripheral conditions. In our previous study, the network of CMV^+ women (that shows multiple connections between nodes of pro- and anti-inflammatory cytokines), exhibited significantly higher levels of the anti-inflammatory factors sTNF-R and IL-1RA. We also found previously that in the CMV^+ group, fluid intelligence, episodic and working memory were negatively associated with the anti-inflammatory factor IL-1RA, the level of which was assumed to be simultaneously increased as a reaction to the elevation of the pro-inflammatory cytokines in the periphery [22]. This phenomenon has also been reported by other investigators [33, 49, 50], showing that individuals with high levels of pro-inflammatory cytokines also tend to display elevated levels of anti-inflammatory factors. The network analyses in the present study allowed the visualization of these multiple and mutual connections between pro- and anti-inflammatory biomarkers, which were only assumed in our previous work [22].

Interestingly, the network of CMV^- males demonstrated some direct connections between DHEA and cognitive nodes, and also to the nodes of anti-inflammatory and metabolic factors. The CMV^+ males, in contrast, displayed multiple connections to cognitive nodes, but no connections to anti-inflammatory nodes, and were connected to the inflammatory cytokine IL-6. A completely different picture was seen in CMV^- females with no connections of DHEA either to pro-inflammatory cytokines or cognition, whereas CMV^+ women had multiple connections to nodes of cytokines and cognition. It is known that inflammatory reactions are, in general, under the influence of different mechanisms including neuroendocrine interactions. Pro-inflammatory mediators and cytokines may lead to the activation of the hypothalamic-pituitary-adrenal axis (HPA) that is in turn capable of modulating the process of inflammation [51–55]. DHEA and cortisol are multifunctional adrenocortical hormones with such immunomodulatory properties. They exert potent and broad influences throughout the body and brain and jointly impact on a variety of processes related to metabolic, immune, and cognitive functions [52]. Being especially abundant in the brain, DHEA exerts a protective effect against the deterioration of mental functioning with aging. Interestingly, both cortisol and DHEA in the CMV^- males are non-hub connectors exhibiting numerous links to diverse modules in the modular organization of the network. This indicates that these nodes play a crucial role in communication between different subsystems. Inverse correlations between DHEA concentrations and neuroinflammatory-related diseases have repeatedly been found in the elderly [52, 56–58]. Similar to DHEA, the cortisol nodes in our study displayed very heterogeneous and group-specific picture concerning their connections. Whereas CMV^- males showed connections from cortisol to the nodes of pro-inflammatory TNF, IGF-1, IGFBP-3, metabolic factors, and immune cells, the cortisol-node of CMV^- females had only one connection to IL-18. In the CMV^+ groups, men showed weak but multiple cortisol-connections to cognitive nodes, neurotrophins, pro- and anti-inflammatory factors. In the network of women, cortisol was connected only to the metabolic factors. The heterogeneous picture seen in these connections may partly be due to the fact that although the effect of cortisol has been typically shown to be immunosuppressive, at certain concentrations it can also induce a biphasic response during a later, delayed systemic inflammatory response [59] through augmentation of inflammation [53]. In other

words, the regulation of inflammation by cortisol may vary from anti- to pro-inflammatory in a time- and concentration-dependent manner and this contributes to further complexity in interpreting results of these already complex interactions.

Pro-inflammatory cytokines are known to be involved in dynamic interactions with the main neurotrophic factor, IGF-1 and its regulator, IGFBP-3 by decreasing IGF-1 signaling and by enhancing the production of IGFBP-3. Conversely, IGF-1 is capable of depressing pro-inflammatory cytokine signaling by increasing anti-inflammatory IL-10 secretion and by directly depressing pro-inflammatory cytokine signaling [23, 60, 61]. Both IGF-1 and IGFBP-3 had relative strong connections to metabolic nodes in the CMV^- men, but only one weak connection to CRP. In contrast, all three of the other networks displayed multiple connections to both pro- and anti-inflammatory cytokines – possibly due to their involvement in the dynamic interactions aiming to balance the pro- and anti-inflammatory equilibrium. Concerning the connections between neurotrophins and cognitive nodes, we can see a relative homogeneous picture: with some connections in the networks of CMV-negative and -positive men, and with only one connection in the networks of CMV-negative and -positive women. There is substantial evidence that IGF-1 deficiency represents a contributing factor for reduced cognitive abilities in aged humans [57, 62], and that supplementation with IGF-1 may reverse this deficit [60, 63–66]. Measures of circulating IGF-1, IGFBP-3 and their ratio, have been proposed for monitoring aged individuals and those at risk of cognitive and functional decline [62]. Thus, we can speculate that the relatively low number of connections between neurotrophins and cognitive nodes, seen in all four networks, might be due to the overall age-related decrease of these neurotrophic factors in peripheral circulation of elderly participants.

Our study has many strengths, including that it is one of the first studies to extensively characterize, prior to any physical, cognitive, and combine interventions, the network topology dynamics in multiple peripheral circulating biomarkers and markers of cognitive functioning. Applying a graph-theory approach allowed us not only to visualize biologically meaningful interconnections between nodes but also for the first time to compare the network topology metrics between different groups of CMV-seronegative and -positive men and women in a statistically sound manner. Inspection of separate nodes in the networks showed that these network topology differences were especially strong for cytokines and cognitive nodes. Modularity analyses showed that the networks under consideration exhibited highly differentiated modular organization in all cases. Moreover, we found that all four networks represented so-called small-world networks (SWNs) at all levels of wiring costs and were identified as SWNs with more random characteristics. We found that the network of the CMV^- males contains more hub nodes but fewer connector nodes than the other three groups. This indicates that the modules in this participants' group are more autonomous and the information flow between the modules may be realized through a small number of connector nodes. Interestingly, three of the four hubs are cognitive variables and the fourth one is IGFBP-3. Thus, cognitive nodes, such as fluid intelligence, working memory, and perceptual speed play a central role in the network of CMV^- males driving or controlling the connections within the corresponding modules.

This is the first study investigating the segregation and integration properties of the individual networks of CMV-seropositive and -negative older men and women by analyzing such network topology measures as clustering coefficient, characteristic path length, local and global efficiency. Using the rewiring procedure for network analyses, we compared network topology dynamics and found that mean clustering coefficient was highest and *CPL* shortest in the network of the CMV^- males. The same network also manifested the highest local and global efficiency, allowing it to be identified as the network with optimal features of segregation and integration. In our previous study, the same group of participants displayed the most balanced inflammatory status in their peripheral circulation (with low levels of pro-inflammatory cytokines and high levels of anti-inflammatory biomarkers) as well as significantly higher cognitive performance in working memory and fluid intelligence [22]. Further studies, however, are required to confirm these findings and to better understand such complex relationships and network topology changes between different groups of older CMV-seropositive and -negative men and women.

There are several limitations to our study that should be acknowledged. The first one has already been mentioned in our previous publication and is "related to the fact that our pre-training cohort consisted of relatively healthy, non-obese, and well-educated Berlin residents with a comparatively low seroprevalence for CMV for this age. For this reason, the generalizability of some of our findings may be limited to the Berlin healthy aging population or to a similar European population in urban areas" [22]. The next limitation concerns the fact that we were not able to disentangle the potential effect of age on the circulating biomarkers and cognitive performance due to the fact that our pre-training cohort consisted exclusively of aged participants with a rather narrow age range from 64 to 79 years old. Another limitation is related to the exploratory character of our study of the network patterns and their relationships. We are

well aware that our choice of variables in the present study, selected on the basis of their involvement in the known age-related functional alterations in the immune, nervous, and other central physiological systems, does not necessarily cover all potential players and, we therefore need further more extended network analyses to obtain a more comprehensive picture on their dynamic interactions.

Conclusions

Network analyses applying a graph-theoretical approach provide a useful strategy for visualization and quantitative description of multiple interactions between various circulating pro- and anti-inflammatory biomarkers, hormones, neurotrophic and metabolic factors, immune cells, and measures of cognitive performance and can be in general applied for analyzing interactions between different physiological systems. Applying this approach, we were able to confirm our previous findings that CMV-infection and sex modulate multiple circulating biomarkers and cognitive performance and that balanced inflammatory and metabolic status in elderly contributes to better cognitive performance. Analyzing the network topology dynamics of circulating biomarkers and cognitive performance in older CMV-seropositive and -seronegative men and women we were able to show that highly integrated and segregated networks have optimal neuroimmune and cognitive interactions.

Methods

Participants

The sample has already been described in [22]. It consisted of 161 older adults (Fig. 1) who had enrolled in a training study that included physical, cognitive, and combined training interventions. Male and female subjects were recruited from volunteer participant pools at the Max Planck Institute for Human Development and by advertisements in the metropolitan area of Berlin, Germany. All the volunteers lived independently at home, leading an active life. Participants were healthy, right-handed adults, aged 64–79 years. All volunteers completed a medical assessment prior to data collection. The medical examination was conducted at the Charité Sports Medicine, Charité Universitätsmedizin Berlin. Of the originally recruited 201 volunteers only 179 individuals met inclusion criteria for study participation after medical assessment. None of the participants had a history of head injuries, medical (e.g., heart attack), neurological (e.g., epilepsy), or psychiatric (e.g., depression) disorders. None of the volunteers had suffered from chronic inflammatory, autoimmune or cancer diseases, nor had clinically evident infections. Moderately elevated and controlled blood pressure was not considered as an exclusion criterion. All subjects completed the informed consent form to the study protocol, which was approved by the Ethics Committee of the German Society of Psychology, UL 072014.

Circulating biomarkers assessment

The assessment of circulating cytokines, receptor antagonist, soluble cytokine receptor, and CMV-serostatus has been described in detail [22]. The blood used for testing of peripheral biomarkers was collected during a medical examination in the timeframe between 11 am and 2 pm. For all analyses, the participants were separated into four groups according to their CMV-serostatus and sex (Fig. 1). The effective sample consisted of 29 CMV-negative males (mean age = 72.4, SD = 3.5, age range = 64.0–77.2), 30 CMV-negative females (mean age = 70.0, SD = 3.6, age range = 64.1–76.9), 50 CMV-positive males (mean age = 70.4, SD = 3.7, age range = 64.0–78.1), and 52 CMV-positive females (mean age = 70.2, SD = 3.6, age range = 63.9–77.1).

Cytokines TNF, IL-10, IL-6, and IL-1β

Serum levels of pro- and anti-inflammatory cytokines (TNF, IL-10, IL-6, and IL-1β) were determined using the high-sensitivity cytometric bead array (CBA) flex system (BD Biosciences, San Jose, CA, USA) that allows multiplex quantification in a single sample. All analyses were performed according to the manufacturer's instructions; to increase accuracy, an additional standard dilution was added. The fluorescence produced by CBA beads was measured on a BD FACS CANTO II Flow Cytometer and analyzed using the software FCAP Array v3 (BD Biosciences).

sTNF-R, IL-1RA, IL-18, cortisol, and DHEA levels, and CMV-serostatus

To gauge sTNF-R (80 kDA), IL-1RA, and IL-18 levels, we used the Sandwich Enzyme-linked Immunosorbent Assay (ELISA), a sensitive method allowing for the measurement of an antigen concentration in an unknown sample. All analyses were conducted according to the manufacturer's instructions. The levels of human circulating sTNF-R (80 kDA), IL-1RA, and IL-18 were determined using the Platinum ELISA kit for the quantitative detection of the three cytokines (ThermoFisher SCIENTIFIC Invitrogen, Vienna, Austria, catalog numbers: BMS211, BMS2080 and BMS267/2).

Serum levels of anti-Cytomegalovirus IgG were determined using a commercial ELISA kit (IBL International GMBH, Hamburg, Germany, catalogue number: RE57061) and according to the manufacturer's instructions. Samples were considered to give a positive signal if the absorbance value exceeded 10% over the cut-off, whereas a negative signal was absorbance lower than 10% below the cut-off.

Quantitative determination of Cortisol and DHEA in serum of participants was performed using Human Cortisol and Human DHEA (sulfate form) ELISA kits (Qarigo Biolabatories, catalog number: ARG81162 and ARG80837). The central mechanism of the competitive ELISA is a competitive binding process performed by sample antigen and add-in antigen. The amount of bound add-in antigen is inversely proportional to the concentration of the sample antigen. The analyses were performed according to the manufacturer's instructions.

All samples were assessed in duplicate at 450 or 450/620 nm using a Multiscan-FC Microtiter Plate Photometer. Protein concentrations were determined in relation to a four-parameter standard curve (Prism 8 GraphPad, San Diego, CA, USA) or calculated using Microsoft Excel 2011.

Levels of IGF-1 and IGFBP-3, CRP, metabolic factors, and immune cells

Serum levels of Insulin-like growth factor 1 (IGF-1) and Insulin-Like Growth Factor-Binding Protein 3 (IGFBP-3) were determined at the Endocrine Routine Laboratory (University Hospital of Würzburg). Measurement of IGF-1 (L2KIGF2) and IGFBP-3 (L2KGB2) was performed according to the manufacturer's instruction, using the Immulite 2000 system - an automated solid-phase, Electrochemiluminescence-Immunoassay (ECLIA) from Siemens Healthcare (Germany). Levels of C-reactive protein (CRP), cholesterol, LDL, HDL, triglyceride, lymphocytes, leukocytes, monocytes, and neutrophils were measured within the clinical diagnostics facility of Berlin, Labor28. Serum concentrations of cholesterols and triglyceride were measured using enzymatic colorimetric tests (Roche, Basel, Switzerland). Counts of the immune cells were determined by flow cytometry (Sysmex, Norderstedt, Germany).

Cognitive assessment

Cognitive assessment was performed 3 months after blood collection, immediately before beginning of training. Participants were invited to a baseline session that lasted about 3.5 h, in which they were tested in groups of four to six individuals. The cognitive battery included a broad range of measures of learning and memory performance, processing speed, working memory, and executive functioning. The group received a standardized session protocol and started, after instructions, each task with practice trials to ensure that all participants understood the task. Responses were collected via button boxes, the computer mouse, or the keyboard. A detailed description of the tasks and scores used in the present study is included in the supplementary material.

Network construction and network properties

For network construction, we used a coefficient of determination (R^2), ranging between 0 and 1, and indicating the extent to which one dependent variable is explained by the other. The coefficient of determination was calculated between all pairs of variables ($N = 33$) for the four experimental groups separately. Thus, the common network in each of the groups contained 33 nodes altogether, covering all possible interactions between the variables or nodes. To be able to construct sparse networks with relatively stable network topology, we first investigated ordered (lattice) and random networks containing the same number of nodes and edges as the real network. To do so, we randomized the edges in the real network to achieve a random network. As for the lattice network, we redistributed the edges such that they were laying close to the main diagonal and in the corner opposite to the main diagonal with increasing order of their weights. The lattice network reconstructed in such a way has the same number of nodes and edges as the initial real network but is characterized by ring or lattice topology incorporating nearest-neighbor connectivity [67]. Random networks were constructed 100 times, and the network topology measures determined each time were averaged for further analyses. To investigate the network topology of the real networks in topology space between regular and random networks with different wiring cost levels, we constructed real and control (i.e., lattice and random) networks in the range of costs between 10 and 60% with a step of 1% of wiring costs (the ratio of the number of actual connections to the maximum possible number of connections in the network). We then decided to set the cost level to 25%, which resulted in sparse and at the same time stable network topology.

Degrees and strengths

The degree of a node provides information about the number of links connected to that node, and the strength reflects the overall strength of that node's connections or weights. Thus, the strength could be considered as a weighted degree. Degree or strength of a node indicates the activity of that node, whereas the sum or mean of all degrees (strengths) represents the overall activity of the network. As R^2 is a weighted symmetric measure, we obtained the node's strength (S_i^w) as the sum of weights of all connections (w_{ij}) to node i, and calculated the mean strength (S) across all nodes in the network:

$$S = \frac{1}{N}\sum_{i \in N} S_i^w = \frac{1}{N}\sum_{i,j \in N} w_{ij} \quad (1)$$

Clustering coefficient and characteristic path length

For an individual node i, the clustering coefficient (CC_i^w) is defined as the proportion of the number of existing

neighbor–neighbor connections to the total number of possible connections within its neighborhood. In the case of a weighted graph, the mean *CC* is calculated as follows [68]:

$$CC = \frac{1}{N}\sum_{i\in N} CC_i^w = \frac{1}{N}\sum_{i\in N}\frac{2t_i^w}{k_i(k_i-1)} \quad (2)$$

with $t_i^w = (w_{ij}w_{ih}w_{jh})^{1/3}$ being the number of weighted closed triangles around a node *i*; k_i is the degree of the node *i*, and *N* is the number of nodes in the network, $N = 33$. The *CC* measures the cliquishness of a typical neighborhood and is thus a measure of network segregation.

The shortest path length or distance d_{ij} between two nodes *i* and *j* is normally defined as the minimal number of edges that have to be passed to go from *i* to *j*. As our networks are weighted graphs, the weight of the links must be considered. The input matrix is then a mapping from weight to length (i.e., a weight inversion), and the distance d_{ij}^w is the minimal weighted distance between the nodes *i* and *j*, but not necessarily the minimal number of edges. To calculate the characteristic path length (*CPL*) of a network, path lengths between all possible pairs of vertices or nodes in the network were determined [69] and then averaged among nodes:

$$CPL = \frac{1}{N}\sum_{i\in N} L^w{}_i = \frac{1}{N}\sum_{i\in N}\frac{\sum_{j\in N, j\neq i} d_{ij}^w}{N-1} \quad (3)$$

whereby $L_i{}^w$ is the shortest path length of a node *i*, and *N* is the total number of nodes in the network. *CPL* shows the degree of network integration, with a short *CPL* indicating higher network integration.

Local and global efficiency

Local efficiency (E_{local}) is similar to the *CC* and is calculated as the harmonic mean of neighbor-neighbor distances [70]:

$$E_{local} = \frac{1}{N_{G_i}(N_{G_i}-1)}\sum_{i\in N} E_{local(i)}^w = \frac{1}{N_{G_i}(N_{G_i}-1)}\sum_{i\in N}\frac{1}{L_{j,h}} \quad (4)$$

where N_{G_i} is the number of nodes in subgraph G_i, comprising all nodes that are immediate neighbours of the node *i* (excluding the node *i* itself), and $E_{local(i)}^w$ is local efficiency of the node *i* determined as the reciprocal of the shortest path length between neighbours *j* and *h*. Thus, E_{local} of node *i* is defined with respect to the subgraph comprising all of *i*'s neighbours, after removal of node *i* and its incident edges (Latora and Marchiori, 2001). Like *CC*, E_{local} is a measure of the segregation of a network, indicating efficiency of information transfer in the immediate neighbourhood of each node.

Global efficiency (E_{global}) is defined as the average inverse shortest path length and is calculated by the formula [70]:

$$E_{global} = \frac{1}{N}\sum_{i\in N} E_{global(i)}^w = \frac{1}{N}\sum_{i\in N}\frac{\sum_{j\in N, j\neq i}\left(d_{ij}^w\right)^{-1}}{N-1} \quad (5)$$

whereby $E_{global(i)}^w$ is a nodal efficiency, d_{ij}^w is the minimal weighted distance between the nodes *i* and *j*, and *N* is the total number of nodes in the network. The nodal efficiency is practically the normalized sum of the reciprocal of the shortest path lengths or distances from a given node to all other nodes in the network. Nodal efficiency quantifies how well a given node is integrated within the network, and global efficiency indicates how integrated is the common network. Thus, like *CPL*, E_{global} is a measure of the integration of a network, but whereas *CPL* is primarily influenced by long paths, E_{global} is primarily influenced by short ones.

Small-Worldness (SW) coefficients

Using graph metrics determined for real and control (i.e., regular and random) networks, specific quantitative small-world metrics were obtained. The first small-world metric, the so-called small-world coefficient σ, is related to the main metrics of a random graph (CC_{rand} and CPL_{rand}) and is determined on the basis of two ratios $\gamma = CC_{real}/CC_{rand}$ and $\lambda = CPL_{real}/CPL_{rand}$ [71]:

$$\sigma = \frac{\gamma}{\lambda} = \frac{CC_{real}/CC_{rand}}{CPL_{real}/CPL_{rand}} \quad (6)$$

The small-world coefficient σ should be greater than 1 in the small-world networks (SWNs). The second SW metric, the so-called small-world coefficient ω, is defined by comparing the characteristic path length of the observed (real) and random networks, and comparing the clustering coefficient of the observed or real network to that of an equivalent lattice (regular) network [72]:

$$\omega = \frac{CPL_{rand}}{CPL_{real}} - \frac{CC_{real}}{CC_{latt}} \quad (7)$$

This metric ranges between − 1 and + 1 and is close to zero for SWN ($CPL_{\mathrm{real}} \approx CPL_{\mathrm{rand}}$ and $CC_{\mathrm{real}} \approx CC_{\mathrm{latt}}$). Thereby, negative values indicate a graph with more regular properties ($CPL_{\mathrm{real}} > > CPL_{\mathrm{rand}}$ and $CC_{\mathrm{real}} \approx CC_{\mathrm{latt}}$), and positive values of ω indicate a graph with more random properties ($CPL_{\mathrm{real}} \approx CPL_{\mathrm{rand}}$ and $CC_{\mathrm{real}} < < CC_{\mathrm{latt}}$). As suggested in [72], the metric ω compared to σ has a clear advantage, i.e., the possibility to define how much the network of interest resembles its regular or random equivalents.

Modularity analyses and *Z-P* parameter space

To investigate the modular organization of the network and the individual role of each node in the emerging modularity or community structure, we partitioned the networks into modules applying modularity optimization algorithm and determined indices of modularity (Q), within-module degree (Z_i), and participation coefficient (P_i) using the Brain Connectivity Toolbox [73]. The optimal community structure is a subdivision of the network into non-overlapping groups of nodes in a way that maximizes the number of within-module edges, and minimizes the number of between-module edges. Q is a statistic that quantifies the degree to which the network may be subdivided into such clearly delineated groups or modules. It is given for weighted networks by the formula [74]:

$$Q^w = \frac{1}{l^w}\sum_{j\in N}\left[w_{ij} - \frac{k_i^w k_j^w}{l^w}\right]\cdot \delta_{m_i m_j}, \quad (8)$$

where l^w is the total number of edges in the network, N is the total number of nodes in the network, w_{ij} are connection weights, k_i^w and k_j^w are weighted degrees or strengths of the nodes, and δ_{m_i,m_j} is the Kronecker delta, where $\delta_{m_i,m_j} = 1$ if $m_i = m_j$, and 0 otherwise. High modularity values indicate strong separation of the nodes into modules. Q^w is zero if nodes are placed at random into modules or if all nodes are in the same cluster. To test the modularity of the empirically observed networks, we compared them to the modularity distribution ($N = 100$) of random networks as described above [75].

The within-module degree Z_i indicates how well node i is connected to other nodes within the module m_i. As shown in Guimerà and Amaral [27], it is determined by:

$$Z_i = \frac{k_i(m_i) - \bar{k}(m_i)}{\sigma^{k(m_i)}}, \quad (9)$$

where $k_i(m_i)$ is the within-module degree of node i (the number of links between i and all other nodes in m_i), and $\bar{k}(m_i)$ and $\sigma^{k(m_i)}$ are the mean and standard deviation of the within-module degree distribution of m_i.

The participation coefficient P_i describes how well the nodal connections are distributed across different modules [27]:

$$P_i = 1 - \sum_{m\in M}\left(\frac{k_i(m_i)}{k_i}\right)^2, \quad (10)$$

where M is the set of modules, $k_i(m_i)$ is the number of links between node i and all other nodes in module m_i, and k_i is the total degree of node i in the network. Correspondingly, P_i of a node i is close to 1 if its links are uniformly distributed among all the modules, and is zero if all of its links lie within its own module. Z_i and P_i values form a so-called Z-P parameter space and are characteristic for the different roles of the nodes in the network [27]. These roles in the Z-P parameter space could be defined as follows: ultra-peripheral nodes ($P_i < 0.05$), provincial nodes (low Z_i and P_i values), connector nodes (low Z_i and high P_i values), hub nodes (high Z_i and low P_i values), and hub connector nodes (high Z_i and P_i values). In this context, hubs are responsible for intra-modular connectivity and contain multiple connections within a module, while connector nodes maintain inter-modular connectivity and are responsible for links between the modules.

Statistical analysis

In order to statistically compare the four different networks at a given cost level, we used a rewiring procedure with a step-by-step replacement of a non-existing edge through an existing one and consecutive determination network topology metrics each time. This procedure can specify the network stability and network topology alteration by very small changes in the network configuration. In a statistical sense, this procedure is similar to bootstrapping with replacement applied to time series. In total, there were about 50,000 rewired networks, on which mean and standard deviation (SD) of the network topology metrics were determined. Because the rewiring distribution showed a normal shape and a small bias, we were able to achieve a 99.7% confidence interval (CI) for the mean by using the empirical rule: $CI = mean \pm 3 \times SD$ ($P < 0.005$).

Supplementary information

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Additional file 1: Figure S1. (A) *CC* is greatest in lattice networks (blue) and lowest in random networks (green), whereas *CC* for the real networks (red) is in-between. In contrast, (B) *CPL* is shortest in random and longest in lattice networks, while the real networks are in-between. CMV, Cytomegalovirus; CMV^- m, CMV-seronegative men; CMV^+ m, CMV-seropositive men; CMV^- f, CMV-seronegative women; CMV^+ f, CMV-seropositive women. **Figure S2.** (A) Local efficiency was highest in regular networks (at least for the cost levels under 45%) and lowest in random networks (at least for the cost levels under 20%), while (B) global efficiency was highest in random (green) and lowest in lattice (blue) networks practically for all levels of wiring costs, with real (red) networks were always in-between. CMV, Cytomegalovirus; CMV^- m, CMV-seronegative men; CMV^+ m, CMV-seropositive men; CMV^- f, CMV-seronegative women; CMV^+ f, CMV-seropositive women.

Abbreviations

CBA: Cytometric bead array; CC: Clustering coefficient; CI: Confidence interval; CMV: Cytomegalovirus; CPL: Characteristic path length; CRP: C-reactive protein; DHEA: Dehydroepiandrosterone; E_{global}: Global efficiency; ELISA: Enzyme-linked Immunosorbent Assay; E_{local}: Local efficiency; EM: Episodic memory; Gf: Fluid intelligence; HDL: High-density lipoprotein; IGF-1: Insulin-like growth factor-1; IGFBP-3: IGF-binding protein; IgG: Immunoglobulin G; IL: Interleukin; IL-1RA: Interleukin 1 receptor antagonist; LDL: Low-density lipoprotein; sTNF-R: Soluble Tumor Necrosis Factor receptor; TNF: Tumor Necrosis Factor; WM: Working memory

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Authors' contributions
Conceptualization: SDB, LM, GP, MS, and VM; methodology: VM, SDB; SR, and TD; software: VM; validation: VM, SR, SDB; TD, formal analysis: SDB, and VM; investigation: SDB, TD, and SR; writing-original draft preparation: SDB; writing-review and editing: GP, LM, VM, and SDB. All authors read and approved the final version of manuscript.

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Availability of data and materials
The datasets for this study will not be made publicly available due to restrictions included in the consent statement that the participants of the study signed only allow the present data to be used for the research purposes within the Max Planck Institute for Human Development in Berlin.

Ethics approval and consent to participate
All participants completed the informed consent form to the study protocol which was approved by the Ethics Committee of the German Society of Psychology, UL 072014.

Consent for publication
The consent forms of all participants are held by the authors' institute. The data in this work have not been published elsewhere. All authors agree to submit this manuscript for publication in this journal.

Competing interests
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Franceschi C, Garagnani P, Vitale G, Capri M, Salvioli S. Inflammaging and 'Garb-aging'. Trends Endocrinol Metab. 2017;28(3):199–212.
2. Di Benedetto S, Müller L, Wenger E, Duzel S, Pawelec G. Contribution of neuroinflammation and immunity to brain aging and the mitigating effects of physical and cognitive interventions. Neurosci Biobehav Rev. 2017;75:114–28.
3. Beydoun MA, Dore GA, Canas JA, Liang H, Beydoun HA, Evans MK, et al. Systemic inflammation is associated with longitudinal changes in cognitive performance among urban adults. Front Aging Neurosci. 2018;10:313.
4. Procaccini C, Pucino V, De Rosa V, Marone G, Matarese G. Neuro-endocrine networks controlling immune system in health and disease. Front Immunol. 2014;5:143.
5. Dantzer R. Neuroimmune interactions: from the brain to the immune system and vice versa. Physiol Rev. 2018;98(1):477–504.
6. Gottesman RF, Albert MS, Alonso A, Coker LH, Coresh J, Davis SM, et al. Associations between midlife vascular risk factors and 25-year incident dementia in the atherosclerosis risk in communities (ARIC) cohort. JAMA Neurol. 2017;74(10):1246–54.
7. Alboni S, Maggi L. Editorial: cytokines as players of neuronal plasticity and sensitivity to environment in healthy and pathological brain. Front Cell Neurosci. 2015;9:508.
8. Ventura MT, Casciaro M, Gangemi S, Buquicchio R. Immunosenescence in aging: between immune cells depletion and cytokines up-regulation. Clin Mol Allergy. 2017;15:21.
9. Fülop T, Larbi A, Dupuis G, Le Page A, Frost EH, Cohen AA, et al. Immunosenescence and Inflamm-aging as two sides of the same coin: friends or foes? Front Immunol. 2017;8:1960.
10. De la Fuente M, Gimenez-Llort L. Models of aging of neuroimmunomodulation: strategies for its improvement. Neuroimmunomodulation. 2010;17(3):213–6.
11. Walker KA, Gottesman RF, Wu A, Knopman DS, Gross AL, Mosley TH Jr, et al. Systemic inflammation during midlife and cognitive change over 20 years: the ARIC study. Neurology. 2019;92(11):e1256–e67.
12. Du Y, Zhang G, Liu Z. Human cytomegalovirus infection and coronary heart disease: a systematic review. Virol J. 2018;15(1):31.
13. Garcia Verdecia B, Saavedra Hernandez D, Lorenzo-Luaces P, de Jesus Badia Alvarez T, Leonard Rupale I, Mazorra Herrera Z, et al. Immunosenescence and gender: a study in healthy Cubans. Immun Ageing. 2013;10(1):16.
14. Kilgour AH, Firth C, Harrison R, Moss P, Bastin ME, Wardlaw JM, et al. Seropositivity for CMV and IL-6 levels are associated with grip strength and muscle size in the elderly. Immun Ageing. 2013;10(1):33.
15. Pawelec G, Derhovanessian E. Role of CMV in immune senescence. Virus Res. 2011;157(2):175–9.
16. Pawelec G, McElhaney JE, Aiello AE, Derhovanessian E. The impact of CMV infection on survival in older humans. Curr Opin Immunol. 2012;24(4):507–11.
17. Lobo-Silva D, Carriche GM, Castro AG, Roque S, Saraiva M. Balancing the immune response in the brain: IL-10 and its regulation. J Neuroinflammation. 2016;13(1):297.
18. Perry RT, Collins JS, Wiener H, Acton R, Go RC. The role of TNF and its receptors in Alzheimer's disease. Neurobiol Aging. 2001;22(6):873–83.
19. Nakagomi A, Seino Y, Noma S, Kohashi K, Kosugi M, Kato K, et al. Relationships between the serum cholesterol levels, production of monocyte proinflammatory cytokines and long-term prognosis in patients with chronic heart failure. Intern Med. 2014;53(21):2415–24.
20. Lee BK, Glass TA, McAtee MJ, Wand GS, Bandeen-Roche K, Bolla KI, et al. Associations of salivary cortisol with cognitive function in the Baltimore memory study. Arch Gen Psychiatry. 2007;64(7):810–8.
21. Wersching H, Duning T, Lohmann H, Mohammadi S, Stehling C, Fobker M, et al. Serum C-reactive protein is linked to cerebral microstructural integrity and cognitive function. Neurology. 2010;74(13):1022–9.
22. Di Benedetto S, Gaetjen M, Muller L. The Modulatory Effect of Gender and Cytomegalovirus-Seropositivity on Circulating Inflammatory Factors and Cognitive Performance in Elderly Individuals. Int J Mol Sci. 2019;20(4).
23. O'Connor JC, McCusker RH, Strle K, Johnson RW, Dantzer R, Kelley KW. Regulation of IGF-I function by proinflammatory cytokines: at the interface of immunology and endocrinology. Cell Immunol. 2008;252(1–2):91–110.
24. Bhavnani SK, Victor S, Calhoun WJ, Busse WW, Bleecker E, Castro M, et al. How cytokines co-occur across asthma patients: from bipartite network analysis to a molecular-based classification. J Biomed Inform. 2011;44(Suppl 1):S24–30.
25. Müller V, Perdikis D, von Oertzen T, Sleimen-Malkoun R, Jirsa V, Lindenberger U. Structure and topology dynamics of hyper-frequency networks during rest and auditory oddball performance. Front Comput Neurosci. 2016;10:108.
26. Müller V, Jirsa V, Perdikis D, Sleimen-Malkoun R, von Oertzen T, Lindenberger U. Lifespan changes in network structure and network topology dynamics during rest and auditory oddball performance. Front Aging Neurosci. 2019;11:138.
27. Guimera R, Nunes Amaral LA. Functional cartography of complex metabolic networks. Nature. 2005;433(7028):895–900.
28. Müller L, Fulop T, Pawelec G. Immunosenescence in vertebrates and invertebrates. Immun Ageing. 2013;10(1):12.
29. Talbot S, Foster SL, Woolf CJ. Neuroimmunity. Annu Rev Immunol. 2016;34:421–47.

30. Morel PA, Lee REC, Faeder JR. Demystifying the cytokine network: mathematical models point the way. Cytokine. 2017;98:115–23.
31. Müller L, Pawelec G. Aging and immunity - impact of behavioral intervention. Brain Behav Immun. 2014;39:8–22.
32. Müller L, Pawelec G. As we age: Does slippage of quality control in the immune system lead to collateral damage? Ageing Res Rev. 2015;23(Pt A):116–23.
33. Kirk GD, Dandorf S, Li H, Chen Y, Mehta SH, Piggott DA, et al. Differential relationships among circulating inflammatory and immune activation biomediators and impact of aging and human immunodeficiency virus infection in a cohort of injection drug users. Front Immunol. 2017;8:1343.
34. McAfoose J, Baune BT. Evidence for a cytokine model of cognitive function. Neurosci Biobehav Rev. 2009;33(3):355–66.
35. Vitkovic L, Bockaert J, Jacque C. "Inflammatory" cytokines: neuromodulators in normal brain? J Neurochem. 2000;74(2):457–71.
36. Tangestani Fard M, Stough C. A review and hypothesized model of the mechanisms that underpin the relationship between inflammation and cognition in the elderly. Front Aging Neurosci. 2019;11:56.
37. Bennett JM, Glaser R, Malarkey WB, Beversdorf DQ, Peng J, Kiecolt-Glaser JK. Inflammation and reactivation of latent herpesviruses in older adults. Brain Behav Immun. 2012;26(5):739–46.
38. Looney RJ, Falsey A, Campbell D, Torres A, Kolassa J, Brower C, et al. Role of cytomegalovirus in the T cell changes seen in elderly individuals. Clin Immunol. 1999;90(2):213–9.
39. Derhovanessian E, Larbi A, Pawelec G. Biomarkers of human immunosenescence: impact of Cytomegalovirus infection. Curr Opin Immunol. 2009;21(4):440–5.
40. Di Benedetto S, Derhovanessian E, Steinhagen-Thiessen E, Goldeck D, Muller L, Pawelec G. Impact of age, sex and CMV-infection on peripheral T cell phenotypes: results from the Berlin BASE-II study. Biogerontology. 2015; 16(5):631–43.
41. Fulop T, Larbi A, Pawelec G. Human T cell aging and the impact of persistent viral infections. Front Immunol. 2013;4:271.
42. Haeseker MB, Pijpers E, Dukers-Muijrers NH, Nelemans P, Hoebe CJ, Bruggeman CA, et al. Association of cytomegalovirus and other pathogens with frailty and diabetes mellitus, but not with cardiovascular disease and mortality in psycho-geriatric patients; a prospective cohort study. Immun Ageing. 2013;10(1):30.
43. McElhaney JE, Zhou X, Talbot HK, Soethout E, Bleackley RC, Granville DJ, et al. The unmet need in the elderly: how immunosenescence, CMV infection, co-morbidities and frailty are a challenge for the development of more effective influenza vaccines. Vaccine. 2012;30(12):2060–7.
44. Solana R, Tarazona R, Aiello AE, Akbar AN, Appay V, Beswick M, et al. CMV and Immunosenescence: from basics to clinics. Immun Ageing. 2012;9(1):23.
45. Whiting CC, Siebert J, Newman AM, Du HW, Alizadeh AA, Goronzy J, et al. Large-scale and comprehensive immune profiling and functional analysis of Normal human aging. PLoS One. 2015;10(7):e0133627.
46. Nikolich-Zugich J, Goodrum F, Knox K, Smithey MJ. Known unknowns: how might the persistent herpesvirome shape immunity and aging? Curr Opin Immunol. 2017;48:23–30.
47. Weltevrede M, Eilers R, de Melker HE, van Baarle D. Cytomegalovirus persistence and T-cell immunosenescence in people aged fifty and older: a systematic review. Exp Gerontol. 2016;77:87–95.
48. Villacres MC, Longmate J, Auge C, Diamond DJ. Predominant type 1 CMV-specific memory T-helper response in humans: evidence for gender differences in cytokine secretion. Hum Immunol. 2004;65(5):476–85.
49. Morrisette-Thomas V, Cohen AA, Fulop T, Riesco E, Legault V, Li Q, et al. Inflamm-aging does not simply reflect increases in pro-inflammatory markers. Mech Ageing Dev. 2014;139:49–57.
50. Tegeler C, O'Sullivan JL, Bucholtz N, Goldeck D, Pawelec G, Steinhagen-Thiessen E, et al. The inflammatory markers CRP, IL-6, and IL-10 are associated with cognitive function–data from the Berlin aging study II. Neurobiol Aging. 2016;38:112–7.
51. Wolkow A, Aisbett B, Reynolds J, Ferguson SA, Main LC. Relationships between inflammatory cytokine and cortisol responses in firefighters exposed to simulated wildfire suppression work and sleep restriction. Physiol Rep. 2015;3(11).
52. Kamin HS, Kertes DA. Cortisol and DHEA in development and psychopathology. Horm Behav. 2017;89:69–85.
53. Marques AH, Silverman MN, Sternberg EM. Glucocorticoid dysregulations and their clinical correlates. From receptors to therapeutics. Ann N Y Acad Sci. 2009;1179:1–18.
54. Alves VB, Basso PJ, Nardini V, Silva A, Chica JE, Cardoso CR. Dehydroepiandrosterone (DHEA) restrains intestinal inflammation by rendering leukocytes hyporesponsive and balancing colitogenic inflammatory responses. Immunobiology. 2016;221(9):934–43.
55. Wu Z, Li L, Zheng LT, Xu Z, Guo L, Zhen X. Allosteric modulation of sigma-1 receptors by SKF83959 inhibits microglia-mediated inflammation. J Neurochem. 2015;134(5):904–14.
56. Shields GS, Moons WG, Slavich GM. Inflammation, self-regulation, and health: an immunologic model of self-regulatory failure. Perspect Psychol Sci. 2017;12(4):588–612.
57. Willis EL, Wolf RF, White GL, McFarlane D. Age- and gender-associated changes in the concentrations of serum TGF-1beta, DHEA-S and IGF-1 in healthy captive baboons (Papio hamadryas anubis). Gen Comp Endocrinol. 2014;195:21–7.
58. Wilson CJ, Finch CE, Cohen HJ. Cytokines and cognition--the case for a head-to-toe inflammatory paradigm. J Am Geriatr Soc. 2002;50(12):2041–56.
59. Elenkov IJ. Neurohormonal-cytokine interactions: implications for inflammation, common human diseases and well-being. Neurochem Int. 2008;52(1–2):40–51.
60. Ashpole NM, Sanders JE, Hodges EL, Yan H, Sonntag WE. Growth hormone, insulin-like growth factor-1 and the aging brain. Exp Gerontol. 2015;68:76–81.
61. Junnila RK, List EO, Berryman DE, Murrey JW, Kopchick JJ. The GH/IGF-1 axis in ageing and longevity. Nat Rev Endocrinol. 2013;9(6):366–76.
62. Wennberg AMV, Hagen CE, Machulda MM, Hollman JH, Roberts RO, Knopman DS, et al. The association between peripheral total IGF-1, IGFBP-3, and IGF-1/IGFBP-3 and functional and cognitive outcomes in the Mayo Clinic study of aging. Neurobiol Aging. 2018;66:68–74.
63. Deijen JB, Arwert LI, Drent ML. The GH/IGF-I Axis and cognitive changes across a 4-year period in healthy adults. ISRN Endocrinol. 2011;2011:249421.
64. Arwert LI, Veltman DJ, Deijen JB, van Dam PS, Drent ML. Effects of growth hormone substitution therapy on cognitive functioning in growth hormone deficient patients: a functional MRI study. Neuroendocrinology. 2006;83(1): 12–9.
65. Molina DP, Ariwodola OJ, Weiner JL, Brunso-Bechtold JK, Adams MM. Growth hormone and insulin-like growth factor-I alter hippocampal excitatory synaptic transmission in young and old rats. Age (Dordr). 2013; 35(5):1575–87.
66. Bozdagi O, Tavassoli T, Buxbaum JD. Insulin-like growth factor-1 rescues synaptic and motor deficits in a mouse model of autism and developmental delay. Mol Autism. 2013;4(1):9.
67. Sporns O, Honey CJ, Kotter R. Identification and classification of hubs in brain networks. PLoS One. 2007;2(10):e1049.
68. Fagiolo G. Clustering in complex directed networks. Phys Rev E Stat Nonlinear Soft Matter Phys. 2007;76(2 Pt 2):026107.
69. Watts DJ, Strogatz SH. Collective dynamics of 'small-world' networks. Nature. 1998;393(6684):440–2.
70. Latora V, Marchiori M. Efficient behavior of small-world networks. Phys Rev Lett. 2001;87(19):198701.
71. Humphries MD, Gurney K, Prescott TJ. The brainstem reticular formation is a small-world, not scale-free, network. Proc Biol Sci. 2006;273(1585):503–11.
72. Telesford QK, Joyce KE, Hayasaka S, Burdette JH, Laurienti PJ. The ubiquity of small-world networks. Brain Connect. 2011;1(5):367–75.
73. Rubinov M, Sporns O. Complex network measures of brain connectivity: uses and interpretations. Neuroimage. 2010;52(3):1059–69.
74. Newman ME. Analysis of weighted networks. Phys Rev E Stat Nonlinear Soft Matter Phys. 2004;70(5 Pt 2):056131.
75. Bassett DS, Khambhati AN. A network engineering perspective on probing and perturbing cognition with neurofeedback. Ann N Y Acad Sci. 2017; 1396(1):126–43.

Publisher's Note

Supplementary material (IV)

Cognitive Tests

Episodic Memory (EM)

Object Location task (Object). In this task, sequences of 12 colored photographs of real-world objects were displayed at different locations in a 6-by-6 grid. After presentation, objects appeared at the side of the screen and had to be moved to the correct locations by clicking on the objects and the locations with the computer mouse. One practice trial and two test trials were included. The sum of correct placements across the two test trials is used as the manifest variable.

Face–Profession task (Face). This task assesses associative binding on the basis of recognition of incidentally encoded face–profession pairs. During the study phase 45 face–profession pairs were each presented for 3.5 s on the computer screen and the participants had to indicate via button presses whether the faces matched the profession or not. After a 3-min delay between study and test phase 54 face–profession pairs consisting of 27 old pairs, 9 new pairs, and 18 newly arranged pairs were presented (in newly arranged pairs the shown face is the same, but is associated with a new profession). The participants were asked to decide whether they had seen a given face–profession combination before or not and to rate the confidence of their decision on a three-point scale ranging from 1 = not sure to 3 = very sure. Recognition memory for the rearranged face–profession pairs (hit minus false alarms) served as the manifest variable in the model.

Working Memory (WM)

Number-N-Back (Number). Three one-digit numbers (ranging from 0 to 9) were presented sequentially in three cells situated horizontally followed by the next sequence of three digits. This cycle was repeated 30 times. In each cycle, two-choice decisions on whether the current stimulus matched the stimulus shown three steps earlier had to be made. Four practice trials including 30 runs were followed by 6 test trials with 30 runs. Subjects made their decision via button-box presses with their left and right index fingers [157].

Letter Updating (LetterU). In this task subjects were presented with 7, 9, 11, or 13 letters in a sequence. Once a sequence stopped, subjects had to report the last three letters in correct order by pressing buttons on the button box corresponding to A, B, C, and D.

Spatial Updating (Spatial). In each block of this task, a display of two or three 3-by-3 grids was shown for 4 s. In each of these, one blue dot was presented in one of the nine locations. Those two or three locations had to be memorized and updated according to shifting operations that were indicated by arrows appearing below the corresponding field. The presentation time of the arrows was 2.5 s with an inter-stimulus interval of 0.5 s. After six updating operations, the two or three grids reappeared and the resulting end positions had to be clicked on. After ten practice blocks with memory loads of two and three grids, ten test blocks with load two and three were conducted and used for scoring. The average percentage of correct placements was used as one of the manifest variables for the working memory (WM) factor [157].

Fluid Intelligence (Gf)

Figural Analogies (Analog). Items in this test followed the format "A is to B as C is to ?". One figure pair was presented in the upper left part of the screen and a single figure was shown beside it. Participants had to use the same rule as the one applying to the complete figure pair to choose one of the five alternative responses presented below. Subjects entered their response by clicking on one of the five alternatives

with the mouse. Before the test phase, instructions and three practice items were given. The test phase was terminated when subjects made three consecutive errors, when they reached the maximum time limit (10 min), or after they had worked on the last test item. Items were ordered by difficulty [158].

Practical Problems (Problem). This task consisted of 12 items depicting everyday problems such as the times in a bus schedule, instructions for medication, a warranty for a technical appliance, a rail map, as well as other forms and tables. For each item, the problems were presented in the upper part of the screen, and five alternative responses were shown in the lower part. Subjects responded by clicking on one of the five alternatives with the computer mouse. A single practice item was provided. The test phase was terminated when subjects made three consecutive errors, or when they reached the maximum time limit of 10 minutes, or after they had answered the last test item. Items were ordered by difficulty [158].

Letter Series (Letter). The task consisted of 22 items. Each item contained five letters followed by a question mark (e.g., c e g i k ?). Items were displayed in the upper half of the screen, and five response alternatives were presented in the lower half. Items followed simple rules such as +1, –1,+2, or +2 +1. Subjects entered their response by touching one of the five answer alternatives. The score was based on the total number of correct responses. Instructions and three practice items were given before the test phase. The test phase was terminated when subjects made three consecutive false responses, when they reached the maximum time limit (6 min), or after they had answered the last item of the test. Items were ordered by difficulty. Sample items were used with respect to tests related to speed, reasoning, and knowledge [158].

Processing Speed / Comparison Task (Speed)

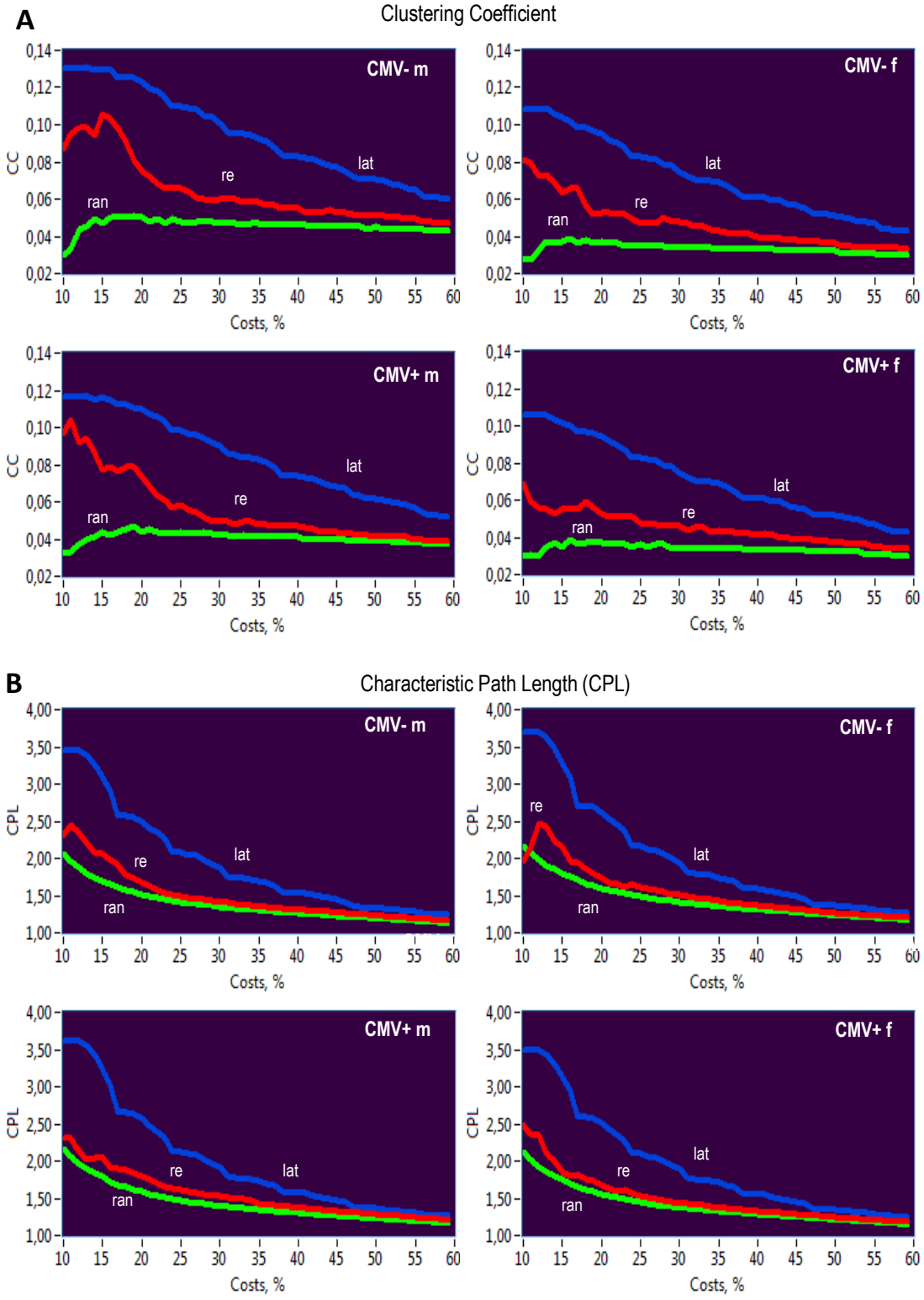
For *the numerical version (number)* of the comparison task, two strings of five numbers each appear on the left and right of the screen, with participants having to decide as quickly as possible whether both strings are exactly the same or different. If different, the strings differ by just one number. Number strings are randomly assembled using digits 1–9.

The verbal version (verbal) of this task is equivalent to the numerical one, using strings of five consonants.

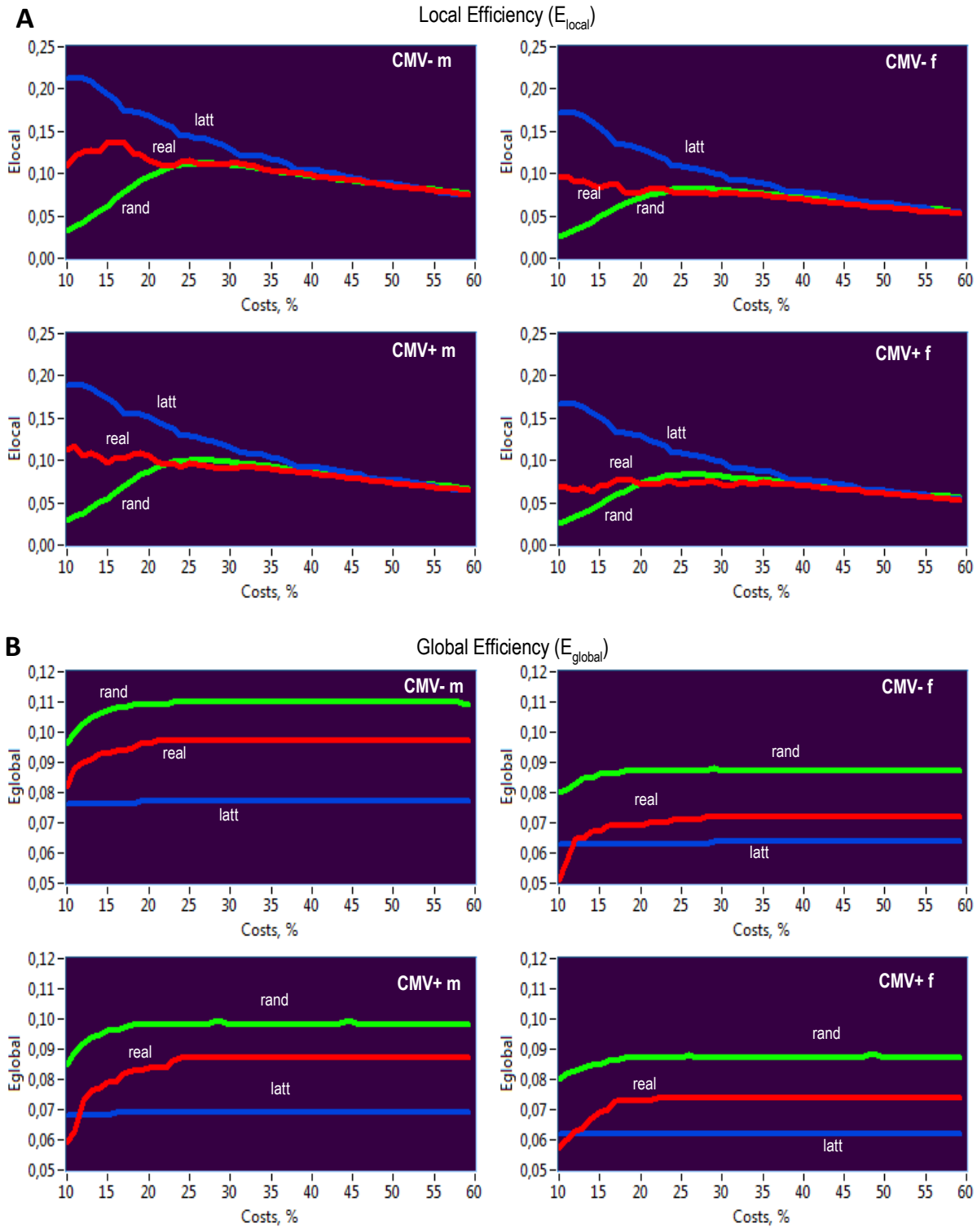
Figural version (figure). Two “fribbles” - the three-dimensional colored objects consisting of several connected parts, are shown to the left and right of the screen, with participants having to decide as quickly as possible whether the two objects are exactly the same or different. If different, the objects differ with respect to one part. The Fribble images in this task are courtesy of Michael J. Tarr, Brown University, http://www.tarrlab.org/). In the session, two trials of 40 items were included for each of the verbal, numerical, and figural tasks.

155. Schmiedek F, Lovden M, Lindenberger U. Hundred Days of Cognitive Training Enhance Broad Cognitive Abilities in Adulthood: Findings from the COGITO Study. *Front Aging Neurosci* 2010; **2**.
156. Lindenberger U, Mayr U, Kliegl R. Speed and intelligence in old age. *Psychol Aging* 1993; **8**(2): 207-20.

Supplementary Figures



Supplementary Figure 1. (**A**) *CC* is greatest in lattice networks (blue) and lowest in random networks (green), whereas *CC* for the real networks (red) is in-between. In contrast, (**B**) *CPL* is shortest in random and longest in lattice networks, while the real networks are in-between. CMV, Cytomegalovirus; CMV^- m, CMV-seronegative men; CMV^+ m, CMV-seropositive men; CMV^- f, CMV-seronegative women; CMV^+ f, CMV-seropositive women.



Supplementary Figure 2. (**A**) Local efficiency was highest in regular networks (at least for the cost levels under 45%) and lowest in random networks (at least for the cost levels under 20%), while (**B**) global efficiency was highest in random (green) and lowest in lattice (blue) networks practically for all levels of wiring costs, with real (red) networks were always in-between. CMV, Cytomegalovirus; CMV^- m, CMV-seronegative men; CMV^+ m, CMV-seropositive men; CMV^- f, CMV-seronegative women; CMV^+ f, CMV-seropositive women.

16 Erklärung nach §5 Abs. 2 Nr. 8 der Promotionsordnung

EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

Mathematisch-
Naturwissenschaftliche
Fakultät

Declaration according to § 5 Abs. 2 No. 8 of the PromO of the Faculty of Science
-Share in publications done in team work -

Name: Svetlana Di Benedetto

List of Publications:

(1) Di Benedetto, S., Derhovanessian, E., Steinhagen-Thiessen, E., Goldeck, D., Müller, L., & Pawelec, G. (2015). Impact of age, sex and CMV-infection on peripheral T cell phenotypes: Results from the Berlin BASE-II Study. Biogerontology. doi:10.1007/s10522-015-9563-2

(2) Di Benedetto, S., Müller, L., Wenger, E., Düzel, S. & Pawelec, G. (2017). Contribution of neuroinflammation and immunity to brain aging and the mitigating effects of physical and cognitive interventions. Neuroscience & Biobehavioral Reviews. *75*, 114-128. doi:10.1016/j.neubiorev.2017.01.044

(3) Di Benedetto, S., Gaetjen, M. & Müller, L. (2019). The Modulatory Effect of Gender and Cytomegalovirus-Seropositivity on Circulating Inflammatory Factors and Cognitive Performance in Elderly Individuals. Int. J. Mol. Sci. 2019, 20(4), 990. doi:10.3390/ijms20040990

(4) Di Benedetto, S., Müller, L., Rauskolb, S., Sendtner, T. Deutschbein, M., Pawelec, G., & Müller, V. (2019). Network topology dynamics of circulating biomarkers and cognitive performance in older Cytomegalovirus-seropositive or -seronegative men and women. Immunity & Ageing. doi:10.1186/s12979-019-0171-x

Nr.	Accepted for publication yes/no	Number of all authors	Position of the candidate in list of authors	Scientific ideas of candidate (%)	Data generation by candidate (%)	Analysis and Interpretation by candidate (%)	Paper writing by candidate (%)
1	Yes	6	1	60	90	70	80
2	Yes	5	1	70	70	70	70
3	Yes	3	1	70	70	60	70
4	Yes	7	1	40	60	50	60

I certify that the above statement is correct.

Date, Signature of the candidate

I/We certify that the above statement is correct.

Date, Signature of the doctoral committee or at least of one of the supervisors