ASC speck formation in primary human keratinocytes

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C O N T E N T S:

Z U S A M M E N F A S S U N G:

Entzündung ist eine Abwehrreaktion auf exogene und endogene (schädliche) Signale und Zustände wie Infektionen und Gewebeverletzungen. Es ist die wichtigste Reaktion des Immunsystems, die sowohl bei Menschen als auch bei Tieren auftritt. Entzündungen können durch eine Vielzahl von Immunzellen ausgelöst werden, darunter Makrophagen, Neutrophile, dendritische Zellen, natürliche Killerzellen (NK) und T- und B-Lymphozyten. Es betrifft überwiegend das angeborene Immunsystem, in späteren Stadien aber auch Komponenten des adaptiven Immunsystems. Die Entzündung kann akut und chronisch sein. Immunzellen können Krankheitserreger und Gefahrensignale durch ein Repertoire verschiedener Rezeptoren wahrnehmen, die als Mustererkennungsrezeptoren (PRRs) bekannt sind. Diese Rezeptoren werden durch pathogen-assoziierte molekulare Muster (PAMPs) aktiviert, evolutionär konservierte Strukturen, die von den meisten Mikroben geteilt werden.

Hautentzündungen treten nach Wahrnehmung verschiedener Reize auf, darunter: UV-Bestrahlung, ionisierende Strahlung, Krankheitserreger, Kontakt mit chemischen Reizstoffen, Allergenexposition, mikrobielle Belastung sowie aufgrund intrinsischer definierter Reize, einschließlich Autoimmunreaktionen. Chronische UV-Bestrahlung führt zu vielen Veränderungen der Haut, einschließlich Hyperplasie, Veränderungen der dermalen Strukturen und Veränderungen der Pigmentierung. UVB dringt nicht viel tiefer als die Epidermisschicht ein und wird von DNA und Protein absorbiert. Daher ist UVB der stärkste Induktor der Apoptose in menschlichen Keratinozyten. Apoptotische Keratinozyten werden histologisch durch eosinophiles Zytoplasma und pyknotische Kerne erkannt und werden als Sonnenbrandzellen (SBC) bezeichnet. Bei menschlichen Keratinozyten führt die Apoptose schließlich zur Permeabilisierung der Plasmamembran, dieser Vorgang wird als sekundäre Nekrose bezeichnet, die zu einer Entzündung führen kann.

Eine lange UVB-Exposition führt zu einer Schädigung der Haut, die zu einer Entzündung führt, die durch eine erhöhte NFkB-Aktivierung, erhöhte Entzündungszytokine wie Tumornekrosefaktor (TNF), Interleukin IL-6, IL-1 sowie IL-8 und IL-10.

Inflammasomen sind im Zytosol lokalisierte Proteinkomplexe, die als Plattformen für die Rekrutierung und Aktivierung der proinflammatorischen Protease Caspase-1 fungieren. Inflammasomen bestehen aus drei Hauptkomponenten: dem Nucleotide Oligomerization Domain (NOD)-like Rezeptor (NLR), der als intrazellulärer Sensor fungiert und die Komplexbildung induziert, dem Adapterprotein ASC (Apoptosis Speck-like Protein, das eine Caspase-Rekrutierungsdomäne enthält (CARD), das Pro-Caspase-1 für den Komplex rekrutiert, und Pro-Caspase 1. Apoptosis speck like protein, ist ein 22 kDa-Protein und besteht aus zwei vohldefinierten Strukturdomänen, der PYD (Pyrine Reach Domain) und der CARD (Caspase requruitment domain), die durch eine 23 aa-Linkerregion verbunden sind. Bei Stimulation lagern sich Inflammasomkomplexe zusammen, um die Spaltung von Caspase-1, die proinflammatorischen Zytokine IL-1β und IL-18 aktiviert, und des zytosolischen Proteins Gasdermin D (GSDMD), das am entzündlichen Zelltod beteiligt ist.

Daher wurde die UVB-induzierte Inflammasomaktivierung untersucht und ob sie zur Bildung von ASC-Flecken führt und ob die Bildung von nuklearen ASC-Flecken zum Zelltod führt. Wir zeigen deutlich, dass UVB die Bildung von ASC-Flecken in primären humanen Keratinozyten provoziert und diese Flecken im Zellkern lokalisiert sind. Die Bildung von ASC-Flecken spiegelt die Inflammasomaktivierung wieder, da ihre Bildung in Keratinozyten vom Vorhandensein eines funktionellen NLRP1- oder NLRP3-Inflammasoms abhängt. Die Blockierung von NLRP1 hat eine schützende Rolle in primären menschlichen Keratinozyten, die UVB-Licht ausgesetzt sind.

Der Schwerpunkt meiner Forschung liegt auf der angeborenen Immunität, neben dem Inflammasom und dem IL-1-Signalweg lag, der Forschungsschwerpunkt auch auf Zytokinen der angeborenen Immunität und deren Regulation bei verschiedenen entzündlichen Erkrankungen. Eine dieser entzündlichen Erkrankungen ist die akute anteriore Uveitis (AAU). Uveitis ist eine intraokulare Entzündung, die alle Teile des Auges betreffen kann. Sie kann idiopathisch, infektiös oder immunvermittelt im Rahmen von systematischen Erkrankungen wie der Behcet-Krankheit (BD), der Vogt-Koyanagi-Harada-Krankheit oder HLA-B27-assoziierten Erkrankungen sein. Nach dem Hauptentzündungsort kann die Uveitis in anterior, intermediär, posterior oder Panuveitis eingeteilt werden. Die anteriore Uveitis zeigt oft eine einseitige Entzündung, die bei folgenden Rezidiven die Seite wechseln kann, niemals beidseitig.

Monozyten spielen eine sehr wichtige Rolle bei der Pathogenese der Krankheit. AAU-Patienten zeigen eine erhöhte Anzahl von Monozyten, aktivierten T-Zellen und hohe Serum (S100A8/A9), heißt Alarmin, die von myeloischen Zellen freigesetzten ist.

Eine bestimmte Population von Monozyten von AAU-Patienten ist durch die erhöhte Expression von TNF, IL-10 und IL-23 gekennzeichnet. Immunantworten über den Th17-Weg spielen eine wichtige Rolle bei der HLA-B27-assoziierten AAU. Humanes Leukozyten-Antigen (HLA)-B27 ist eine Prädisposition für akute anteriore Uveitis (AAU), aber es gibt auch Patienten, die HLA-B27-negativ sind, daher wird diese Krankheit als „HLA-B27-typisch“ bezeichnet.

S U M M A R Y:

Inflammation is a defense response to exogenous and endogenous (harmful) stimuli, and conditions, such as infection and tissue injury. It is the most important response of the immune system that occurs both in humans and animals. Inflammation can be triggered by a variety of immune cells, including macrophages, neutrophils, dendritic cells, natural killer (NK) cells, and T and B lymphocytes. It predominantly involves the innate immune system, but in later stages also includes components of the adaptive immune system. Inflammation could be acute and chronic. Immune cells, can sense pathogens and danger signals through a repertoire of different receptors, known as pattern recognition receptors (PRRs). These receptors are activated by pathogen-associated molecular patterns (PAMPs), evolutionally conserved structures that are shared by most microbes.

Skin inflammation occurs after sensing of different stimuli that include: UV irradiation, ionizing radiation, pathogens, contact with chemical irritants, allergen exposure, microbial challenge, as well as due to intrinsic defined stimuli including autoimmune responses. Chronic UV irradiation results in many changes in the skin, including hyperplasia, changes in dermal structures, and alteration of pigmentation. UVB does not penetrate much deeper than the epidermal layer, and is absorbed by DNA and protein. Therefore, UVB is the most potent inducer of apoptosis in human keratinocytes. Apoptotic keratinocytes are recognized histologically by eosinophilic cytoplasm and pyknotic nuclei and are known as the sun burn cells (SBC). In human keratinocytes, apoptosis will finally result in permeabilization of the plasma membrane, and this process is known as secondary necrosis, which can lead to inflammation.

Long exposure to UVB leads to damage to the skin, which results in inflammation characterized by increased NF*k*B activation, increased inflammatory cytokines, such as tumor necrosis factor (TNF), interleukin IL-6, IL-1 as well as IL-8 and IL-10. Inflammasomes are protein complexes located in the cytosol that function as platforms for the recruitment and activation of the pro-inflammatory protease caspase-1. Inflammasomes consist of three major components: the nucleotide oligomerization domain (NOD)-like receptor (NLR), which acts as an intracellular sensor and induces complex formation, the adaptor protein ASC (apoptosis speck-like protein containing a caspase recruitment domain (CARD), which recruits pro-caspase-1 to the complex, and pro-caspase-1. Apoptosis speck like protein, is a 22 kDa protein, and it is composed of two well-defined structural domains the PYD (pyrine reach domain) and the CARD (Caspase reqruitment domain), which are connected by a 23 aa linker region. Upon stimulation, inflammasome complexes assemble to process the cleavage of caspase-1, which activates the proinflammatory cytokines IL-1β and IL-18, and the cytosolic protein gasdermin D (GSDMD), which is involved in inflammatory cell death. Therefore, UVB induced inflammasome activation has been studied, and whether it leads to the formation of ASC specks, and if nuclear ASC speck formation results in cell death. Data clearly show that UVB provokes ASC speck formation in primary human keratinocytes, and those specks are localized in the nucleus. Formation of ASC specks reflect inflammasome activation as their formation in keratinocytes depends on the presence of a functional NLRP1 or NLRP3 inflammasome. Blocking of NLRP1 have protective role in primary human keratinocytes exposed to UVB light.

Main focus of my research is the innate immunity, beside the inflammasome and IL-1 pathway, research focus was also on innate immunity cytokines and their regulation in different inflammatory conditions. One of those inflammatory disorders is acute anterior uveitis (AAU). Uveitis is an intraocular inflammation that can affect all parts of the eye. It can be idiopathic, infectious or immune mediated as part of systematic diseases such as Behcet´s disease (BD), Vogt-Koyanagi-Harada disease or HLA-B27 associated diseases. According to the main site of inflammation uveitis can be classified into anterior, intermediate, posterior or panuveitis. Anterior uveitis often shows a unilateral inflammation, which during the following recurrences may change sides, never bilateral at the same time point.

Monocytes play a very important role in the pathogenesis of the disease. AAU patients show an elevated number of monocytes, activated T cells, and high S100A8/A9 serum levels, an alarmin released by myeloid cells. A particular population of monocytes of AAU patients is characterized by the elevated expression of TNF, IL-10, and IL-23. Immune responses via the Th17 pathway play an important role in HLA-B27- associated AAU. Human Leukocyte Antigen (HLA)-B27 is a predisposition for acute anterior uveitis (AAU) but there are also patients who are HLA-B27 negative, therefore calling this disease “HLA-B27-typical AAU”. They respond to the same treatment with the exception that sulfasalazine seems to be only in HLA-B27+ patients effective.

Treatment of AAU includes topical corticosteroids (CS), mydriatic eye drops, systemic disease modifying anti-rheumatic drugs (DMARDs) such as methotrexate (MTX) or sulfasalazine (SSZ), and the more recently introduced anti-TNF antibodies. Anti-inflammatory drugs such as sulfasalazine or its metabolite mesalazine (5-ASA) are widely used for the treatment of various inflammatory diseases including uveitis for several decades, but its mechanism of action still remains unclear, but one of its suggested modes of action is toxic reactive oxygen intermediate (ROI) scavenging by neutrophils, monocytes, and macrophages. ROIs are involved in the activation of nuclear factor kappa B (NF*k*B), with sulfasalazine being a potent inhibitor of NF*k*B. Later it was postulated that the anti-inflammatory effects of 5-ASA are mediated, at least in part, by peroxisome proliferator-activated receptor gamma (PPARγ). Some other possible mechanisms described include inhibition of the platelet activating factor, and inhibition of the production of ROS. The aim of this work was to reveal the mechanism of 5-ASA on proinflammatory cytokines in PBMCs of patients with HLA-B27 associated acute anterior uveitis (AAU), and whether the effect and mechanism of 5-ASA depends on the HLA-B27 status of patients. Data clearly show that 5-ASA inhibits the transcription of proinflammatory and endoplasmatic reticulum (ER) stress associated cytokines and ER stress associated markers, (such as IL-6, IL-1β, IL-23A, TNF, ATF6 and CHOP) independently of the HLA-B27 status. This supports to treat both groups identical regarding corticosteroids, but does not explain the different response to sulfasalazine, therefore other mechanisms have to be investigated in the future.

1. I N T R O D U C T I O N:

1.1 Inflammation:

Inflammation is a defense response to exogenous and endogenous (harmful) stimuli, and conditions, such as infection and tissue injury (Majno G. and Joris I. 2004), (Kumar V. 2003). It is the most important response of the immune system, that occurs both in humans and animals. Inflammation is closely connected to many diseases, such as neurodegenerative diseases (Pinto A.et.al. 2018), cancer (Coussens L.M. and Werb Z. 2002), and cardiovascular diseases (Libby P.et.al. 2002). It plays important role in constitution of a protective response, that is crucial for the recruitment of immune cells, which mediates pathogen clearance and tissue repair (Medzhitov M .2008). Inflammation can be triggered by a variety of immune cells, including macrophages, neutrophils, dendritic cells, natural killer (NK) cells, and T and B lymphocytes (de Visser K.E. et.al. 2006). It predominantly involves the innate immune system, but in later stages also includes components of the adaptive immune system. Inflammation could be acute and chronic.

Typical symptoms of inflammation include fever, pain and dysfunction.

Immune cells, can sense pathogens and danger signals through a repertoire of different receptors, known as pattern recognition receptors (PRRs). These receptors are activated by evolutionally conserved structures known as pathogen-associated molecular patterns (PAMPs). PRRs are also activated by cellular stress signals, and markers of tissue and cell homeostasis disruption, that are known as damage-associated molecular patterns (DAMPs) (Matzinger P. 1994). DAMPs are multifunctional molecules that includes heat shock proteins (HSPs), single strand RNA (ssRNA), and small fragments of extracellular matrix that are released into the extracellular environment following tissue and cellular injury (Kwon BK.et.al. 2015). The two major and most important PRRs are TLRs (Toll-like receptors) and NLRs (NOD-like receptors) (Baccala R.et.al. 2009). Some NLRs can detect PAMPs and stimulate the expression of pro-inflammatory genes (Welter-Stahl L.et.al. 2006), while other NLRs require the participation of other PRRs that can be able to promote processing and secretion of key proinflammatory cytokines such as IL-1β and IL-18 (Dinarello C. 2006).

Skin inflammation occurs after sensing of different stimuli that include: UV irradiation, ionizing radiation, pathogens, contact with chemical irritants, allergen exposure, microbial challenge, as well as due to intrinsic defined stimuli including autoimmune responses. (Behrends U.et.al. 1994), (Fuchs J.et.al. 1998), (Martin S. 2012), (Miller JD. and Cho JS. 2011), (Rustemeyer T.et.al. 2011).

Exposure to UV light is harmful to the skin and leads to skin inflammation and to cancer. UV radiation is dangerous and has very negative effects on human health. In the year 2000, excessive exposure to UV light led to 60,000 deaths world-wide [Lucas R.et.al. 2006] . There are three types of UV light (200-400nm): UVA, UVB and UVC, but two types of UV radiation are primarily responsible for damage and inflammation in the skin: UVA (314-400nm) and UVB (280-315nm). Chronic UV irradiation results in many changes in the skin, including hyperplasia, changes in dermal structures, and alteration of pigmentation [D'Orazio J. et.al. 2013] . In solar radiation UVA is much more abundant than UVB, accounting about 95% of solar UV radiation [van Weelden H.et.al. 1988]. UVA penetrates through the epidermis into the dermis, and it is weakly absorbed by DNA and protein. In contrast, UVB does not penetrate significantly through the epidermis, and it is strongly absorbed by DNA and protein [Bijl M.and Kallenberg CGM. 2006]. Therefore UVB light is one of the most potent inducers of apoptosis in human keratinocytes.

Apoptotic keratinocytes are recognized histologically by eosinophilic cytoplasm and pyknotic nuclei [Daniels F.et.al. 1961]. Sun burn cells (SBC), as apoptotic cells induced by acute UV-exposure can be detected eight hours after UVB exposure, with maximal numbers of the cells at 24-48 hours and disappearance by 60-72 hours [Murphy G.et.al. 2001].

UVB induced apoptosis is a complex mechanism that includes different signaling pathways: apoptosis induced by direct DNA damage, death receptor mediated apoptosis and apoptosis via formation of reactive oxygen species (ROS) [Reefman E.et.al. 2005].

Apoptosis plays an essential role in response to sunburn, although it is known that apoptosis is an anti-inflammatory or immunosuppressive event [Voll R.E.et.al. 1997), (Freire-de-Lima C.G. et.al. 2006]. In human keratinocytes, apoptosis will finally result in permeabilization of the plasma membrane, and this process is known as secondary necrosis, which can lead to inflammation. However, it is believed that this will not occur under physiological circumstances due to rapid clearance of apoptotic cells [Savill J.et.al. 2002].

Mammone et al. showed that dose dependent increasing of UVB exposure causes increases in apoptosis [Mammone T.et.al. 2000]. Long exposure to UVB light leads to damage to the skin, which results in inflammation characterized by increased NF-*k*B activation, increased inflammatory cytokines, such as tumor necrosis factor (TNF), IL-6, IL-1 as well as IL-8 and IL-10.

In addition increased level of prostaglandins, reactive oxygen species (ROS), cis-urocanic acid and histamine have been observed [Ichihashi M.et.al. 2003), (Black AT.et.al. 2008), (Gludsdale GJ.et.al. 2001].

Another type of cell death, which is caspase-1 dependent, that occurs after UVB damage is pyroptosis. Pyroptosis is an inflammatory type of cell death, resulting in IL-1β and IL-18 secretion and in the release of cellular compartments [(Magna M. and Pisetsky D.S..2015)].

It is also known as gasdermin-mediated programmed necrosis [(Jinjin S. et.al.2017)]. Pyroptosis is a general innate immune effector mechanism in vertebrates [(Jorgensen I. and Miao E.A. 2015)]. Although initially identified as a type of cell death in monocytes, macrophages and dendritic cells, pyroptosis can also occur in other cell types, including CD4+ T cells, neurons, microglia, hepatocytes and haematopoietic stem/progenitor cells [(Wree A.et.al. 2014), (Doitsh G.et.al. 2014), (Lamkanfi M.and Dixit V.M. 2012)].

1.2. Inflammasome and types of inflammasomes:

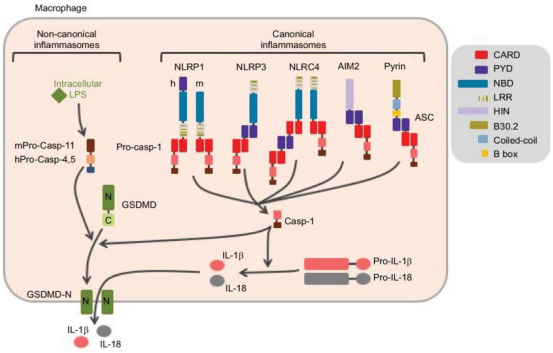
Inflammasomes are protein complexes located in the cytosol that function as platforms for the recruitment and activation of the pro-inflammatory protease caspase-1 [(Chavarría-Smith J.et.al. 2015)]. They are innate immune sensors involved in response to cytosolic pathogens or stress signals [(Lamkanfi M.et.al. 2012)]. Inflammasomes recognize intracellular PAMPs and DAMPs [(Volpe CMO.et.al. 2016)], and they are well characterized in immune cells of myeloid origin, but they are also expressed by some non-professional immune cells (for example keratinocytes) [(Broz P. and Dixit V. M. 2016)]. Inflammasomes consist of three major components: the nucleotide oligomerization domain (NOD)-like receptor (NLR), which acts as an intracellular sensor and induces complex formation [(Lomedico PT.et.al. 1984)], the adaptor protein ASC (apoptosis speck-like protein containing a caspase recruitment domain (CARD), which recruits pro-caspase-1 to the complex [(Ogilvie AC. et.al.1996)], and pro-caspase-1 [(Yazdi AS.et.al. 2010)]. Upon stimulation, inflammasome complexes assemble to process caspase-1, which activates the proinflammatory cytokines interleukin-1β (IL-1β) and interleukin-18 (IL-18), and the cytosolic protein gasdermin D (GSDMD) [(Ming Man S.et.al. 2017)]. Cleaved GSDMD translocates from the cytosol to the cell membrane where it forms pores which induce pyroptotic cell death, and permit the release of IL-1β and IL-18 into the extracellular space [(Evavold CL.et.al. 2018)]. Due to their ability to form inflammasomes the best characterized NLRs are NLRP1, NLRP3, and NLRC4. Recent studies have revealed some additional protein families that can assemble inflammasomes: the AIM2-like receptor (ALR) family, which contains a HIN200 (DNA binding domain) instead of an NLR [(Schattgen SA. and Fitzegrald K.A. 2011)] and the RIG-I-like receptor (RLR) family [(Ireton RC. and Gale M.J. 2011)].

According to the type of their activation inflammasomes can be:

1) canonical

2) non-canonical.

The canonical inflammasome is a multimodular complex that stimulates the activation of caspase-1[(Lamkanfi M.et.al. 2012)]. Canonical inflammasomes form activation platforms for caspase-1 recruitment, and they are assembled by PRRs, that belong to the nucleotide-binding oligomerization domain-like receptor (NLR) family [(Bürckstümmer T.et.al. 2009)], but also belong to Pyrin [(Xu H. et.al. 2014)], or the HIN200 protein AIM2 [(Bürckstümmer T.et.al. 2009)].

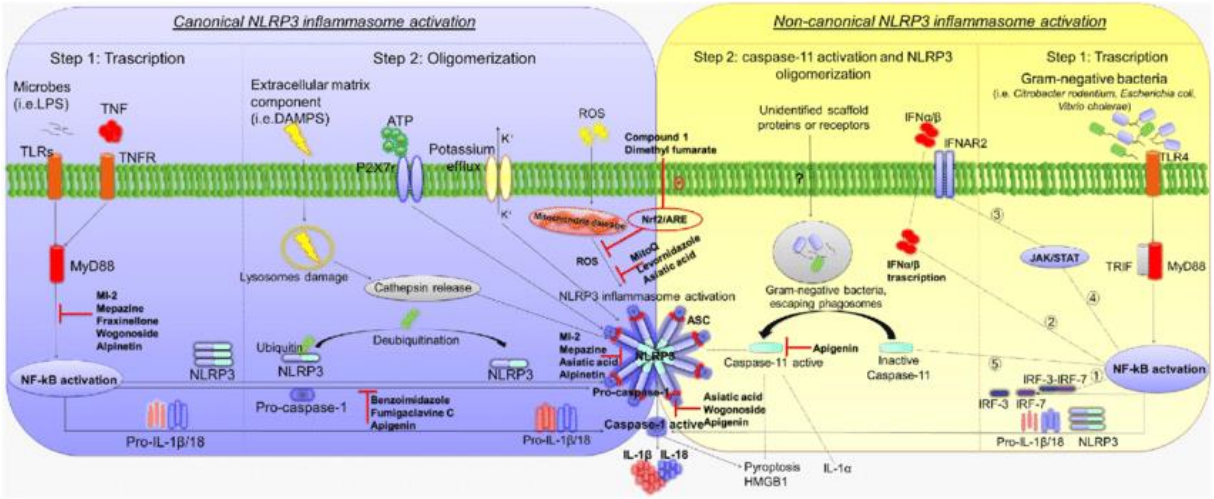


Picture 1. Canonical and non-canonical inflammasomes [(Groslambert M. and Benedicte F.P. 2018)]

Their assembly is dependent on cytosolic PRRs, that belong to the nucleotide-binding domain leucin-rich repeat (NLR) family including, NLR and pyrin domain containing receptor 1 (NLRP1), NLRP3, and NLR and caspase recruitment domain containing receptor 4 (NLRC4) [(Medzhitov R. and Janeway C.A. 1997)].

2) Non-canonical inflammasomes are inflammasomes that control the activation of caspase-11 in mice and caspase-4 and -5 in humans in response to cytosolic lipopolysaccharide (LPS). Studies have shown that non-canonical inflammasome can induce caspase-1-dependent maturation and secretion of IL-1β and IL-18, through recruitment of caspase-4/5 in human or caspase-11 in mouse [(Kayagaki N. et.al. 2011)]. Caspase-11 cannot directly cleave pro-IL-1β, but it promotes the secretion of bioactive IL-1β and IL-18 indirectly through engagement of the NLRP3 inflammasome [(Kayagaki N.et.al. 2011)].

Direct binding of cytosolic LPS promotes caspase-11-mediated pyroptosis as a host defense response, that seems to be important for disposal of macrophages that are infected with *Escherichia coli, Citrobacter rodentium, Vibrio cholerae* and other Gram-negative pathogens (Kayagaki N.et.al. 2011).



Picture 2. Scheme of canonical and non-canonical inflammasome pathway (Pellegrini C.et.al. 2017)

Non-canonical inflammasomes promote pyroptosis in a TLR4- and caspase-1-independent manner in response to infection with cytoplasmic Gram-negative bacteria (Kayagaki N.et.al. 2013). Lipid moiety of bacterial LPS has been proposed to directly interact with the CARD domain of caspase-11, -4, and -5 which then undergoes oligomerization, resulting in the activation of the caspases (Kayagaki N.et.al. 2013).

Those facts show that non-canonical inflammasome assembly is not dependent on dedicated PRRs, because caspase-11, -4, and -5 can act as receptors themselves (Ratsimandresy RA. et.al. 2013).

Many evidence shows that murine caspase-11 activation triggers an NLRP3–ASC–caspase-1-dependent signaling pathway, also known as "non-canonical NLRP3 inflammasome activation pathway," which is different from the before mentioned "canonical" NLRP3 inflammasome activation pathway" (Schroder K. and Tschopp J. 2010).

Even though it appears that caspase-4/5 and -11 can directly detect intracellular LPS derived from Gram-negative bacteria (Pellegrini C. et.al. 2017), the exact mechanism of the non-canonical inflammasome activation is not yet understood.

The most important and best characterized types of inflammasomes in humans and mice are the NLRP1, NLRP3 among NLRs and AIM2 inflammasome that belongs to ALRs.

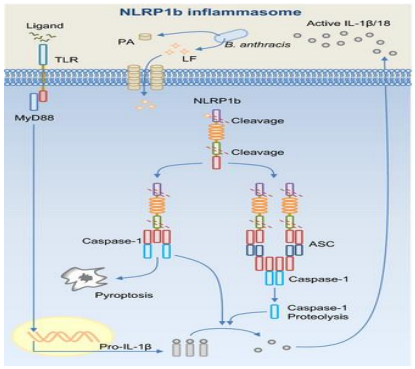
1.2.1. NLRP1 inflammasome:

The NLRP1 inflammasome was the first inflammasome to be discovered in 2002. NLRP1 was the first member of NLR family that has been shown to form a cytosolic complex named the inflammasome, (Martinon F. et.al. 2002). NLRP1-like genes are found in most, mammalian species, including primates, rodents, ungulates, and even marsupials.

The human NLRP1 protein is composed of a PYD, NBD, LRR, FIIND, and CARD domains. Unlike human NLRP1, mice carry three NLRP1 paralogs (Nlrp1a, -b, and -c), and all of them lack a PYD domain (Boyden ED. and Dietrich W.F.) 2006). NLRP1, like all NLRs, contains a central NBD and a short leucine rich repeat domain (LRR), and it also contains a domain termed a "function to find" domain (FIIND) (Martinon F.et.al. 2009) which is similar to a ZU-5 and UPA domain pair, found in non-NLR family proteins, such as PIDD and UNC5B (D'Osualdo A.et.al. 2011). This domain undergoes a spontaneous self-processing (maturation) event, that process is necessary before NLRP1 can response to stimuli (Finger JN.et.al. 2012).

NLRP1 contains two effector- recruitment domains. One of these domains is an N-terminal pyrin domain (PYD), and the other is a C-terminal caspase activating and recruitment domain (Mitchell P.S.et.al. 2019). These two effector domains are part of the death domain superfamily, and they are structurally related to each other.

It is generally believed that the PYD and CARD domains mediate homotypic interactions with other downstream PYD/CARD containing proteins, for example, ASC and Caspase 1 (Frew BC.et.al. 2012). Even though initial studies showed that the NLRP1 inflammasome could assemble spontaneously in cell lysates, more recent studies have described two natural activation ligands for NLRP1: muramyl dipeptide (MDP), a peptidoglycan fragment from both Gram positive and negative bacteria, and the lethal toxin (LeTx) produced by *Bacillus anthracis* (Faustin F.et.al. 2007). It seems that those ligands are species-specific because MDP can activate human, but not mouse NLRP1, whereas anthrax toxin has been shown to activate mouse NLRP1 (Kovarova M.et.al. 2012). Anthrax lethal toxin (LeTx) is the causative agent of anthrax disease. LeTx is a toxin composed of two components first is protective antigen (PA) and second is lethal factor (LF). LF is a zinc metalloprotease that cleaves and inactivates all mitogen-activated protein kinases (MAPKKs) (also called MKKs or MEKs), with the exception of MAPKK5 (Chavarría-Smith J.et.al. 2015).



Picture 3. Activation of the NLRP1b inflammasome by *Bacillus anthracis* Anthrax lethal toxin (Ming Man S.and Devi-Kanneganti T. 2015).

The protease activity of LF is critical for the activation of NLRP1B and CASP1-dependent pyroptosis, and it has been shown that catalytically inactive mutants of LF fail to trigger pyroptosis (Fink SL.et.al. 2008). This observation suggested that LF was unable to activate NLRP1B by acting simply as a ligand, and instead of that NLRP1B could sense the protease activity of LF, directly or indirectly.

1.2.2. NLRP3 inflammasome:

The best studied inflammasome from the NLR family, is the pyrin domain containing 3 (NLRP3, also known as NALP3 or cryopyrin) inflammasome. NLRP3 is a cytosolic pattern recognition receptor that senses microbes and endogenous danger signals. NLRP3 is known as a global sensor of PAMPs and DAMPs and forms an inflammasome in response to diverse stimuli. NLRP3 is composed of an N-terminal PYD, a central NBD, and a C-terminal LRR domain (Agostini L.et.al. 2004). The LRR domain contributes to autoinhibition of NLRP3, whereas the NBD is responsible for its oligomerization (Dowds TA.et.al. 2003). Messenger (mRNA) for Nlrp3 is mostly expressed in lymphoid organs and in organs which are highly populated by immune cells, and constitute to the first line of defense against pathogen-mediated or sterile damage (Sutterwala FS.et.al. 2006).

It is mainly expressed in immune cells notably antigen presenting cells (APCs), macrophages, dendritic cells (Baumgart DC), neutrophils in the spleen and monocytes (Zhong Y.et.al. 2013). NLRP3 inflammasome can sense a wide range of different stimuli such as ATP, toxins, RNA viruses, and crystals (Latz E.et.al. 2013).

Upon activation, the NLRP3 inflammasome oligomerizes and triggers the helical fibrillar assembly of the adapter ASC via pyrin domain PYD–PYD interactions (Latz E.et.al. 2013), (Lu A.et.al. 2014). ASC fibrils form large structures, called ASC specks (Franklin BS.et.al. 2014), (Schmidt FI.et.al. 2016) and recruit pro-caspase-1, leading to its autoproteolytic activation. Caspase-1 mediates activation and release of proinflammatory cytokines of the IL-1 family and triggers pyroptosis. Resting macrophages express insufficient amounts of NLRP3 and require a priming signal for NLRP3 inflammasome activation (Guarda G.et.al. 2011). NLRP3 expression can be induced in macrophages via MyD88- and Trif-dependent pathways (Kelley N. et.al. 2019). NLRP3 expression can also be induced in monocytes and macrophages upon exposure to inflammatory stimuli (O'Connor W.et.al. 2003), (Franchi L.et.al. 2009). There are some reports of NLRP3 expression in eosinophils and in B and T lymphocytes (Kummer JA.et.al. 2007), (Bakele M.et.al 2014), (Schroder K.et.al. 2012).

Canonical NLRP3 inflammasome:

For the activation of the canonical NLRP3 inflammasome normally two signals are needed. The first signal, known as “priming” signal requires the engagement of TLRs, and it up-regulates the expression of the NLRP3 protein, and second “activation” signal leads to activation and assembling of NLRP3 inflammasome (Bauernfeind FG.et.al. 2009), (Franchi L.et.al. 2009).

However, there is also evidences that suggest that the activation of the canonical NLRP3 inflammasome can be achieved without extensive priming (Bakele M.et.al. 2014), (Schroder K.et.al. 2012). Simultaneous exposure of unprimed mouse BMDMs to TLR and NLRP3 activators (LPS plus ATP, LPS plus nigericin, or infection with *Listeria monocytogenes*) leads to the activation of the NLRP3 inflammasome in 15 or 20 min, dependent on kinases IRAK1 and IRAK4 (Schroder K.et.al. 2012), (Lin KM.et.al. 2014). NLRP3 can sense a variety of DAMPs, including ATP, uric acid Simultaneous exposure of unprimed mouse BMDMs to TLR and NLRP3 activators (LPS plus ATP, LPS plus nigericin, or infection with *Listeria monocytogenes*), lysophosphatidylcholine, aluminum hydroxide, and bee venom leads to the activation of the NLRP3 inflammasome in 15 or 20 min, dependent on kinases IRAK1 and IRAK4, (Mariathasan S.et.al. 2006), (Hornung V.et.al. 2008) (Allam R.et.al. 2013).

There are three major molecular mechanisms that could lead to activation of the NLRP3 inflammasome. One mechanism includes ATP that is sensed by the P2X7 purinergic receptor, and results in potassium efflux and recruitment of pannexin that induces NLRP3 activation (Mariathasan S.et.al. 2006), (Pétrilli V.et.al. 2007).

Pannexin is a membrane pore that allows the delivery of extracellular PAMPs and DAMPs into the cytosol (Kanneganti TD. et.al. 2007). Although potassium efflux was proposed to be the required converging signal, there are pathways leading to the activation of the NLRP3 inflammasome that do not require potassium efflux (Muñoz-Planillo R. et.al. 2013).

A second proposed mechanism of NLRP3 activation includes crystals or large particles such as silica, asbestos, aluminium, amyloid, monosodium urate, and cholesterol (Eisenbarth SC.et.al. 2008), (Dostert C. et.al. 2008), (Duewell P. et.al. 2010). As already known, LPS which is major component of bacterial cell walls of Gram-negative bacteria binds to TLR4 and leads to inflammation and the release of cytokines (Poltorak A. et.al. 1998). Some data showed that LPS does not only bind to TLR4, but additionally activates the inflammasome without previous priming, in a TLR4 independent manner (Kelley, N. et.al. 2019). Schotte et.al reported that the lysosomal cysteine protease cathepsin B acts as a caspase-11 activating enzyme *in vitro* (Schotte P.et.al. 1998).

Chen N.et.al showed that disruption of the lysosomal membrane caused by cytoplasmatic LPS or by phagocytosis resulted in cathepsin B release, which then mediates the activation of proinflammatory caspase-11 and leads to non-canonical NLRP3 inflammasome activation (Chen N.et.al. 2018).

As third major activator of NLRP3 inflammasome reactive oxygen species (ROS) are discussed (Fubini B. and Hubbard A. 2003), (Zhou R.et.al. 2010). Recent studies postulated that the kinase NEK7 is specifically required for the activation of the NLRP3 inflammasome (Shi H. et.al. 2016). Phosphorylated NEK7 binds to the LRR domain of NLRP3 (He Y.et.al. 2016), suggesting that NEK7 may impact autoinhibition of NLRP3. Some evidence demonstrates that the NLRP3 inflammasome and cell death components regulate each other, especially in relation to the apoptotic caspase-8 (Yang Y.et.al. 2019). The activity of the NLRP3 inflammasome can be positively and negatively regulated by caspase-8. Caspase-8 and its adaptor Fas-associated protein with death domain, are required for priming and possibly activation of NLRP3 inflammasome in macrophages and DCs (Hu WH.et.al. 2010).

Caspase-8 is recruited to the ASC-inflammasome speck, indicating that it is a component of the canonical NLRP3 inflammasome complex (Ming Man Si.et.al. 2013), (Gurung P.et.al. 2014).

Direct activation of caspase-1 by caspase-8 or redundant activation of both caspase-1 and caspase-8, lead to the proteolytic processing of pro-IL-1β (Maelfait J.et.al. 2008), (Pierini R.et.al. 2012).

In mouse DCs lacking caspase-8 treated with LPS only, there is already activation of the canonical NLRP3 inflammasome (Kang TB.et.al. 2013).

This effect was mediated by the kinases RIPK1 and RIPK3, the necroptotic effector MLKL, and the phosphatase called phosphoglycerate mutase (Lu A.et.al. 2014).

There is evidence linking the NLRP3 inflammasome to necroptotic cell death. In response to necroptotic activators, the kinase RIPK3 and the necroptotic effector MLKL are required to activate the NLRP3 inflammasome and induce IL-1β release (Conos SA.et.al. 2017). In this case, MLKL oligomerizes on the cell membrane to induce pore formation, resulting in necroptosis and a reduction in the level of intracellular potassium, triggering activation of the NLRP3 inflammasome (Gutierrez KD.et.al. 2017).

Several different mechanisms of NLRP3 inflammasome activation were described, but there is just one known mechanism of its inactivation. NLRP3 reaches its inactive form via binding of its LRR domain to the ubiquitin ligase associated protein, suppressor of the G2 allele of skp1 (SGT1) (Mayor A.et.al. 2007).

NLRP3 is ubiquitinated and undergoes proteasomal degradation mediated by ubiquitin ligases, named S-phase kinase-associated protein 1– cullin–F-box protein and FBXL2 (Hye Han S.et.al. 2015).

Noncanonical NLRP3 inflammasome:

For the activation of the noncanonical NLRP3 inflammasome pathway, caspase-11 is needed (Kayagaki N.et.al. 2011). Many Gram-negative bacteria, such as *Citrobacter rodentium, Escherichia coli, Vibrio cholerae, Salmonella enterica* serovar *Typhimurium, Legionella pneumophila, and Yersinia pseudotuberculosis,* are the major activators of the noncanonical NLRP3 inflammasome pathway (Aachoui Y.et.al. 2013).

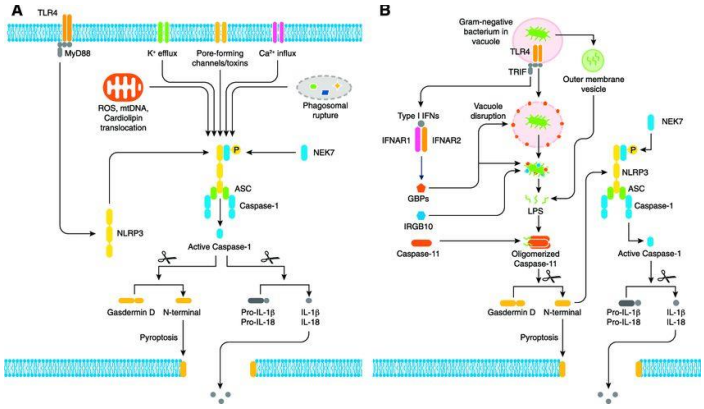
Caspase-11, a murine inflammatory caspase, senses LPS in the cytoplasm (Hagar JA.et.al. 2013), (Shi J.et.al. 2014).

Human analogs of murine caspase-11, such as caspase -4, and -5 can sense LPS (Shi J. et.al. 2014), and initiate activation of the NLRP3 inflammasome and secretion of IL-1β and IL-18 (Schmid-Burgk JL.et.al. 2015), (Baker PJ.et.al. 2015). The mechanisms which are involved in the activation of the noncanonical NLRP3 inflammasome are still not completely clear.

One of the mechanisms that was recently proposed, suggests that LPS that gained entry into the cytoplasm activates inflammatory caspases (Vanaja SK.et.al. 2016), allowing them to cleave and release directly the N-terminal fragment of gasdermin D to drive pyroptosis (Case C.L.et.al. 2013).

Activation of caspase-11 leads to a decrease in intracellular potassium levels sufficient to activate NLRP3, highlighting that the noncanonical pathway might also have a dependency on potassium efflux.

Some further studies shed light on this mechanism and showed that LPS-induced activation of caspase-11 leads to caspase-11-dependent cleavage of the large pore channel pannexin-1 (Yang D.et.al. 2015). As a result, ATP is released from the cell, activating the purinergic receptor P2X7R to drive pyroptosis (Yang D.et.al. 2015). The opening of the pannexin-1 pore also generates potassium efflux that induces IL-1β release (Yang D.et.al. 2015).



Picture 4. Mechanisms of canonical (A) and noncanonical (B) NLRP3 inflammasome activation (Mathur A.et.al. 2018)

1.2.3. AIM2 (absent in melanoma 2) inflammasome:

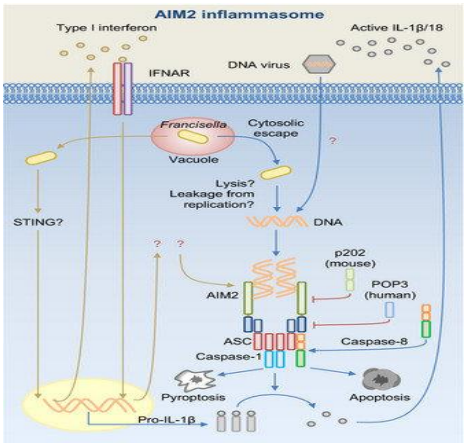
AIM2 is a cytoplasmic innate immune sensor which recognizes double stranded (dsDNA) or its analogues (Poly (dA:dT), Poly I:C, CpG)), and is able to form an inflammasome complex with ASC and pro-caspase-1 (Fernandes-Alnemri T.et.al. 2009), (Hornung V.et.al. 2009). AIM2 consists of a C-terminal dsDNA-binding HIN-200 domain and an N-terminal PYD (pyrine reach domain). The HIN domain is present in one copy in AIM2, but some ALRs such as IFI16 harbors two HIN domains at the C-terminus (Shaw N. and Liu ZJ.2014).

The AIM2 inflammasome can recognize dsDNA from a subset of bacteria and the dsDNA viruses CMV and vaccinia virus (Rathinam VAK.et.al. 2010). Infection with the malaria parasite *Plasmodium* (Kalantari P.et.al. 2014), and the fungal pathogen *A. fumigatus* also activates AIM2 inflammasome (Suzuki T.et.al. 2007). Inactive conformation of the HIN-200 domain of AIM2 interacts with the PYD in an autoinhibitory fashion (Jin T.et.al. 2013).

The release of the AIM2-PYD which happens upon dsDNA binding allows oligomerization of AIM2 along the DNA [(Morrone SM.et.al. 2015)]. PYD facilitates self-oligomerization of AIM2 and induces activation of the AIM2 inflammasome [(Lu A.et.al. 2014)].

Upon activation, AIM2 binds and recruits ASC via PYD-PYD interactions [(Bürckstümmer T.et.al. 2009), (Fernandes-Alnemri T.et.al. 2009)].

AIM2 expression is mostly detected in the spleen, small intestine, and in peripheral blood [(Cridland JA.et.al. 2012)]. Expression of AIM2 can be rapidly induced by treatment that promote type I IFNs [(Landolfo S.et.al. 1998)].



Picture 5. Activation of the AIM2 inflammasome. [(Ming Man S.et.al. 2015)]

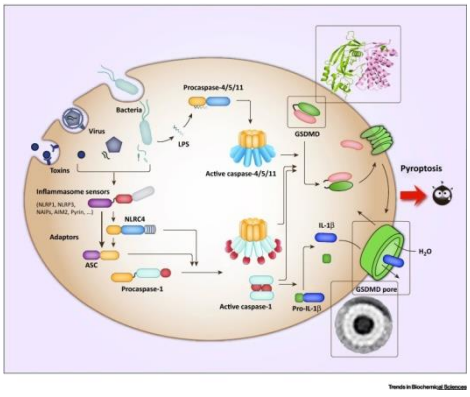
It has been reported until now that just the few DNA viruses activate AIM2. Among them are the mouse cytomegalovirus and Vaccinia virus [(Rathinam V.A.K.et.al. 2010)]. Also AIM2 cleaved caspase-1, and cleaved IL-1β were detected in the skin lesion of patients infected with Human papillomavirus 16 (HPV16) [(Reinholz M.et.al. 2013)].

Primary human keratinocytes silenced for AIM2 and transfected with HPV16 DNA, display a great reduction of IL-1 and IL-18 [(Reinholz M.et.al. 2013)].

Abundant cytosolic DNA observed in keratinocytes in psoriatic lesions behave like a “disease-associated molecular pattern” that can elicit AIM2 inflammasome leading to IL-1β maturation and the autoinflammation signs [(Dombrowski Y.et.al. 2011)].

1.2.4. Caspase-1 and pyroptosis:

An activated inflammasome is necessary for Caspase-1 recruitment, leading to cleavage of pro-IL-1β and pro-IL-18 into their active forms (Martinon F.et.al. 2004). The function of caspases is best studied in apoptosis where they play an essential role in the activation and implementation of cellular demise (Martinon F.et.al. 2007). Members of caspase protein family such as human caspase-1, -4, and -5 and murine caspase-1, -11 and -12 are involved in the processing and secretion of pro-inflammatory molecules and are known as "pro-inflammatory caspases" (Nicholson D.W. 1999).
It was shown that UVB radiation can induce inflammasome activation and inflammasome-dependent IL-1 activation and secretion, through membrane pore formation by Gasdermin D (GSDMD) (Feldmeyer L.et.al. 2007), leading to pyroptosis which can also be induced by formation of an protein structure called pyroptosome (Fernandes-Alnemri T.et.al. 2007). GSDMD contains 480 amino acids which forms two domains, N-terminal gasdermin N-domain and C-terminal gasdermin C-domain, and they are linked by a long loop. Activated caspase-1 and caspase-11 cleave GSDMD at an aspartate site in the long loop (Shi J.et.al. 2017).
All characteristics of the pyroptosis are a result of caspase-1 activity and include DNA fragmentation, nuclear condensation, lysosome exocytosis, disappearance of organelles, pore formation in the plasma membrane, cellular swelling, and disintegration of plasma membrane (Magna M.et.al. 2015).



Picture 6. Pyroptosis pathway and scheme of Gasdermin D (GSDMD) pore formation (Shi J.et.al. 2017)

Pyroptotic cells can also release HMGB1 (high mobility group box 1). HMGB1 is a highly conserved non-histone nuclear protein, which functions as a DNA binding element in the nucleus.

HMGB1 however, can serve as damage-associated molecular pattern (DAMP) in the extracellular space, and is an important component of immune complexes [(Magna M.et.al. 2015), (Xu J.et.al. 2014)]. HMGB1 is released from both cultured keratinocytes as well as keratinocytes present in murine skin following UVB exposure [(Johnson KE.e.t.al. 2013)].

1.2.5. Inflammasome and disease:

Defects in normal regulation of the inflammasome causes many different diseases. Some studies found that the activation of the NLR inflammasome family is associated with the pathogenesis of several diseases including type 1 and type 2 diabetes, multiple sclerosis (MS), rheumatoid arthritis (RA), and Systemic Lupus Erythematosus [(Duewell P.et.al. 2010)].

Hereditary defects in inflammasome (usually gain of function mutations in inflammasome genes) can cause rare types of defects called inflammasompathies. Many immune and autoimmune diseases and cancer are associated with dysfunctions and mutations in NLRP1 inflammasome. Single nucleotide polymorphisms (SNPs) in the NLRP1 gene are associated with autoimmune or autoinflammatory diseases including vitiligo, rheumatoid arthritis, systemic sclerosis, Crohn's disease, and melanoma [(Finger JN.et.al. 2012)]. Mutation in the MEFV gene lead to increased caspase-1 activity and increased levels of IL-1β [(Park H.et.al. 2012)].

The most common inflammasompathies are Familial Mediterranean fever (FMF), cryopyrin-associated periodic syndromes (CAPS), that includes three clinical forms: cold-associated autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS) and chronic infantie neurological cutaneous articular syndrome (CINCA) [(Goldbach-Mansky R. 2011)].

CAPS are autosomal dominantly inherited inflammsompathies that are caused by a gain of function mutation in the NLRP3 gene (also known as CIAS1) on chromosome 1q44, that encodes cryopyrin protein which is the key molecule of NLRP3 inflammasome [(Miyamae T. 2012)]. Symptoms of this disorders are recurrent episodes of fever and serosal inflammation due to increased production of IL-1β and can be successfully treated with the IL-1 receptor antagonist IL-1ra or Canakinumab [(Dinarello CA.et.al. 2012)] which is an antibody directed against IL-1beta.

In patients who harbor gain of function mutations in NLRP3, this inflammasome is constitutively activated. This leads to persistent IL-1β and IL-18 secretion by myeloid cells and to the systemic inflammation.

Dysfunctions in NLRP3 inflammasome contribute to the chronic liver disease and cirrhosis (Szabo G. and Csak T. 2012). Furthermore, inflammasomes have been directly involved in autoimmunity. For example, NLRP1 is overexpressed in T-cells and Langerhans cells, presented in the vitiligo skin, leading to increased IL-1β production and activation of the Th17 response (Wang CQ.et.al. 2011).

Also NLRP3 expression and NLRP3-mediated IL-1β secretion are increased in RA patients (Choulaki C.et.al. 2015), and also NLRP3 is involved in the pathogenesis of experimental autoimmune encephalomyelitis (Inoue M.et.al. 2013).

1.3. Apoptosis speck like protein (ASC, TMS1)-structure and function:

ASC apoptosis speck like protein (Andrade F.et.al.2005), is a 22 kDa protein, that was first discovered in 1999 by Masumoto et.al. (Masumoto J.et.al. 1999). It contains three exons composed of 195 amino acids (aa). ASC is composed of two well-defined structural domains the PYD (pyrine reach domain) and the CARD (Caspase recruitment domain), which are connected by a 23 aa linker region. The linker region restrains flexibility and inter-domain dynamics between PYD and CARD (Davis BK.et.al. 2011).

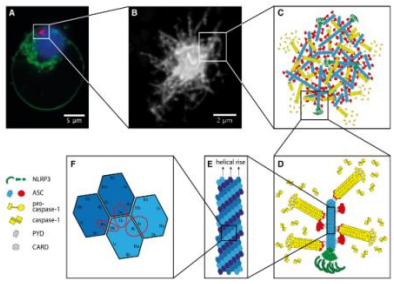
ASC is highly expressed in the spleen and lungs, but also in the digestive tract, skin, urinary bladder, and bone marrow (Masumoto J.et.al. 2001). Peripheral blood leukocytes show high expression of ASC. Among them, CD14 monocytes have the highest expression levels of ASC, whereas polymorphonuclear cells and CD3 T lymphocytes express lower levels of ASC (Masumoto J.et.al. 2001), (Conway KE.et.al. 2000). ASC protein is constitutively expressed in most cell lines of the myeloid lineage (e.g.,THP-1, HL-60, or U937). Other cell lines, such as Jurkat T cells, Burkitt's lymphoma, or HeLa, did not express ASC (Pelegrin P.et.al. 2008). Regarding its localisation in the cell, many publications report that in resting cells ASC is distributed throughout the cytosol, (Masumoto J T. et.al. 2001), (Conway KE.et.al. 2000), while some report a both cytosolic and the nuclear presence (Fernandes-Alnemri T.et.al. 2007). Other even report ASC being mainly in the nucleus (Martin BN.et.al. 2014), (Bryan NB.et.al. 2009).

ASC was originally described to have a function as a pro-apoptotic protein (Masumoto J.et.al. 1999) and (Conway KE.et.al. 2000), but further studies have revealed that ASC also plays an important role in cell death, which is very important in cancer (Trang Ha Thi H., and Hong S. 2017).

ASC is soluble as a monomer at low pH and in solution with chaotropic agents, but it can form long and well-defined fibrils at higher pH (De Alba E. 2009).

Also at physiological potassium concentrations, ASC remains soluble, whereas reducing the potassium to half-physiological concentration leads to ASC oligomerization at 37 ^{o}C but not at 4 ^{0}C (Groß CJ.et.al. 2016).

The ASC fibrils are highly conserved between human and mouse (Sborgi L.et.al. 2015). It has been shown that ASC oligomers „prefer" to form disk-like particles rather than filaments, with a diameter of 12 nm and a height of 1 nm (De Alba E. 2009). In cells which are not activated, ASC is found in its soluble form, and high thermodynamic energy barrier prevents spontaneous oligomerization (Lu A.et.al. 2014). However, inflammasome sensors are capable to form an oligomeric PYD cluster, which recruits the first ASC molecules to form a helical filament (Lu A.et.al. 2014).



Picture 7. Structure and assembly of ASC specks (A) (Hoss F.et.al. 2017)

It is not yet clear whether filamentous structures indeed make up the ASC specks and if they occur at physiological conditions (Cai X.et.al. 2014). ASC also enhances inflammation, since its recruitment of pro-caspase 1 leads to the cleavage and secretion of mature IL-1β (Wang L.et.al. 2002), (Srinivasula M.et.al. 2002).

1.3.1. Assembly and function of ASC specks:

As already mantioned ASC consists of two conserved domains: a pyrin reach domain (PYD) and a caspase recruitment domain CARD (Franklin B.S. et.al. 2018). ASC has one central function, being the central adapter for the inflammasome formation (Wang L.et.al. 2002).

The pyrin domain also functions as a sensor and can assemble inflammasomes [(Latz E.et.al. 2013), (Broderick L.et.al. 2015), (Dick MS.et.al. 2016)]. Sensor proteins induce ASC to form long filaments through oligomerization with other ASC proteins via interactions of the ASC-PYD domain. Pro-caspase-1 contains a CARD domain that plays a crucial role in the recruitment of pro-caspase-1 to the ASC filament, as well as for the oligomerization with other pro-caspase-1 molecules. [(Dorfluenter A. et.al. 2015)].

Using this mechanism, several units of pro-caspase-1 can be recruited to the ASC speck and could auto-catalyze their cleavage at two positions, thereby generating one p20 and one p10 subunit per one pro-caspase-1 molecule. This two p20 subunits together with two p10 subunits form an active heterotetramer that processes pro-IL-1β to produce mature IL-1β [(Broz P.et.al. 2016), (Guo H.et.al. 2015)]. Protein fragments of ASC–PYD alone and ASC–CARD alone, could both form fibrils with similar structure when expressed separately [(Richards N.et.al. 2001)], it was shown in some data that the ASC–PYD and ASC–CARD do not only form homotypic interactions, but also heterotypic interactions, when expressed as single-domain fragments [(Zhihao, X. et.al. 2021)]. However, the most recent studies indicate that they form mutually exclusive filaments of only one domain fragment [(Sahillioglu AC.et.al. 2014)].

This phenomenon supports the current model of speck formation [(Sborgi L.et.al. 2015), (Lu A.et.al. 2016)]. The ASC speck is composed of multiple filamentous structures which bind together and form a molecular globular complex that looks like a bird's nest with filaments placed in the periphery [(Richards N.et.al. 2001)]. Full-length ASC filaments have a much rougher surface compared to the smooth surfaces of the PYD-only filament [(Sborgi L.et.al. 2015)].

Those findings indicates that the CARDs are located on the outside of the filaments. The two domains of ASC are not only independently of each other in the monomeric state [(De Alba E.et.al. 2009)], but also in filaments [(Schmidt FI.et.al.2016)].

The formation of ASC specks is regulated via Popeye domain-containing protein (POPs) or through the post-translational protein modifications such as ubiqitination and phosphorylation [(Yang J., et.al.2017)].

1.3.2. ASC in health and disease:

ASC protein dysfunctions and modifications play an important role in many diseases including cancer [(Kelley N.et.al 2019)]. Formation of ASC specks correlates with chronic inflammatory diseases. It has been shown that ASC is down-regulated in numerous human cancers, suggesting its role as a tumor-suppressor [(Levine JJ.et.al. 2003), (Stone AR.et.al. 2004), (Riojas MA.et.al. 2007), (Collard RL.et.al. 2006)].

It was shown that in many disease models, the epigenetic regulation of ASC/TMS1 expression is very important in the function of major host defense systems, cellular housekeeping, and carcinogenesis [(Salminen A.et.al. 2014)]. Ippagunta et al. revealed an independent role of ASC in T cell priming in a murine model of collagen-induced arthritis [(Ippagunta SK.et.al. 2010)].

Drexler et.al., demonstrate that the conditional knockout of ASC/TMS1 in keratinocytes or bone marrow-derived macrophages in an inflammatory SCC model (DMBA/TPA) promotes tumorigenesis in ASC deficient keratinocytes, but suppresses tumor formation by lacking ASC in myeloid cells [(Drexler SK.et.al. 2012)]. Increased expression of human IL-1β in the stomach induces spontaneous gastric inflammation and tumorigenesis [(Tu S.et.al. 2008)].

In inflammatory diseases, extracellular ASC specks accumulation was detected in a patient with COPD [(Franklin BS.et.al. 2014)], a chronic inflammatory airway disease in which inflammasome activation and IL-1β play a significant role [(Botelho FM.et.al. 2011), (De Nardo D.et.al. 2014)]. ASC plays a role in kidney damage in autoinflammatory diseases such as Familial Mediterranean fever.

1.4. Behcet's disease and Uveitis

1.4.1. Behcet's disease:

Behcet's disesase is a chronic systematic inflammatory disesase, first described by Turkish dermatologist Hulusi Behçet in 1937, that can affect multiple parts of the body [(Baumgart D.C. and Sandborn W.J. 2012)]. BD is characterized by recurrent oral aphthous, genital ulcers and numerous systematic manifestations such as: cutaneous, ocular, neurologic, articular and gastrointestinal involvement [(Boualia E.et.al 2015)]. BD also affects blood vessels of all kinds and sizes [(Ciccarelli F. et.al. 2014)].

Behcet's disease usually affects men and women in their 20s and 30s, but the disease is usually more common in men. BD also can affect children before the age of 16 years. In childhood-onset gastrointestinal symptoms of disease are more common [(Koné-Paut I. 2016)].

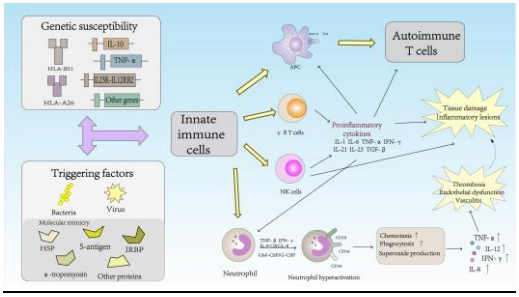
The etiopathogenesis of BD is not well understood, but microbial agents, environmental functions, genetic predisposition, endothelial cell dysfunction, neutrophil hyperfunction, and autoimmune mechanisms are reported to play the major role (Pineton de Chambrun M.et.al. 2012), (Özgüçlü S.et.al. 2019).

Although the etiology of the disease is still unknown, BD is closely related to the high prevalence of HLA-B51 (human leukocyte antigene B51) allele (Gul A. and Ohno S. 2012), than increased expression of heat shock protein 60 (HSP 60) and Th1 dominant immune responses (İnal EE.et.al. 2015).

There is a lots of data showing that autoantigens play a key role in the development of BD via molecular mimicry, (Pineton de Chambrun M.et.al. 2012). This molecular mimicry is based on sequence homology between microbial and human HSP peptides (Ergun T. et.al. 2001), (Direskeneli H. and Saruhan-Deriskeneli G. 2003).

Interaction among genetics and environmental factors play an essential role in the pathogenesis of BD. The environmental trigger factors for BD include bacterial and viral infections, and also presence of abnormal autoantigens.

There is also evidence that non-infectious neutrophil activation is associated with BD (Suzuki Kurokawa M. and Suzuki N. 2004).



Picture 8. The role of innate immune cells in the pathogenesis of Behcet's disease (Tong B.et.al. 2019).

Proinflammatory cytokines like IL-1, IL-6 and TNFα were found in the ocular fluid of patients with BD and it is believed that they are major inflammatory mediators responsible for pathogenesis of disease (Wakefield D. and Lloid A. 1992).

Some studies that were published recently addressing the role IL-37 (member of IL-1 cytokine family) in BD. It might contribute to manifestations with moderate clinical symptoms [(Özgüçlü S.et.al. 2019)].

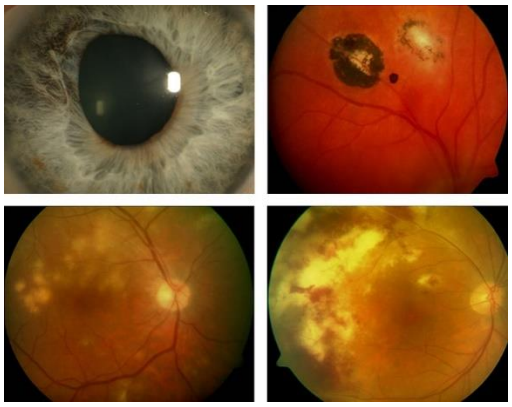
BD may cause either anterior uveitis (inflammation in the front of the eye) or posterior uveitis (inflammation in the back of the eye), and sometimes causes both at the same time [(Tugal-Tutkun I. 2009)].

1.4.2. Uveitis:

Uveitis represents a group of conditions defined as infectious or noninfectious inflammation of the uvea, that can affect one or both eyes [(Darell RW.et.al. 1962)]. The term "uveitis" is used because the diseases often affect a part of the eye called the “uvea”, but uveitis not only affects the uvea, it can also affect the lens, retina, optic nerve, and vitreous body. Uveitis can be idiopathic or related to systemic inflammatory disorders including Behcet’s disease, [(Kornbluth A.and Sachar D.B. 1997)] Vogt-Koyanagi-Harada disease, sarcoidosis and inflammatory bowel diseases and it is also common as a manifestation of spondyloarthropathies (SpAs), ankylosing spondylitis (AS), reactive arthritis (ReA) and psoriasis arthritis (PsA) [(Jabs DA and Rosenbaum JT. 2001)].

According to the main site of inflammation uveitis is classified into anterior, intermediate, posterior or panuveitis [(Jabs DA.et.al. 2005)]. Uveitis primarily affects people between 20-60 years old, and occurs in about 33% of people with SpA [(Zeboulon N.et.al. 2008)].

In general there is no gender prevalence, but in some for example juvenile idiopathic arthritis related uveitis is more common in females and HLA-B27 associated uveitis is more common in males [(Amur S.et.al. 2012)].



Picture 9. Typical types of infectous uveitis: Herpses virus infection (upper left), iris damage and increase in intraocular pressure (left down), toxoplasmosis with typical scars; these can become active again after years of rest (upper right), and Tuberculosis with retinal infiltrates, but can affect all structures of the uvea (right down). (https://www.augenklinik-sulzbach.de/patienten/52-behandlungsspektrum/uveitis-zentrum/263-ursachen-einer-uveitis).

A major complication of the disease can be blindness, due to secondary complications such as vasculitis, macular edema, cataract, glaucoma, and optic nerve atrophy (Onal C.et.al. 2011). Characteristic signs of uveitis include cellular infiltration and fibrin formation in the anterior chamber (AC) and spill-over of infiltrating cells into the anterior vitreous (Agraval RW. et al. 2010).

1.5.3. Acute anterior uveitis (AAU):

Acute anterior uveitis is acute inflammation of the iris and/or ciliary body (Zeboulon N.et.al. 2008), in the general population, uveitis accounts for approximately 75-90% of cases in Western countries (Chang JH. et.al. 2005). The major cause of uveitis still remains unknown, but human leukocyte antigen (HLA)-B27 seems to play important role in pathogenesis of acute anterior uveitis (Chang JH.et.al. 2005).

HLA-B27-associated AAU is a type of uveitis occuring in a proportion of approximately 50% of all cases of anterior uveitis in Western Europe and the USA (Haroon M.et.al. 2015).

It's annual incidence is about 12/100,000 up to 23/100,000 in the population in Finland, where a high frequency of HLA-B27 in the population has been found (Chang JH.et.al. 2005). HLA-B27 allele is the most frequently associated with human acute anterior uveitis. Despite the significant association between HLA-B27 and AAU, the great majority of persons carrying the HLA-B27 allele are healthy, and only about 1% develop AAU (Linssen A.et.al. 1991).

The HLA-B27 genotype is also independently associated with isolated cases of AAU, where it increases the risk of AAU by 23-fold and also homozygosity for HLA-B27 doubles the risk for AAU (Robinson PC.et.al. 2016), (Linssen A.et.al. 1991).

Picture 10. Acute anterior uveitis (AAU)
(https://www.atlasophthalmology.net/photo.jsf?node=7850&locale=en)

AAU associated with HLA-B27 allele has characteristic clinical features, including a young age of onset (peak 36 years), male preponderance, mostly unilateral or unilaterally alternating uveitis flares, nongranulomatous appearance, and frequent recurrences (Zeboulon N.et.al. 2008), (Braakenburg AMD.et.al. 2008).

Typical symptoms of AAU are redness, periorbital pain, photophobia, and blurred or decreased vision (Eneh AA. 2010). The majority of HLA-B27-positive [acute anterior uveitis (AAU)] patients develop clinically symptoms that include acute symptomatic onset of flare and a recurrent disease course characterized by a massive cellular ocular infiltrate during uveitis relapse.
Somethimes disease course may result in complications such as posterior synechiae formation, cataract, band keratopathy, secondary ocular hypertension or glaucoma, papillitis, and cystoid macular edema (CME) (D'Ambrosio EM.et.al. 2017).
HLA-B27 AAU may occur as a solitary condition, but it is usally associated with HLA-B27-associated spondyloarthropathy (SpA) (Brewerton DA.et.al. 1973), (Monnet D.et.al. 2004).
The frequency of SpA in patients with AAU is approximately 50%, whereas AAU has been reported with the freqency of about 25–40% of patients with SpA (Linder R.et.al. 2004).

In contrast, uveitis in HLA-B27-negative [idiopathic anterior uveitis (IAU)] patients tends to develop a clinically less fulminant, more chronic, and typically asymptomatic disease course (Kasper M. et.al. 2018). IAU patients showed increased frequency of CD56- and CD163-positive monocytes and also both granulocytic myeloid-derived suppressor cells and Th17 cells during active uveitis (Kasper M. et.al. 2018).
In contrast to HLA-B27 negative (IAU), AAU patients showed an elevated frequency of monocytes, activated T cells, and elevated S100A8/A9 serum levels an alarmin during clinically quiescent disease (Kasper M. et.al. 2018).

Those data suggested that monocytes could possibly play a very important role in pathogenesis of disesase. In juvenile idiopathic arthritis-associated uveitis (JIAU) or non-infectious uveitis monocytes are involved in the course of the disease (Walscheid K. et.al. 2018).

This monocytic population in particular is characterized by elevated expression of TNF-α, IL-10, and IL-23-/-, as well as an increased exertion of effector functions toward T cells (Krasselt M.et.al. 2013). Monocytes of AAU patients were phenotypically stable during uveitis activity (Kasper M. et.al. 2018). IAU patients showed increased frequencies of CD56+ monocytes during uveitis activity, and such an increase in CD56+ monocytes is also present in patients suffering from Crohn's disease or rheumatoid arthritis (Sconocchia G.et.al. 2005).

Furthermore, CD163+ monocytes were increased and elevated frequencies of granulocytic CD11b+ CD14− CD15+ CD33+ (MDSC), were found in IAU patients during uveitis activity and might be linked to the increased presence of Th17 cells in IAU (Kasper M. et.al. 2018).

Elevated frequency of CD56+ and CD163+ monocytes, granulocytic MDSC, and Th17 cells during uveitis activity represents unique features in IAU patients, suggesting that Th17-driven immune response play important role in HLA-B27- associated AAU (Grip O.et.al. 2007).

The therapy against AAU include topical corticosteroids (CS), mydriatic eye drops, and also disease modifying anti-rheumatic drugs (DMARD) such as methotrexate (MTX) or sulfasalazine (SSZ), and also more recently introduced anti-TNF agents (anti-TNF antibodies) (Fabiani C. et.al. 2016).

1.5.4. Sulfasalazine (SSZ):

Sulfasalazine is an antiinflammatory drug that was synthesized in 1942 as combination of the antibiotic sulfapyridine, and an anti-inflammatory agent, 5-aminosalicylic acid (5-ASA, mesalazine) (Grange LK.et.al. 2014). It is used for the treatment of inflammatory bowel disease (IBD), ulcerative colitis (UC), rheumatoid arthritis and Crohn's disease (CD), and it belongs to disease-modifying antirheumatoid drugs (DMARD) (Pinals RS.et.al. 1986) .

Sulfasalazine consists of 5-aminosalicylic acid (5-ASA, mesalazine) and sulfapyridine joined by an azo bond (Ye B. and van Langenberg DR. 2015). The sulfapyridine component is the carrier of the 5-ASA to the colon (Karagozian R.et.al. 2007).

The azo bond is broken down by gut microorganisms and the 5-ASA exerts its anti-inflammatory action, while the sulphur moiety is slowly absorbed and excreted in the urine. 70% of sulfapyridine is absorbed opposite to 5-ASA, which appears mostly unchanged in the feces (Sutherland LR.et.al. 1991).

The mode of action of sulfasalazine still remains unclear but many pharmacological and biochemical effects have been described, including immunosuppressive and modulatory actions on lymphocytes and leukocyte functions [(Hirohata S. et.al.2002)].

However, it is still unclear which actions are essential for the observed clinical outcome. Sulfasalazine can inhibit granulocyte activation, as assessed by superoxide production [(Stenson WF.et.al. 1984)] and IL-2 synthesis and lymphocyte proliferation as well as IL-1 production by monocytes [(Carlin G.et.al. 1989), (Gaginella TS. and Walsh RE. 1992)]. One of the suggested modes of action of sulfasalazine, and 5-ASA includes toxic reactive oxygen intermediate (ROI) scavenging by neutrophils, monocytes, and macrophages [(Grisham MB. and Granger DN. 1988)].

ROIs play an important role in activation of nuclear factor kappa B (NFkB) and sulfasalazine is a potent inhibitor of the transcription factor NFkB [(Wahl C.et.al. 1998)], and treatment with sulfasalzine inhibits its translocation into the nucleus [(Wahl C.et.al. 1998)].

It has been shown by Wahl C et.al., that sulfasalazine inhibits TNFα-, LPS-, or PMA induced activation of NF*k*B and suppresses NF*k*B–dependent transcription [(Wahl C.et.al. 1998)].

Inhibitory actions of sulfasalazine were specifically directed against NF*k*B activation, since it did not inhibit DNA binding activity and transactivation of some other transcription factors like AP-1 [(Wahl C.et.al. 1998)]. Sulfasalazine inhibitory effect is the result of prevention of nuclear translocation of RelA, due its ability to inhibit I*k*Bα phosphorylation and subsequent degradation. Sulfasalazine exerts either a direct effect on an I*k*Bα kinase or on another upstream signal [(Miller JD.et.al. 1985)].

1.6.7. 5-Aminosalicylic acid (5-ASA, mesalazine, mesalamine):

5-aminosalicylic acid (5-ASA) was discovered in 1977 as a therapeutically active moiety of sulfasalazine (SSZ) and was launched for topical and oral therapy of ulcerative colitis (UC) in 1984 [(Dignass A.et.al. 2012)]. It has been also widely used in treatment of inflammatory bowel disease and rheumatoid arthritis [(Feagan BG. and Sandborn WJ.002)].

Mesalazine is, anti-inflammatory compound, that acts locally within the colonic mucosa to reduce inflammation, and is recommended as the first-line treatment for patients with active mild-to-moderate UC and despite of that it was introduced in UC therapy 30 years ago, it still remains one of the major drugs for treatment in both indications [(Sutherland L.and MacDonald JK. 2003)].

It is therefore recommended for induction of remission, oral 5-ASA alone or in combination with topical application to menage active mild to moderate left-sided or extensive UC [(Kornbluth A. et.al. 1997)].

5-ASA together with sulfapyridine is the part of sulfazalacine molecule. 5-ASA bound to sulfapyridine via a diazobond, and that bond is cleaved by bacterial azoreductases in the colon, resulting in release of the two components and thus deliver 5-ASA to the intended site of action (Ham M. and Moss CA. 2012). Sulfapyridine is metabolized in the liver and excreted in the urine, whereas in the case of the 5-ASA, it is acetylated by the colonic epithelium (Rochester J. and Abreu MT. 2005).

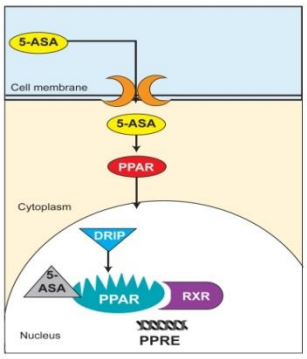
Ingested 5-ASA is acetylated in intestinal epithelial cells by the N-acetyltransferase 1 (NAT1) to form the inactive metabolite N-Ac-5ASA (Allgayer H.et.al. 1989). This metabolite is then either absorbed and excreted in the urine, or secreted back into the colonic lumen and excreted in the faeces (D'Incà R.et.al. 2013).

Some ammounts of 5-ASA are also absorbed directly into the bloodstream and undergo metabolism by the NAT1 enzyme in liver cells, followed by the elimination in the urine (Schröder H. and Campbell DE. 1972), (Myers B.et.al. 1987). Mesalazine is almost completely absorbed from the small intestine, and just a small percentage of intact drug reaches the diseased overtaken target regions (Peppercorn MA. and Goldman P. 1973).

To solve this problem, different approaches have been applied to achieve selective drug delivery to the diseased regions.

Until now various mechanisms of 5-ASA action have been proposed, but exact mechanism still remains poorly understood. One of those mechanisms that has been proposed, includes inhibition of the activity of the nuclear factor-kappa B (NF*k*B) pathway (Egan LJ.et.al. 1999).

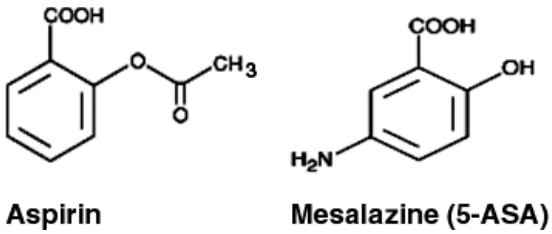
Some evidences suggesting that the anti-inflammatory effects of 5-ASA are mediated, at least in part, by peroxisome proliferator-activated receptor gamma (PPARγ) (Desreumaux PD. 2008).



Picture 11. 5-ASA binds to cytoplasmic PPARγ, inducing its translocation to the nucleus and conformational change. This re-arrangement promotes the recruitment of the co-activator DRIP and the assembly of the active heterodimer PPAR-RXR, leading to the activation of PPRE (Desreumaux PD. 2008).

PPARγ is a nuclear receptor that modulates the inflammatory response of monocytes and macrophages by inhibiting the production of nitric oxide (iNOS) and cytokines produced by macrophages such as tumor necrosis factor TNF-α, interleukin IL-1, and IL-6 (Parameswaran N. and Patial S. 2010). Novel PPARγ modulators having similarities to 5-ASA have been developed, one of them is (GED-0507-34-Levo) (Bertin B. et.al. 2013).

Furthermore, Santucci L.et.al showed that mesalazine may have antioxidant properties, that reduce tissue injury and play a role in inhibition of T cell activation and proliferation (Santucci L.et.al. 2005). Mesalazine shares structural similarity with acetylsalicylic acid (ASA) differing from it only by the presence of an amino group at position 5 of the benzene ring **(Picture 12).**



Picture 12. Similar structure of acetylsalicylic acid (ASA) and mesalazine (Chu EC.et.al. 2007)

Mezalazine (5-ASA) may have therefore antineoplastic and chemopreventive properties similar to acetylsalicylic acid (ASA)(Williams C.et.al. 2011). Limited studies on animals, colon cancer cell lines and human tissues have also demonstrated antiproliferative and pro-apoptotic effects of mesalazine (Narisawa T. and Fukaura Y. 2003).

The molecular mechanisms responsible for the potential chemo preventive effect of mesalazine are not fully understood, but it seems to include both COX-dependent and COX-independent mechanisms (Allgeyer H. 2003).

In vitro experiments in different cell systems and also using cultures from normal and neoplastic cell lines, showed that mesalazine inhibits COX-1 and COX-2, NF*k*B activation, MAP kinases, cell cycle progression and Bcl-2 (Bantel H et.al. 2000).

2. MATERIALS AND METHODS:

2.1. Chemicals and reagents:

2.1.1. Cell culture material:

1) CnT-PR cell culture media (CELLnTEC Advanced Cell Systems, Bern, Switzerland).
2) RPMI 1640 media (Biochrom, Berlin, Germany).
3) Biocoll reagent gradient (Biochrom, Berlin, Germany).
4) Dulbeco´s Phosphate Buffer Saline (Sigma Aldrich, Darmstadt, Germany).
5) Fetal Bovine Serum (FBS), (Gibco, Thermo Fisher Scientific, Karlsruhe, Germany)
6) Trypsin/EDTA solution, 0,05%/0.02% (Biochrom, Berlin, Germany).
7) T75 cell culture flasks (Greiner Bio-One GmbH, Frickenhausen, Germany).
8) Cell well plates (Greiner Bio-One GmbH, Frickenhausen, Germany).
9) Chamber polystyrene slides (8 chamber), (Corning Incorporated, NY 14831, USA).
10) Dispase II (neutral protease, grade II) solution, (Roche, Basel, Switzerland).
11) Petri dishes for cell culture 100x15mm (Corning Incorporated, NY 14831, USA).
12) Millipore cell culture filters 100µM (Sigma Aldrich, Darmstadt, Germany).
13) Poly-L-Ornithine Solution (0.01%), (Sigma Aldrich, Darmstadt, Germany).
14) ACK lysis buffer (Lonza, Verviers, Belgium).
15) HBSS media (Hank's Balanced Salt Solution), (Sigma Aldrich, Darmstadt, Germany).

2.1.2. Reagents and antibodies:

1) 1.2mM Calcium chloride ($CaCl_2$)
2) Pan-Caspase inhibitor-Z-VAD-FMK (InvivoGen, San Diego, CA 92121-USA).
3) MCC950 (NLRP3-inflammasome inhibitor), (InvivoGen, San Diego, CA 92121-USA).
4) Poly (dA:dT) naked-Double stranded B-DNA (InvivoGen, San Diego, CA 92121-USA).
5) Mezalazine (5-ASA), (Sigma Aldrich St.Louis, USA).
6) Lypopolisacharide (LPS), (InvivoGen, San Diego, CA 92121-USA).
7) ATP (InvivoGen, San Diego, CA 92121-USA).
8) Thapsigargin (Tsg), (Tocris, Bristol, UK)
9) Anti-Asc, rabbit pAb (AL177), antibody (Adipogen AG, Liestal, Switzerland).
10) Normal Donkey Serum (Jackson ImmunoResearch, Hamburg, Germany).

11) Cy3 F $(ab)_2$ Donkey-anti Rabbit IgG antibody (Jackson ImmunoResearch, Hamburg, Germany).
12) Anti p-IKKε antibody-Phospho-IKKε (Ser172), (D1B7), Rabbit-mAb #8766, (Cell signaling, Frankfurt am Main, Germany).
13) Anti-IKKε- IKKε (D20G4) Rabbit mAb #2905, (Cell signaling, Frankfurt am Main, Germany).
14) Anti-IKKα/β - IKKα/β Antibody (H-470): sc-7607, (Cell signaling, Frankfurt am Main, Germany).
15) Anti-p-IκBα-Phospho-IκBα (Ser32/36) (5A5) Mouse mAb #9246, (Cell signaling, Frankfurt am Main, Germany).
16) Anti-IκBα-IκB-α Antibody (C-21): sc-371, (Santa Cruz Biotechnology, Inc., Heidelberg, Germany).
17) Anti-p-p65-Phospho-NF-κB p65 (Ser536) (7F1) Mouse mAb #3036, (Cell signaling, Frankfurt am Main, Germany).
18) Anti-Caspase-3-Anti-rabbit pAb#9662 (Cell signaling, Frankfurt am Main, Germany),
19) Anti-Caspase-3-Anti-mouse mAb #9746 (Cell signaling, Frankfurt am Main, Germany),
20) Actin-A2228 Monoclonal Anti-β-Actin antibody produced in mouse (Sigma Aldrich, Darmstadt, Germany).
21) AURION BSA-c (AURION 6709 PD Wageningen, The Netehrlands).
22) Alexa Fluor™ 488 Phalloidin (Thermo Fisher Scientific, Karlsruhe, Germany).
23) DAPI (4′,6-Diamidine-2′-phenylindole dihydrochloride), (Sigma Aldrich, Darmstadt, Germany).
24) Formaldehyde 37%, (AppliChem GmbH, Darmstadt, Germany).
25) Tween 20 (Sigma Aldrich, Darmstadt, Germany).
26) Triton X 100 (Carl Roth GmbH, Karlsruhe, Germany).
27) Fam-Flica-Caspase-1 Kit (Bio-Rad Laboratories GmbH, D-85622 Feldkirchen, Germany).
28) Opti-MEM (1X) Reduced Serum Media (Gibco, Thermo Fisher Scientific, Karlsruhe Germany).
29) Lipofectamine RNAiMax reagent (Thermo Fisher Scientific, Karlsruhe, Germany).
30) Protein quantification assay kit (Thermo Fisher Scientific, Karlsruhe, Germany).
31) Pierce™ BCA™ Protein-Assay Reagent A (Thermo Fisher Scientific, Karlsruhe , Germany).

32) Roti-Quant universal reagent 2 (Carl Roth GmbH, Karlsruhe, Germany).
33) Intercept (TBS) Blocking Buffer (LI-COR Biosciences-GmbH, Lincoln, Nebraska, USA)
34) Complete tablets-Protease inhibitor cocktail (Roshe, Basel, Switzerland).
35) Peq Gold total RNA Kit Sline, (VWR, Erlangen, Germany).
36) RNA lyses buffer T (Radnor, Pennsylvania, USA).
37) Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Thermo Fischer Scientific, Waltham, Massachusetts).
38) 5X siRNA Buffer (Dharmacon Lafayette, CO 80026 USA).
39) R&D ELISA kits (Minneapolis, Canada).
40) HRP-Conjugated Streptavidin (Thermo Fischer Scientific, Waltham, Massachusetts).
41) PCR master mix (Thermo Fisher Scientific, Karlsruhe, Germany).
42) siGENOME Human NLRP1 siRNA, 17p13.2, 22861 (Dharmacon Lafayette, CO 80026 USA).
43) siGenome Human NLRP3 siRNA, 1q44, 114548 (Dharmacon Lafayette, CO 80026 USA).
44) Single guide RNA (sgRNA), (New England Biolabs GmbH, Frankfurt am Main Germany).
45) UV irradiation machine (Medisun® Psori-Kamm, Schulze&Böhm, Brühl, Germany).
46) Odyssey Infrared Imagging system with Odyssey imaging Software (LI-COR Biosciences-GmbH, Lincoln, Nebraska, USA).
47) Zeiss LSM 800 confocal laser scanning microscope with Zeiss ZEN 2.3 (blue edition) Software (Carl Zeiss Microscopy GmbH, Jena, Germany).
48) qRT-PCR LightCycler 480 instrument (Roche, Basel, Switzerland).

2.2. Cell culture of primary human keratinocytes:

Primary human keratinocytes were isolated from human foreskin of 2-4 years old children. Foreskin samples were provided by Loretto Clinic (Tübingen, Germany) in HBSS media (Hank's Balanced Salt Solution), (Sigma Aldrich, Darmstadt, Germany). Epidermis from dermis was separated using Dispase II (neutral protease, grade II) solution, (Roche, Basel, Switzerland) overnight at 37^0C with 5% CO_2 supply. The next day epidermis was cut into pieces and was incubated in Trypsin/EDTA solution, 0,05%/0.02% (Biochrom, Berlin, Germany), on 37^0C for 10 min.

For blocking of the Trypsin/EDTA, RPMI 1640 media (Biochrom, Berlin, Germany) containing 5% FBS (Gibco, Thermo Fisher Scientific, Karlsruhe, Germany) was used. Cells were filtered using Millipore cell culture filters 100µM (Sigma Aldrich, Darmstadt, Germany). Then cells were centrifuged and resuspended in CnT-PR cell culture media (CELLnTEC Advanced Cell Systems, Bern, Switzerland) and cultivated in T75 flasks containing collagen (Greiner Bio-One GmbH, Frickenhausen, Germany) untill they reach 100% confluency.

2.2.1. Transfection of primary human keratinocytes:

Cells were seeded on 12-well plates (Greiner Bio-One GmbH, Frickenhausen, Germany) until they reach 60-70% of confluency. Then cells were transfected in the Opti-MEM (1X) Reduced Serum Media (Gibco, Thermo Fisher Scientific, Karlsruhe Germany), with siGENOME Human NLRP1 siRNA and siGenome Human NLRP3 siRNA (Dharmacon, Lafayette, CO 80266 USA), using Lipofectamine RNAiMax reagent (Thermo Fisher Scientific, Karlsruhe, Germany), and also with single guide RNA (sgRNA), (New England Biolabs GmbH, Frankfurt am Main, Germany) as the positive control.

As negative control Opti-MEM media had been used. Transfected keratinocytes were incubated for 24h at 37^0C.

After 24h cells were irradiated with UVB light (50 mJ/cm^2) using Medisun® Psori-Kamm, Schulze & Böhm, Brühl, Germany, and incubated after irradiation for 4 hours and 8 hours.*2.2.2.*

Caspase blocking with pan-caspase inhibitor zVAD:

hPKs were seeded on 8-chamber polystyrene slides (Corning Incorporated, NY 14831, USA) and cultured in CnT-PR cell culture media (CELLnTEC Advanced Cell Systems, Bern Switzerland), until they reach 100% confluency. Than cells were differentiated using 1.2mM $CaCl_2$ over night. Next day cells were treated with 20µM zVAD-FMK pan-caspase inhibitor (InvivoGen, San Diego, CA 92121-USA) for 1h, and than were irradiated with UVB light 50 mJ/cm^2 using Medisun® Psori-Kamm, Schulze & Böhm, Brühl, Germany, irradiation machine and incubated for 4h and 8h. CnT-PR medium alone was used as a negative control. Cells were than routinously prepared for ASC specks staining, and cytokine analyses has been performed in cell free supernatants using ELISA.

2.2.3. NLRP3 inflammasome blocking using MCC950:

Keratinocytes were seeded on 8-chamber polystyrene slides (Corning Incorporated, NY 14831, USA), and cultured in CnT-PR cell culture media (CELLnTEC Advanced Cell Systems, Bern, Switzerland), until they reach 100% confluency. Cells were than differentiated using 1.2mM $CaCl_2$ overnight. Next day keratinocytes were treated with 5µM MCC950 (NLRP3-inflammasome inhibitor), (InvivoGen, San Diego, CA 92121-USA) for 1h, and then were irradiated with UVB light 50 mJ/cm^2 using Medisun® Psori-Kamm, Schulze & Böhm, Brühl, Germany, irradiation machine, and incubated after irradiation for 4 hours and 8 hours.

CnT-PR medium alone was used as a negative control. Cells were than routinously prepared for ASC specks staining, and cell supernatants were analysed using ELISA.

2.2.4. AIM2 inflammasome activation via double stranded DNA analogue Poly (dA:dT):

Cells were seeded on 8-chamber polystyrene slides (Corning Incorporated, NY 14831, USA) (for ASC stainig), and on 12-well plates (Greiner Bio-One GmbH, Frickenhausen, Germany), (for Western Blot analyses) until they reach 60-70% confluency. Than cells were transfected in the Opti-MEM (1X) Reduced Serum Media (Gibco, Thermo Fisher Scientific, Karlsruhe Germany), with 5µg/ml Poly (dA:dT) naked-Double stranded B-DNA (InvivoGen, San Diego, CA 92121-USA) for 24h, using Lipofectamine RNAiMax reagent (Thermo Fisher Scientific, Karlsruhe, Germany). Opti-MEM medium were used as a negative control. Transfected keratinocytes were incubated for 24h on 37^{0}C in incubator.

Non-transfected group of cells were irradiated with UVB light 50 mJ/cm^2 using Medisun® Psori-Kamm, Schulze & Böhm, Brühl, Germany, irradiation machine, and were incubated for 4 and 8h after irradiation. Opti-MEM media had been used as a negative control. Cells were than routinously prepared for ASC specks staining and Western Blot analyses, cytokine analyses have been performed in cell free supernatants using ELISA.

2.2.5. Immunofluorescence staining-ASC protein:

Primary human keratinocytes were seeded on 8-chamber polystyrene slides (Corning Incorporated, NY14831, USA). Cell were fixed using 2% Formaldehyde (37% Stock), (AppliChem GmbH, Darmstadt, Germany).

Perforations on cell membrane to enable antibody binding to the ASC protein, has been made using 0.5% Triton X 100 (Carl Roth GmbH, Karlsruhe, Germany). For blocking 1-3% Normal Donkey Serum (Jackson ImmunoResearch, Hamburg, Germany) + AURION BSA-c (AURION 6709 PD Wageningen, The Netehrlands) had been used. ASC protein has been stained using Anti-Asc, rabbit pAb (AL177), antibody (Adipogen AG, Liestal, Switzerland), 1:200 diluted in washing buffer containing Dulbeco´s Phosphate Buffer Saline (Sigma Aldrich, Darmstadt, Germany), and Tween 20 (Sigma Aldrich, Darmstadt, Germany). For visualization of ASC aggregates Cy3 F $(ab)_2$ Donkey-anti Rabbit IgG secondary antibody 1:200 (Jackson ImmunoResearch, Hamburg, Germany) had been used. Cytoskeleton (F-actin) had been stained using Alexa Fluor™ 488 Phalloidin (Thermo Fisher Scientific, Karlsruhe, Germany).

Cell nucleus had been stained using DAPI (4′,6-Diamidine-2′-phenylindole dihydrochloride), (Sigma Aldrich, Darmstadt, Germany) fluorescent dye.

Pictures has been taken using Zeiss LSM 800 confocal laser scanning microscope with Zeiss ZEN 2.3 (blue edition) Software (Carl Zeiss Microscopy GmbH, Jena, Germany), using 43x magnification.

2.2.6. FAM-FLICA active caspase 1 staining:

hPKs were seeded on 8-chamber polystyrene slides (Corning Incorporated, NY 14831, USA). Active caspase 1 were stained using Fam-Flica-Caspase-1 Kit (Bio-Rad Laboratories GmbH, D-85622 Feldkirchen, Germany). Hoechst staining has been performed using 0.5% Hoechst reagent diluted in the apoptosis wash buffer from the kit.

Propidium iodide (PI) staining has been done using 0.5% PI diluted in the apoptosis wash buffer from the kit Fam-Flica-Caspase-1 Kit (Bio-Rad Laboratories GmbH, D-85622 Feldkirchen, Germany).

Than cells were fixed using Fixative solution diluted 1:10 in apoptosis wash buffer. Pictures has been taken using Zeiss LSM 800 confocal laser scanning microscope with Zeiss ZEN 2.3 (blue edition) Software (Carl Zeiss Microscopy GmbH, Jena, Germany), using 43x magnification.

2.3. Isolation and cultivation of human PBMCs:

Human PBMCs were isolated from human blood (healthy donors and patients with HLA-B27 positive or negative AAU) using Biocoll gradient centrifugation (Biochrom, Berlin, Germany) to separate the PBMCs fraction. For adherence of PBMCs, cells were cultured for 1 hour in RPMI (Biochrom, Berlin, Germany) without FCS, and the adherent cells were further cultivated in RPMI 1640 medium with 10% FCS (Merk Life Sciences, Darmstadt, Germany) for 24h.

2.3.1. Patients:

All uveitis patients were recruited at the Department of Ophthalmology at University Eye Hospital, Tübingen, Germany.

None of the patients showed disease activity at the time of the blood draw. The study protocol was approved by the local ethics committee (737/2018B02). The study followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from all patients before study entry. Information about patients included in the study is shown in **Table 1.**

Patient number	HLA-B27 status	Complications	Previous treatment
1	+	-	Topical NSAIDs
2	+	-	Topical NSAIDs
3	+	-	-
4	+	-	Topical NSAIDs
5	-	Macular edema	Acetacolamid, Avastin (intravitreal)
6	+	Secondary ocular hypertension, Scleritis	Systemic+Topical NSAIDs
7	+	Macular edema	-
8	+	Posterior scleritis, Catarct	Sulfasalazine, Topical NSAIDs, Systemic corticosteroids (4mg)
9	+	Macular edema	-
10	-		Topical steroids, Systemic NSAIDS
11	+		-
12	-	Macular edema	Topical NSAIDs, Systemic corticosteroid, Methotrexate (10mg), Adalimumab
13	+	-	-
14	-	-	Topical NSAIDs
15	+	-	Topical, NSAIDs, Topical corticosteroids, Acetacolamid
16	-	-	Methotrexate

Table 1. Information about AAU patients (HLA-B27-status, complications and previous treatment) used in the study

2.3.2. RNA isolation and gene expression analysis:

Total RNA was isolated using Peq Gold total RNA Kit Sline, (VWR, Erlangen, Germany) and reverse transcription (Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Thermo Fischer Scientific, Waltham, Massachusetts) was performed according to the manufacturer's instructions.

To determine gene expression quantitative real-time-PCR (Roche LightCycler 480 system) was performed using the LightCycler 480 instrument (Roche, Basel, Switzerland).

The expression of the specified genes was calculated relative to the expression of the housekeeping gene β-actin. Primer and probe sequences are listed in **Table 2**.

2.3.4. Determination of cell viability:

Toxicity of 5-ASA and thapsigargin (Tsg) was determined using MTT cell viability assay (MTT cell viability Kit, Abcam, Cambridge, UK) according to the manufacturer’s instructions.

2.3.5. Induction of proinflammatory cytokines in human PMBCs:

Human PBMCs were preincubated with mesalazine (5-ASA) (100µM and 500µM) (Sigma Aldrich St. Louis, USA) for 30 minutes, prior to LPS (InvivoGen, San Diego, CA 92121-USA) stimulation for additional 2.5h. Inflammasome activation was performed by 5mM ATP (InvivoGen, San Diego, CA 92121-USA) for 30 min. To induce endoplasmic reticulum stress, leading to unfolded protein response (UPR), thapsigargin (Tsg) (4µM) (Tocris, Bristol, UK) was used.

Target gene	Accession no.	Primer	Primer sequence	Purpose
β-actin	P60709	ACTIN F ACTIN R ACTIN TM	AGCCTCGCCTTTGCCGA CTGGTGCCTGGGGCG 6FAM-CCGCCGCCCGTCCACACCCGCC--BBQ	qPCR qPCR Hybridisation probe
IL-6	P05231	hu IL-6 U hu IL-6 B hu IL-06 TM	CCAGAGCTGTGCAGATGAGTACA CCTGCAGCTTCGTCAGCA 6FAM-CATTTGTGGTTGGGTCAGGGGTGGT--BBQ	qPCR qPCR Hybridisation probe
IL-1β	P01584	IL-01b S IL-01b rev IL-01b TM	CAGGGACAGGATATGGAGCAA ATGTACCAGTTGGGGAACTG 6FAM-AGAATCTGTACCTGTCCTGCGTGTTGAA--BBQ	qPCR qPCR Hybridisation probe
TNFα	P01375	TNFa S TNFa A TNFa TM	CTTCTCCTTCCTGATCGTGGC GGGTTTGCTACAACATGGGC 6FAM-CGCCACCACGCTCTTCTGCCT--BBQ	qPCR qPCR Hybridisation probe
IFNϒ	P01579	IFNg-S IFNg-A IFNg-Probe	GCATCCAAAAGAGTGTGGAG GGACATTCAAGTCAGTTACCgG 6FAM-ATCAAGGAAGACATGAATGTCAAGTTTTTCAA--BB	qPCR qPCR Hybridisation probe
IL-23A	Q9NPF7	IL23A S IL23A A IL23A P	TAGTGGGACACATGGATCTAAG GCAAGCAGAACTGACTGTTG 6FAM-CCATCTCCACACTGGATATGGGGA--BBQ	qPCR qPCR Hybridisation probe
CHOP (DDIT3)	F8VS99	DDIT3 F DDIT3 S DDIT3 A DDIT3 R DDIT3 P	ACCTCCTGGAAATGAAGAGGAAGAAT CTCCTGGAAATGAAGAGGAAGAATCA ACTGGAATCTGGAGAGTGAGGGC GCTCTGACTGGAATCTGGAGAGTGAG F-CTGGGAGGTGCTTGTGACCTCTGCTGG--BBQ	qPCR qPCR Hybridisation probe
ATF6	O15139	ATF6 F ATF6 S ATF6 A ATF6 R ATF6 P	ACAAAGCCCTGATGGTGC ACTGAAGAACCATTGCTTTACATTC CTCGAAGTTCATGATTTAACCTGAG CTTTCTACTTCATGTCTATGAACCCA F-CACCTCCTTGTCAGCCCCTAATTAACAC—BBQ	qPCR qPCR Hybridisation probe
NLRP1	Q9C000	NLRP1 F NLRP1 S NLRP1 A NLRP1 R NLRP1 P	CACTTTATATGGGCTGTCGTTACA GGGTCTGGTTCAGGGATGC CCAACGTAGAACTCCGAGAACA CTCATCTTTCTTGTCTTTCACTTGC 6FAM-CTCCAGGGCTTCGATAGCAGAGCT—BBQ	qPCR qPCR Hybridisation probe
NLRP3	Q1JQ87	NLRP3 F NLRP3 S NLRP3 A NLRP3 R NLRP3	CACTTCTGACCTCCAGCCA CAACAATGACCTGGGCGA TCTTCTTGAAGTGTTTCTAACGCA AGGCTCAAAGACGACGGT 6FAM-CTGAAACAGCAGAGCTGCCTCCTG--BBQ	qPCR qPCR Hybridisation probe

Table 2. Oligonucleotide primers and LightCycler hybridization probes.

2.4. Cytokine analysis:

Human IL-1α and IL-1β secretion was measured in cell-free supernatants using R&D ELISA kits (Minneapolis, Canada), (Human IL-1 beta/IL-1F2 Duoset ELISA, Human IL-1α DuoSet ELISA) according to the manufacturer's instructions.

2.5. Western Blot analysis:

hPKs were seeded on 12-well plates (Greiner Bio-One GmbH, Frickenhausen, Germany). Cell supernatants had been removed and cell pellets were collected in Western Blot lysis buffer containing protease inhibitor cocktail (complete tablets), (Roshe, Switzerland). Standards for protein quantification measurement has been made using Protein quantification assay kit (Thermo Fisher Scientific, Karlsruhe, Germany). Quantification measurement has been done using Pierce™ BCA™ Protein-Assay Reagent A (Thermo Fisher Scientific, Karlsruhe, Germany) +Roti-Quant universal reagent 2 (Carl Roth GmbH, Karlsruhe, Germany). Proteins of interests has been separated on 10% Western Blot separation gel and were detected using Anti p-IKKε antibody-Phospho-IKKε (Ser172), (D1B7), Rabbit-mAb #8766, (Cell signaling, Frankfurt am Main, Germany), Anti-IKKε- IKKε (D20G4) Rabbit mAb #2905, (Cell signaling, Frankfurt am Main, Germany), Anti-IKKα/β-IKKα/β Antibody (H-470):sc-7607, (Cell signaling, Frankfurt am Main, Germany), Anti-p-IκBα- Phospho-IκBα (Ser32/36) (5A5) Mouse mAb #9246, (Cell signaling, Frankfurt am Main, Germany), Anti-IκBα-IκB-α Antibody (C-21): sc-371, (Santa Cruz Biotechnology, Inc., Heidelberg, Germany), Anti-p-p65-Phospho-NF-κB p65 (Ser536) (7F1) Mouse mAb #3036, (Cell signaling, Frankfurt am Main, Germany), Anti-caspase-3-Anti-rabbit pAb#9662 (Cell signaling, Frankfurt am Main, Germany), Anti Caspase-8-Anti-mouse mAb#9746 (Cell signaling, Frankfurt am Main, Germany). As the control housekeeping gene Actin -A2228 Monoclonal Anti-β-Actin antibody produced in mouse (Sigma Aldrich, Darmstadt, Germany) has been used. For the blocking of the antibody 5ml Intercept (TBS) Blocking Buffer (LI-COR Biosciences-GmbH, Lincoln, Nebraska, USA) had been used. Protein detection had been performed using Odyssey Infrared Imagging system with Odyssey imaging Software (LI-COR Biosciences-GmbH, Lincoln, Nebraska, USA).

2.6. LDH cell viability assay:

Primary human keratinocytes were seeded on 96 well plate until 100% confluency, then cells were differentiated using 1.2mM $CaCl_2$ overnight. On the next day cells were treated with 10µl of 10X Lysis buffer from the kit (Thermo Fisher Scientific, Karlsruhe, Germany), and were incubated for 45min with 5% CO_2 supply. Then cells were treated with 50µl LDH solution (Thermo Fisher Scientific, Karlsruhe, Germany. The plate was incubated on room temperature for 30min, and than 50µl of stop solution has been added. Absorbance has been measured at 490nm and 680nm. Cytotoxicity is calculated using the following formula:

$$\% \text{ cytotoxicity} = \frac{\text{Compound-treated LDH activity- Spontanous LDH activity}}{\text{Maximum LDH activity- Spontaneous LDH activity}} \text{ x100}$$

2.7. Statistics:

For statistical analyses One-Way ANOVA and Student t-test were used. Statistics was done in GraphPad Prism 8 (San Diego, CA, USA) software.

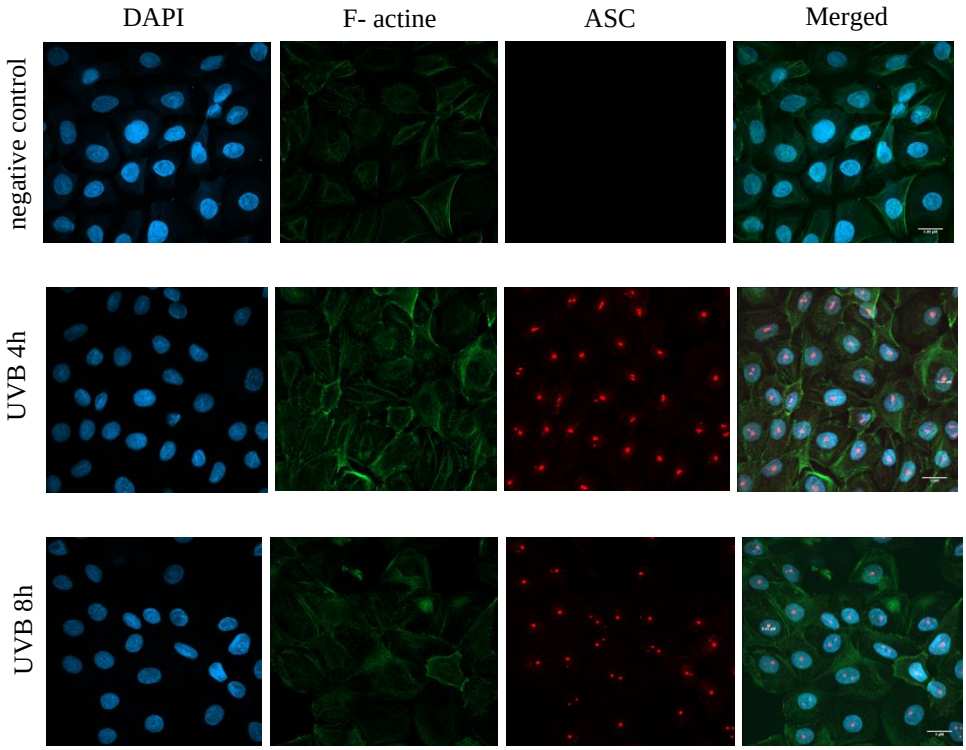
3. R E S U L T S:

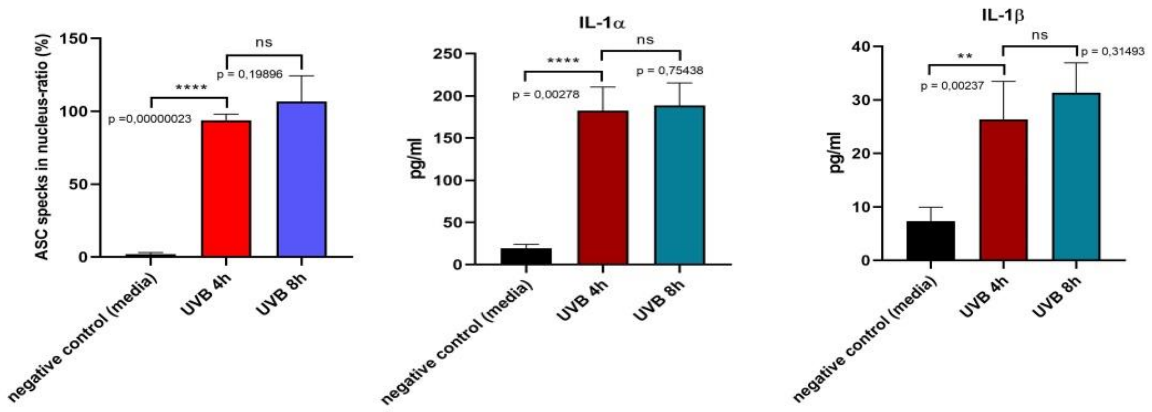
3.1. *Inflammasome activation via UVB light irradiation in primary human keratinocytes:*

UV irradiation can also lead to the activation of inflammasomes, that is responsible for Caspase 1 recruitment and processing of pro-IL-1β and pro-IL-18 to their active forms (Chavarría-Smith J.et.al. 2015), (Ming Man S.et.al. 2017). A crucial role in the recruitment of caspase-1 to the inflammasome complex plays ASC (Andrade F. et.al. 2005), (Ogilvie AC.et.al. 1996).

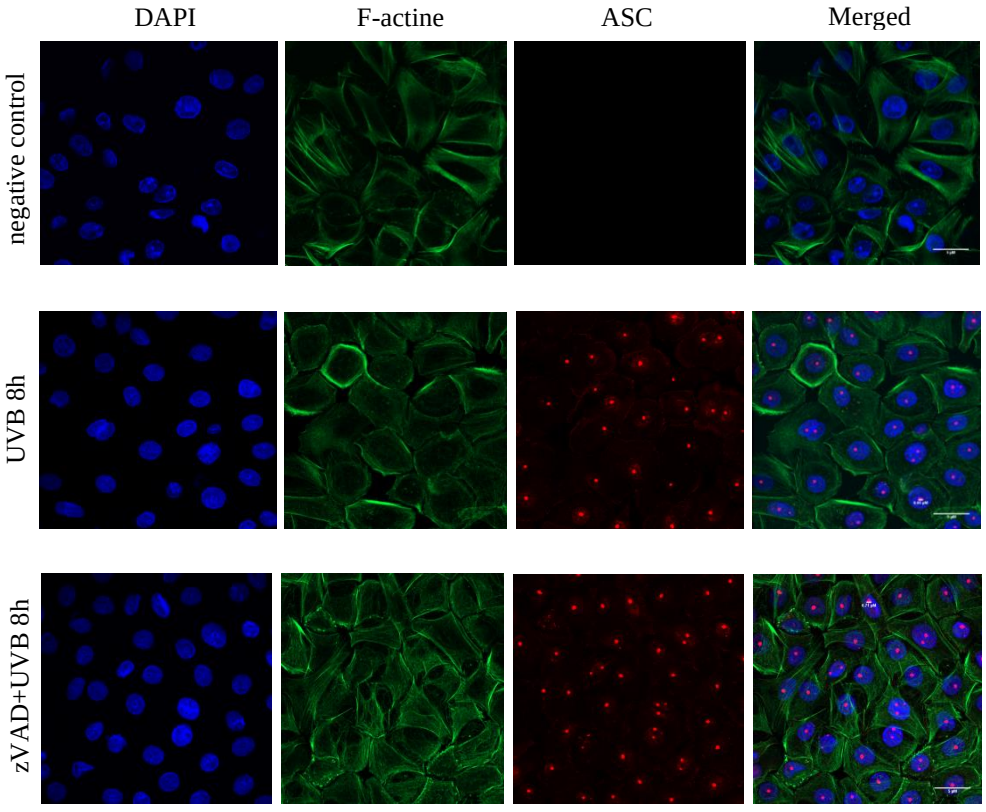
Activation and assembling of the inflammasome can by visualized by the formation of specific protein structures called the ASC "specks".

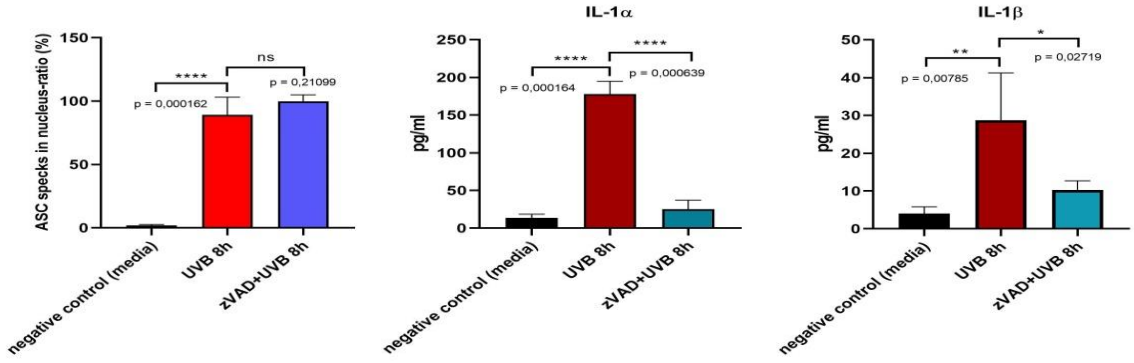
(a)




ASC specks in nucleus-ratio (%)
150
100
50
0
****
p =0,00000023
ns
p = 0,19896
negative control (media)
UVB 4h
UVB 8h
IL-1α
pg/ml
250
200
150
100
50
0
****
p = 0,00278
ns
p = 0,75438
negative control (media)
UVB 4h
UVB 8h
IL-1β
pg/ml
40
30
20
10
0
**
p = 0,00237
ns
p = 0,31493
negative control (media)
UVB 4h
UVB 8h

(b)


DAPI
F-actine
ASC
Merged
negative control
UVB 8h
zVAD+UVB 8h


ASC specks in nucleus-ratio (%)
150
100
50
0
****
p = 0,000162
ns
p = 0,21099
negative control (media)
UVB 8h
zVAD+UVB 8h
IL-1α
pg/ml
250
200
150
100
50
0
****
p = 0,000164
****
p = 0,000639
negative control (media)
UVB 8h
zVAD+UVB 8h
IL-1β
pg/ml
50
40
30
20
10
0
**
p = 0,00785
*
p = 0,02719
negative control (media)
UVB 8h
zVAD+UVB 8h

(c)

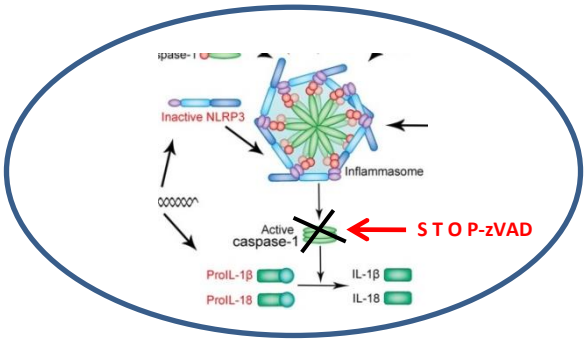


Figure 1. (a). UVB irradiation leads to formation of ASC specks in primary human keratinocytes. Primary human keratinocytes were irradiated with UVB (50 mJ/cm^2). Exposure to UVB light leads to the formation of nuclear ASC "specks". **(b) zVAD does not inhibit ASC "specks" formation, but suppresses IL-1 secretion**. Keratinocytes were treated with 20μM zVAD for 1h, and were irradiated with UVB (50 mJ/cm^2). ASC (red), F-actine (green), DAPI (dark blue). IL-1α and IL-1β was measured in cell free supernatants using ELISA. Data represents at least 3 independent experiments and are presented as means ± SD. Statistics: One-Way ANOVA statistical test. Symbols for P-values used in the figures *P<0,05, **P<0,01, ***P<0,001, ****P<0,0001 NS-not significant. Magnification x43.

As UVB irradiation leads to the activation of the inflammasome in primary human keratinocytes, the formation of the ASC "specks" as the marker of inflammasome assembly and activation was observed. Exposure to UVB light leads to the formation of ASC "specks" (red dots) after 4h and after 8h **(Fig. 1a)**, and there was no significant difference in the number of "specks" formed after 4h and after 8h **(Fig. 1a)**. A significant amount of IL-1α was secreted after exposure to UVB, while IL-1β secretion were closed to background **(Fig. 1a)**.

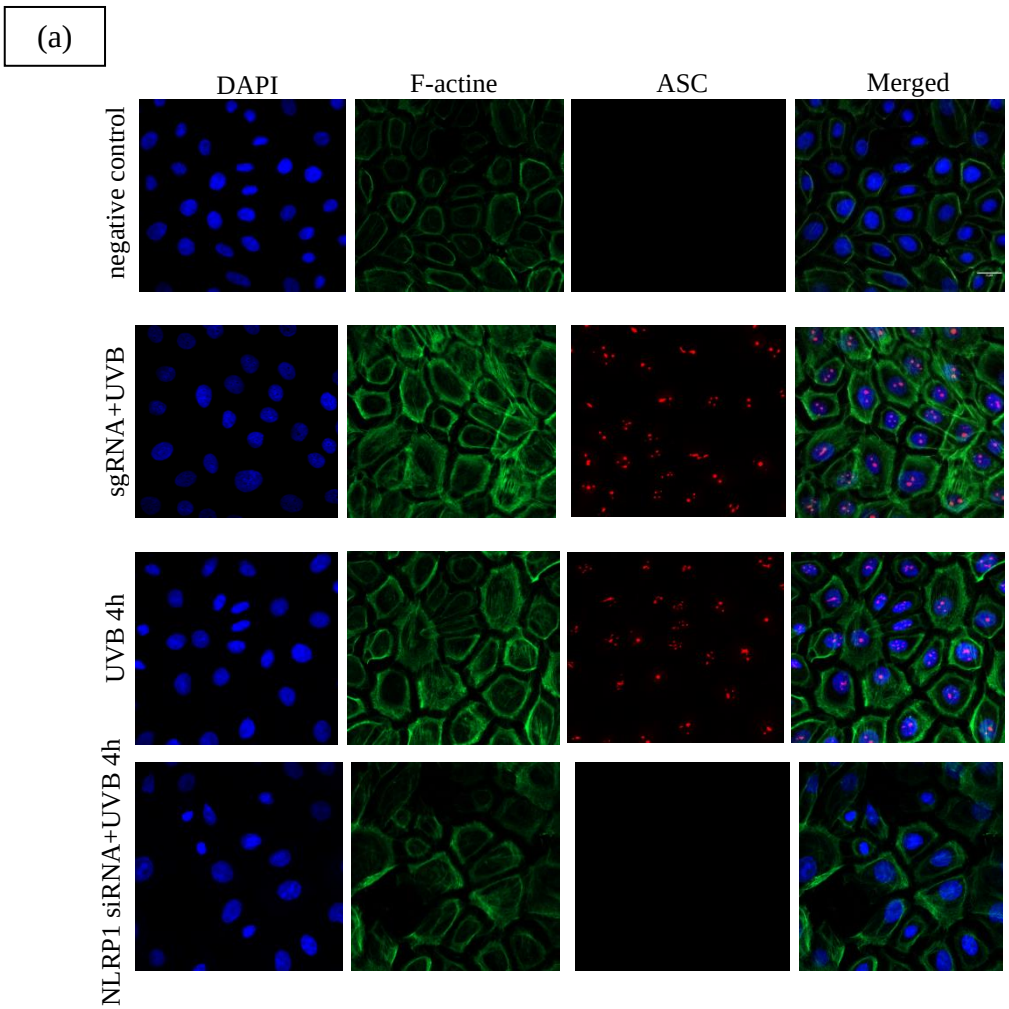
Using the pan-caspase inhibitor zVAD, ASC specks were not altered, as zVAD blocks caspase activation, inflammasome complexes are still formed, however, they are not able to activate inflammasomes. Therefore ASC-speck formation is not influenced, while IL-1 secretion is reduced upon zVAD-treatment. **(Fig. 1b**, **Fig. 1c)**.

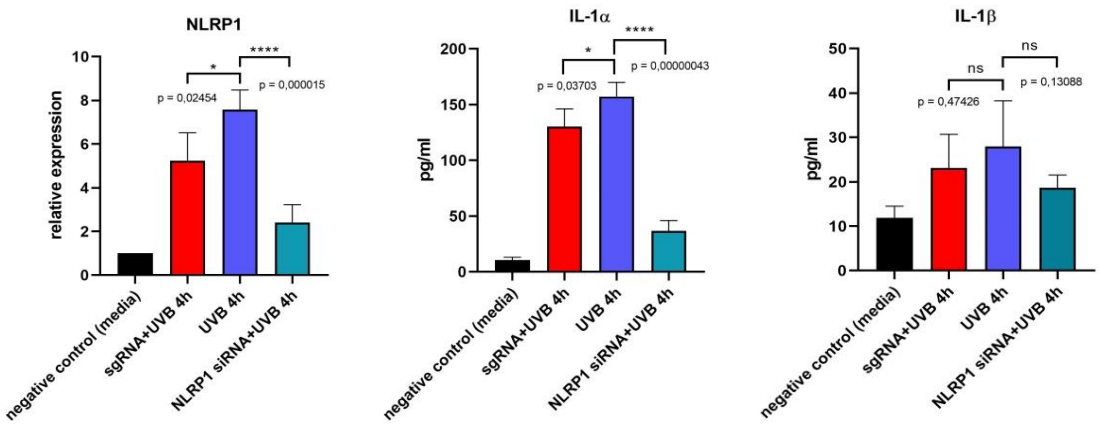
3.2. NLRP1 is more important for UVB sensing in hPKs:

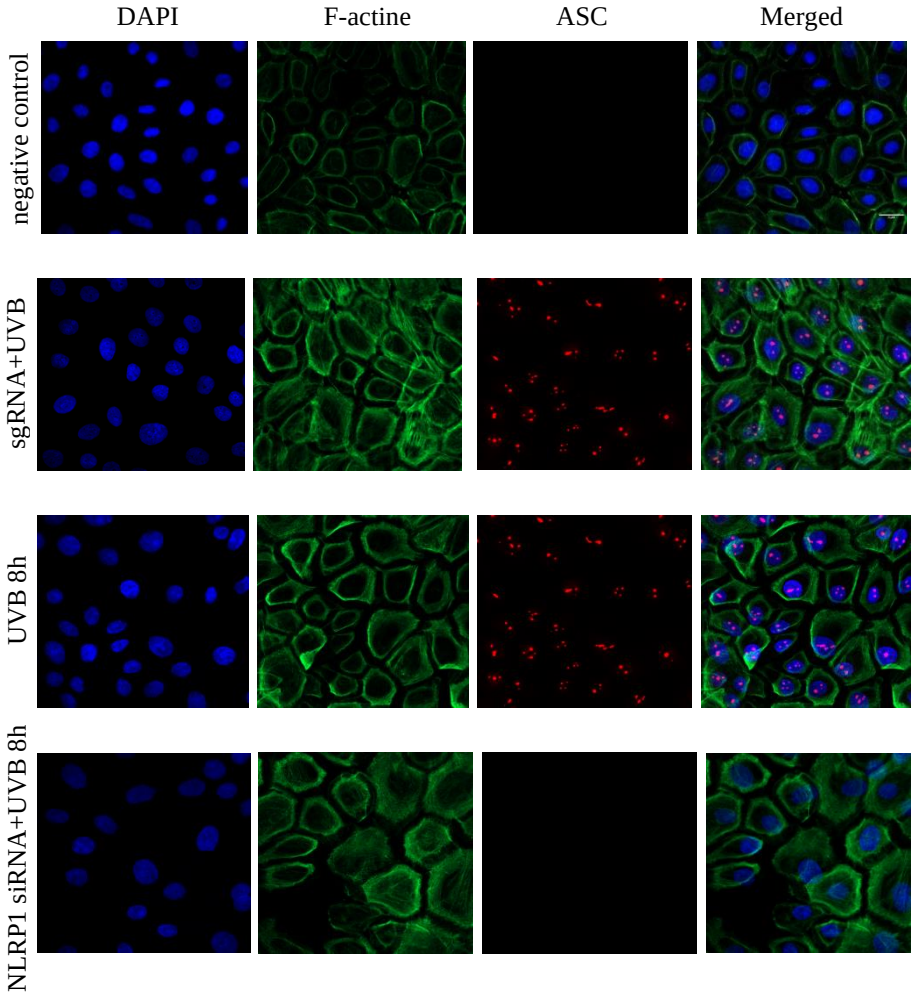
Fenini et.al., showed that for UVB sensing in hPKs the NLRP1 dominates over NLRP3 inflammasome (Fenini G. et.al. 2018).

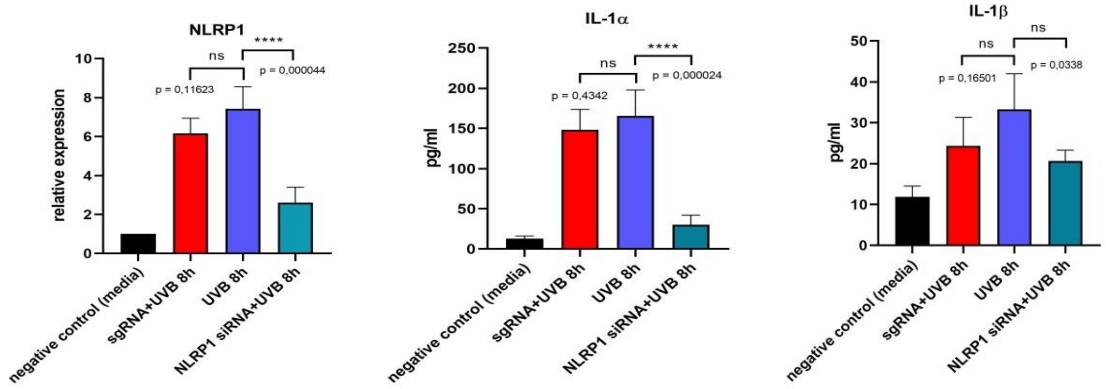
Cells were transfected with NLRP1 siRNA to specifically silence NLRP1. Keratinocytes were transfected with siRNA and incubated for 24h, and then irradiated with UVB (50 mJ/cm^2).

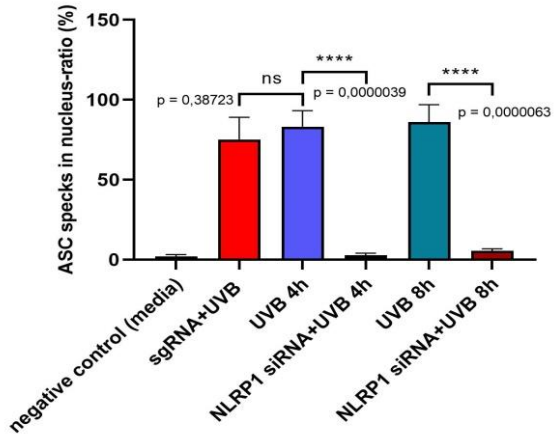
To be sure that silencing with siRNA was successful qRT-PCR has been performed (oligonucleotides are listed in **Table 2.** When NLRP1 was absent, the formation of ASC "specks" was almost completely suppressed and a decrease of IL-1α was observed (**Fig. 2a)**. This suggests that when NLRP1 is needed for the the binding of ASC as adaptor protein for caspase 1 recruitment. In the absence of active caspase-1, pro-IL-1 processing is blocked.



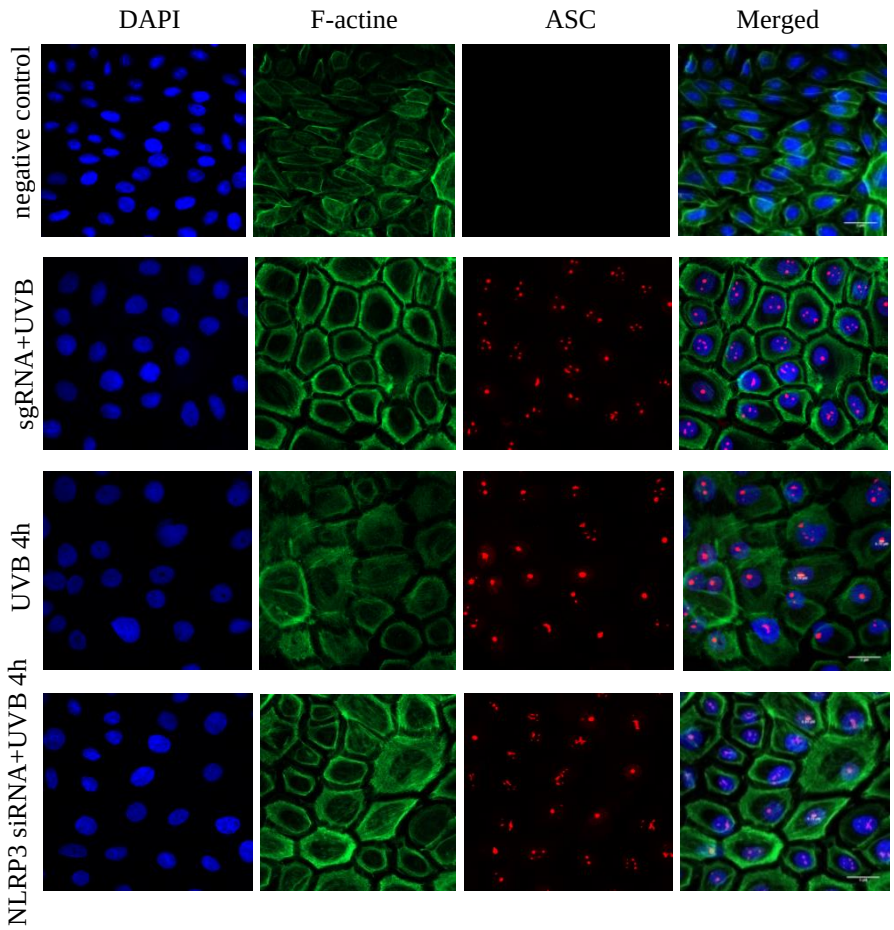

NLRP1
relative expression
p = 0,02454
p = 0,000015
IL-1α
pg/ml
p = 0,03703
p = 0,00000043
IL-1β
p = 0,47426
p = 0,13088
ns
negative control (media)
sgRNA+UVB 4h
UVB 4h
NLRP1 siRNA+UVB 4h

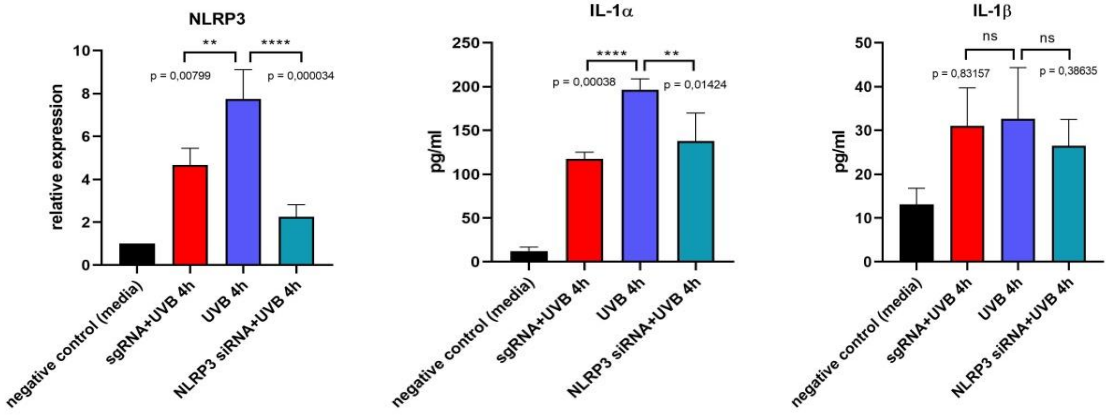

DAPI
F-actine
ASC
Merged
negative control
sgRNA+UVB
UVB 8h
NLRP1 siRNA+UVB 8h

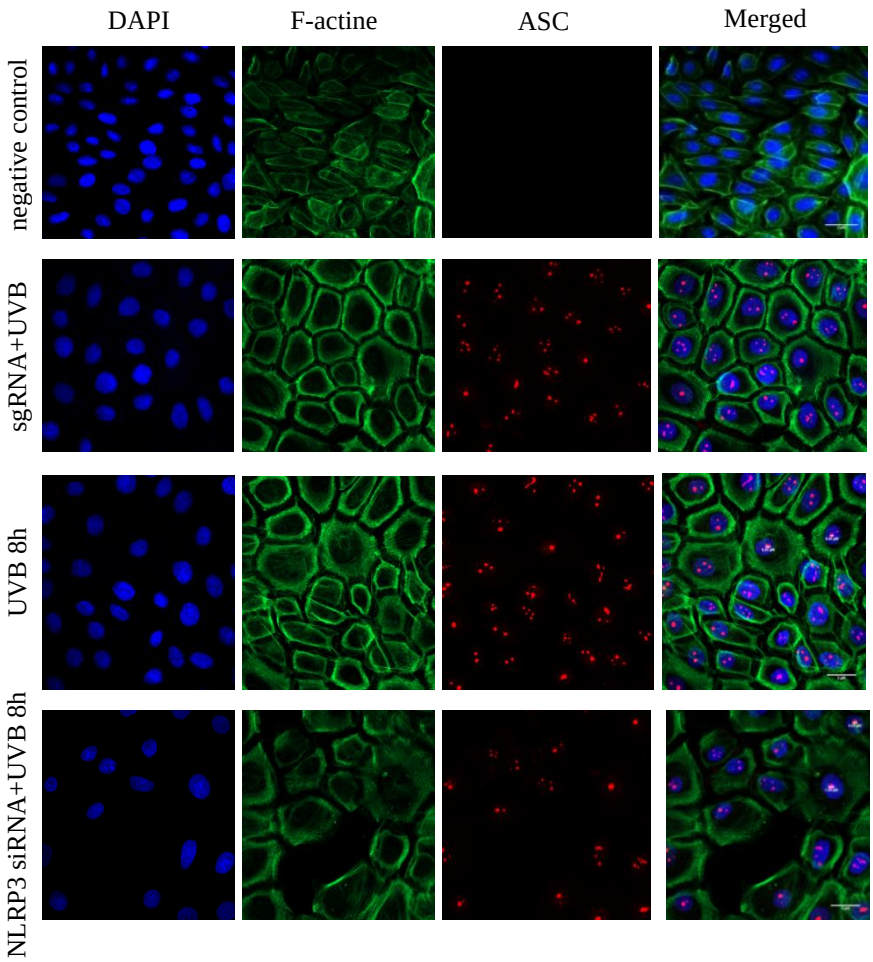

NLRP1
relative expression
ns
****
p = 0,11623
p = 0,000044
negative control (media)
sgRNA+UVB 8h
UVB 8h
NLRP1 siRNA+UVB 8h
IL-1α
pg/ml
ns
****
p = 0,4342
p = 0,000024
IL-1β
pg/ml
ns
ns
p = 0,16501
p = 0,0338

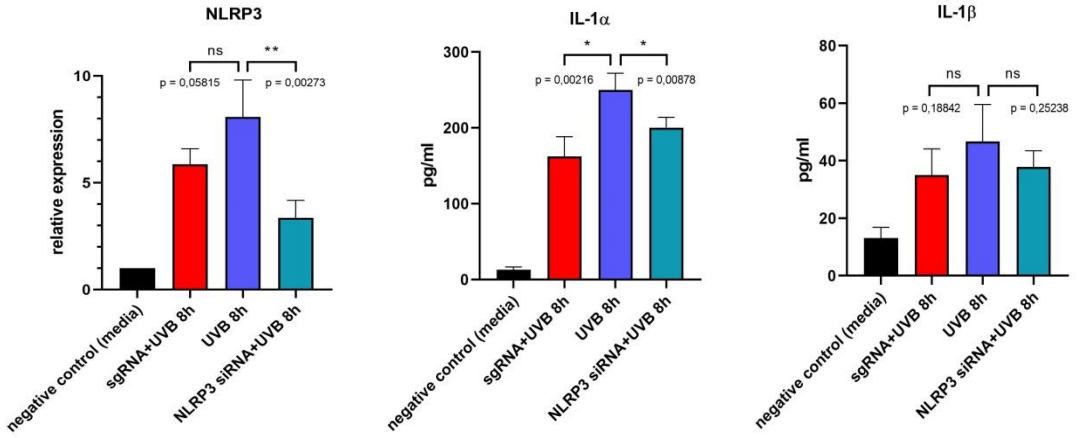

ASC specks in nucleus-ratio (%)
ns
****
****
p = 0,38723
p = 0,0000039
p = 0,0000063
negative control (media)
sgRNA+UVB
UVB 4h
NLRP1 siRNA+UVB 4h
UVB 8h
NLRP1 siRNA+UVB 8h

(b)


DAPI
F-actine
ASC
Merged
negative control
sgRNA+UVB
UVB 4h
NLRP3 siRNA+UVB 4h


NLRP3
relative expression
p = 0,00799
p = 0,000034
IL-1α
pg/ml
p = 0,00038
p = 0,01424
IL-1β
ns
p = 0,83157
p = 0,38635
negative control (media)
sgRNA+UVB 4h
UVB 4h
NLRP3 siRNA+UVB 4h


DAPI
F-actine
ASC
Merged
negative control
sgRNA+UVB
UVB 8h
NLRP3 siRNA+UVB 8h


NLRP3
relative expression
ns
**
p = 0,05815
p = 0,00273
IL-1α
pg/ml
*
*
p = 0,00216
p = 0,00878
IL-1β
pg/ml
ns
ns
p = 0,18842
p = 0,25238
negative control (media)
sgRNA+UVB 8h
UVB 8h
NLRP3 siRNA+UVB 8h

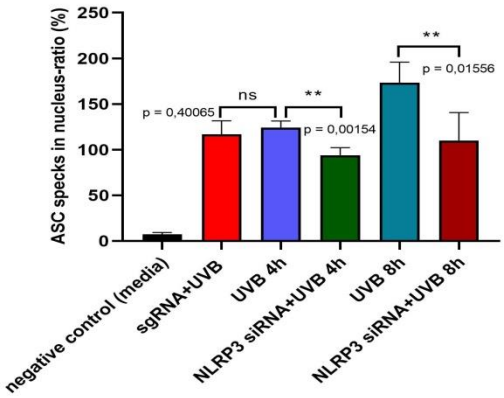


Figure 2. (a) NLRP1 is the most important inflammasome in primary human keretinocytes responsible for UVB sensing. Cells were transfected with NLRP1 and NLRP3 siRNA and were irradiated with UVB (50 mJ/cm^2) for 14s. NLRP1 siRNA completely blocked formation of ASC aggregates. ASC (red), F-actine (green), DAPI (blue) **(b) NLRP3 is dispensable for UVB-induced ASC "specks" in hPKs.** IL-1α and IL-1β were measured in cell free supernatants by ELISA. Data represent at least 3 independent experiments and are presented as means ± SD. Statistics: One-Way ANOVA statistical test. Symbols for P-values used in the figures *P<0,05, **P<0,01, ***P<0,001, ****P<0,0001 NS-not significant. Magnification x43

In contrast, the NLRP3 inflammasome is mainly expressed in immune cells notably antigen presenting cells (APCs), macrophages, dendritic cells (Baumgart DC. et.al. 2013), neutrophils and monocytes (Zhong Y.et.al. 2013). Activated NLRP3 inflammasome oligomerizes and triggers the assembly of the ASC protein (Latz E.et.al. 2013) (Lu A.et.al. 2014).

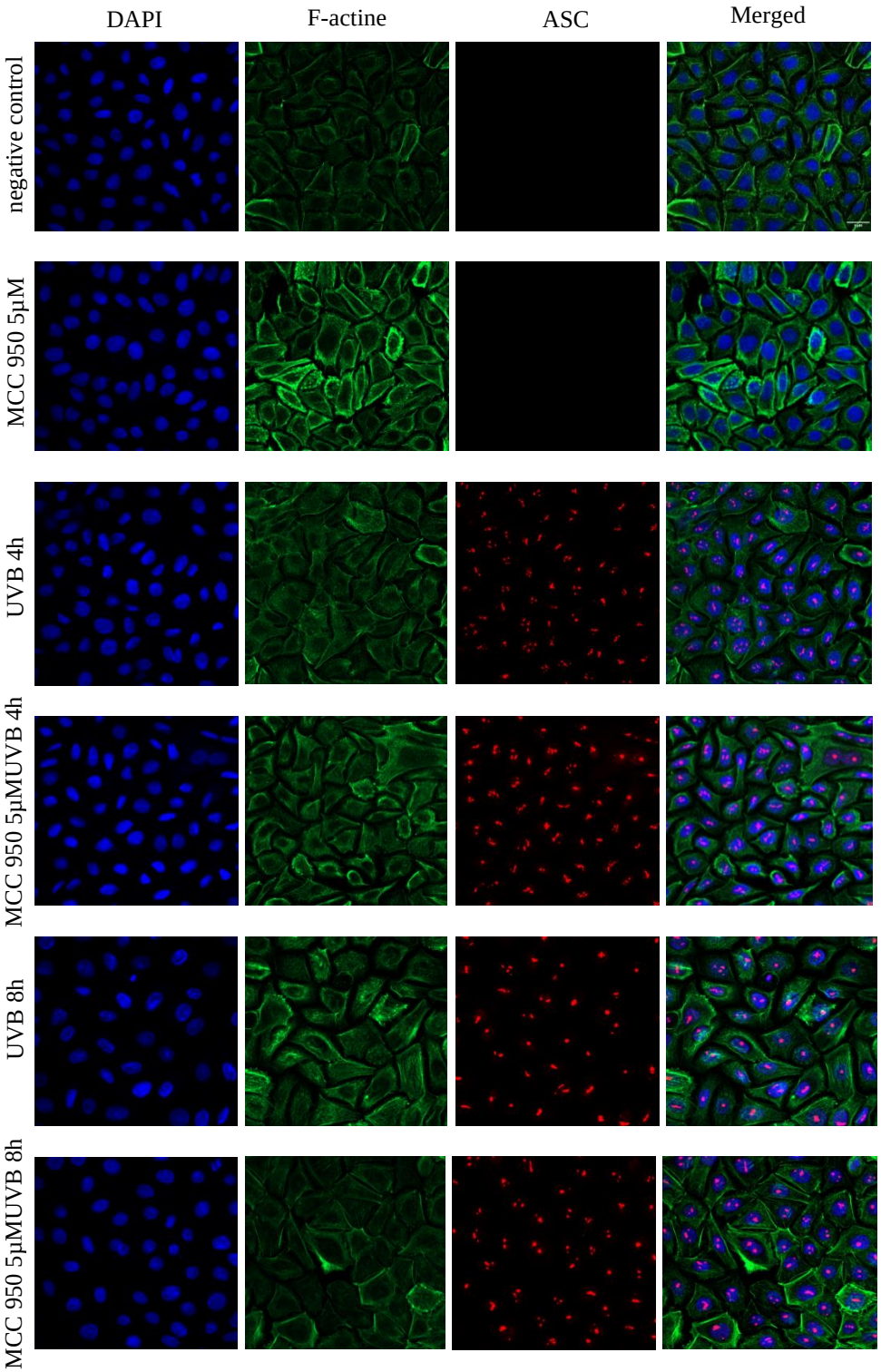
Upon silencing of NLRP3 using siRNA pharmacological inhibition via the small molecule MCC950, (**Fig 3)** inflammasome activation and formation of ASC "specks" in the nucleus (red dots on the pictures) is reduced, but to a much lesser extent than in the absence of NLRP1. IL-1a secretion is also slightly diminished, IL-1β values were closed to background.

3.3. Pharmacollogical blocking of NLRP3 inflammasome with MCC950:

Next, we blocked NLRP3 inflammasome activation by MCC950. hPKs were treated with 5μM MCC950 for 1 hour, and than were irradiated with UVB light (50 mJ/cm^2). Again ASC "specks" staining was performed at 4h and 8h after exposure to UVB light.

The formation of ASC "specks" was partially suppressed by MCC 950 and IL-1α secretion was diminuished, but not as prominent as in the absence of NLRP1. Again IL-1β secretion was close to background **(Fig. 3)**.

These data suggest that NLRP3 is partially needed for ASC speck formation in keratinocytes, but in a lesser extent compared to NLRP1.



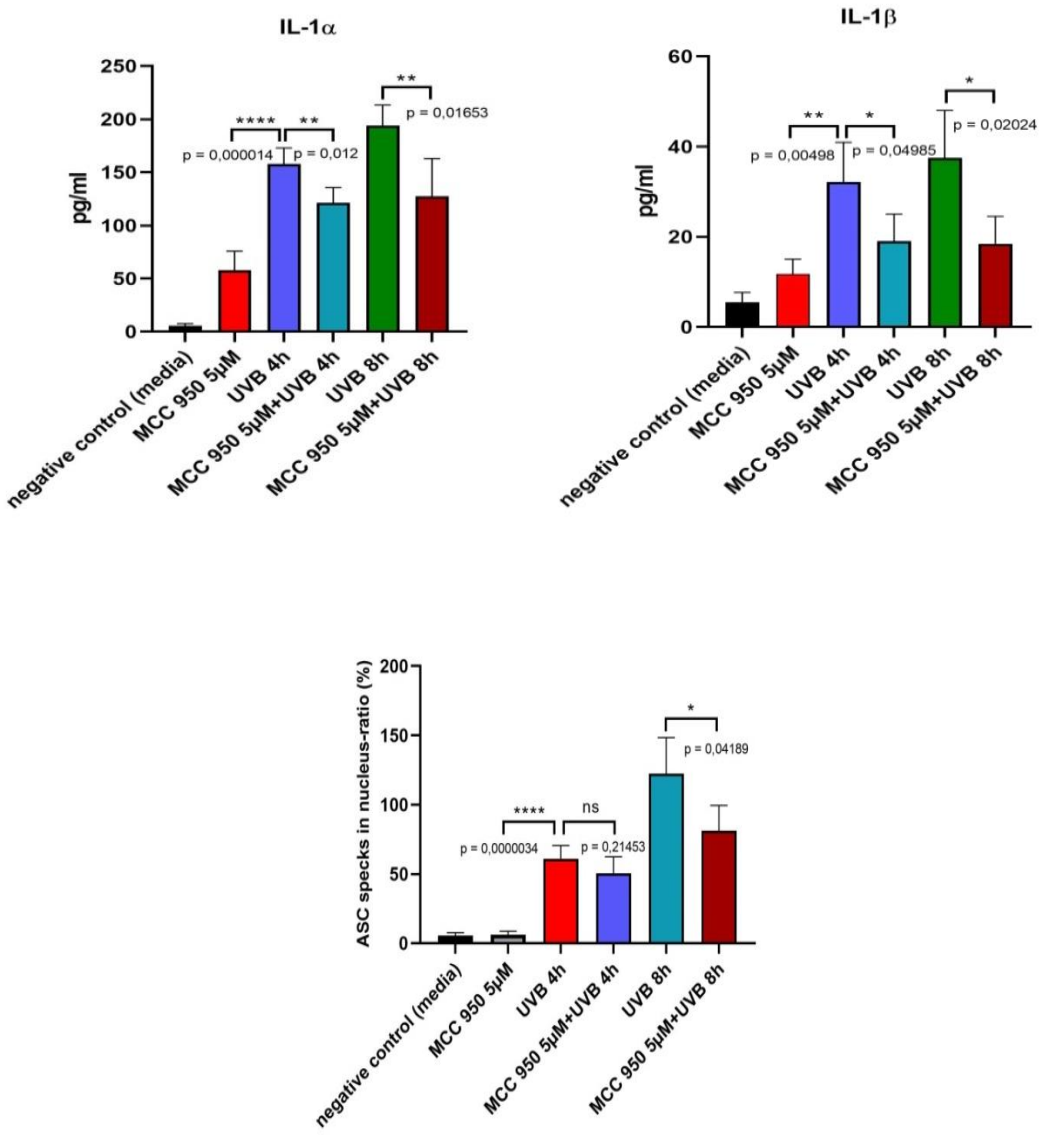


Figure 3. Pharmacological blocking of NLRP3 via MCC950 does not suppress significantly UVB-induced ASC specks formation and IL-1 secretion. Cells were treated with 5μM MCC950 for 1h prior to UVB treatment (50 mJ/cm^2) IL-1α and IL-1β were measured in cell free supernatants using ELISA. Data represents at least 3 independent experiments and are presented as means ± SD. Statistics: One-Way ANOVA statistical test. Symbols for P-values used in the figures *P<0,05, **P<0,01, ***P<0,001, ****P<0,001 NS-not significant. Magnification x43.

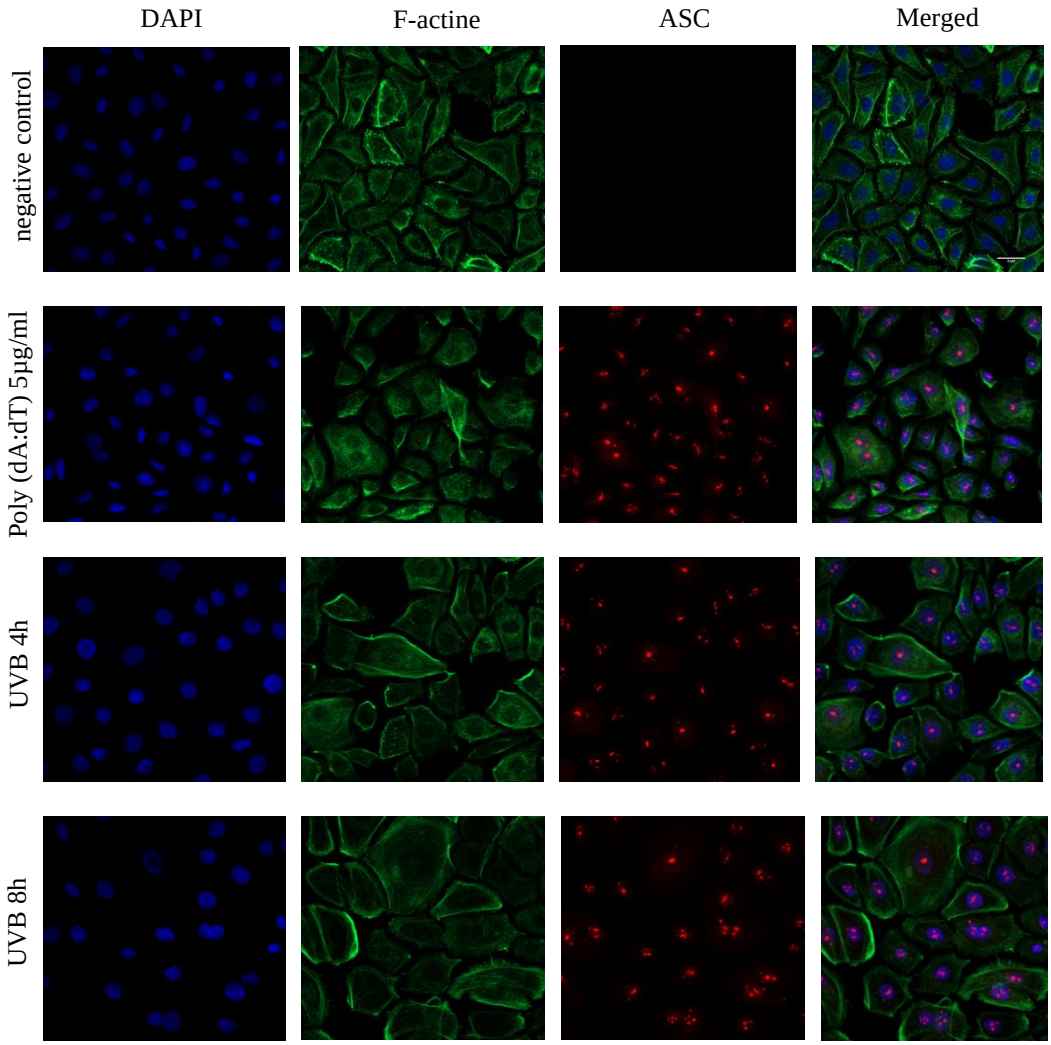
3.4. AIM2 inflammasome activation via UVB and poly(dA:dT):

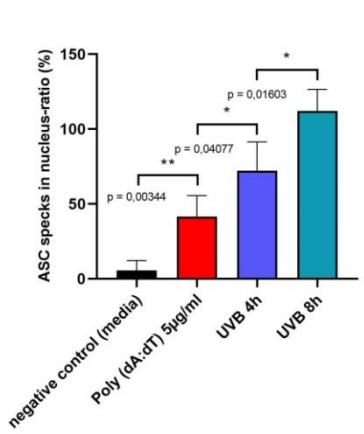
AIM2 inflammasome is asensor which recognizes double stranded (ds) DNA), either bacterial or viral or its analogues (poly(dA:dT)), and it is able to form an inflammasome complex with ASC and pro-caspase-1 (Fernandes-Alnemri T. et.al. 2009), (Hornung V.et.al. 2009).

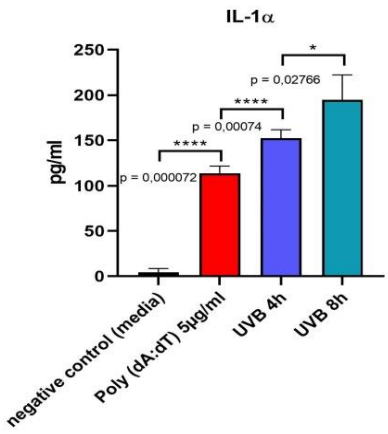
Therefore, we investigated, whether the activation of AIM2 inflammasome via dsDNA analogue poly(dA:dT), leads to ASC "specks" formation and IL-1 secretion. hPKs were transfected with poly(dA:dT) incubated for 24h and then irradiated with UVB (50 mJ/cm^2). ASC "specks" were observed both after exposure to UVB and to a lesser extent after poly(dA:dT) transfection. Secreted IL-1α confirms the ASC "specks" staining pattern, showing that the activation of AIM2 leads to IL-1α and IL-β secretion, but weaker compared to UVB **(Fig. 4a)**. This suggests that both NLRP1/NLRP3-activation via UVB and AIM2 stimulation by intracellular dsDNA leads to the formation of ASC specks in hPKs. The sensing of bacterial and viral DNA leads to the activation of the NF*k*B pathway (Liu T. et.al. 2017).

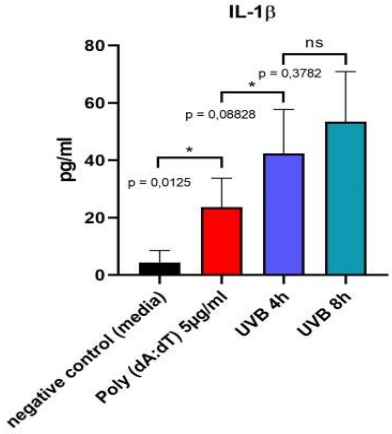
Different proteins that are a part of NF*k*B pathway had been observed by Western blot after transfection with poly(dA:dT) and irradiation with UVB light. Phosphorylated form of I*k*Bα (p-I*k*Bα), I*k*Bα, IKKα/β, and p-p65 (Rella) were upregulated with poly(dA:dT) and also in combination of poly(dA:dT) and UVB. IKKα/β long form was not upregualated at all, and IKKε were upregulated with poly(dA:dT) but not with UVB, confirming some previous findings that poly(dA:dT) and UVB light, induces NF*k*B and also proteins involved in the regulation of NF*k*B pathway **(Fig 4b)**.

(a)


DAPI
F-actine
ASC
Merged
negative control
Poly (dA:dT) 5µg/ml
UVB 4h
UVB 8h


ASC specks in nucleus-ratio (%)
150
100
50
0
p = 0,00344
**
p = 0,04077
*
p = 0,01603
*
negative control (media)
Poly (dA:dT) 5µg/ml
UVB 4h
UVB 8h


IL-1α
pg/ml
250
200
150
100
50
0
p = 0,000072
****
p = 0,00074
****
p = 0,02766
*
negative control (media)
Poly (dA:dT) 5µg/ml
UVB 4h
UVB 8h


IL-1β
pg/ml
80
60
40
20
0
p = 0,0125
*
p = 0,08828
*
p = 0,3782
ns
negative control (media)
Poly (dA:dT) 5µg/ml
UVB 4h
UVB 8h

(b)

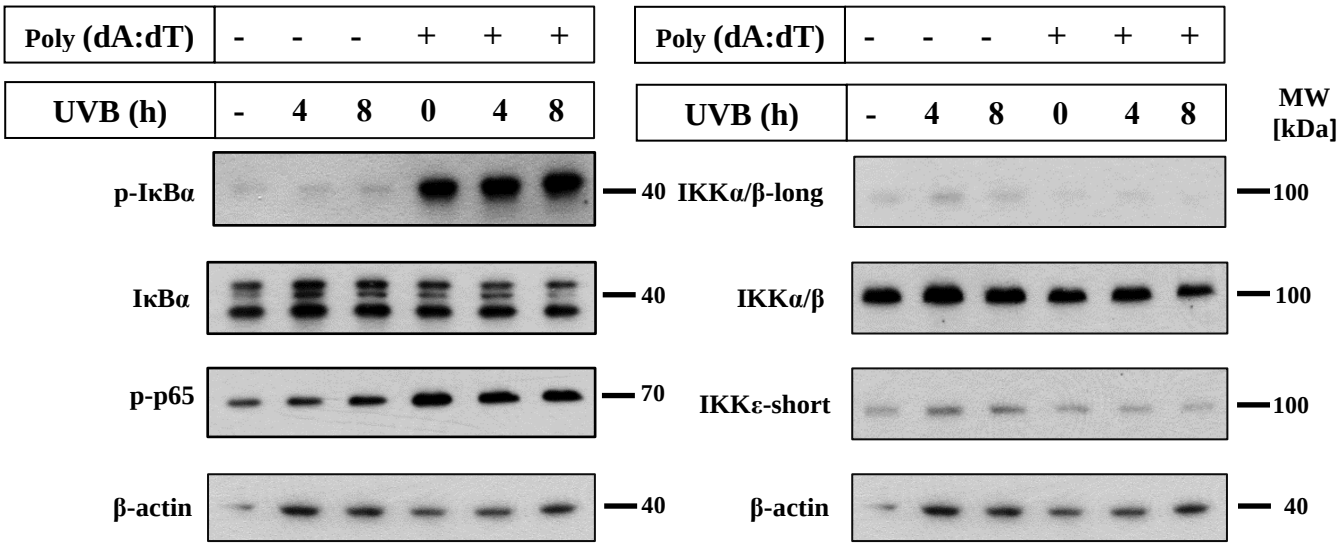


Figure 4 (a) Poly (dA:dT) leads to AIM2 inflammasome and ASC "specks" formation. Human keratinocytes were transfected with dsDNA analogue Poly (dA:dT) using Lipofectamine RNAiMax reagent, and incubated for 24h, and than were irradiated with UVB light (50 mJ/cm^2). ASC specks had been stained 4h and 8h after irradiation using Anti-Asc, rabbit pAb (AL177), antibody (red), F-actine (green), DAPI (blue). IL-1α and IL-1β has been measured in cell free supernatants using ELISA. **(b) UVB light and poly (dA:dT) leads to activation of NF*k*B pathway assocciated proteins**. Data represents at least 3 independent experiments and were normalized to β-actin levels, and are presented as (means ± SD). Statistics: One-Way ANOVA statistical test . Symbols for P-values used in the figures *P<0,05, **P<0,01, ***P<0,001, ****P<0,0001, NS-not significant. Magnification x43.

3.5 ASC "specks"and IL-1 secretion follow a similar kinetics:

To evaluate the kinetics of ASC speck formation, keratinocytes were irradiated with UVB (50 mJ/cm^2), and ASC "specks" staining was performed at 30min, 1h, 2h, 4h, and 8h after exposure. The formation of ASC specks starts 2 hours after irradiation, with the peak between 4 and 8 hours **(Fig.5)**.

Analysis on protein level using ELISA, supports immunoflourescence findings, showing the peak of IL-1α secretion between 4h and 8h after exposure to UVB light **(Fig.5)**.

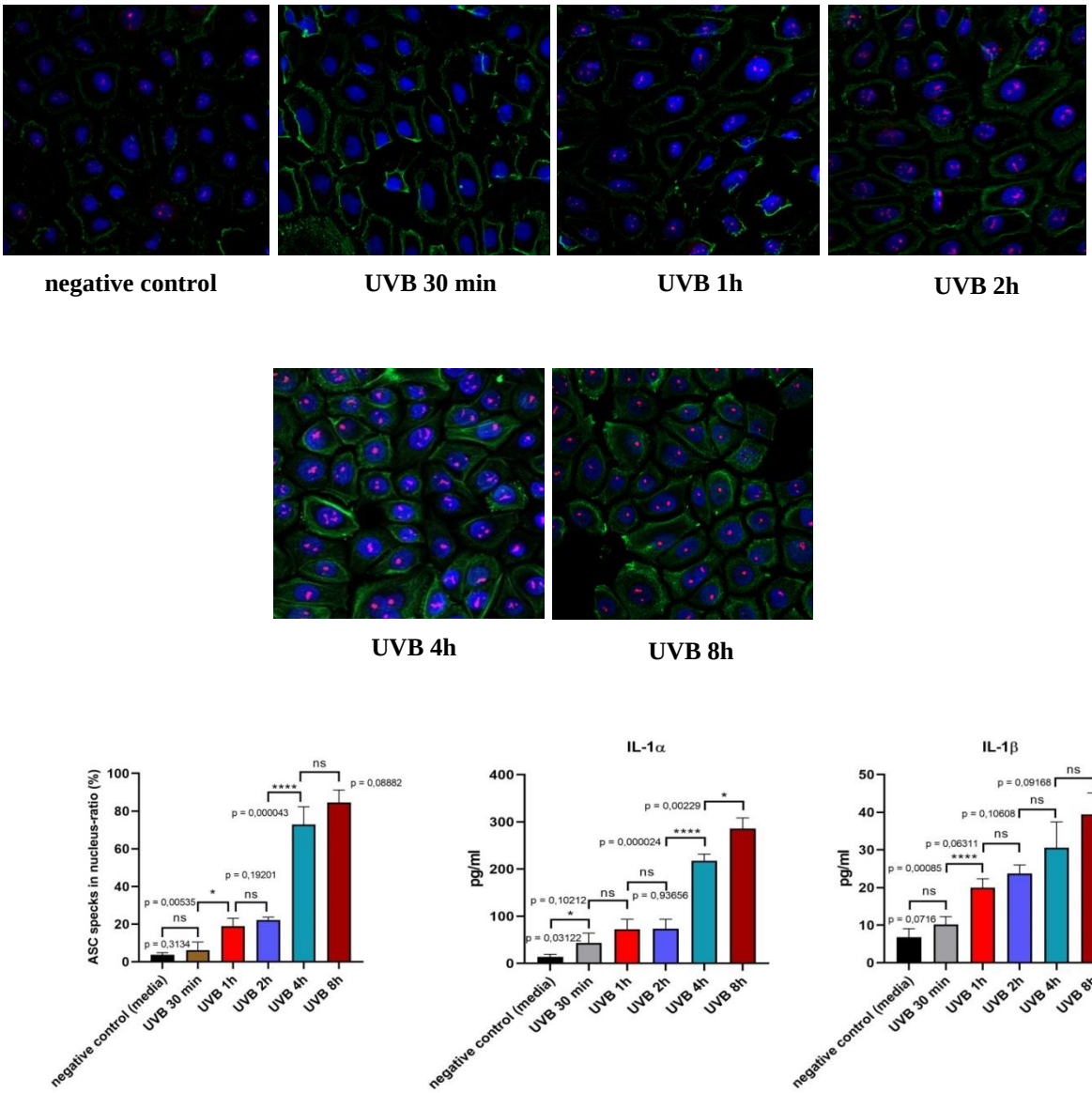


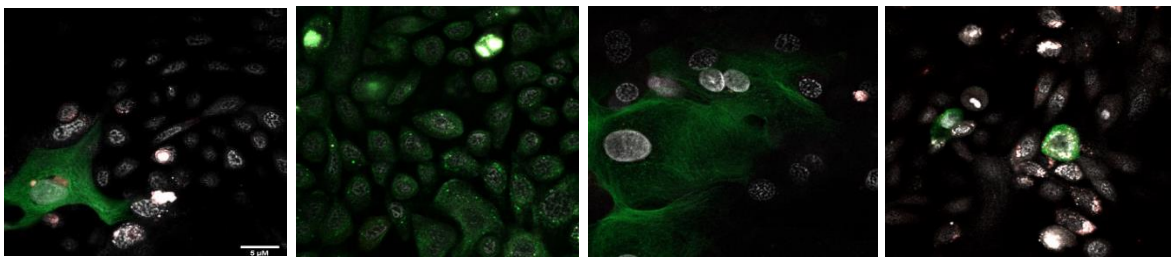
Figure 5. UVB light leads to inflammasome activation, followed by ASC „specks" formation and IL-1α secretion in the time dependent manner. Human keratinocytes had been irradiated with UVB light (50 mJ/cm^2) .ASC specks has been stained 30min, 1h, 2h, 4h and 8h after UV irradiation using Anti-Asc, rabbit pAb (AL177), antibody (red), F-actine (green), DAPI (blue). IL-1α and IL-1β has been measured in cell free supernatants using ELISA. Data represents at least 3 independent experiments, and are presented as (means ± SD). Statistics: One-Way ANOVA statistical test . Symbols for P-values used in the figures *P<0,05, **P<0,01, ***P<0,001, ****P<0,0001 NS-not significant. Magnification x43.

3.6. ASC "specks" lead to the apoptosis:

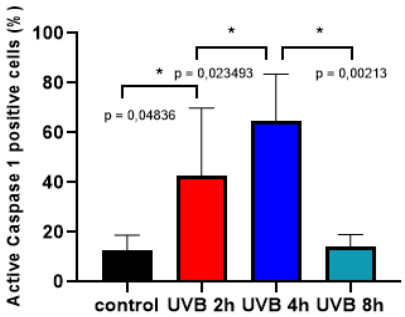
As ASC is not only involved inflammasome activation, but also in apoptosis, we aimed to check whether ASC "specks" could putatively lead to the apoptosis. To investigate whether UVB irradiation leads to the apopotosis, FAM-Flica staining for active caspase-1 was performed. Caspase FLICA kits measures active caspase 1 in whole, living cells. FLICA is cell-permeant and can efficiently diffuse in and out of cells. If there is an active caspase-1 enzyme inside the cell, it will covalently bind with FLICA and retain the green fluorescent signal within the cell. Cells were also stained with propidium-iodide, that can diffuse in the nucleus. PI binds to DNA of dying cells by intercalating between the bases with little or no sequence preference. By this means, caspase-1 activitiy can correlated to cell death.

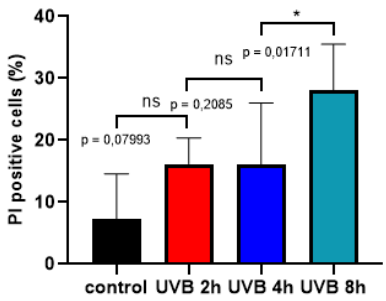
Caspase-1 activation is visible starting 2 hours after expossure to UVB with the peak of activity 4 hours after UVB irradiation. As expected, most PI positive cells were observed 8 hours after UVB exposure. This indicates that inflammasome activation and ASC-speck formation ultimately results in cell death in human keratinocytes. UVB exposure first leads to inflammasome assembly and finally to cell death in primary human keratinocytes. **(Fig.6a)**. To support Flica stainings an LDH assay was performed. LDH leakage from dying cells increases over time, correlating with the FAM-FLICA results **(Fig.6b).**

(a)



medium control **UVB 2h** **UVB 4h** **UVB 8h**





(b)

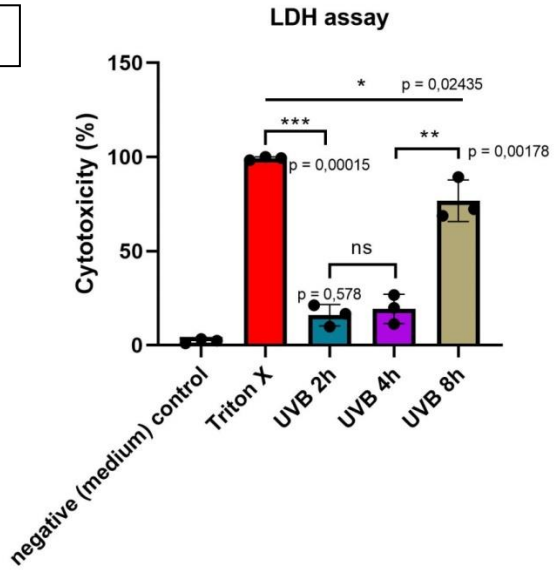


Figure 6. (a) UVB irradiation causes cell death in primary human keratinocytes. Primary human keratinocytes were irradiated with UVB (50 mJ/cm^2). To detect active caspase-1 FAM-Flica staining (green) was done. Propidium iodide (PI) stainig (red) was performed to detect dying cells. **(b) LDH cell death assay**. Data represent at least 3 independent experiments and are presented as means ± SD. Statistics: One-Way ANOVA statistical test. Symbols for P-values used in the figures *P<0,05, **P<0,01, ***P<0,001, NS-not significant. Magnification x43.

3.7. NLRP1 inflammasome activation leads to apoptosis in hPKs:

As shown before, ASC-speck formation depends on NLRP1. To investigate which inflammasome leads to cell death after the formation of ASC aggregates in keratinocytes, NLRP1 and NLRP3 were shut down using siRNA. When NLRP1 is absent, LDH release is significantly reduced, showing that NLRP1 inflamamsome activation is crucial for caspase-1 mediated cell death in human hPKs **(Fig.7)**.

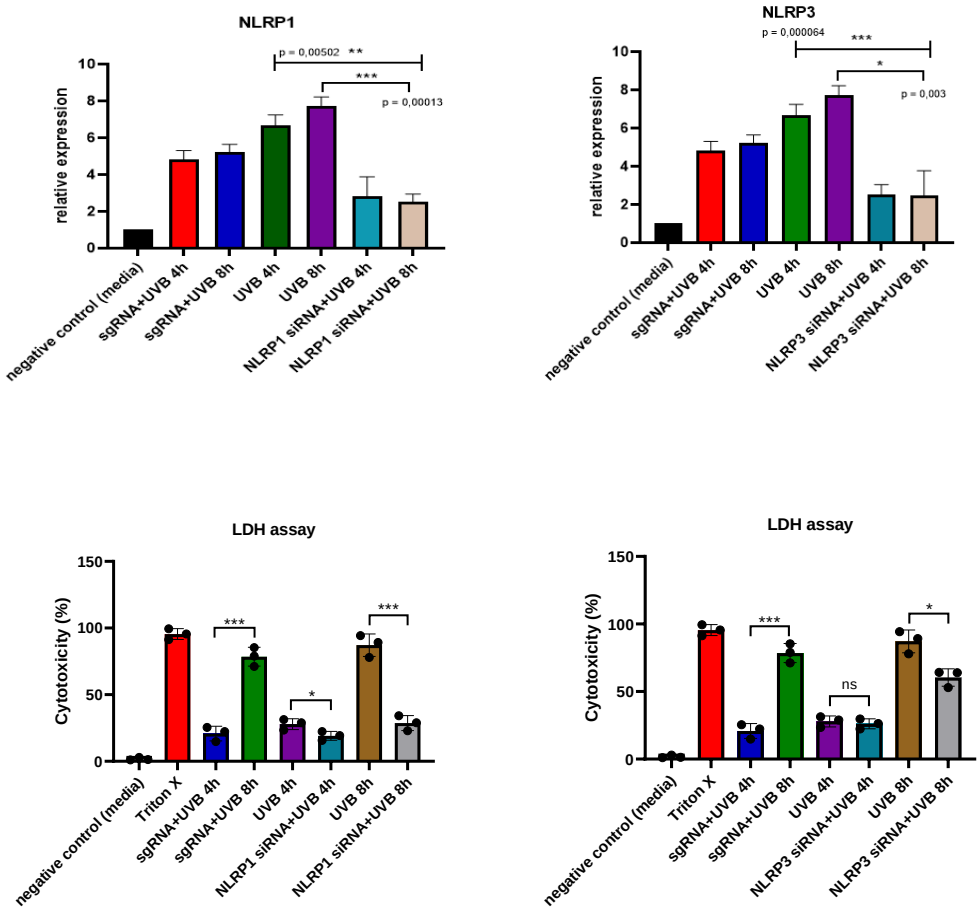


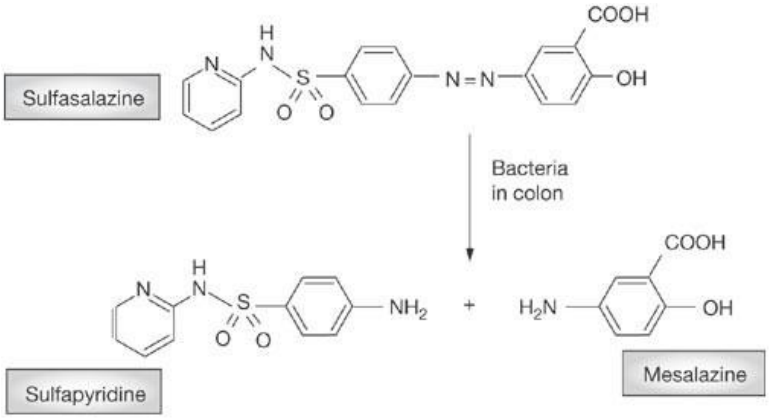
Figure 7. NLRP1 inflammasome blocking protects from cell death: hPKs were transfected with NLRP1 and NLRP3 siRNA and were irradiated with UVB (50 mJ/cm^2). Lack of NLRP1 leads to a reduction of LDH release. Data represent at least 3 independent experiments and are presented as means ± SD. Statistics: One-Way ANOVA statistical test. Symbols for P-values used in the figures *P<0,05, **P<0,01, ***P<0,001, NS-not significant

As presented above, innate immunity plays a key role in keratinocytes. How these cytokines are regulated in an inflammatory disease was examined in acute anterior uveitis (AAU), a disease that affects the uvea, and can be presented on one or both eyes. AAU can be HLA-B27 positive and negative. Cytokine immunology, especially the mechanism how mesalazine (5-ASA) influences proinflammatory cytokines in patients with AAU is poorly understood It has been shown clinically that HLA-B27 positive patients differ in their response to mesalazine, compared to HLA-B27 negative patients (HLA-B27 positive patients respond to sulfasalazine therapy and HLA-B27 negative not), one reason for clinicians to differentiate both types.

Therefore it is very important to decipher the impact of mesalazine, on cytokine expression in the PBMCs of HLA-B27 AAU patients**.** To rule out toxicity of the concentrations of 5-ASA (100µM and 500µM) used, an MTT cell viability assay has been performed **(Fig. 8b)**.

3.8. 5-ASA suppress IL6 but not IL1 in PBMCs derived from healthy donors:

(a)



(b)

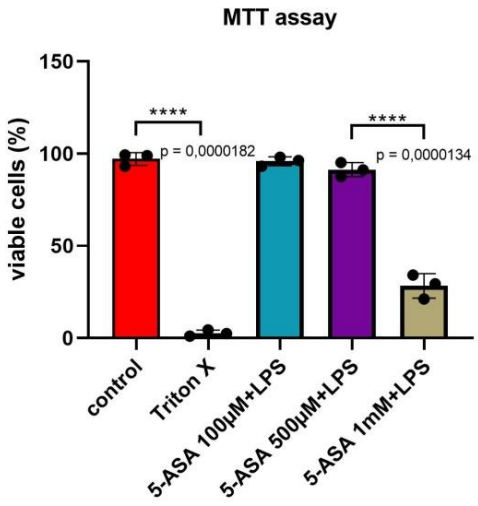


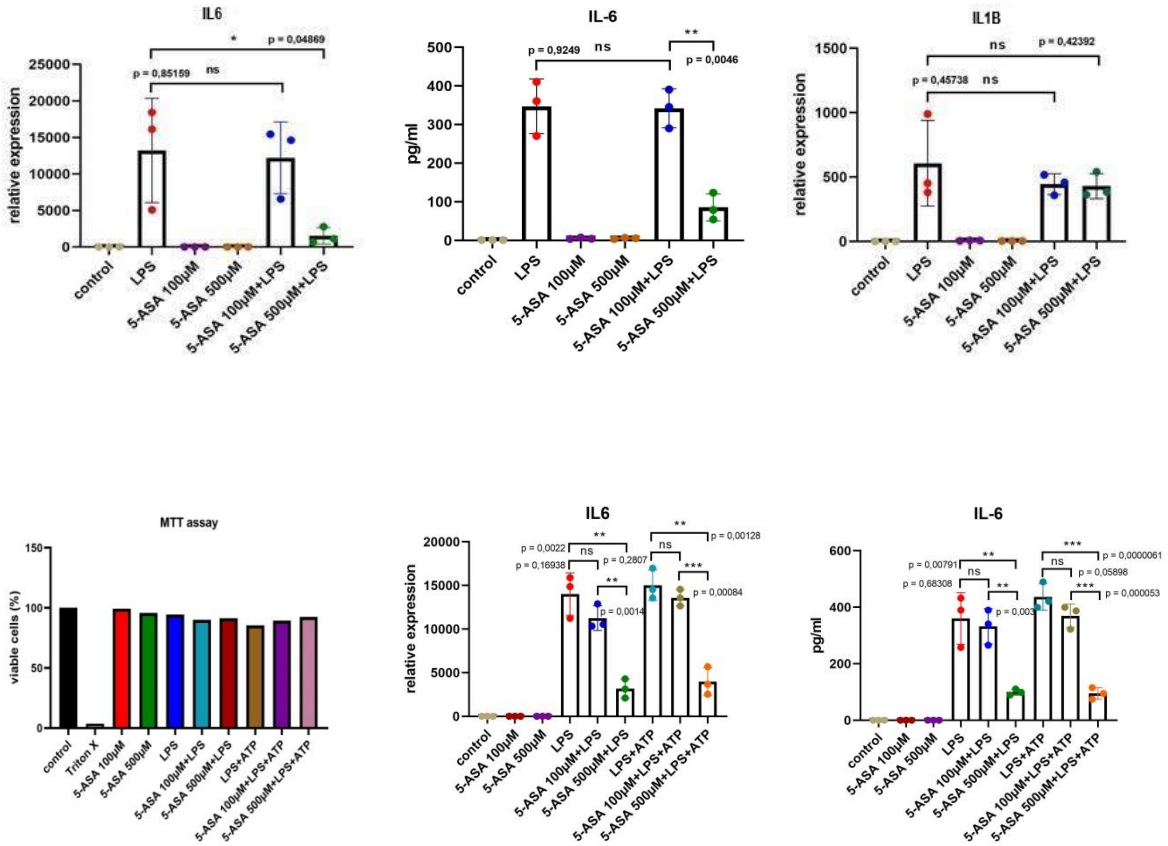
Figure 8. (a) Scheme of mesalazine (5-ASA) metabolism. **(b)** MTT cell viability assay ruled out toxicity of the two concentrations of mesalazine (5-ASA) used. Statistics: One-Way ANOVA statistical test. Symbols for P-values used in the figures *P<0,05, **P<0,01, ***P<0,001, ****P<0,0001 NS-not significant

To investigate a possible antinflammatory role of mesalazine (5-ASA) in myeloid cells, human PBMCs isolated from healthy donors were preincubated with 5-ASA and stimulated with the TLR4 agonist LPS. Analyzing the pro-inflammatory innate cytokines IL-6 and IL-1β, it has been found that 5-ASA suppressed both IL6 mRNA induction and secretion, while IL1B mRNA was not suppressed. **(Fig. 9a).**

For the secretion of IL-1β, caspase-1 activation is needed to cleave pro-IL-1β to its active form. Caspase-1 activation is achieved via inflammasome assembly (Schroder K.et.al. 2010).

For this "two signals" are needed, the first signal known as "priming" signal and the second known as "activation" signal (Bauernfeind F.et.al. 2009). To induce the secretion of IL-1β, we used LPS as the "priming" signal for IL1B transcription and ATP for inflammasome activation. In this setting it has been clearly observed that IL1B mRNA and IL-1β secretion were not suppressed by 5-ASA, **(Fig.9b).**

(a)



(b)

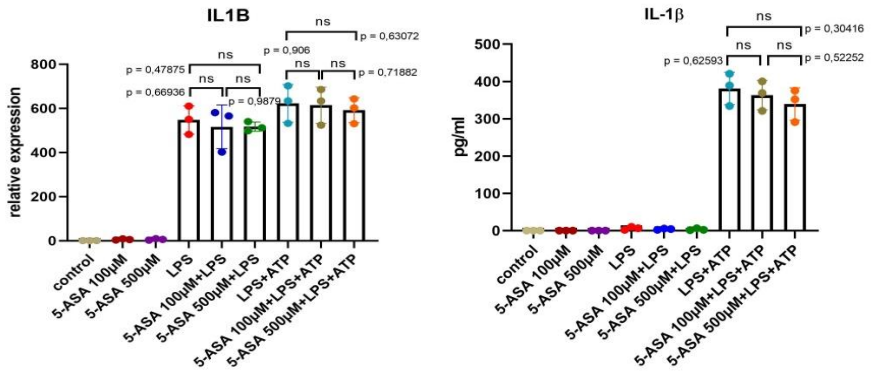
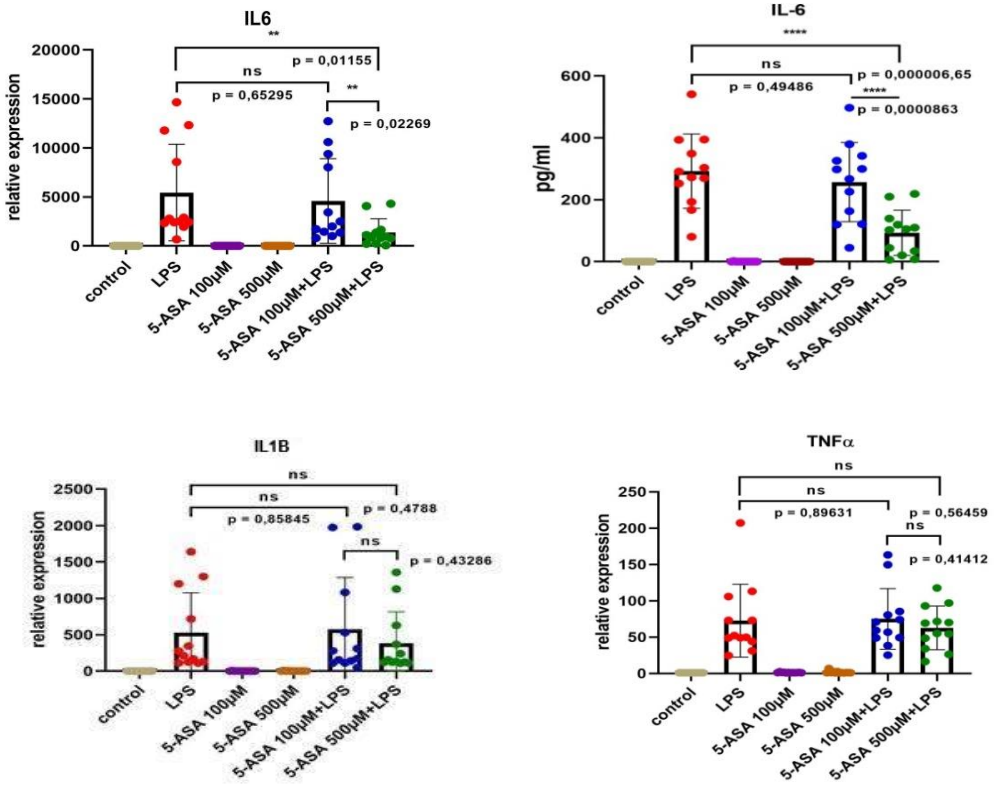


Figure 9. Mesalazine (5-ASA) suppresses IL-6 mRNA expression and protein secretion, but did not suppress IL-1β mRNA in PBMCs of healthy donors. (a) PBMCs from healthy donors (n=3) were treated with TLR4 receptor ligand LPS (100nM) for 3h (positive control), and cells were preincubated with 5-ASA (100µM and 500µM) for 30 min and then treated with LPS (100nM) for 2.5h. Medium was used as a negative control **(b)** PBMCs from healthy donors (n=3) were preincubated with mesalazine (5-ASA) (100µM) for 30 min and treated with LPS (100nM) and ATP (5mM) 30 min. Data represents at least 3 independent experiments and were normalized to β-actin levels, and are presented as (means ± SD). Statistics: One-Way ANOVA statistical test. Symbols for P-values used in the figures *P<0,05, **P<0,01, ***P<0,001, NS-not significant. (Smatlik N. et.al. 2021. *Ocular immunology and inflammation.* In press)

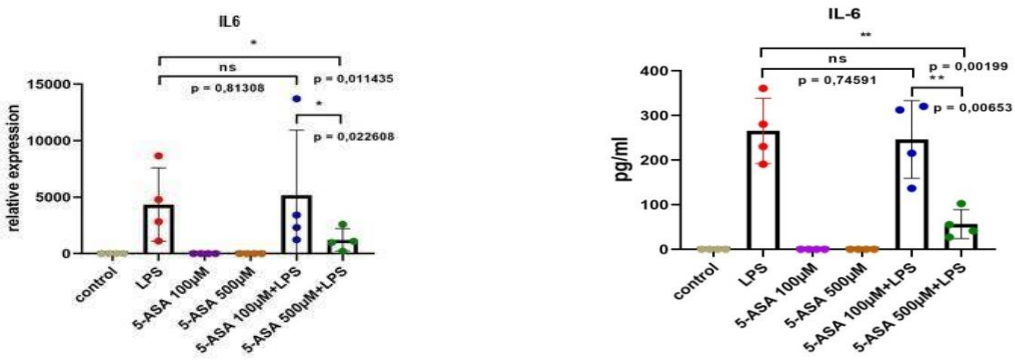
3.9. 5-ASA suppress proinflammatory cytokine IL-6, but not IL-1 and TNF in PBMCs derived from HLA-B27+ and HLA-B27- AAU patiens:

Mesalazine is one of the treatment options in HLA-B27 associated acute anterior uveitis, with only HLA-B27 positive patients showing a good response. Therefore, the effect of 5-ASA on PBMCs isolated form AAU patients were investigated. Patients included in the study were under topical NSAIDs or systemic steroid treatment **(Table 1)**. It has been observed that 5-ASA suppressed IL6, but neither TNF nor IL1B mRNA induction. The suppression of IL-6 secretion and synthesis did not dependent on the HLA-B27 status of the patients **(Fig. 10).**

HLA-B27+



HLA-B27-



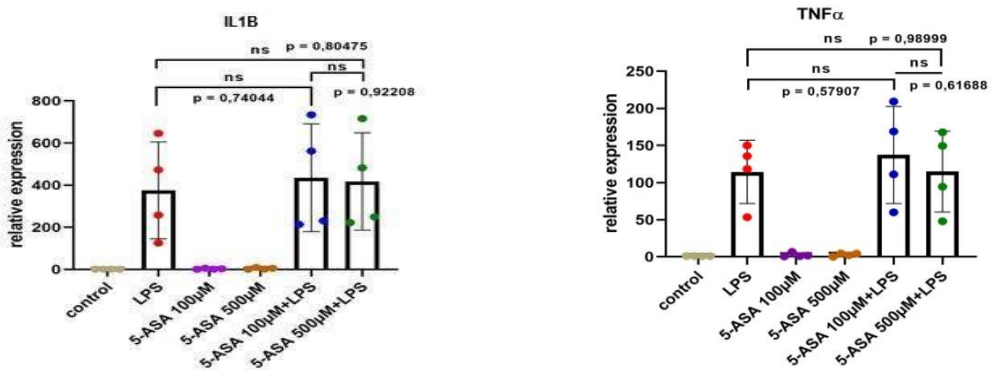


Figure 10. IL-6, but not IL-1β and TNF is supressed by 5-ASA in PBMCs from HLA-B27+ and HLA-B27- AAU patients. PBMCs from HLA-B27+ (n=12) and HLA-B27- (n=4) associated (AAU) patients were preincubated with mesalazine (5-ASA), (100µM and 500µM) concentrations for 30 min, and then treated with LPS (100 nM) for 2.5h Data represents at least 3 independent experiments and were normalized to β-actin levels and are presented as (means ± SD). Statistics: One-Way ANOVA statistical test. Symbols for P-values used in the figures *P<0,05, **P<0,01, ***P<0,001, ****P<0,001, NS-not significant.

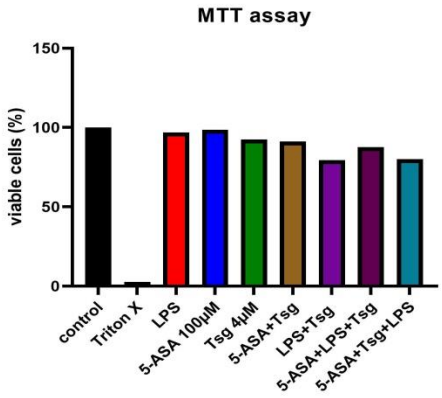
3.10. 5-ASA suppress proinflammatory and ER stress associated cytokines and markers in PBMCs derived from healthy donors and HLA-B27+ AAU patients:

The suppressive impact of 5-ASA is mediated via the inhibition of ER stress. ER stress leads to the unfolded protein response (UPR), leading to the induction of proinflammatory cytokines including IL-6. Endoplasmic reticulum stress differentially modulates the IL-6 family of cytokines in murine astrocytes and macrophages (Santiago MF. et.al 2003).
In this experiment we investigated whether 5-ASA could suppress UPR, and if this serves as explanation for the anti-inflammatory effect of mesalazine.

To artificially induce ER stress (Tsg), a non-competitive inhibitor of the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) pump was used. Then its impact on the ER stress associated cytokines (IL-6, IL-1β, TNF, IFNγ and IL-23A) and ER stress associated markers (CHOP and ATF6) was measured.MTT cell viability ruled out a toxic effect **(Fig. 11a)**. 5-ASA suppressed significantly, thapsigargin-induced ER-stress mediated IL6 mRNA induction and consecutive IL-6 secretion. Interestingly, in opposition to LPS-treatment, ER-stress induced IL1B, TNF and IL23A mRNA expression was diminuished upon 5-ASA treatment, while IFNG was not altered. However, 5-ASA was able to suppress the superinduction via both LPS and thapsigargin of IL6, IL1B, TNF and IL23A, but not of IFNG, demonstrating that the effect of 5-ASA on ER-stress dominates **(Fig. 11b).**

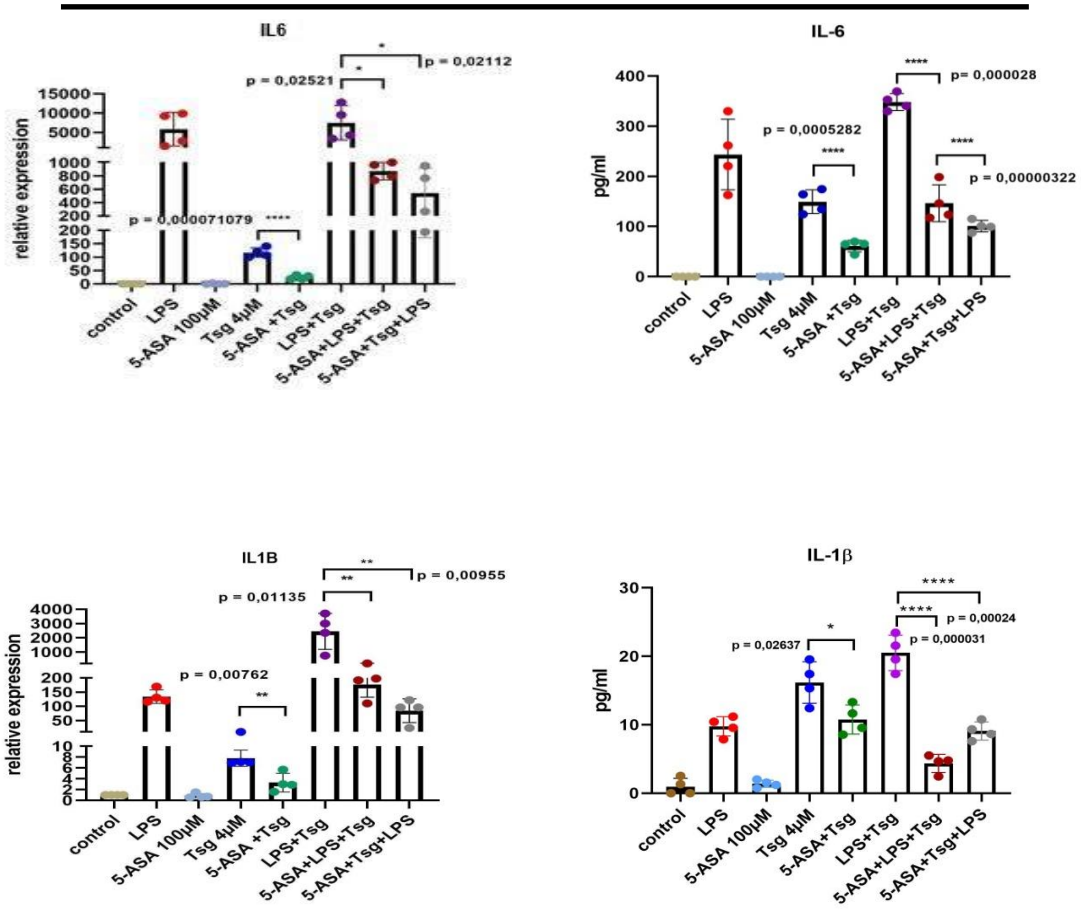
The inhibition of ER by 5-ASA was proven by the suppression of ER-stress associated markers, such as CHOP (DDIT3) and ATF6 in PBMCs **(Fig. 11c).**

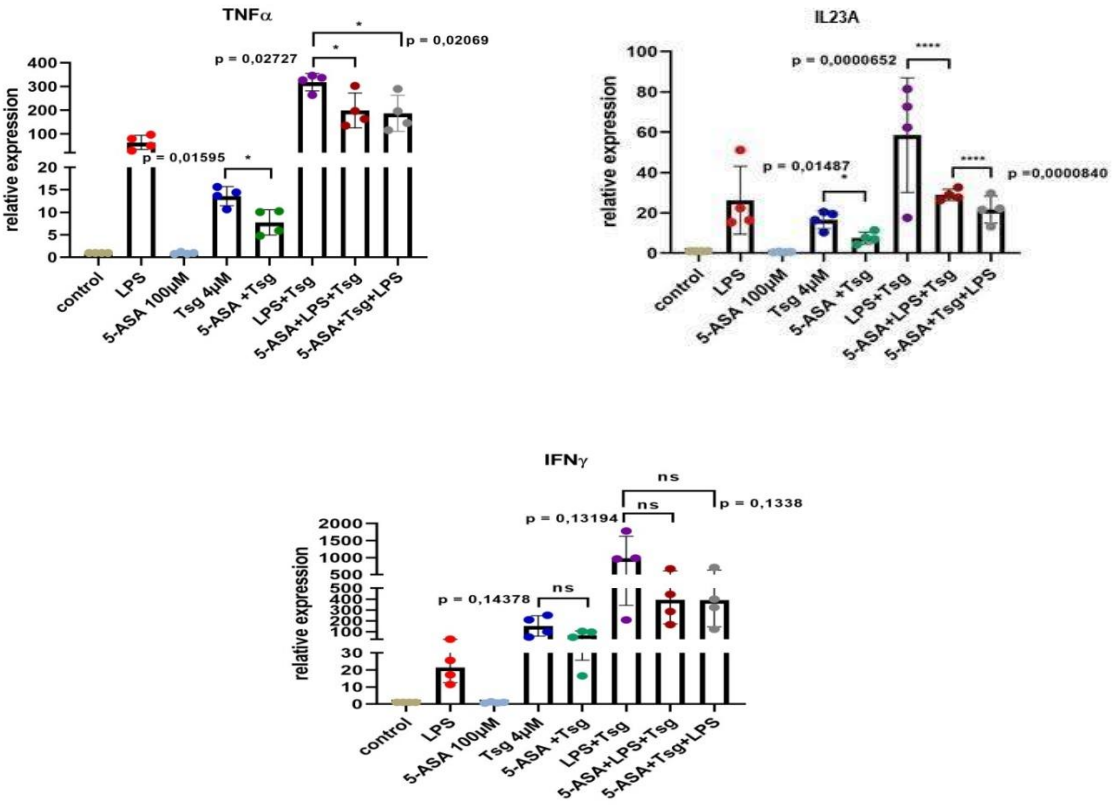
(a)



(b)

HLA-B27+





(c)

Healthy donors

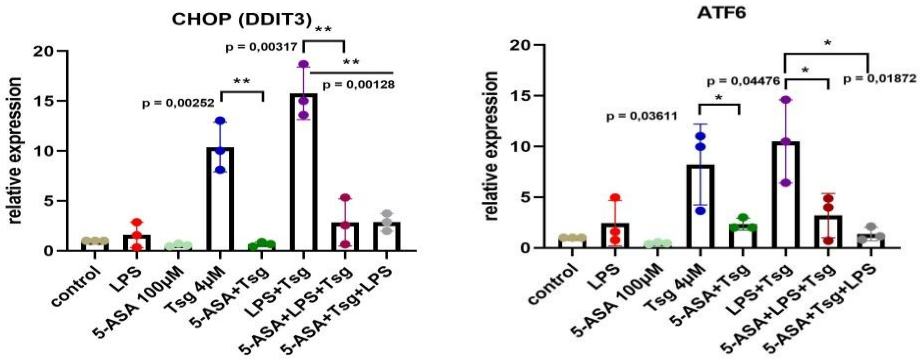


Figure 11. (a) ER stress associated cytokines and markers are suppressed by 5-ASA. (a) MTT cell viability assay was performed to detect whether concentrations of 5-ASA, LPS and Tsg are toxic for the cells. **(b) IL-6, IL-1β, TNF*α*, and IL-23A are suppressed by 5-ASA in PBMCs from HLA-B27+ associated acute anterior uveitis (AAU) patients, while IFNγ is not altered**. Human peripheral blood mononuclear cells (PBMCs) from HLA-B27+ (AAU) patients (n=4) were preincubated with mesalazine (5-ASA), (100µM) for 30 min, then were treated with LPS (100 nM) for 2.5h. To induce ER stress leading to UPR response cells were treated with (4µM) thapsigarin (Tsg). **(c)** ER stress associated markers CHOP (DDIT3) and ATF6 were suppressed by 5-ASA in the PBMCs derived from healthy donors (n=3). Data represents at least 3 independent experiments and were normalized to β-actin levels and are presented as (means ± SD). Statistics: One-Way ANOVA statistical test. Symbols for P-values used in the figures *P<0,05, **P<0,01, ***P<0,001, ****P<0,001, NS-not significant.

4. D I S C U S S I O N:

Skin inflammation can take place after sensing of different stimuli such as UV irradiation, ionizing radiation, pathogens, contact with chemical irritants (soap, hair dyes etc.), allergen uptake or microbial challenge (Behrends U.et.al. 1994), (Fuchs J.and Kern H..1998), (Martin S.et.al. 2012), (Miller S.et.al. 2011), (Rustemeyer T.et.al. 2011). Exposure to UV light is harmful for the skin and leads to skin inflammation and cancer.

UVB light is one of the most potent inducers of apoptosis in human keratinocytes. Apoptotic keratinocytes are cells characterized by eosinophilic cytoplasm and pyknotic nuclei, also known as sunburn cells (SBC) (Daniels F.et.al. 1961). In human keratinocytes *in vitro,* apoptosis results in permeabilization of the plasma membrane, and this process is known as secondary necrosis, which can lead to inflammation. However, this will not occur under physiological circumstances due to rapid clearance of apoptotic cells (Savill J.et.al. 2002).

The aim of this work was to investigate the impact of UVB exposure on inflammasome assembly and formation of ASC “specks” in primary human keratinocytes and whether formation of ASC “specks” leads to cell death in hPKs.

Exposure to UVB light leads to damage to the skin, which results in inflammation characterized by increased in NF*k*B activation and eventually increased inflammatory cytokines, such as TNF, IL-6, IL-1 as well as IL-8 and IL-10 (Ichihashi M.et.al. 2003), (Black A.T.et.al. 2008), (Gludsdale G. J.et.al. 2001).

One of the protein complexes that is involved in UVB sensing in primary human keratinocytes is complex called the “inflammasome”.

Inflammasomes are protein complexes located in the cytosol that function as platforms for the recruitment and activation of pro-inflammatory caspase-1 (Chavarría-Smith J.et.al. 2015).

Inflammasome complexes form upon stimulation to process the cleavage of caspase-1, which activates the proinflammatory cytokines interleukin-1β (IL-1β) and interleukin-18 (IL-18), and the cytosolic protein gasdermin D (GSDMD) (Ming Man S.et.al. 2017). Cleaved GSDMD translocates from the cytosol to the cell membrane where it forms pores which induce pyroptotic cell death and permit the release of IL-1β and IL-18 into the extracellular space (Evavold CL.et.al. 2018).

Activation of the inflammasome protein complex can be visualized by the formation of ASC "specks", which reflect the recruitment of ASC into the complex.

Here, we clearly show that UVB irradiation in primary human keratinocytes leads to the inflammasome activation and the formation of ASC specks in the cell nucleus, with the peak in number of "specks" between 4 and 8 hours after exposure to UVB light.

This finding is opposite to the findings in myeloid cells where ASC "specks" are located in the perinuclear area, but is similar to findings of Kuri et.al. in Zebra fish, where ASC "specks" are also located in the nucleus of keratinocytes (Kuri P. et.al.2017).

The presence of nuclear ASC was also observed by Kerur et.al. in HMVEC-cells. These non-myeloid, endothelial cells react upon infection with Kaposi-Sarcoma-Virus first with nuclear ASC-aggregates (Kerur N. et.al. 2011).

Differences between ASC localization might exist between myeloid cells and resident cells, such as endothelial cells or keratinocytes.

The pan-caspase inhibitor zVAD did not lead to the suppression of ASC "specks" formation because it does not block recruitment of ASC and pro-caspase 1 to the inflammasome complex, but rather the activation of caspase-1. Therefore, the secretion of IL-1 is blocked by zVAD as it needs active form caspase-1 for processing.

NLRP1 contains two effector-recruitment domains. One of these domains is an N-terminal pyrin domain (PYD), and the other is a C-terminal caspase activating and recruitment domain (D'Incà R. et.al. 2013). These two effector domains are part of the death domain superfamily, and they are structurally related to each other. It is generally believed that the PYD and CARD domains mediate homotypic interactions with other downstream PYD/CARD containing proteins, for example, ASC and Caspase 1 (Frew BC.et.al. 2012). NLRP1 contains his own CARD domain but it still needs ASC for its activation and caspase-1 cleavage (Sandstrom A. et.al. 2019).

NLRP3 inflammasome is a cytosolic pattern recognition receptor that senses microbes and endogenous danger signals. It is mainly expressed in immune cells notably antigen presenting cells (APCs), macrophages, dendritic cells (Baumgart DC.et.al.2013), neutrophils in the spleen and monocytes (Zhong Y.et.al. 2013).

Both inflammasomes NLRP1 and NLRP3 are expressed in primary human keratinocytes, but which one is responsible for UV sensing is still matter of debate. Recent data from Fenini et.al. suggests that NLRP1 plays the crucial role in UVB sensing in hPKs (Fenini G. et.al. 2018).

Therefore, we questioned which inflammasome is crucial for UVB sensing in hPKs. When NLRP1 is silenced by siRNA, the formation of ASC "specks" in human keratinocytes was almost completely blocked, same as the secretion of IL-1. In contrast, when NLRP3 is absent, the number of ASC "specks" is reduced, but less prominent compared to NLRP1.

Again, the secretion of IL-1alpha correlates with ASC "specks" staining as IL-1 secretion was suppressed, but not as much as in the absence of NLRP1. These findings support Fenini et.al., where NLRP1 dominated regarding caspase-1 activation and IL-1 secretion.

Here we showed, the NLRP1 inflammasome plays more important role than NLRP3 in UVB sensing in hPKs.

Activation of AIM2 inflammasome via bacterial/viral dsDNA analogues leads to formation of ASC specks in myeloid cells (Malik A. et.al. 2017).

To investigate whether ASC speck formation needs an NLR-inflammasome, we stimulated keratinocytes with transfected poly(dA:dT) to mimic cytosolic dsDNA. Again, ASC specks were observed, similar to NLRP1/NLRP3 activation located in the nucleus.

Therefore, nuclear ASC specks are a general feature in keratinocytes. Maybe on their nuclear functions is NF*k*B pathway activation.

Phosphorylated form of I*k*Bα (p-I*k*Bα), I*k*Bα, IKKα/β, and p-p65 (Rella) were upregulated with poly(dA:dT) and also in combination of poly(dA:dT) and UVB. IKKα/β long form was not upregualated at all, and IKKε were upregulated with poly(dA:dT) but not with UVB.

Those findings confirm the previous findings from different groups that poly(dA:dT) and UVB light, induces NF*k*B and also proteins involved in the regulation of NF*k*B pathway (Bauernfeind F. et.al. 2009), (Lewis DA.et.al. 2006), (Abe T. and Barber GN. 2014).

ASC is not only involved in inflammasome activation, but also in apoptosis or pyroptosis (Ohtsuka T. et.al. 2004), (Zheng D. et.al. 2020). Formation of ASC specks in the nucleus after caspase-1 activation in primary human keratinocytes might be involved in the induction of cell death.

To investigate whether UVB induced ASC specks leads to cell death, active caspase-1 FAM-Flica stainig was performed. Caspase-1 activation clearly proceeds cell death which started to take place at 8 hours, while active caspase-1 was visible after 1 hour.

To see which type of inflammasome in hPKs plays a crucial role in cell death NLRP1 and NLPR3 were silenced by siRNA. Cells without functional NLRP1 inflammasome were protected from LDH release, showing that NLRP1 is needed for the execution of cell death.

One prominent disease, where innate inflammation might play a key role is acute anterior uveitis. It is defined as acute onset of inflammation of the iris and/or ciliary body [(Zeboulon N. et .al. 2008)]. The pathogenesis of AAU is still not well understood but Human Leukocyte Antigen (HLA)-B27 is often detectable in acute anterior uveitis [(Pichi F. et.al. 2015)]. Also, myeloid cells including monocytes might play a crucial role in the pathogenesis of the disease. AAU patients show an elevated number of monocytes, activated T cells, and high S100A8/A9 serum levels, an alarmin released by myeloid cells [(Kasper M. et.al. 2018)].

A particular population of monocytes of AAU patients is characterized by the elevated expression of TNF, IL-10, and IL-23 [(Krasselt M et.al. 2013)].

One of the treatment options for AAU is sulfasalazine and its metabolic derivate mesalazine. For more than 30 years it is known that sulfasalazine inhibits granulocyte activation and chemotaxis [(Stenson WF. et.al.1984)]. More than 10 years later Wahl et.al. claimed that it can suppress TNFα-, LPS-, or PMA- induced activation of NF*k*B and suppresses NF*k*B–dependent transcription [(Wahl C. et.al. 1998)].

Our data shows the inhibitory effect of mesalazine, the active metabolite of sulfasalazine, on IL-6 and IL-1 transcription, which can both be activated via NF*k*B. Due to less transcription, less IL-1 is secreted after in inflammasome activation, however inflammasome activation itself seems to be unaltered. Here, we demonstrate the anti-inflammatory effect of 5-ASA via the inhibition of TLR-activated IL-6 transcription in PBMCs of healthy donors. Approximately 50% of all cases of (AAU) are associated with the HLA-B27 antigen. This HLA is also associated with other inflammatory conditions such as psoriasis, reactive arthritis, or inflammatory bowel disease, where 5-ASA is used as a treatment option. Due to the clinical experience that the HLA-B27 status decides about the outcome of this treatment, we investigated PBMCs derived from patients with positive and also negative HLA-B27 associated AAU.

Interestingly, the HLA-status did not change the suppressive capacity on IL-6 both on mRNA and protein level. IL-1β and TNF were not suppressed in both groups. This might explain the identical antinflammatory response with corticosteroids on HLA-B27 positive and negative AAU.

One mechanism to initiate innate immune responses in myeloid cells is ER stress [(Menu P. et.al. 2012)]. This results in an elevated expression of TNF, IL-10, and IL-23A [(Krasselt M et.al. 2013)]. A different response of HLA-B27 + and – AAU patients could be based on this mechanism.

By measuring ER stress associated cytokines like IL-6, IL-1β, TNF, and IL-23A we observed their suppression by 5-ASA. ER-stress is blocked by 5-ASA, as CHOP (DDIT3) and ATF6 as ER-stress markers were suppressed upon treatment, linking the effect of 5-ASA to ER-stress. Taken together, our data support the use of the term “HLA-B27- “typical” AAU, because both groups show not only identical clinical findings, but also the same effect on proinflammatory cytokines after 5-ASA treatment. The innate immune response is not influenced by the HLA-B27-status. Other mechanisms may explain the different response to sulfasalazine.

5. C O N C L U S I O N S.

Inflammasomes especially NLRP1 play very important roles in UVB sensing in primary human keratinocytes. UVB induces inflammasome assembling, caspase 1 recruitment and IL-1 secretion. Considering previous data, it can be concluded that UVB light leads to ASC speck formation in primary human keratinocytes, and those specks are localized in the nucleus. These findings are opposite to the findings in myeloid cells, where ASC "specks" localize in the perinuclear area, but are similar to the findings in Zebra fish, where ASC specks also localize in the nucleus (Kuri P. et.al. 2017). ASC specks reflect inflammasome activation as their formation in hPKs is dependent on the presence of NLRP1 and NLRP3 inflammasomes. In opposition to cytosolic ASC specks, nuclear ASC speck formation putatively links cell death to immune activation, as nuclear ASC are involved in cell death. Blocking of NLRP1 impairs IL-1 secretion and cell death in primary human keratinocytes exposed to UVB light. A further disease of the innate immune system might be acute anterior uveitis.

Acute anterior uveitis as an inflammatory disease of the eye that is characterized by a strong infiltration of myeloid cells, especially monocytes to the uvea, and one of the treatment options for AAU is sulfasalazine and its methabolic derivate mesalazine, which mode of action is still metter of debate.

Our data clearly shows an inhibitory effect of mesalazine on IL6 transcription and translation, which can be activated via NF*k*B in myeloid cells. 5-ASA additionally inhibits the transcription of proinflammatory and endoplasmatic reticulum (ER) stress associated cytokines and ER stress associated markers (CHOP and ATF6), independently of the HLA-B27 status linking 5-ASA mode of action to ER stress.

As the innate immune response is not influenced by the HLA-B27-status, other mechanisms are therefore needed to decipher the molecular reason of the different response to mesalazine.

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References:

[1] Aachoui, Y., Laef, IA., Hagar JA.,et.al. (2013). "Caspase-11 protects against bacteria that escape the vacuole". *Science*. **6122**: 975-978.

[2] Abe, T and Barber GN. (2014)." Cytosolic-DNA-mediated, STING-dependent proinflammatory gene induction necessitates canonical NF-κB activation through TBK1". *J Virol*. 10:5328-5341.

[3] Agostini, L., Martinton, F., Burns, K., et.al. (2004). "NALP3 forms an IL-1beta-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder". *Immunity*. **3**: 319-325.

[4] Agrawal, RV., Murthy, S., Sangwan V., and Biswas, J.(2010)." Current approach in diagnosis and management of anterior uveitis". *Indian J Ophtalmol*. **1**:11-19.

[5] Allam, R., Darisipudi, MN., Tschopp, J., and Anders, HJ. (2013). "Histones trigger sterile inflammation by activating the NLRP3 inflammasome". *Eur J Immunol*. **12**: 3336-3342.

[6] Allgayer, H., Ahnfel, NO., Kruis, W, Klotz, U. (1989)."Colonic N-acetylation of 5-aminosalicylic acid in inflammatory bowel disease". *Gastroenterology*. 1: 38-41.

[7] Allgeyer, H. (2003)."Mechanisms of action of mesalazine in preventing colorectal carcinoma in inflammatory bowel disease". *Aliment Pharmacol Ther*. **2**: 10-14.

[8] Amur, S., Parekh, A., A., and Mummaneni, P. (2012)."Sex differences and genomics in autoimmune diseases". *J Autoimmun*. **3**: 254-265.

[9] Andrade, F., Casciola-Rosen, LA., and Rosen, A. (2005). "Generation of novel covalent RNA–protein complexes in cells by ultraviolet B Irradiation: Implications for Autoimmunity". *Arthritis Rheum*. **4**: 1160–1170.

[10]Baccala,R., Gonzales-Quintal, R.,Lawson, B.R., et.al. (2009). "Sensors of the innate immune system:their mode of action". *Nat Rev Rheumatol*. **5**: 448-456.

[11] Bakele, M., Joos, M., Burdi, S., et.al. (2014). "Localization and functionality of the inflammasome in neutrophils". *J Biol Chem*. **8:** 5320-5329.

[12] Baker, PJ., Boucher, D., Bierschenk, D., Tebartz, C., et.al. (2015). "NLRP3 inflammasome activation downstream of cytoplasmic LPS recognition by both caspase-4 and caspase-5". *Eur J Immunol*. **10**: 2918-2926.

[13] Bantel, H., Berg, C., et.al. (2000)."Mesalazine inhibits activation of transcription factor NF-kappaB in inflamed mucosa of patients with ulcerative colitis". *Am J Gastroenterol*. **12**: 3452-3547.

[14] Bauernfeind, FG., Horvath, G., Stutz, A., et.al. (2009). "Cutting edge: NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression". *J Immunol*. **2**: 787-791.

[15] Baumgar,t DC, and Sanborn, WJ. (2012). "Crohn's disease". *Lancet*. **9853**:1590-1605.

[16]Behrends, U., Peter, R.U., Hintermeier-Knabe, R., et.al. (1994). "Ionizing Radiation Induces Human Intercellular Adhesion Molecule-1 In Vitro". *J Invest Dermatol*. **103**: 726-730.

[17] Bertin, B., Dubuqouy, L., Colombe.l JF, and Desreumaux, P. (2013). "PPAR-gamma in ulcerative colitis: a novel target for intervention". *Curr Drug Targets*. **12**: 1501–1507.

[18] Bijl, M., and Kalenberg., CGM., (2006). "Ultraviolet light and cutaneous lupus". *Lupus*. **11**: 724-727.

[19] Black A.T., Gray, JP., Shakarjian, MP., et.al. (2008). "UVB light upregulates prostaglandin synthases and prostaglandin receptors in mouse keratinocytes". *Toxicol Appl Pharmacol*. **1**: 14-24.

[20] Botelho, FM., Bauer, CMT., Finch, D., et.al. (2011). "IL-1α/IL-1R1 expression in chronic obstructive pulmonary disease and mechanistic relevance to smoke-induced neutrophilia in mice". PLoS One. **12**: doi: 10.1371/journal.pone.0028457.

[21] Boualia, E., Kaabachi, W.J, Hamzaouia, A., and Hamzaouia, K. (2015). "Interleukin-37 expression is decreased in Behc¸ et's disease and isassociated with inflammation". *Immunology Letters*. **167**: 87-94.

[22] Boyden, ED., and Dietrich, F. (2006). "Nalp1b controls mouse macrophage susceptibility to anthrax lethal toxin". *Nat Genet*. **2**: 240-244.

[23] Braakenburg, AMD., de Valk, VD., de Boer, J., and Rothova, A. (2008)."Human leukocyte antigen-B27-associated uveitis: long-term follow-up and gender differences". *Am J Ophthalmol*. **3**: 472-479.

[24]Brewerton, DA., Caffrey, M., Nicholls, A., et.al. (1973)."Reiter's disease and HL-A 27". *Lancet*. **7836**: 996-998.

[25] Broderick, L., De Nardo, D., Franklin, BS., Hoffman, HM., and Latz, E. (2015). "The inflammasomes and autoinflammatory syndromes". *Annu Rev Pathol*. **10**: 395-424.

[26] Broz, P., and. Dixit, VM. (2016). "Inflammasomes: mechanism of assembly, regulation and signalling". *Nat Rev Immunol*. **7**: 407-420.

[27] Bürckstümmer T., Baumann, C., Blüml, S., Dixit, E., et.al. (2009). "An orthogonal proteomic-genomic screen identifies AIM2 as a cytoplasmic DNA sensor for the inflammasome". *Nat Immunol*. **3**: 266-272.

[28] Cai, X., Chen, J., Xu, H., et.al. (2014)."Prion-like polymerization underlies signal transduction in antiviral immune defense and inflammasome activation". *Cell*. **6**: 1207-1222.

[29] Carlin, G., Djursäter, R., and Smedegård, G. (1989)."Inhibitory effects of sulfasalazine and related compounds on superoxide production by human polymorphonuclear leukocytes". *Pharmacol Toxicol*. **2**: 121-127.

[30] Case, CL., Kohler, LJ., Lima, JB., et.al.(2013)." Caspase-11 stimulates rapid flagellin-independent pyroptosis in response to Legionella pneumophila". *Proc Natl Acad Sci U S A*. **5**:1851-1856.

[31] Chang, JH., McKluskey, PJ., and Wakefield, D. (2005). "Acute anterior uveitis and HLA-B27". *Surv Ophthalmol*. **4**: 364-388.

[32] Chavarría-Smith, J., and Russel, EV. (2015). "The NLRP1 inflammasomes". *Immunol Rev*. **1**: 22-34.

[33] Chen, N., Ou, Z., Zhang, W., Zhu X., Li, P., and Gong, J. (2018). "Cathepsin B regulates non-canonical NLRP3 inflammasome pathway by modulating activation of caspase-11 in Kupffer cells". *Cell Prolif*. **6**: doi: 10.1111/cpr.12487

[34] Choulaki. C., Papadaki, G., Repa, A., et.al. (2015). "Enhanced activity of NLRP3 inflammasome in peripheral blood cells of patients with active rheumatoid arthritis". *Arthritis Res Ther*. **1**: 1-11.

[35] Chu, EC., Chai, J., Ahluwalia, A., and Tarnawski, AS. (2007). "Mesalazine downregulates c-Myc in human colon cancer cells. A key to its chemopreventive action?." *Aliment Pharmacol Ther*. **12**: 1443-1449.

[36] Ciccarelli F., De Martinis M., Ginaldi, L. (2014). "An update on autoinflammatory diseases". *Curr Med Chem*. **3**: 261-269.

[37] Collard RL., Harya, NS., Monzon, FA., Maier, CE., and O'Keefe, DS. (2006). "Methylation of the ASC gene promoter is associated with aggressive prostate cancer". *Prostate*. **7**: 687-695.

[38] Conos, SA., Chen, CW., De Nardo, D. (2017)."Active MLKL triggers the NLRP3 inflammasome in a cell-intrinsic manner". *Proc Natl Acad Sci U S A*. 6: 961-969.

[39] Conway KE., McConell, BB., Bowring, CE.,et.al. (2000). "TMS1, a novel proapoptotic caspase recruitment domain protein, is a target of methylation-induced gene silencing in human breast cancers". *Cancer Res*. **22**: 6236-6242.

[40] Coussens L.M., and Werb, Z. (2002). "Inflammation and cancer." *Nature*. **420**: 860-868.

[41] Cridland, JA., Curley, EZ., Wykes, MN., Schroder, K., Sweet, MJ., et.al (2012)."The mammalian PYHIN gene family: Phylogeny, evolution and expression". *BMC Evol Biol*. **12**: doi: 10.1186/1471-2148-12-140.

[42] D'Ambrosio, EM., La Cav,a M., Tortorella P. (2017)."Clinical Features and Complications of the HLA-B27-associated Acute Anterior Uveitis: A Metanalysis". *Semin Ophthalmol*. **6**: 689-701.

[43] D'Incà, R., Paccagnella, M., Cardin, R., and Pathak, S. (2013). "5-ASA colonic mucosal concentrations resulting from different pharmaceutical formulations in ulcerative colitis". *World J Gastroenterol*. **34**: 5665-5670.

[44] D'Osualdo, A., Weichenberger, CX., Wagner, RN., et.al. (2011). "CARD8 and NLRP1 undergo autoproteolytic processing through a ZU5-like domain". *PLoS One*. **6**: 11-19.

[45] D'Orazio J., Jarett,. S., Amaro-Ortiz, A., and Scott, T., (2013). "UV Radiation and the Skin". *Int J Mol Sci*. **14**: 12222-12248.

[46] Daniels, F., Brophy, D., and. Lobitz, WC. (1961). "Histohemical responses of human skin following ultraviolet irradiation". *J Investigative Dermatology*. **37**: 351-357.

[47] Darell, RW., Wagener, HP., H., and Kurtland, LT. (1962). "Epidemiology of uveitis. Incidence and prevalence in a small urban community". *Arch Ophthalmo*l. **68**: 502-514.

[48] Davis, BK., Wen, H., and P-Y Ting, J. (2011). "The inflammasome NLRs in immunity, inflammation, and associated diseases". *Annu Rev Immunol*. **29**: 707-735.

[49] De Alba, E. (2009). "Structure and interdomain dynamics of apoptosis-associated speck-like protein containing a CARD (ASC)". *J Biol Chem*. **47**: 32932-32941.

[50] De Nardo, D., De Nardo, CM., and Latz, E. (2014). "New insights into mechanisms controlling the NLRP3 inflammasome and its role in lung disease". *Am J Pathol*. **1**: 42-54.

[51] de Visser K.E., Eichten, A., and Coussens, L.M. (2006). "Paradoxical roles of the immune system during cancer development". *Nat Rev Cancer*. **6**: 24-37.

[52] Desreumaux, P. (2008)."Understanding the Mechanism of 5-ASA in Treating Colonic Inflammation". *Gastroenterol Hepatol (N Y)*. **5**: 319-320.

[53] Dick, MS., Sborgi, L., Rühl, S., Hiller, S., and Broz, P. (2016). "ASC filament formation serves as a signal amplification mechanism for inflammasomes". *Nat Commun*. **7**: doi: 10.1038/ncomms11929.

[54] Dignass, A., Lindsay, JO., Sturm, A., et.al. (2012)."Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 2: current management". *J Crohns Colitis*. **10**: 991-1030.

[55] Dinarello, C. (2006). "Interleukin 1 and interleukin 18 as mediators of inflammation and theaging process". *Am J Clin Nutr*. **83**: 447–455.

[56] Dinarello, CA., Simon, A., and van der Meer, JWM. (2012). "Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases". *Nat Rev Drug Discov*. **8**: 633–652.
[83] Direskeneli, H., and Saruhan.-Deriskeneli, G. (2003). "The role of heat shock proteins in Behcet's disease". *Clin Exp Rheumatol*. **21**: 44-48.

[57] Doitsh, G., Galloway, NLK., Geng, X. (2014). "Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection". *Nature*. **7484**: 509-514.

[58] Dombrowski Y., Peric, M., Koglin, S., et.al (2011)."Cytosolic DNA triggers inflammasome activation in keratinocytes in psoriatic lesions". Sci Transl Med. **82**: doi: 10.1126/scitranslmed.3002001.

[59] Dorfleutner, A., Chu, L., and Stehlik, C. (2015)."Inhibiting the inflammasome: one domain at a time". *Immunol Rev*. **1**: 205-216.

[60] Dostert, C., Petrilli. V., Van Bruggen, R.,et.al. (2008). "Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica". *Science*. **5876**: 674-677.

[61] Dowds, TA., Masumoto, J., Chen, FF., et.al. (2003). "Regulation of cryopyrin/Pypaf1 signaling by pyrin, the familial Mediterranean fever gene product". *Biochem Biophys Res Commun*. **3**: 575-580.

[62] Drexler, SK., Bonsignore, L., Masin, M., et.al. (2012). "Tissue-specific opposing functions of the inflammasome adaptor ASC in the regulation of epithelial skin carcinogenesis". *Proc Natl Acad Sci U S A*. **45**: 18384-18389.

[63] Duewell, P., Kono, H., Rayner, KJ., et.al. (2010). "NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals". *Nature*. **7293**: 1357-1361.

[64] Egan, LJ., Mays, DC. D., Huntoon, CJ., and Bell, MP. (1999)."Inhibition of interleukin-1-stimulated NF-kappaB RelA/p65 phosphorylation by mesalamine is accompanied by decreased transcriptional activity". *J Biol Chem*. **37**: 26448-26453.

[65] Eisenbarth, SC., Colegio, OR., O'Connor, W., Sutterwala, FS., and Flavell, RA. (2008). "Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminium adjuvants". *Nature*. **7198**: 1122-1126.

[66] Ergun, T., Unce, I., Ekşioğlu-Demiralp, E., et.al.(2001)."HSP 60 expression in mucocutaneous lesions of Behçet's disease". *J Am Acad Dermatol*. **6**: 904-909.

[66] Eneh, AA. (2010)." Answer: Can you identify this condition?". *Can Fam Physician*. 9: 896–897.

[67] Fabiani, C., Vitale, A., Lopalco, G., et .al. (2016)." Different roles of TNF inhibitors in acute anterior uveitis associated with ankylosing spondylitis: state of the art". *Clin Rheumatol*. 11: 2631-2638**.**

[68] Evavold, CL., Ruan, J., Tan, Y., et.al. (2018). "The Pore-Forming Protein Gasdermin D Regulates Interleukin-1 Secretion from Living Macrophages". *Immunity*. **1**: 35-44.

[69] Faustin, F., Lartigue, L., Bruey, JM., et.al. (2007). "Reconstituted NALP1 inflammasome reveals two-step mechanism of caspase-1 activation". *Mol Cell*. **5**: 713-724.

[70] Feagan, BG., and Sandborn, WJ. (2002;). "Initial therapy for mild to moderate Crohn's disease: mesalamine or budesonide?". *Rev Gastroenterol Disord*. **2**: 9-15.

[71] Feldmeyer, L., Keller, M., Niklaus, G., et.al. (2007). "The Inflammasome mediates UVB-inuced ativation and secretion of interleukin-1b by keratinocytes". *Curr Biol*. **17**: 1140-1145.

[72] Fenini, G., Grossi, S., and Contassot, E., et .al. (2018). " Genome Editing of Human Primary Keratinocytes by CRISPR/Cas9 Reveals an Essential Role of the NLRP1 Inflammasome in UVB Sensing". *J Invest Dermatol*. **12** :2644-2652.

[73] Fernandes-Alnemri, T., Wu, J., Yu, J.W., et.al. (2007)."The pyroptosome: a supramolecular assembly of ASC dimersmediating inflammatory cell death via caspase-1 activation". *Cell Death Differ*. **14**: 1590-1604.

[74] Fernandes-Alnemri, T., Je-Wook, Yu., Datta, P., Wu, J., and Alnemri, ES. (2009). "AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA". *Nature*. **7277**: 509-513.

[75] Finger, JN., Lich, DJ., J., Dare, LC., et.al. (2012). "Autolytic proteolysis within the function to find domain (FIIND) is required for NLRP1 inflammasome activity". *J Biol Chem*. **30**: 25030-25037.

[76] Fink, SL., Bergsbaken, T., and Cookson, BT. (2008). "Anthrax lethal toxin and Salmonella elicit the common cell death pathway of caspase-1-dependent pyroptosis via distinct mechanisms". *Proc Natl Acad Sci U S A*. **11**: 4312-4317.

[77] Franchi, L., Egenbrod,. T., and Núñez, G. (2009). "Cutting edge: TNF-alpha mediates sensitization to ATP and silica via the NLRP3 inflammasome in the absence of microbial stimulation ". *J Immunol*. **2**: 792-796.

[78] Franklin, BS., Bossaller. L., De Nardo, D., et.al. (2014). "ASC has extracellular and 'prionoid' activities that propagate inflammation". *Nat Immunol*. **8**: 727-737.

[79] Freire-de-Lima, CG., Qun Xiao, Yi., Gardai, SJ., et.al. (2006). "Apoptotic cells, through transforming growth factor, coordinately induce anti-inflammatory and suppress pro-inflammatory eicosanoid and NO synthesis in murine macrophages". *J Biol Chem*. **281**: 38376–38384.

[80] Frew, BC., Joag, VR., and Mogridge, J. (2012). "Proteolytic processing of Nlrp1b is required for inflammasome activity". *PLoS Pathog*. **8**: DOI: 10.1371/journal.ppat.1002659

[81] Fubini, B., and Hubbard, A. (2003). "Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generation by silica in inflammation and fibrosis". *Free Radic Biol Med*. **12**: 1507-1516.

[82] Fuchs, J., and Kern H. (1998). "Modulation of UV light-induced skin inflammation by d-alpha tocopherol and I-ascorbic acid : a clinical study using solar simulated radiation". *Free Radical Biology & Medicine*. **25**: 1006-1012.

[83] Gaginella, TS., and Walsch, RE. (1992). "Sulfasalazine. Multiplicity of action". *Dig Dis Sci*. **6**: 801-812.

[84] Gludsdale, GJ., Dandie, GW., and Muller, HK. (2001). "Ultraviolet light induced injury: Immunological and inflammatory effects". *Immunology and Cell Biology*. **6**: 547–568.

[85] Goldbach-Mansky, R. (2011). "Current status of understanding the pathogenesis and management of patients with NOMID/CINCA". *Curr Rheumatol Rep*. **2**: 123-131.

[86] Grange, LK., Kouchouk, A., Dalal, MD., et.al. (2014)."Neoplastic masquerade syndromes in patients with uveitis". *Am J Ophthalmol* . **3**: 526-531.

[87] Grip, O., Bredberg, A., Lindgren, S., and Henriksson, G. (2007)."Increased subpopulations of CD16(+) and CD56(+) blood monocytes in patients with active Crohn's disease". *Inflamm Bowel Dis*. **5**: 566-572.

[88] Grisham, MB., and. Granger, DN. (1988)."Neutrophil-mediated mucosal injury. Role of reactive oxygen metabolites". *Dig Dis Sci*. **3**: 6-15.

[89] Groß, CJ., Mishra, R.,Schneider, KS.,et al. (2016). "K+Efflux-Independent NLRP3 Inflammasome Activation by Small Molecules Targeting Mitochondria". *Immunity*. **45**: 761–773.

[90] Groslambert, M., and Benedicte, FP., (2018). "Spotlight on the NLRP3 inflammasome pathway". *J Inflamm Res*. **11**: 359-374.

[91] Guarda, G., Zenger, M., Yazdi, AS., et.al. (2011). "Differential expression of NLRP3 among hematopoietic cells". *J Immunol*. **4**: 2529-2534.

[92] Gul, A., and Ohno, S., (2012). "HLA-B*51 and Behçet Disease". *Ocul Immunol Inflamm*. **1**: 37-43.

[93]Guo, H., Callaway, JB., and P-Y Ting, J. (2015). "Inflammasomes: mechanism of action, role in disease, and therapeutics". *Nat Med*.**7**: 677-687.

[94] Gurung, P., Anand, PK., Subbarao Malireddi, RK., et.al. (2014). "FADD and caspase-8 mediate priming and activation of the canonical and noncanonical Nlrp3 inflammasomes". J *Immunol*. **4**: 1835-1846.

[95] Gutierrez, KD., Davis, MA., M., Daniels, BP., et.al. (2017). "MLKL Activation Triggers NLRP3-Mediated Processing and Release of IL-1β Independently of Gasdermin-D". *J Immunol*. **5**: 2156-2164.

[96] Hagar, JA., Powell, DA., Aachoui, Y., Ernst, RK., and Miao, EA. (2013). "Cytoplasmic LPS activates caspase-11: implications in TLR4-independent endotoxic shock". *Science*. **6151**: 1250-1253.

[97] Haroon, M., O'Rurke, R.M., Ramasamy, P.,et.al.(2015)."A novel evidence-based detection of undiagnosed spondyloarthritis in patients presenting with acute anterior uveitis: the DUET (Dublin Uveitis Evaluation Tool)". *Ann Rheum Dis*. **11**: 1990-1995.

[98] Ham, M., and Moss, CA. (2012)." Mesalamine in the treatment and maintenance of remission of ulcerative colitis". *Expert Rev Clin Pharmacol*. **2**: 113-123.

[99] He, Y., Zeng, MY., Yang, D., Motro, B., and Núñez, G. (2016). "NEK7 is an essential mediator of NLRP3 activation downstream of potassium efflux". *Nature*. **7590**: 354-357.

[100] Hirohata, S., Ohshima, N., Yanagida, T., and Aramaki, K.(2002):" Regulation of human B cell function by sulfasalazine and its metabolites". *Int Immunopharmacol*. 5:631-640.

[101] Hornung, V., Ablasser, A., Charrel-Dennis, M., et.al. (2009). "AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC". *Nature*. **7237**: 514-518.

[102] Hornung, V., Bauernfeind, F., Halle, A., et.al. (2008). "Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization". *Nat Immunol*. **8**: 847-856.

[103] Hoss, F., Rodriguez-Alcazar, JF., and Latz, E. (2017). "Assembly and regulation of ASC specks". *Cell Mol Life Sci*. **74**: 1211-1229.

[104] Hu, WH., Johnson, H., and Shu, HB. (2010). "Activation of NF-kappaB by FADD, Casper, and caspase-8". *J Biol Chem*. **15**: 10838-10844.

[105] Hye Han, S., Lear, TB., Jerome, JA., et.al. (2015). "Lipopolysaccharide Primes the NALP3 Inflammasome by Inhibiting Its Ubiquitination and Degradation Mediated by the SCFFBXL2 E3 Ligase". *J Biol Chem*. **29**: 18124-18133.

[106] Ichihashi M., Ueda, M., Budiyanto, A. et.al. (2003). "UV-induced skin damage". *Toxicology*. **189**: 21-39.

[107] İnal, EE., Rüstemogly, A., İnanır, A., et.al. (2015)."Associations of rs4810485 and rs1883832 polymorphisms of CD40 gene with susceptibility and clinical findings of Behçet's disease". *Rheumatol Int*. **5**: 837-843.

[108] Inoue, M., and Shinohara, ML. (2013)."NLRP3 Inflammasome and MS/EAE". Autoimmune Dis: **2013**: doi: 10.1155/2013/859145

[109] Ippagunta SK., Brand DD., Luo, K., Boyd KL., et.al. (2010)."Inflammasome-independent role of apoptosis-associated speck-like protein containing a CARD (ASC) in T cell priming is critical for collagen-induced arthritis . *J Biol Chem*. **16**: 12454-12462.

[110] Ireton, RC., and Gale, MJ. (2011). "RIG-I like receptors in antiviral immunity and therapeutic applications". *Viruses*. **6**: 906-919.

[111] Jabs, DA., and Rosenbaum JT., (2001). "Guidelines for the use of immunosuppressive drugs in patients with ocular inflammatory disorders: recommendations of an expert panel". *Am J Ophthalmol*. **5**: doi: 10.1016/s0002-9394(01)00830-3.

[112] Jabs, DA., Nussenblatt, RB., and Rosenbaum, JT., (2005)."Standardization of uveitis nomenclature for reporting clinical data. Results of the First International Workshop". *Am J Ophthalmol*. **3**: 509-516.

[113] Jin, T., Perry, A., Smith P., Jiang, J., and Xiao, TS. (2013). "Structure of the absent in melanoma 2 (AIM2) pyrin domain provides insights into the mechanisms of AIM2 autoinhibition and inflammasome assembly". *J Biol Chem*. **19**: 13225-13235.

[114] Jianjin, S., Wenqing, G., and Feng, S. (2017). "Pyroptosis: "Gasdermin-Mediated Programmed Necrotic Cell Death". *Cell*. **42**: 245-254.

[115] Johnson, K.E ., Wulf. BC,, Oberyszyn, TM., and Wilgus, TA. (2013). "Ultraviolet light exposure stimulates HMGB1 release by keratinocytes". *Arch Dermatol Res*. **305**: 805-815.

[116] Jorgensen, I., and Miao, EA. (2015). "Pyroptotic cell death defends against intracellular pathogens". *Immunol Rev*. 1:130-142.

[117] Kalantar,i P., DeOliveira, RB., R., Chan, J., et.al (2014). "Dual engagement of the NLRP3 and AIM2 inflammasomes by plasmodium-derived hemozoin and DNA during malaria". *Cell Rep*. **1**: 196-210.

[118] Kang, TB., Yang, SH., Toth, B., Kovalenko, A., and Wallach, D. (2013). "Caspase-8 blocks kinase RIPK3-mediated activation of the NLRP3 inflammasom". *Immunity*. **1**: 27-40.

[119] Kanneganti, TD., Lamakanfi, M., Kim, YG., et.al. (2007)."Pannexin-1-mediated recognition of bacterial molecules activates the cryopyrin inflammasome independent of Toll-like receptor signaling". *Immunity*. **4**: 433-443.

[120] Kasper, M, Walscheid, K,, Laffer, B,. (2018)."The Phenotype of Monocytes in Anterior Uveitis depends on the HLA-B27 Status". *Frontiers in Immunology*. **9**: doi: 10.3389/fimmu.2018.01773.

[121] Kayagaki. N., Wong, MT., Stowe, IB., et.al. (2013). "Noncanonical inflammasome activation by intracellular LPS independent of TLR4". *Science*. **6151**: 1246-1249

[122] Kayagaki. N., Warming. S., Lamkanfi, M., Walle, VL., et.al. (2011). "Non-canonical inflammasome activation targets caspase-11". *Nature*. **7371**: 117-121.

[1823] Kelley, N., Jeltema, D., Duan, Y., and He, Y. (2019). " The NLRP3 Inflammasome: An Overview of Mechanisms of Activation and Regulation". *Int J Mol Sci*. **13**:1-24.

[124] Kerur, N., Monhan, M., Veetil, V., et.al. (2011). “IFI16 acts as a nuclear pathogen sensor to induce the inflammasome in response to Kaposi Sarcoma-associated herpesvirus infection “. *Cell Host Microbe*. **5**: 363-375.

[125]Kone-Paut, I. (2016). "Behçet's disease in children, an overview". *Pediatr Rheumatol Online J*. 1: doi: 10.1186/s12969-016-0070-z.

[126] Kornbluth, A., and Sachar, DB. (1997). "Ulcerative colitis practice guidelines in adults: American College Of Gastroenterology, Practice Parameters Committee". *Am J Gastroenterol*. **2**: 204-211.

[127] Kovarova, M., Hesker, PR., Jania, L. (2012). "NLRP1-dependent pyroptosis leads to acute lung injury and morbidity in mice". *J Immunol*. **4**: 2006-2016.

[128] Krasselt, M., Baerwald, C., Wagner, U., and Rossol, M. (2013). "CD56+ monocytes have a dysregulated cytokine response to lipopolysaccharide and accumulate in rheumatoid arthritis and immunosenescence". *Arthritis Res Ther*. **5**: doi: 10.1186/ar4321.

[129] Kumar V. (2003). "Robbins Basic Pathology." *Saunders*.

[130] Kummer, JA., Broekhuizen, R., Everett, H., et.al. (2007). "Inflammasome components NALP 1 and 3 show distinct but separate expression profiles in human tissues suggesting a site-specific role in the inflammatory response". *J Histochem Cytochem*. **5:** 443-452.

[131] Kuri, P., Schieber, NL., Thumberger, T., et.al. (2017). " Dynamics of in vivo ASC speck formation". *J Cell Biol*. **9**: 2891-2909.

[132] Kwon BK., Streijger, F., Hill, CE., Anderson, AJ., et al. (2015). "Large animal and primate models of spinal cord injury for the testing of novel therapie". *Exp Neurol*. **269**: 154-168.

[133] Lamkanfi, M., and Dixit VM. (2012). "Inflammasomes and their roles in health and disease". *Annu Rev Cell Dev Biol*. **28**: 137–161.

[134] Lamkanfi, M., and Dixit. VM. (2014). "Mechanisms and Functions of Inflammasomes". *Cell*. **5**: 1113–1122.

[135] Landolfo, S., Gariglio, M., Gribaudo, G., and Lemb,o D. (1998). "The Ifi 200 genes: an emerging family of IFN-inducible genes". *Biochimie*. **8**: 721-728.

[136] Latz, E., Sam Xiao, T., and Stutz, A. (2013). "Activation and regulation of the inflammasomes". *Nat Rev Immunol*. **6**: 397-411.

[137] Levine, JJ., Stimson, KM., and Vertino, PM. (2003)."Effects of methylation on expression of TMS1/ASC in human breast cancer cells". *Oncogene*. **22**: 3475-3488.

[138**]** Lewis, DA., Hangletraub SF., Gao FC., and Leviant MA.(2006). " Aberrant NF-κB activity in HaCaT cells alters their response to UVB signaling". *Journal of Investigative Dermatology*. **8**: 1885-1892.

[139] Libby, P., Ridker, PM., and Maseri, A. (2002). "Inflammation and atherosclerosis." *Circulation*. **105**: 1135-1143.

[140] Lin KM., Hu, W., Troutman, TD., et.al. (2014). "IRAK-1 bypasses priming and directly links TLRs to rapid NLRP3 inflammasome activation". *Proc Natl Acad Sci U S A*. **2**: 775-780.

[141] Linder, R., Hoffman, A., and Brunner, R. (2004)."Prevalence of the spondyloarthritides in patients with uveitis". *J Rheumatol*. **11**: 2226-2229.

[142] Linssen, A., Rothova, A., Valkenburg, HA., et.al. (1991)."The lifetime cumulative incidence of acute anterior uveitis in a normal population and its relation to ankylosing spondylitis and histocompatibility antigen HLA-B27". *Invest Ophthalmol Vis Sci*. **9**: 2568-2578.

[143] Lomedico, PT., Gubler, U, Hellmann, CP., et.al. (1984). "Cloning and expression of murine interleukin-1 cDNA in Escherichia coli". *Nature*. **5993**: 458-462.

[144] Lu, A., Magupali VG., Ruan J., et.al (2014). "Unified polymerization mechanism for the assembly of ASC-dependent inflammasomes". *Cell*. **6**: 1193-1206.

[145] Lu, A., Kabaleesweran, V., Fu, T., Magupalli, VG., and Wu, H. (2014). "Crystal structure of the F27G AIM2 PYD mutant and similarities of its self-association to DED/DED interactions". *J Mol Biol*. **7**: 1420-1427.

[146] Lu A.,Girri Mangupalli, V., Ruan, J.,et.al. (2014). "Unified polymerization mechanism for the assembly of ASC-dependent inflammasomes". *Cell*. **6**: 1193-1206.

[147] Lucas, R., McMichael, T., Smith, W., Armstrong, B., et .al. (2006). "Solar ultraviolet radiation: Global burden of disease from solar ultraviolet radiation". *Environmental Burden of Disease Series*. 13:1-250.

[148] Maelfait, J., Vercammen, E., Janssens, S., et.al. (2008). "Stimulation of Toll-like receptor 3 and 4 induces interleukin-1beta maturation by caspase-8". *J Exp Med*. **9**: 1967-1973.

[149] Magna, M., and Pisetsky, DS. (2015). "The role of cell death in the pathogenesis of SLE: Is pyroptosis the missing link?". *Scand.J.Immunol*. **3**: 218-224.

[150] Majno, G., and Joris, I. (2004). "Cells, Tissues and Disease". *Oxford Univ. Press*.

[151]Mammone, T., Gan, D., Collins, D., Lockshin, R.A., Marenus, K., and Maes, D. (2000). "Successful separation of apoptosis and necrosis pathways in HaCaT keratinocyte cells induced by UVB irradiation". *Cell Biol Toxicol*. **5**: 293-302.

[152] Malik A., and Devi Kaneganti T. (2017). " Inflammasome activation and assembly at a glance". *J Cell Sci*. **23**: 3955–3963

[153] Mariathasan, S., Weiss, DS., Newton, K.,et.al. (2006)."Cryopyrin activates the inflammasome in response to toxins and ATP". *Nature*. **7081**: 228-232.

[154] Martin, BN., Wang, C., Willette-Brown, J., Herjan T., et.al. (2014)."IKKα negatively regulates ASC-dependent inflammasome activation". *Nat Commun*. **5**: doi: 10.1038/ncomms5977

[155] Martin, S. F. (2012). "Contact dermatitis: from pathomechanisms to immunotoxicology". *Experimental Dermatology*. **25**: 382-389.

[156] Martinon, F., and Tschopp, J. (2004). "Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases". *Cell*. **5**: 561-574.

[157] Martinon, F., and. Tschopp, J. (2007). "Inflammatory caspases and inflammasomes: master switches of inflammation". *Cell Death Differ*. **1**: 10-22.

[158] Martinon, F., Burns, K., and Tschopp, J. (2002). "The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta". Mol *Cell*. **2**: 417-426.

[159] Martinon, F., Mayor, A., and Tschopp, J. (2009). "The inflammasomes: guardians of the body". *Annu Rev Immunol*. **27**: 229-265.

[160] Masumoto, J., Taniguchi, S., Ayukawa, K., et.al. (1999). "ASC, a novel 22-kDa protein, aggregates during apoptosis of human promyelocytic leukemia HL-60 cells." *J Biol Chem*. **48**: 33835-33838.

[161] Masumoto, J., Taniguchi, S., Nakayama, J., et.al (2001). "Expression of apoptosis-associated speck-like protein containing a caspase recruitment domain, a pyrin N-terminal homology domain-containing protein, in normal human tissues". *J Histochem Cytochem*. **10**: 1269-1275.

[162] Matzinger, P. (1994). "Tolerance, danger, and the extended family". *Annu Rev Immunol*. **12**: 991–1045.

[163] Mayor A., Matrinton, F., De Smedt, T., Pétrilli, V., and Tschopp, J. (2007). "A crucial function of SGT1 and HSP90 in inflammasome activity links mammalian and plant innate immune responses". *Nat Immunol*. **5**: 497-503.

[164] Medzhitov, R., (2008). "Origin and physiological roles of inflammation". *Nature*. **454**: 428-435.

[165]Medzhitov R., Janeway, CA., (1997). "Innate immunity: the virtues of a nonclonal system of recognition". *Cell*. **3**: 295-298.

[166] Menu,' P., Mayor, A., Zhou, R., et.al. (2012)." ER stress activates the NLRP3 inflammasome via an UPR-independent pathway". *Cell Death Dis*.**1**: doi: 10.1038/cddis.2011.132.

[167] Miller, JD., Ganguli S., Artal, R, and Sperling, MA. (1985). "Indomethacin and salicylate decrease epinephrine-induced glycogenolysis". *Metabolism*. **2**: 148-153.

[168] Miller, SL., and Cho, JS. (2011). "Immunity against Staphylococcus aureus cutaneous infections". *Nat Rev Immunol*. **11**: 505-517.

[169] Ming Man, S., and Devi-Kanneganti, T. (2015). "Regulation of inflammasome activation". *Immunol Rev*. **1**: 6-21.

[170] Ming Man, S., Karki, R., and Kanneganti, TD. (2017)."Molecular mechanisms and functions of pyroptosis, inflammatory caspases and inflammasomes in infectious diseases". *Immunol Rev*. **1**: 61-75.

[171] Ming Man Si., Torlomousis, P., Hopkins L.,et.al. (2013). "Salmonella infection induces recruitment of Caspase-8 to the inflammasome to modulate IL-1β production". *J Immunol*.**10**: 5239-5246.

[172] Mitchell, PS., Sandstrom, A., and Vance, RE.(2019). "The NLRP1 inflammasome: new mechanistic insights and unresolved mysteries"**.** *Curr Opin Immunol*. **60**: 37-45.

[173] Miyamae, T. (2012). "Cryopyrin-associated periodic syndromes: diagnosis and management". *Paediatr Drugs*. **2**: 109-117.

[174] Monnet, D., Breban, M., Hudry, C., Dougados, M., and Brézin, AP. (2004). "Ophthalmic findings and frequency of extraocular manifestations in patients with HLA-B27 uveitis: a study of 175 cases". *Ophthalmology*. **4**: 802-809.

[175] Morrone, SM., Matyszewski, M., Yu, X., et.al. (2015). "Assembly-driven activation of the AIM2 foreign-dsDNA sensor provides a polymerization template for downstream ASC". *Nat Commun*. **7827**: doi: 10.1038/ncomms8827.

[176] Muñoz-Planillo, R., Kuffa, P., Martínez-Colón, G., et.al. (2013). "K^+ efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter". *Immunity*. **6**: 1142-1153.

[177] Murphy, G., Young, AR., Wulf, HC., Kulms, D., and Schwarz, T. (2001). "The molecular determinants of sunburn cell formation". *Exp Dermatol*. **10**: 155-160.

[178] Myers, B., Evans, DN., Rhodes, J., et.al. (1987). "Metabolism and urinary excretion of 5-amino salicylic acid in healthy volunteers when given intravenously or released for absorption at different sites in the gastrointestinal tract". *Gut*. **2**: 196-200.

[179] Narisawa, T., and Fukaura, Y. (2003). "Prevention by intrarectal 5-aminosalicylic acid of N-methylnitrosourea-induced colon cancer in F344 rats". *Dis Colon Rectum*. **7**: 900-903.

[180] Nicholson, DW. (1999). "Caspase structure, proteolytic substrates, and function during apoptotic cell death". *Cell Death Differ*. **11**: 1028-1042.

[181]O'Connor,W.,Harton,JA.,Zhu,X.,(2003)."Cuttingedge:CIAS1/cryopyrin/PYPAF1/NALP 3/CATERPILLER 1.1 is an inducible inflammatory mediator with NF-kappa B suppressive properties". *Immunol Rev*. **12**: 6329-6333.

[182] Ogilvie, AC., Hack, CE., Wagstaff, J.,et.al. (1996). "IL-1 beta does not cause neutrophil degranulation but does lead to IL-6, IL-8, and nitrite/nitrate release when used in patients with cancer". *J Immunol*. **1**: 389-394.

[183] Onal, S., Kazokogly, H,. Koc, A., et.al. (2011). "Long-term efficacy and safety of low-dose and dose-escalating interferon alfa-2a therapy in refractory Behçet uveitis".*Arch Ophthalmol*. **3**: 288-294.
[184] Özgüçlü, S., Duman, T., Özalp Ateş, FS., and Küçükşahin, O. (2019). "Serum interleukin-37 level and interleukin-37 gene polymorphism in patients with Behçet disease". C*lin Rheumatol*. **2**: 495-502.

[185] Parameswaran, N., and Patial, S. (2010). "Tumor necrosis factor-α signaling in macrophages". *Crit Rev Eukaryot Gene Expr*. **2** :87-103

[186] Park, H., Bourla, AB., Kastner, DL., Colbert, RA., and Siegel, RM. (2012). "Lighting the fires within: the cell biology of autoinflammatory diseases". *Nat Rev Immunol*. **8**: 570-580.

[187]Peppercorn, MA., and Goldman P. (1973). "Distribution studies of salicylazosulfapyridine and its metabolites". *Gastroenterology*. **2**: 240-245.

[188] Pelegrin, P., Baroso-Gutierrez, C., and Surprenant, A. (2008)."P2X7 receptor differentially couples to distinct release pathways for IL-1beta in mouse macrophage. J *Immunol*. **11**: 7147-7157.

[189] Pellegrini, C., Antonioli. L., Lopez-Castejon, G., Blandizzi, C., and Fornai, M. (2017). "Canonical and Non-Canonical Activation of NLRP3 Inflammasome at the Crossroad between Immune Tolerance and Intestinal Inflammation". *Front Immunol*. **8**: 1-12.

[190] Pétrilli, V., Papin, S., Dostert, C.,et.al. (2007). "Activation of the NALP3 inflammasome is triggered by low intracellular potassium concentration". *Cell Death Differ*. **9**: 1583-1589.

[191] Pichi, F., Carrai, P., Srivastava SK., et.al. (2015). " Genetic of uveitis". *Int Ophthalmol.* **36**: 419-433.

[192] Pierini, R., Juruj, C., Perret, M.,et.al. (2012). "AIM2/ASC triggers caspase-8-dependent apoptosis in Francisella-infected caspase-1-deficient macrophages". *Cell Death Differ*. **10**: 1709-1721.

[193] Pinals, RS., Kaplan, SB., and Hepburn, B. (1986)."Sulfasalazine in rheumatoid arthritis. A double-blind, placebo-controlled trial". *Arthritis Rheum*. **12**: 1427-1434.

[194] Pineton de Chambrun, M., Wehcler, B., Geri, G., Cacoub, P., and Saadoun, D. (2012). "New insights into the pathogenesis of Behçet's disease". *Autoimmun Rev.* **10**: 687-698.

[195] Pinto, A., Bonucci, A., Maggi, E., Corsi, M., and Businaro, R. (2018). "Anti-oxidant and anti-inflammatory activity of ketogenic diet: new perspectives for neuroprotection in Alzheimer's disease." *Antioxidans* **7**: 63-68.

[196] Poltorak, A., He, X., Smirnova, I., et.al. (1998). "Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene". *Science*. **5396**: 2085-2088.

[197] Rathinam, VAK., Jiang, Z., Waggoner, SN., et.al (2010). "The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses". *Nat Immunol*. **5**: 395-402.

[198] Ratsimandresey, RA., Dorfluenter,. A., and Stehlik, C. (2013). "An Update on PYRIN Domain-Containing Pattern Recognition Receptors: From Immunity to Pathology". *Front Immunol*. **4**: 1-20.

[199] Reefman E., Limburg, PC., Kallenberg, CGM., and Bijl, M. (2005). "Apoptosis in Human Skin: Role in Pathogenesis of Various Diseases and Relevance for Therapy". *Ann NY Acad Sci*. **1051**: 52–63.

[200] Reinholz, M., Kawakami, Y., Salzer, S., et.al. (2013). "HPV16 activates the AIM2 inflammasome in keratinocytes". *Arch Dermatol Res*. **8**: 723-732.

[201] Richards, N., Schaner, P., Diaz, A., Stuckey J., et.al. (2001). "Interaction between pyrin and the apoptotic speck protein (ASC) modulates ASC-induced apoptosis". *J Biol Chem*. **42**: 39320-39329.

[202] Riojas, MA., Guo, M., Glöckner, SC., et.al. (2007)."Methylation-induced silencing of ASC/TMS, a pro-apoptotic gene, is a late-stage event in colorectal cancer". *Cancer Biol Ther*. **11**: 1710-1716.

[203] Robinson, PC., Leo, PJ., Pointon, JJ., et.al. (2016)."The genetic associations of acute anterior uveitis and their overlap with the genetics of ankylosing spondylitis". *Genes Immun*. **1**: 46-51.

[204] Rochester, J., and Abreu, MT. (2005). "Ulcerative colitis therapy: importance of delivery mechanisms". *Rev Gastroenterol Disord*. **4**: 215-222.

[205] Rustemeyer T., van Hoogstraten, IMV., von Blomberg BME., et.al. (2011). "Mechanisms of Irritant and Allergic Contact Dermatitis". *Springer*. **1**: 43-90.

[206] Sandstrom, A., Mitchell, PS., Goers, L., et.al., (2019). " Functional degradation: A mechanism of NLRP1 inflammasome activation by diverse pathogen enzymes". *Science*. **30**:1-13.

[207] Sahillioglu, AC., Sumbul, F., Ozoren, N., and Haliloglu, T. (2014)."Structural and dynamics aspects of ASC speck assembly". *Structure*. **20**: 1722-1734.

[208] Salminen, A., Kauppinen, A., Hiltunen, M, Kaarniranta, K. (2014)."Epigenetic regulation of ASC/TMS1 expression: potential role in apoptosis and inflammasome function". *Cell Mol Life Sci*. **10**: 1855-1864.

[209] Santiago Muñoz, F., Ventura, H., Fernández-Melón, J., et al. (2003). "Sulfasalazine reduces the number of flares of acute anterior uveitis over a one-year period." *The Journal of Rheumatology*. **30**: 1277-1279.

[210] Santucci, L., Wallace, J., Mencarelli, et.al. (2005). "Different sensitivity of lamina propria T-cell subsets to nitric oxide-induced apoptosis explains immunomodulatory activity of a nitric oxide-releasing derivative of mesalamine in rodent colitis". *Gastroenterology.* **5**:1243-1257.

[211] Savill J., Dransfield, I., Gregory, C., and Haslett, C. (2002). "A blast from the past: clearence of apototic cells regulates immune responses". *Nat Rev Immunol*.**12**: 965-967.

[212] Sborgi, L., Ravotti, F., Dandey, VP., et.al. (2015). "Structure and assembly of the mouse ASC inflammasome by combined NMR spectroscopy and cryo-electron microscopy". *Proc Natl Acad Sci U S A*. **43**: 13237-13242.

[213] Schattgen, SA., and. Fitzgerald, KA. (2011). "The PYHIN protein family as mediators of host defenses". *Immunol Rev*. **1**: 109-118.

[214] Schmid-Burgk, JL., Gaidt, M., Schmidt, T.,et.al. (2015). "Caspase-4 mediates non-canonical activation of the NLRP3 inflammasome in human myeloid cells". *Eur J Immunol*. **10**: 2911-2917.

[215] Schmidt, FI., Lu, A., Chen, JW., et.al. (2016). "A single domain antibody fragment that recognizes the adaptor ASC defines the role of ASC domains in inflammasome assembly". *J Exp Med*. **5**: 771-790.

[216] Schotte, P., Criekinge, VW., Van de Craen, M., et.al. (1998). "Cathepsin B-mediated activation of the proinflammatory caspase-11". *Biochem Biophys Res Commun*. **1**: 379-387.

[217] Schröder, H., and Campbell, DE. (1972)."Absorption, metabolism, and excretion of salicylazosulfapyridine in man". *Clin Pharmacol Ther*. **4**: 539-551.

[218] Schroder. K., and. Tschopp, J. (2010). "The inflammasomes". *Cell*. **6**: 821-832.

[219] Schroder, K., Sagulenko, V., Zamoshnikova, A., et.al. (2012). "Acute lipopolysaccharide priming boosts inflammasome activation independently of inflammasome sensor induction". *Immunobiology*. **12**: 1325-1329.

[220] Sconocchia, G., Keyvanfar, K., Ouriaghli, FE., et.al. (2005)."Phenotype and function of a CD56+ peripheral blood monocyte". *Leukemia*. **1**: 69-76.

[221] Shaw N., and Liu ZJ. (2014). " Role of the HIN Domain in Regulation of Innate Immune Responses". *Mol Cell Biol*. **1**:2-15.

[222] Shi, H., Wang, Y., Li, X., et.al (2016). "NLRP3 activation and mitosis are mutually exclusive events coordinated by NEK7, a new inflammasome component". *Nat Immunol*. **3**: 250-258.

[223] Shi, J., Zha, Y., Wang, Y., et.al. (2014). "Inflammatory caspases are innate immune receptors for intracellular LPS". *Nature*. **7521**: 187-192.

[224] Srinivasula, M., Poyet, JL., J., Razmara, M., et.al. (2002). "The PYRIN-CARD protein ASC is an activating adaptor for caspase-1". *J Biol Chem*. **24**: 21119-21122.

[225] Stenson, WF., Mehta, J., and Spilberg, I. (1984)."Sulfasalazine inhibition of binding of N-formyl-methionyl-leucyl-phenylalanine (FMLP) to its receptor on human neutrophils". *Biochem Pharmacol*. **3**: 407-412.

[226] Stone, AR., Bobo, W., Brat, DJ., et.al. (2004). "Aberrant methylation and down-regulation of TMS1/ASC in human glioblastoma". *Am J Pathol*. **4**: 1151-1161.

[227] Sutherland, L., and MacDonald, JK. (2003). "Oral 5-aminosalicylic acid for induction of remission in ulcerative colitis". *Cochrane Database Syst Rev*. **3**: doi: 10.1002/14651858.

[228] Sutherland, LR., May, GR., and Shaffer, EA. (1991). "Sulfasalazine revisited: a meta-analysis of 5-aminosalicylic acid in the treatment of ulcerative colitis". *Ann Intern Med*. **7**: 540-549.

[229] Sutterwala FS., Ogura, Y., Szczepanik, M., Lara-Tejero, M., et.al. (2006). "Critical role for NALP3/CIAS1/Cryopyrin in innate and adaptive immunity through its regulation of caspase-1". *Immunity*. **3**: 317-327.

[300] Suzuki Kurokawa, M., and Suzuki, N. (2004). "Behcet's disease". *Clin Exp Med*. **1**: 10-20.

[301] Suzuki T., Franchi, L. T., Toma, C., Ashida, H., et.al. (2007). "Differential regulation of caspase-1 activation, pyroptosis, and autophagy via Ipaf and ASC in Shigella-infected macrophages". *PLoS Pathog*. **8**: doi: 10.1371/journal.ppat.0030111

[302] Szabo, G., and Csak, T. (2012)."Inflammasomes in liver diseases". *J Hepatol*. **3**: 642-654.

[303] Tong, B., Liu, X., Xiao, J., and Su, G. (2019). "Immunopathogenesis of Behcet's Disease". *Front Immunol*. **10**: doi: 10.3389/fimmu.2019.00665.

[304] Trang Ha Thi, H., and Hong, S. (2017)." Inflammasome as a Therapeutic Target for Cancer Prevention and Treatment". *J Cancer Prev*. **2**: 62-73.

[305] Tu, S., Bhagat, G., Cui, G., et.al. (2008). "Overexpression of interleukin-1beta induces gastric inflammation and cancer and mobilizes myeloid-derived suppressor cells in mice". *Cancer Cell*. **5**: 408-419.

[306] Tugal-Tutkun, I. (2011). "Pediatric uveitis". *J Ophthalmic Vis Res*. **4**: 259-269.

[307] van Weelden H., de Grujil, van der Putte SCJ., et.al. (1988). "The carcinogenic risks of modern tanning equipment: Is UV-A safer than UV-B?". *Archives of Dermatological Research*. **280**: 300-307.

[308] Vanaja SK, Russo, AJ., Behl, B., Banerjee, I., et.al. (2016). "Bacterial Outer Membrane Vesicles Mediate Cytosolic Localization of LPS and Caspase-11 Activation". *Cell*. **5**: 1106-1119.

[309] Voll, RE., Hermann, M., Roth, EA., Stach C., et.al. (1997). "Immunosuppressive effects of apoptotic cells". *Nature*. **390**: 350-351.

[310] Volpe, CMO., Anjos, PMF., and Nogueira-Machado, JA. (2016). "Inflammasome as a new therapeutic target for diabetic complications". *Recent Pat Endocr Metab Immune Drug Discov*. **1**: 56-62.

[311] Wahl, C., Liptay, S., Adler G., and Schmid, RM. (1998). "Sulfasalazine: a potent and specific inhibitor of nuclear factor kappa B". *J Clin Invest* . **5**: 1163-1174.

[312] Walscheid, K., Neekamp, L., and Heiligenhaus, A. (2018). "Peripheral blood monocytes reveal an activated phenotype in pediatric uveitis". *Clin Immunol*. **190**: 84-88.

[313] Wakefield, D., and Lloud, A. (1992). "The role of cytokines in the pathogenesis of inflammatory eye disease". *Cytokine*. **1**: 1-5.

[314] Wang, CQF., Cruz.-Inigo, AE., Fuentes-Duculan, J., et.al. (2011). "Th17 cells and activated dendritic cells are increased in vitiligo lesions". *PLoS One*. **4**: doi: 10.1371/journal.pone.0018907.

[315] Wang, L., Manji, GA., Grenier, JM., et.al. (2002). PYPAF7, a novel PYRIN-containing Apaf1-like protein that regulates activation of NF-jB and caspase-1-dependent cytokine processing". *J.Biol.Chem*. **33**: 29874–29880.

[316] Welter-Stahl L., Ojcius, D.M., Viala, J., Girardin, S., et.al. (2006). "Stimulation of the cytosolic receptor for peptidoglycan, Nod1, by infection with Chlamydia trachomatis or Chlamydia muridarum". *Cellular Microbiology*. **6**: 1047–1057.

[317] William, C., Panaccione, R., Ghos, S., and Rioux, K. (2011). "Optimizing clinical use of mesalazine (5-aminosalicylic acid) in inflammatory bowel disease". *Therap Adv Gastroenterol*. **4**: 237-248.

[318] Wree, A., Eguchi, A.,McGeough, MD., et.al. (2014). "NLRP3 inflammasome activation results in hepatocyte pyroptosis, liver inflammation, and fibrosis in mice". *Hepatology*. **3**: 898–910.

[319] Xu, H., Yang, J., Gao, W., Lin, L., Li, P., et.al. (2014). "Innate immune sensing of bacterial modifications of Rho GTPases by the Pyrin inflammasome". *Nature*. **7517**: 237-241.

[320] Xu, J., Jiang, Y., Wang, J., et.al. (2014). "Macrophage endocytosis of high-mobility group box 1 triggers pyroptosis". *Cell Death Differ*. **8**: 1229-1239.

[321] Yang, D., He, Y., Muñoz-Planillo, R., Liu, Q., and Núñez, G. (2015). "Caspase-11 Requires the Pannexin-1 Channel and the Purinergic P2X7 Pore to Mediate Pyroptosis and Endotoxic Shock". *Immunity*. **5**: 923-932.

[322] Yang, J., Liu Z., and Xiao, TS. (2017):"Post-translational regulation of inflammasomes". *Cell Mol Immunol*. **1**:65-79.

[323] Yazdi, AS., Guarda, G., D'Ombrain, MC., and Drexler, SK. (2010)."Inflammatory Caspases in Innate Immunity and Inflammation". *J Innate Immun* **3**: 228–237

[324] Ye, B., and van Langenberg, LD. (2004). "Mesalazine preparations for the treatment of ulcerative colitis: Are all created equal?". *World J Gastrointest Pharmacol Ther*. **4**: 137-144.

[325] Zeboulon, N., Dougados M., and Gossec L. (2008)."Prevalence and characteristics of uveitis in the spondyloarthropathies: a systematic literature review." *Ann Rheum Dis*. **7**: 955-999.

[326] Zhong, Y., Kinnio, A., and Saleh, M. (2013). "Functions of NOD-Like Receptors in Human Diseases". *Front Immunol*. **4**: doi: 10.3389/fimmu.2013.00333.

[327] Zhou, R., Tardivel, A., Thorens, B., Choi, I., and Tschopp, J. (2010). "Thioredoxin-interacting protein links oxidative stress to inflammasome activation". *Nat Immunol*. **2**: 136-140.