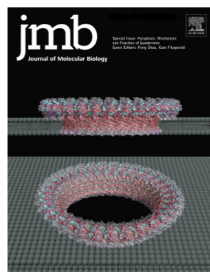
Review Article



Inflammasomes in T cells

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https://doi.org/10.1016/j.jmb.2021.167275
Edited by Kate Fitzgerald

Abstract

The concept of non-self recognition through germ-line encoded pattern recognition receptors (PRRs) has been well-established for professional innate immune cells. However, there is growing evidence that also T cells employ PRRs and associated effector functions in response to certain non-self or damage signals. Inflammasomes constitute a special subgroup of PRRs that is hardwired to a signaling cascade that culminates in the activation of caspase-1. Active caspase-1 processes pro-inflammatory cytokines of the IL-1 family and also triggers a lytic programmed cell death pathway known as pyroptosis. An increasing body of literature suggests that inflammasomes are also functional in T cells. On the one hand, conventional inflammasome signaling cascades have been described that operate similarly to pathways characterized in innate immune cells. On the other hand, unconventional functions have been suggested, in which certain inflammasome components play a role in unrelated processes, such as cell fate decisions and functions of T helper cells. In this review, we discuss our current knowledge on inflammasome functions in T cells and the biological implications of these findings for health and disease.

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Introduction - inflammasomes

The detection of a pathogen is key to mount immune responses and to fight infection. To this end, the innate immune system relies on germline-encoded receptors that have evolved to discriminate non-self from self. These so-called pattern recognition receptors (PRRs) specifically engage with pathogen associated molecular patterns (PAMPs) such as cell membrane components or nucleic acids that are absent or restricted to certain subcellular compartments in the host under normal conditions.[1] The activation of a PRR can trigger diverse effector programs such as cytokine secretion or the upregulation of co-stimulatory molecules but also the programmed cell death (PCD) of an affected cell.

On the one hand, PCD-induction can be considered an altruistic effort to contain infection in a multicellular organisms by disrupting the replicatory niche of an invading pathogen.[2] On the other hand, lytic cell death programs result in the release of highly proinflammatory cytosolic components that function to initiate and orchestrate antimicrobial immune responses. One family of PRRs hardwired to induce a unique PCD called pyroptosis are the so-called inflammasomes.[3] Inflammasomes are macromolecular complexes which form upon activation of a sensor molecule that then recruits the enzyme pro-caspase-1. Most inflammasome

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sensors utilize the adapter molecule ASC to recruit pro-caspase-1, which forms a large filamentous signaling hub that serves to amplify the signal.[4] Initiation of inflammasome signaling typically involves the oligomerization of the sensor protein. For most inflammasome sensors, this is effectuated through a sensor-intrinsic function that is activated in the course of sensor activation. At the same time, the ligand of the inflammasome sensor itself can serve as an oligomerization-inducing scaffold. Oligomerization is critically required to generate a signalosome of homotypic interaction domains that serve to recruit ASC or directly pro-caspase-1. Upon recruitment pro-caspase-1 is auto-processed, which in turn activates it to cleave its substrates. On the one hand, caspase-1 cleaves cytokines of the IL-1 family, most prominently pro-IL-1β, which will be rendered bioactive by this processing step.[5] On the other hand, caspase-1 also cleaves Gasdermin D (GSDMD) that forms a large pore in the plasma membrane, which will eventually result in plasma membrane rupture and thus release of cytosolic content, including processed IL-1 family cytokines.[6] Of note, the PCD of pyroptosis is not restricted to inflammasome activation, as other gasdermin molecules have been shown to be cleaved by other proteases in the context of other signaling cascades.[6]

The largest group of inflammasome sensors is part of the NLR (nucleotide-binding domain and leucine-rich repeat containing) protein family (e.g. NLRP1, NLRP3, NLRC4, NLRP6, NLRP12), yet members outside this family also exist (e.g. CARD8, PYRIN and AIM2). In the following, we briefly discuss individual inflammasome sensors that have been associated with Tcell intrinsic functions. For a comprehensive review on all inflammasome-forming sensors, we refer to recent review articles.[3,7–11]

NLRP3. NLRP3 consists of an N-terminal PYRIN domain that is followed by a NACHT and leucine rich repeat (LRR) domain. NLRP3 is, without doubt, the most-studied inflammasome sensor in that it plays a prominent role in host defense against a broad array of pathogens.[8] Further, NLRP3 engagement has been documented in a large number of sterile inflammatory conditions, such as Gout, Alzheimer's disease and type 2 diabetes.[12] The pleiotropic involvement of NLRP3 in these conditions can be explained by its highly sensitive, but non-specific mode of action. As such, NLRP3 is indirectly activated by the dissipation of the physiological potassium gradient over the plasma membrane. However, to avoid untimely activation, NLRP3 requires a so-called priming step, which usually occurs through the preceding activation of an additional PRR such as a Toll-like receptor. As such, conditions that induce potassium efflux in a cell, in which NLRP3 was primed, typically trigger NLRP3 activation. This can be specific triggers, e.g. the activation of the ATP-gated cation channel P2X7 that is permeable for potassium upon activation or certain ionophoric compounds such as nigericin that selectively induce potassium permeability across membranes. However, it appears that physiological triggers of NLRP3 primarily engage this pathway by disrupting membrane integrity.[13] Consequently, any lytic cell death occurring in an NLRP3-competent cell results in secondary activation of this inflammasome pathway by dissipating the potassium gradient following the induction of plasma membrane rupture.[14] The exact molecular mode of NLRP3 activation remains unclear. However, it has been shown that the dispersal of the trans-Golgi network is a uniform signal upstream of NLRP3 activation. This dispersal was shown to result in NLRP3 recruitment via a conserved polybasic region that binds to phosphatidylinositol-4-phosphates on the dispersed trans-Golgi network.[15] How this dispersal is induced by NLRP3 stimuli and how this recruitment initiates NLRP3 activation still remains unclear. In either way, it is assumed that NLRP3 activation results in its oligomerization through its central NACHT domain, which then results in a formation of a signalosome disc of its N-terminal PYD that results in ASC recruitment and subsequent pro-caspase-1 activation.

NLRP1 and CARD8. NLRP1 and CARD8 are closely related proteins. While no T cell intrinsic role has so far been identified for NLRP1, CARD8 has emerged as a master regulator of T cell pyroptosis. Nevertheless, major advances in understanding the function of NLRP1 have also fueled research on CARD8. Therefore, also the current understanding of NLRP1 will be summarized in brief. Unlike other NLR-proteins, NLRP1 contains a C-terminal extension that consists of a FIIND (function to find domain) and CARD (caspase activating and recruitment domain). Within its FIIND, NLRP1 is autoprocessed between the ZU5 and UPA subdomains, while the C-terminal UPA-CARD and the N-terminal fragment remain non-covalently associated with one another.[7] Unlike the other inflammasome-forming NLRs, NLRP1 employs its C-terminal CARD to initiate inflammasome signaling with the UPA domain constituting the oligomerization seed. A protein that negatively regulates NLRP1 is DPP9 (dipeptidyl-peptidase 9).[16] Under steady state conditions, DPP9 together with a full length autocleaved NLRP1 and a C-terminal NLRP1 fragment, forms a ternary complex.[17,18] In this complex, DPP9 prevents the UPA domain dependent oligomerization of the C-terminal fragment and thereby blocks the initiation of inflammasome signaling. The DPP9 inhibitor Val-boroPro (VbP) disrupts the interaction of DPP9 and NLRP1 and liberates the C-terminal fragment and thereby initiates signaling. Next to VbP, the NLRP1 inflammasome can also be activated by stimuli that destabilize its N-terminal fragment.[19,20] This can be achieved by certain proteases that cleave the N-

terminal portion of NLRP1, which exposes a neo N-terminus that functions as a degron for the N-end rule pathway. Following its ubiquitination, the N-terminal portion of NLRP1 is then subjected to proteasomal degradation, which appears to destabilize the ternary DPP9-NLRP1 complex and thereby sets of signal transduction via the C-terminus. Murine Nlrp1b, one of several Nlrp1 paralogs, is cleaved by anthrax lethal factor,[19,20] whereas human NLRP1 has been shown to be cleaved by enteroviral proteases.[21,22] Further, human but not murine NLRP1 also can serve as a classical PRR that binds to and becomes activated by cytoplasmic dsRNA.[23] A protein that is related to NLRP1 but has only recently been characterized as inflammasome sensor proteins is CARD8.[24] Overall, CARD8 resembles a truncated form of NLRP1 that is lacking the N-terminal portion of NLRP1 up to the FIIND.[7] Instead, it harbors an unstructured region at its N-terminus that is essential for it to be activatable.[25] Analogous to NLRP1, CARD8 is also restrained by DPP9.[26] Blocking this interaction also triggers CARD8 activation via the release of if the C-terminal fragment which - unlike NLRP1 – forms an oligomerization seed for direct pro-caspase-1 activation.[27,28] While human NLRP1 is largely confined to cells of epithelial barrier tissues,[29] CARD8 is highly expressed in bone marrow-derived cells of the myeloid and lymphoid lineage.[24,30,31]

AIM2. AIM2 is a direct receptor for double stranded DNA (dsDNA)[32–34] that is part of the PYHIN protein family, which is characterized by an N-terminal PYD that is followed by one or two HIN (hematopoietic interferon-inducible nuclear) domain(s). Of note, certain members of the PYHIN family only contain the PYHIN-characteristic PYD or HIN domain. While there are five PYHIN genes in a cluster on human chromosome 1, the equivalent gene locus in mice has undergone duplications and rearrangements and harbors 13 PYHIN genes.[35,36] Interestingly, AIM2 is the only one for which true orthology exists across these species. AIM2 is expressed in the cytoplasm and harbors one HIN domain with which it binds double stranded DNA (dsDNA) with high affinity.[37] DNA binding involves the phosphate backbone of the dsDNA molecule without sequence specificity.[37] In the absence of dsDNA, AIM2 is in an autoinhibited state in which its HIN domain interacts with the PYD.[38] In the presence of dsDNA, several AIM2 molecules wrap around the double stranded helix. This reliefs the autoinhibiton of the HIN domains towards the PYD and brings several PYD into close proximity so that they can form a nucleation cluster for ASC. AIM2 senses the presence of cytosolic non-self dsDNA as it occurs in the context of viral or bacterial infection. However, in the course of cell damage also endogenous DNA can be sensed by AIM2.[11]

IFI16. The PYHIN family member IFI16 is predominantly expressed in the nucleus, yet it can also shuttle between the nucleus and the cytoplasm.[39] Unlike AIM2, IFI16 has two HIN domains that differ in their structure and also their affinity towards DNA.[37] In contrast to AIM2, IFI16 does not assume an autoinhibited state, in which the PYD binds to the HIN domains. Instead, it has been shown that the PYD participates in the stabilization of IFI16 binding to DNA by promoting oligomerization.[40] Predating the discovery of cGAS, IFI16 was originally described as a DNA-sensor that could elicit interferon-responses through STING.[39] However, human cells deleted for IFI16 question its role in DNA-sensing with regard to type I IFN induction.[41,42] Moreover, while there is no orthologous PYHIN protein in the murine system, deleting the entire PYHIN gene cluster has no impact on DNA-triggered antiviral gene expression.[41] Subsequent work suggested that IFI16 acts as a nuclear inflammasome sensor that detects infection with Kaposi sarcoma herpes virus[43] and a role in HIV-1 recognition leading to inflammasome activation has been reported (see below). On the other hand, it has been shown that IFI16 and other human PYHIN proteins restrict HIV-1 and other viruses by sequestrating the key transcription factor Sp1[44] without engaging inflammasome components. This latter functionality would be well in line with their predominant nuclear localization.

Inflammasomes in T cells?

Since most inflammasomes have been identified and characterized in “professional innate immune cells” such as macrophages, most of our knowledge on the mechanisms of inflammasome sensing and signaling and its role in health and disease stems from studies focusing on these cell types. However, it has become clear that also cells that are not traditionally considered to exert innate immune functions entertain functional PRR systems.[45] Indeed, even T cells, as bona fide adaptive lymphocytes, have been shown to sense non-self through certain PRR systems.[46–49] The tropism of certain viruses for T cells (e.g., HIV) provides a conceptual framework for why T cells should be equipped with cell-autonomous defense systems that are triggered by non-self-recognition. On the other hand, it is also conceivable that these PRRs operate to enhance antigen-dependent functions of T cells or that they function to trigger the release of effector cytokines to activate bystander cells.[50] All in all, there is increasing evidence that pyroptosis can occur in T cells and that certain inflammasomes are expressed and functional. Since some of this work predates the identification of key inflammasome components and the identification of the mechanism underlying pyroptosis, it is important to revisit these studies and discuss them alongside our current understanding of inflammasome biology. In addition, diverse "moonlighting" functions in T helper cell differentiation have been ascribed

to prominent inflammasome proteins such as NLRP3, AIM2, ASC or caspase-1. In this review article, we revisit the diverse roles of inflammasome-related proteins in shaping T cell function as well as the role of inflammasome triggered pyroptosis.

The role of inflammasome-components in T helper cell differentiation

In order to commit to a certain T helper cell lineage, naïve CD4 + T cells interrogate the cytokines and other cues provided by the immediate milieu upon encounter with their cognate antigen. T cells then interpret these signals and differentiate into particular T helper cell subsets.[51] At the center of the regulatory network of each lineage is a master transcription factor. For example, T-bet orchestrates the commitment towards the Th1 lineage, whereas GATA3 governs Th2 differentiation. But additional factors are at play that finetune or maintain the lineage commitment, e.g. by impacting on metabolic pathways. More recently, it was shown that components of various inflammasome pathways can exert T cell intrinsic functions in either an inflammasome-dependent or -independent manner. Conceptually, the former implies the formation of T cell intrinsic inflammasomes with the consecutive activation of caspase-1 or in some instances caspase-8, while the latter means that inflammasome-components have acquired additional functions irrespective of their conventional roles. T cell intrinsic inflammasome activity is usually proposed when the genetic perturbation of various components of an inflammasome pathway show congruent phenotypes in T cell dependent disease models. Inflammasome-independent functions are inferred when T cell intrinsic deficiency for upstream or downstream inflammasome signaling components show discrepant phenotypes.

The role of cytokines of the IL-1 family in promoting differentiation of T helper cells towards the proinflammatory Th1 and Th17 subsets is well documented.[52] As such, IL–18 is a potent inducer of IFNγ[53] and therefore considered to promote Th1 responses in concert with IL-12. In contrast, IL-1β cooperates with IL-6, TGFβ and IL-23 to drive and sustain Th17 differentiation.[54,55] The maturation and release of IL-1β and IL-18 is regulated by inflammasomes. The main cell types providing IL-1 family cytokines to shape adaptive immune responses are cells of myeloid origin, even though pyroptosis of APCs might even limit adaptive responses.[56] Next to the plausible model of myeloid-intrinsic inflammasome pathways shaping Th differentiation, the concept has emerged that also T cell intrinsic expression and inflammasome-dependent release especially of IL-1β can contribute to Th1 or Th17 lineage commitment and thereby impact on the pathology of diverse diseases (Figure 1).

A commonly used paradigm to study Th17 lineage commitment in vivo is the experimental autoimmune encephalitis (EAE) model, which recapitulates certain features of multiple sclerosis in humans.[57] A commonly used EAE model is the immunization of mice with myelin oligodendrocyte glycoprotein (MOG) peptides, which induces autoreactive Th17 cells. This model was not only instrumental in substantiating the importance of IL-1-receptor-signalling in Th17-differentiation,[55] but has been tested extensively in animals deficient for various inflammasome-related proteins such as NLRP3, ASC or caspase-1. Animals with global deficiency in ASC or caspase-1 display a milder disease phenotype in EAE,[58–60] with the involvement of NLRP3 remaining controversial as some studies do[61–63] and others do not[59] report protection from EAE when NLRP3 is absent. More recently, another study addressed the role of ASC in EAE by employing a T cell specific knock out of ASC through LCK-(lymphocyte-specific protein tyrosine kinase) promoter-driven Cre-expression in mice with a floxed ASC locus.[64] This approach uncovered the surprising finding that an NLRP3-ASC-CASP8-IL-1β axis operates within T cells. Here, it was suggested that T cell intrinsic ASC is critically involved in maintenance of pathogenic Th17 cells, while early steps of Th17 differentiation in secondary lymphatic organs were unaffected. The authors could trigger the release of mature IL–1β from Th17 cells by exposing them to the known NLRP3-activator ATP, although this specific finding could not be recapitulated by a later study in a similar experiment.[65] The engagement of caspase-8 but not caspase-1 with ASC was unexpected, but it is in line with an emerging role for caspase-8 in substituting caspase-1.[52] While the T cell specific knock-out of ASC – partially even in combination with adoptive transfer of such T cells – as employed by Martin et al. represents a high standard to proof T cell intrinsic action of ASC, other studies report conflicting results. As such, expression of the hyperactive NLRP3 variant D301N under the control of CD4 – and therefore preferentially in CD4 T cells – leads to a defect in Th1 differentiation and a moderate protection from EAE.[66] Another study proposed NLRP3-dependent production of IL-1β from T cells to perpetuate a Th1 phenotype through the induction of IFNγ. In this scenario, NLRP3 and IL-1β are transcriptionally primed downstream of the complement receptor CD46, while the signal activating NLRP3 remained undefined.[67] The involvement of IL-1β but not IL-18 was unexpected, as it is reported to promote Th17 rather than Th1 differentiation. With few exceptions,[68,69] NLRP3-dependent secretion of IL-1β is linked to pyroptosis and therefore the demise of the secreting cell. But in these aforementioned studies IL-1β was proposed

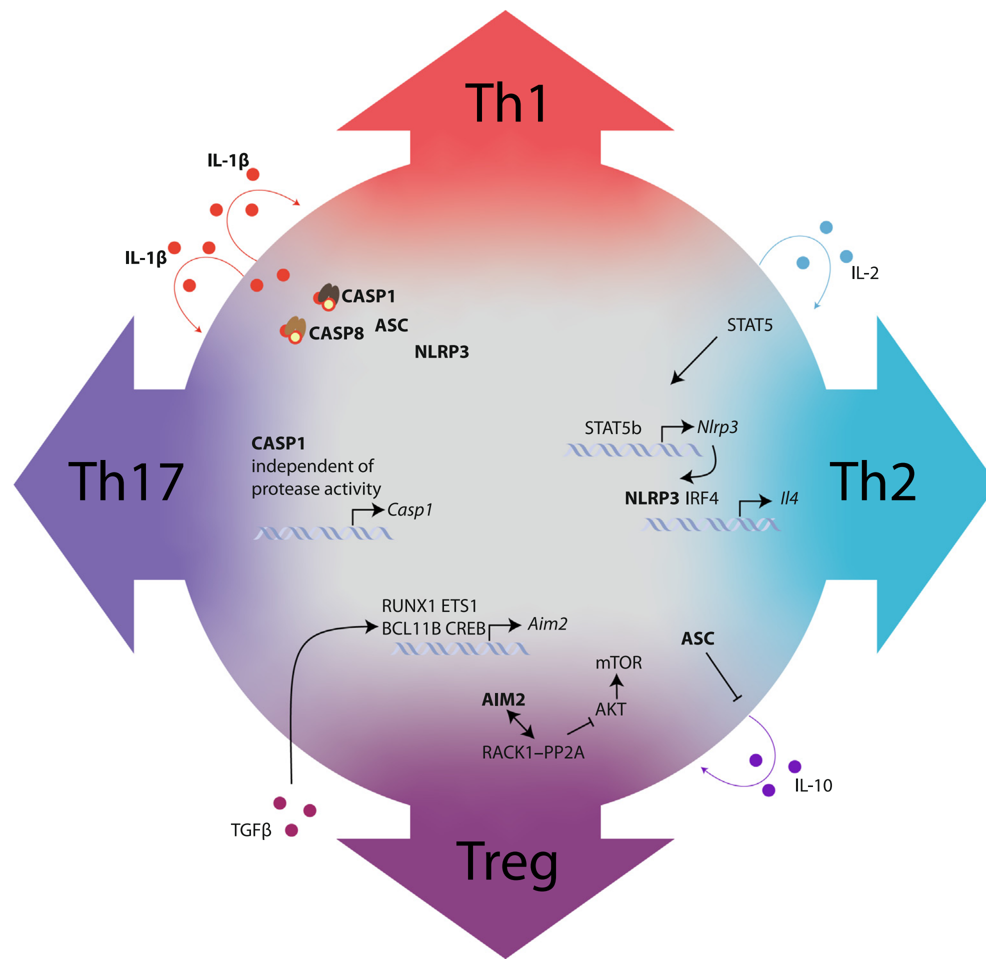

Figure 1. The role of inflammasome-components in T helper cell differentiation. T cell derived IL-1β was proposed to support Th1 differentiation in a scenario that involves the activation of caspase-1 through NLRP3. T cell derived IL-1β was also proposed to be involved in the maintenance of Th17 cells in a scenario that involves the activation of caspase-8 downstream of NLRP3. In addition, an inflammasome-independent function of caspase-1 in Th17 differentiation was proposed for which its enzymatic activity was obsolete. NLRP3 was found to be induced through IL-2 signaling in T cells and to act as a transcription factor in Th2 differentiation through regulating the expression of IL-4. ASC was shown to interfere with IL-10 production by an undefined mechanism. TGFβ signaling induces the expression of AIM2 in T cells and AIM2 in concert with RACK1 facilitates the dephosphorylation of AKT through PP2A which restrains mTOR-signaling and promotes metabolic reprogramming in favor of regulatory T cells.

to act in an auto- and paracrine fashion to support Th1 or Th17 differentiation, which would suggest its release from viable cells. And indeed, in the study by Martin et al., ATP treatment increased viability of Th17 cells. Nevertheless, whether the T cells in these scenarios secrete IL-1β as living cells or commit to GSDMD-dependent pyroptosis has not been addressed in detail. In the study by Arbore et al., the defect in IFNγ-production and a shift towards a Th17 phenotype upon adoptive transfer of NLRP3- (and IL-1α/β deficient T cells was found to be also reflected in a LCMV-infection model, in a colitis model (induced by the transfer of naïve T cells into Rag2$^{-/-}$ mice) as well as a graft-versus-host disease (GVHD) model (induced by the transfer of bone marrow and naïve T cells into lethally irradiated mice). However, the reported worsened outcome upon transfer of NLRP3$^{-/-}$ T cells in the colitis model or the GVHD model was not recapitulated in very similar experimental set-ups.[65,70] While these studies agree among themselves on the lack of a phenotype for T cell intrinsic NLRP3- or CASP1-deficiency in the related models, they report opposing findings with regard to ASC-deficiency. In the colitis model, the transfer of ASC-deficient T cells increases colitis[65] but mice receiving ASC-deficient T cells in the GVHD-model are protected.[70] The dissociation of the phenotype elicited by ASC-deficient T cells from NLRP3- or CASP1-deficient T cells indicates inflammasome-independent functions of ASC in both studies.

Interestingly, a dissociation of the phenotype of (global) ASC-deficiency from NLRP3- or CASP1-deficiency has also been reported in collagen- and

antigen-induced arthritis which are T cell-dependent disease models.[71,72] While in the collagen-induced arthritis model the effect was attributed to a DC- and not T cell intrinsic inflammasome-independent function of ASC,[71] in the antigen-induced arthritis model a T cell intrinsic function of ASC was proposed. As such, ASC was found to promote the production of Th1-related cytokines, contribute to T cell proliferation and to restrain IL-10 production in a T cell intrinsic, inflammasome independent manner,[72,73] While a defect in survival of ASC-deficient T cells upon adoptive transfer, which would be in line with a role of ASC in promoting T cell proliferation, was reported independently,[59] another study found improved survival of adoptively transferred ASC-deficient T cells.[65]

Many of the studies that promote a T cell intrinsic, inflammasome-independent role of ASC, neglect the existence of inflammasome sensor proteins other than NLRP3 as well as the potential redundancy of caspase-1 and caspase-8. As such, in the absence of a clear mechanism of how ASC impacts on T cells in a cell autonomous fashion, inferring an inflammasome-independent phenotype from the fact that there is no concordant phenotype of NLRP3- or CASP1-deficient T cells appears insufficient. Nevertheless, additional inflammasome-independent functions in CD4 T cell biology have been ascribed to NLRP3,[74] caspase-1[75] and AIM2[60] for which more mechanistic groundwork exists. Specifically, NLRP3 was found to contribute to Th2 differentiation by acting as a transcription factor of the Th2 hallmark cytokine IL-4 in concert with IRF4. NLRP3 itself was induced through IL-2 signaling in a STAT5-dependent manner.[74] In vivo, global NLRP3- but not caspase-1- or ASC-deficiency was protective in an ovalbumin (OVA)-induced asthma model and reintroducing OVA-specific wildtype T cells into NLRP3-deficient animals sufficed to restore lung pathology. This finding was further corroborated by studying Th2-dependent cancer models. In these models, melanoma or lung cancer cells elicit a Th2 response which is required to sustain tumor growth, which was found to be fully dependent on T cell intrinsic NLRP3. Interestingly, the same study also reported an impaired Th17 differentiation in vitro, potentially by NLRP3 impacting on IL1R1 transcription.

In vitro differentiation into the various T helper subsets is usually studied by activating T cells through their T cell receptor in the presence of a distinct cytokine milieu that is under physiological conditions provided by the antigen-presenting cell (APC). As such, IL-12 serves to induce Th1, IL-4 to induce Th2 and IL-1β, IL23, IL-6 and TGFβ to induce Th17 cells. These cytokines are often combined with blocking antibodies that antagonize cytokines which would induce an opposing T helper lineage. Interestingly, Th17 differentiation proceeded unabated in caspase-1 deficient T cells when such cytokine-induced polarization was employed.[74] On the contrary, caspase-1 was attributed a critical role in Th17 differentiation when generating Th17 cells through priming with pathogen-exposed DCs which potentially provide additional information to the T cells other than the aforementioned cytokines.[75] An inflammasome-independent role for caspase-1 in Th17 differentiation was inferred from the fact that ASC and IL-1β deficient T cells did not phenocopy caspase-1 deficient T cells as well as that enzymatic activity of caspase-1 was dispensable to sustain Th17 differentiation. Gao et al. employ a model in which adoptive transfer of naïve T cells into Rag1- deficient mice induces colitis and report a partial protection when transferring caspase-1 but not IL-1β deficient T cells. This is at odds with what was reported by another study that could not determine a phenotype for T cell intrinsic caspase-1 in T cell induced colitis,[65] as well as with the concept of the NLRP3-caspase-1-IL-1β axis promoting Th1 and surpressing Th17 functions in this model.[67]

With the exception of ASC, which was shown to negatively impact on IL-10 secretion, little has been known about the involvement of inflammasome-related proteins such as NLRP3 or caspase-1 in regulatory T cell (Treg) biology. This changed recently, when AIM2 was found to positively regulate the function of Treg cells.[60] Opposed to caspase-1/11- and ASC-deficiency, global AIM2-deficiency does not protect mice from EAE but instead leads to a deteriorating clinical phenotype and an increase of Th17 cells and a decrease in Treg cells in the central nervous system. This phenotype was also reflected in an adoptive transfer set-up in the EAE model, which indicated a T cell intrinsic role of AIM2 independent of its conventional role as an inflammasome sensor. Additionally, AIM2 deficient regulatory T cells failed to mitigate colitis induced through the parallel transfer of naïve wildtype T cells to RAG-1 deficient mice, while wildtype or ASC-deficient Treg cells readily reversed the induction of colitis. Mechanistic analyses revealed that AIM2 in T cells is similarly regulated as the Treg master transcription factor FOXP3. Specifically, TGFβ signaling was required for the induction of AIM2 and the promoter region of the AIM2 locus was found to be occupied with the known FOXP3-regulators RUNX1, EST1, BCL11B and CREB. Once transcribed, AIM2 enters into a complex with RACK1 and PP2A which are known to negatively regulate mTOR signaling through the dephosphorylation of AKT. Downregulation of mTOR signaling and a consecutive metabolic switch in favor of oxidative phosphorylation and fatty acid oxidation over glycolysis is considered one of the key metabolic hallmarks of Tregs.[76]

As more and more studies are published addressing T cell intrinsic functions of inflammasome components, very similar in vivo models have been explored by independent

Table 1 Studies that address a T cell intrinsic involvement of inflammasome components in mouse models.

Main inflammasome component and relevant cell type	Inflammasome dependent or independent signaling	Mouse model, main genotype studied and associated phenotype	Additional genotypes studied and phenotype	Ref.
ASC in Th17	dependent	**EAE**, transfer of LCK-Cre ASC$^{fl/fl}$ Th17 cells with protected phenotype	**EAE,** transfer of IL1$\beta^{-/-}$ T cells with protected phenotype	64
NLRP3 in Th1	dependent	**LCMV infection**, transfer of NLRP3$^{-/-}$ T cells with decrease in IFNγ production in virus-specific T cells **Colitis model** and **GVHD** transfer of NLRP3$^{-/-}$ T cells with increase in Th17 cells and aggravated phenotype	**LCMV infection**, IL1$\alpha/\beta^{-/-}$ and IL-1R1$^{-/-}$ with decrease in IFNγ production	67
NLRP3 in Th1/ Th17	undetermined	**EAE,** transfer of T cells with CD4-Cre driven expression of NLRP3$^{gain-of-function}$ on a NLRP3$^{-/-}$ background with protected phenotype	**EAE**, global NLRP3$^{-/-}$ with protected phenotype	66
NLRP3 in Th2	independent	**Asthma model,** transfer of NLRP3$^{-/-}$ T cells with protected phenotype **Th2-dependent cancer models,** transfer of NLRP3$^{-/-}$ T cells with protected phenotype	**Asthma model,** global NLRP3$^{-/-}$ with protected phenotype, global CASP1$^{-/-}$ or ASC$^{-/-}$ with unaltered phenotype **Th2-dependent cancer models,** global NLRP3$^{-/-}$ with protected phenotype, CASP1$^{-/-}$ and ASC$^{-/-}$ with unaltered phenotype	74
CASP1 in Th17	independent	**Colitis model,** transfer of CASP1$^{-/-}$ T cells with protected phenotype	**Colitis model,** transfer of IL1$\beta^{-/-}$ T cells with unaltered phenotype	75
AIM2 in Treg cells	independent	**EAE,** transfer of AIM2$^{-/-}$ T cells with aggravated phenotype **Colitis model**, transfer of AIM2$^{-/-}$ Treg cells fails to mitigate phenotype	**EAE**, global AIM2$^{-/-}$ with aggravated phenotype, global ASC$^{-/-}$ and CASP1$^{-/-}$ with protected from EAE	60
ASC in CD4 T cells	independent	**Colitis model**, transfer of ASC$^{-/-}$ T cells with aggravated phenotype	**Colitis model,** transfer of NLRP3$^{-/-}$ or CASP1$^{-/-}$ with unaltered phenotype	65
ASC in CD8 T cells	independent	**GVHD**, transfer of ASC$^{-/-}$ T cells with protected phenotype	**GVHD**, transfer of NLRP3$^{-/-}$, CASP1/CASP11$^{-/-}$, MYD88/TRIF$^{-/-}$, IL-1R1$^{-/-}$ T cells with unaltered phenotype	70

For simplicity, the common molecule names have been used to indicate the studied genotypes rather than the official gene symbols.

groups (Table 1). However, as outlined above, the reported results are often not congruent and the inferred concepts sometimes even contradictory. Although many studies try to address potential confounding factors for example by employing NLRP3-deficient or ASC-deficient animals on various genetic backgrounds,[70,74] the question emerges whether additional confounding factors could influence the observed results. A potential confounder could be that NLRP3 and other inflammasomes also impact on the composition of the gut microbiome, which plays an important role in priming T cell functions.[77] For example, mice carrying a gain-of-function variant of NLRP3 not only harbor a significantly altered gut microbiome but also increased numbers of regulatory T cells that render these animals less susceptible to colitis and colorectal cancer.[78] It is conceivable that the microbiome of the donor animal deficient for the gene under study influences the function of adoptively transferred T cells. One study addressing inflammasome function in T cells addressed this potential confounding factor by co-housing wildtype and ASC-deficient animals prior to their experiments, while they reassuringly did not observe an effect on T cell functions upon adoptive transfer.[70] However, the composition of the gut microbiome under the co-housing conditions was not monitored. In summary, more mechanistic studies are required to provide conclusive concepts on how inflammasome components impact on T helper cell differentiation. Here, more reductionist settings under which environmental factors can better be accounted for would be desirable. Moreover, there is a lack of knowledge about which of these findings can be applied to the human system. Among all studies that propose an involvement of NLRP3 or other inflammasome components in T helper differentiation, only one provides affirmative data studying human cells.[67] In this study, the small molecule NLRP3-inhibitor MCC950 inhibits IFNγ production from T cells, which can be restored by the addition of exogenous

IL-1β and T cells isolated from patients carrying an NLRP3 gain-of-function mutation show increased IFNγ and decreased IL-17 production.

Activation of the CARD8 inflammasome triggers T cell pyroptosis

While the aforementioned studies, that describe an inflammasome-dependent release of IL-1 family cytokines in shaping T cell biology, do not unambiguously address whether this implies pyroptosis and therefore the death of the involved cell, we and others could recently show that T cells are faithfully capable of committing to pyroptosis.[30,31] In these studies, it was shown that inflammasome activation in T cells recapitulates morphological, biochemical and genetic features of pyroptosis. Screening a diverse set of inflammasome stimuli, it was found that the DPP8/9 inhibitor VbP triggered a lytic type of cell death in human T cells. Morphologically, T cells treated with VbP acquired a pyroptotic phenotype with characteristic cell swelling and rapid loss of membrane integrity. Biochemically, cleavage of GSDMD into its pore-forming fragment as well as characteristic caspase-1 cleavage products could be detected. Genetically, T cells deficient for CARD8, caspase-1 or GSDMD were fully protected from VbP-induced pyroptosis, while NLRP1- and ASC-deficiency did not show such a phenotype. At odds with the prominent T cell intrinsic role attributed to the NLRP3 inflammasome by some studies, this pathway could not be activated in human T cells, just as much as the expression or secretion of IL-1 family cytokines remained undetectable. Altogether, these studies provide clear evidence of a functional CARD8 inflammasome pathway in human primary T cells.

Remarkably, a study predating the discovery of this pathway had already suggested that DPP inhibition through VbP results in apoptosis in peripheral blood mononuclear cells.[79] Interestingly, here it was also found that only resting, but not activated lymphocytes succumbed upon VbP treatment. A drop in the ratio of cleaved over full length CARD8 in activated T cells could point towards a regulatory mechanism acting on the level of CARD8 by influencing its autoprocessing.[30,31] Very recently structural insight on NLRP1 and CARD8 was gained, which shows that DPP9 cooperates with the full-length molecules of NLRP1 or CARD8 to limit the activity of free UPA-CARD fragments in a ternary complex.[17,18,26] Apparently, a delicate equilibrium of the three components exists, that on the one hand prevents the UPA-CARD from oligomerizing and thus becoming active but on the other hand also keeps the inflammasome activatable. Studying the mechanism that renders activated T cells resistant to CARD8, potentially by impacting on the autoprocessing of CARD8 and thereby disrupting this equilibrium, could unveil another layer of regulation of the CARD8 inflammasome in T cells.

While the activity of the CARD8 inflammasome in human T cells is now well documented, the question is what physiological trigger it might recognize. In line with the recently established role of human NLRP1 detecting RNA virus protease activity,[21,22] the question emerged whether CARD8 could also detect viral infection by acting as a decoy substrate of a viral protease activity. Given the importance of HIV, for which T cells constitute the pathophysiologically most relevant target cell type, studying a potential involvement of CARD8 in the detection of HIV appeared the logical next step. However, a different inflammasome sensor had previously been proposed to govern pyroptosis of HIV infected cells.

Evidence of pyroptosis in T cells driven by HIV

Ever since the emergence of HIV, the cell death mechanism that depletes T cells has been subject of intense research and various mechanisms of how T cells die during HIV infection have been proposed.[80] In vitro infection of lymphoblastic cell lines was shown to elicit two distinct cytopathic effects.[81] One was the induction of multinucleate giant cells, an effect that was later attributed to be induced by the gp120 glycoprotein.[82] The other was a lytic form of cell death that was preceeded by cell swelling. The identification of the latter as a form of programmed cell death, dates back to a time when any programmed cell death was equated with apoptosis.[83] The analysis of ex vivo specimen of lymph nodes from infected individuals suggested that the cell death triggered by HIV occurs primarily in non-productively infected bystander cells (not staining positive for viral RNA) rather than in productively infected cells.[84] Based on this observation, it was furthermore proposed that human lymphatic tissue rather than lymphoblastic cell lines or peripheral blood derived T cells constitute the relevant model system to study the cell death caused by HIV. Indeed, massive cell death of non-productively infected T cells can be recapitulated in this model.[85] Nevertheless, it was not until the work from Greene and colleagues that the observed cell death could be linked to T cell pyroptosis rather than apoptosis. In a series of publications, the group first showed that the cell death is triggered by products of incomplete reverse transcription prior to viral integration.[86] Small-hairpin-RNA (shRNA) mediated suppression of the expression of caspase-1 and ASC (but not NLRP3) rendered abortively infected T cells fully resistant to this kind of cell death.[87] Finally, the group proposed IFI16 as the inflammasome sensor that directly engages with ssDNA products from incomplete reverse transcription.[88] Central to this concept is the recognition of reverse transcription intermediates through IFI16. This implies that HIV-derived nucleic acids are

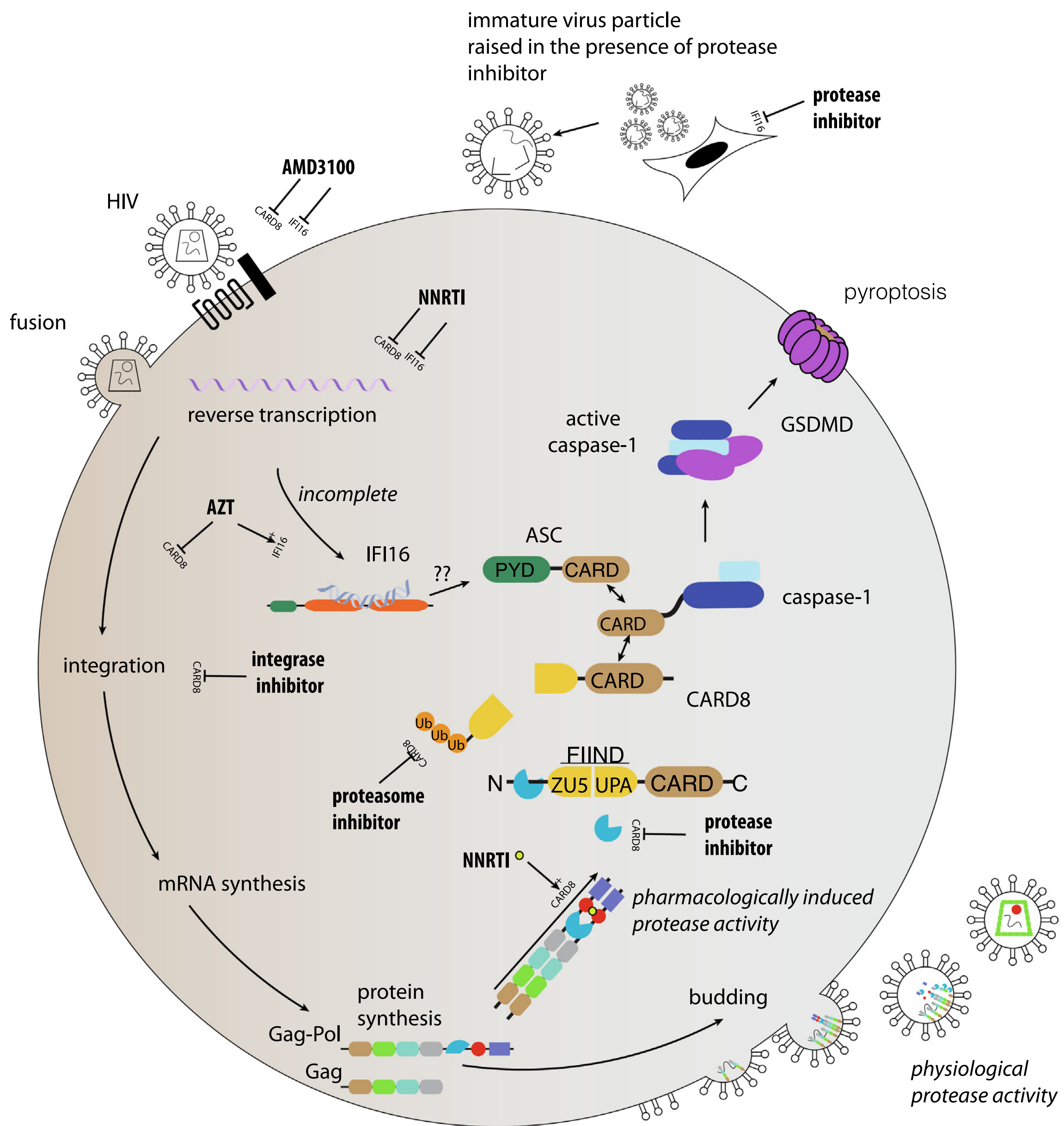

Figure 2. Mechanisms of HIV triggered T cell pyroptosis. Two mechanism have been put forward of how HIV can trigger pyroptosis of T cells which can be induced or inhibited by various pharmacological interventions that interfere with different stages of the HIV replication cycle. The first mechanism involves the activation of IFI16 which serves as an inflammasome sensor for incomplete reverse transcription products of HIV. While non-nucleoside reverse transcription inhibitors (NNRTI) inhibit this mechanism, the reverse transcription inhibitor azidothymidine (AZT) that competitively interrupts nucleic acid chain elongation promotes IFI16 activation. IFI16 forms an inflammasome with ASC and activates caspase-1. The concept of IFI16 dependent pyroptosis in HIV infection predates the discovery of GSDMD as the executioner of pyroptosis. The CARD8 inflammasome becomes active when its N-terminus is cleaved by the HIV protease. This deems the destabilized N-terminus of CARD8 for proteasomal degradation and the UPA-CARD fragment is released. UPA-CARD then interacts with caspase-1 which triggers pyroptosis through the cleavage of GSDMD. Under physiological conditions, the HIV protease does not become active intracellularly but only during the process of budding. Adding NNRTI to cells that have integrated the HIV provirus and express the Gag-Pol polyprotein triggers premature protease activity by dimerizing Gag-Pol. Thereby, the protease can cleave and activate CARD8. Inhibition of protease activity blocks the destabilizing cleavage of the N-terminus of CARD8 and subsequent pyroptosis. Viral particles generated in the presence of protease inhibition were incapable of activating IFI16-dependent pyroptosis. Proteasome inhibition interferes with CARD8 activation as it blocks the degradation of the destabilized N-terminus of CARD8 which restrains the activity of the UPA-CARD fragment. Entry inhibition through the compound AMD3100 blocks both mechanisms of HIV triggered T cell pyroptosis.

accessible to the detection by innate immune sensors and that IFI16 can act as an inflammasome sensor protein (Figure 2).

At odds with the concept that the sensing of reverse transcription products through IFI16 is the main driver of T cell loss in the course of HIV infection, is the fact that it assumes a post-entry mechanism for the loss of T cells. Yet, while T cell loss is an early event preceding the emergence of CXCR4-tropic variants (reviewed in[89]), the transmission of HIV is exclusive to CCR5 tropic variants which can only infect a small fraction of T cells due to the limited expression of the CCR5 co-receptor.[90,91] As such, the infection of the minor CCR5-expressing target cell population can hardly account for the global loss of CD4 T cells observed in HIV infected individuals. Opposed to the notion that peripheral blood derived T cells are considered resistant to pyroptosis triggered during abortive infection,[92] a recent study suggests that the NLRP3 inflammasome triggers pyroptosis of HIV infection of productively infected T cells in the peripheral blood.[93] In this scenario NLRP3 was proposed to be activated through mitochondrial reactive oxygen species. A role of NLRP3 in activating T cell pyroptosis is nevertheless difficult to reconcile with older studies, that observed that intracellular potassium concentrations increase[94] rather than decrease during HIV infection as it would be required for NLRP3 activation. Further, infection in the presence of high extracellular potassium – a method commonly used to inhibit the potassium-efflux dependent activation mechanism of NLRP3 - does not block but rather increases the cytopathic effects of HIV.[95] In addition, in pyroptosis of non-productively infected lymphatic T cells, a role for NLRP3 was excluded, even though it was reported that these cells can be activated through nigericin-treatment.[87] Interestingly, similar to the studies finding NLRP3 to be critically involved in Th1 and Th17 differentiation, the release of IL-1β from T cells is thought to play an integral role in the pathology driven by HIV-induced pyroptosis. As such it was proposed, but not experimentally addressed, that the inflammation instilled by the release of IL-1β from T cells leads to the recruitment of additional T cells to the affected lymphatic tissue. These in turn become infected themselves and depleted through pyroptosis in a vicious cycle.[96] The expression of IL-1β was most pronounced in a CCR5-positive subset of effector T cells,[87] while its transcriptional control is believed to be inherently different in T cells from what has been shown for myeloid cells as it occurs independently of Spi1.[97] Of note, the reported concentrations of IL-1β released from T cells in standard inflammasome activation assays are often orders of magnitude lower than what is usually observed in monocytes and macrophages, which stresses that special care has to be taken with the regard of the purity of the studied cell population, as trace amounts of myeloid cells might lead to confounding results.

The CARD8 inflammasome as a sensor of HIV

While the activity of the CARD8 inflammasome in human T cells is now well documented, its physiological trigger remained undefined. Based on the recent extension of the pathogens detected by NLRP1 through their protease activity to RNA viruses,[21,22] the question emerged whether CARD8 could also detect viral infection by acting as a decoy substrate of a viral protease. The HIV-encoded protease appeared as a plausible candidate that could reconcile the lack of a physiological trigger of the CARD8 inflammasome and the concept of HIV-induced pyroptosis. A recent study now established that CARD8 can serve as a substrate of the HIV protease and that cleavage of CARD8 through the HIV protease triggers pyroptosis of T cells and myeloid cells.[98] However, HIV protease does not become active intracellularly during the natural replication cycle of the virus (Figure 2). It is only during the process of budding that the Gag-Pol protein that harbors the protease activity is locally enriched in a way that allows for it to activate the protease. While this can be mimicked through the overexpression of the Gag-Pol protein which suffices to induce its dimerization and subsequent cleavage of co-expressed CARD8, the authors employed a pharmacological treatment to induce intracellular protease activity and CARD8 activation in T cells. Wang et al. took advantage of the fact, that non-nucleoside reverse transcription inhibitors (NNRTIs) such as Efavirenz or Rilpivirine, in addition to their canonical function in blocking the reverse transcriptase, can induce dimerization of the Gag-Pol polyprotein.[99] The Gag-Pol polyprotein harbors both, the reverse transcriptase to which the inhibitor binds and the pro-form of the protease in its inactive state. The dimerizing effect of the NNRTI on Gag-Pol brings the protease into proximity so that it turns active. Subsequently, the HIV protease can engage with cytoplasmic CARD8 as its substrate. Interestingly, the fact that NNRTI treatment post viral integration can induce a rapid cell death in HIV infected T cells had been noted before with the mechanism not having been further characterized.[100] To this end, the pharmacological effect of NNRTIs is twofold. While NNRTIs readily block viral replication when added early during infection, adding it after the virus has integrated into the host genome and started to produce the Gag-Pol polyprotein, it can induce CARD8-dependent pyroptosis. Opposed to the concept that pyroptosis constitutes a driver of HIV pathology, the authors propose that the intentional activation of the CARD8 inflammasome in latently HIV infected T cells could serve as a therapeutic opportunity as part of a shock and kill strategy. Whether the active protease in the incoming viral particle can be sensed by CARD8 early in the replication cycle under physiological conditions, is an interesting open question.

Concluding remarks

As described in this review, there is currently a plethora of different models proposing unique functionalities of inflammasomes in T cells. These range from "conventional" signaling cascades leading to GSDMD-dependent pyroptosis to "moonlighting" functions of inflammasome components affecting seemingly unrelated processes. Needless to say, given the fact that some of these results are in conflict to another, further work is needed to clarify whether these models are indeed correct. Moreover, it would be desirable if inflammasome functions would be validated using orthogonal approaches thus increasing the confidence in the proposed models. In this regard, it has to be considered that concepts purely inferred from genetic studies can be misleading in light of the fact that passenger mutations can impact on the phenotype of interest.[101] With the availability of efficient genome engineering methodologies to manipulate human primary T cells, it will be also be feasible to address some of these concepts directly in the human system.

Among the here-discussed models, the CARD8 inflammasome represents the most advanced concept of a T cell intrinsic inflammasome function, as both loss-of-function genetic and biochemical data are in clear agreement. However, the physiological role of this pathway is not yet entirely clear. While HIV protease-dependent cleavage of CARD8 clearly triggers CARD8 activation in the context of certain drugs, it remains unclear whether such a mechanism is also important during the course of natural infection, which may have been a selection pressure during evolution. Here, it might be worthwhile to study other T cell tropic viruses, such as certain Herpesviruses, for their CARD8 stimulatory capacity.

CRediT authorship contribution statement

Andreas Linder: Conceptualization, Funding acquisition, Visualization, Writing – original draft, Writing – review & editing. **Veit Hornung:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Acknowledgements

A.L. is supported by a fellowship of the Else Kröner-Forschungskolleg: Cancer Immunotherapy. V.H. is supported by the Deutsche Forschungsgemeinschaft: project grant ID 318287247 - SPP-1923, project grant ID 452881907 - TRR338 and project grant ID 210592381 – CRC 1054.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Received 26 July 2021;
Accepted 22 September 2021;
Available online xxxx

Keywords:
inflammasome;
T cells;
T helper cells;
NLRP1;
CARD8;
pyroptosis

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