POP goes the inflammasome

Jayendra Kumar Krishnaswamy, Dong Liu & Stephanie C Eisenbarth

The PYRIN domain–only protein POP3 sets limits for activation of the AIM2 inflammasome after cytosolic double-stranded DNA is sensed.

Encoded in the human genome are dozens (probably hundreds) of receptors that detect a wide range of molecular motifs derived from pathogens or from damaged host tissue, collectively called 'pattern-recognition receptors' (PRRs). As part of the innate immune response, the triggering of specific PRRs orchestrates a swift inflammatory response that includes alterations in cellular transcriptional programs, the release of cytokines and even cell death. However, the robust immune response that ensues can lead to the collateral damage of host tissues. Therefore, numerous molecules also exist that limit the activity of PRRs once triggered. In this issue of *Nature Immunology*, Khare *et al.* identify one such regulator, POP3, as a previously unknown member of a family of inhibitors of PRRs that is important in regulating the response to double-stranded DNA (dsDNA)[1].

There are many cytoplasmic sensors of nucleic acids that detect invading viruses and bacteria[2]. AIM2 ('absent in melanoma 2') and its closely related family member IFI16 are two such sensors and are members of the DNA-binding PYHIN ('PYRIN and HIN-200 domain–containing') family. The PYHIN family (or, in keeping with the dominant nomenclature in the PRR field, the 'AIM2-like receptor' (ALR) family) has 4 members in humans and 13 predicted members in mice. AIM2 directly binds to long stretches of cytosolic dsDNA regardless of its sequence[3]. Therefore, AIM2 activity can be triggered by and is crucial for the immune response to pathogens ranging from *Francisella tularensis*

to vaccinia virus and cytomegalovirus but could theoretically also be activated by host DNA[2]. IFI16 senses modified viral dsDNA, also via its HIN-200 domain, but does so in the nucleus[4].

After being activated, certain members of the ALR family and the Nod-like receptor (NLR) family (also PRRs) orchestrate an inflammatory response through the formation of an inflammasome—a large multimolecular complex—in the cytosol[5]. This platform is composed of sentinel receptors such as AIM2 or NLRP3, the adaptor ASC and an effector caspase, such as caspase-1. Activation of those cysteine proteases results in the cleavage and release of the potent cytokines interleukin 1β (IL-1β) and IL-18 or can induce a unique form of cell death called 'pyroptosis'. ASC is an important scaffold for the formation of inflammasomes and acts as a bridge between the PRR and caspase by providing homotypic domain interactions for all partners involved. For example, the PYRIN domain of AIM2 binds to the PYRIN domain of ASC to nucleate the AIM2 inflammasome after recognition of dsDNA.

Clear evidence exists showing that dysregulated inflammasome activity induces human disease, most notably the autoinflammatory disorders driven by excessive production of IL-1β. Inappropriate recognition of self nucleic acids can also potentially promote particular autoimmune diseases. Therefore, a system of negative regulators of inflammasome activity is crucial. Humans have two families of molecules whose main function is to regulate inflammasome activity: PYRIN domain–only proteins (POPs) and caspase-recruitment domain–only proteins (COPs). Again through homotypic domain interactions, COPs and POPs act as decoys for various components of the inflammasome to inhibit the formation of inflammasomes through a straightforward sequestration mechanism[6]. The potency of

such inhibitors is evident in the identification of POP mimics encoded by certain viruses such as poxviruses[7].

Genes encoding POPs and COPs seem to have originated in the human genome through gene duplications that resulted in proteins containing only PYRIN domains (POPs) or just caspase-recruitment domains (COPs); accordingly, most genes encoding COPs cluster with those encoding the inflammatory caspases that the COPs regulate, whereas genes encoding POPs are located throughout the genome but are proximal to genes encoding the molecular targets of the POPs. For example, POP1, the negative regulator of ASC, has 64% sequence identity to the PYRIN domain of ASC, and the gene encoding POP1 is located on human chromosome 16 next to the gene encoding ASC[8]. The PYRIN domain of POP2 shares identity with the PYRIN domain in NLRP2 (and others) and inhibits formation of the NLRP2 inflammasome by blocking its interaction with ASC[9]. Stehlik and colleagues now show that a third member of the POP family, aptly named 'POP3', is encoded by a gene region containing the genes encoding the inflammasome-forming ALRs that respond to intracellular DNA and likewise negatively regulates its neighbors[1] (**Fig. 1**).

The story begins with identification of a previously undescribed human POP-encoding gene nestled within an interferon-inducible gene region flanked by genes encoding various ALRs. In contrast to POP1 and POP2, which have high sequence similarity to the PYRIN domain of ASC, POP3 shows greater sequence similarity to the PYRIN domain of AIM2, encoded by a neighboring gene. The authors predict that POP3 might regulate activity of the AIM2 inflammasome in the cells with the highest POP3 expression: human macrophages. Indeed, they find association of POP3 with the PYRIN domain of

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both AIM2 and its related family member IFI16 in monocytes. Interestingly, and consistent with its degree of homology to the ASC PYRIN domain, POP3 does not associate with ASC as do other members of the POP family. Instead, the direct association of POP3 with AIM2 diminishes the ability of AIM2 to associate with ASC and therefore to form an inflammasome. To determine if POP3 indeed inhibits ALR-dependent inflammasome activity, they infect human macrophages with modified vaccinia virus Ankara or mouse cytomegalovirus. In line with its apparent molecular targets, POP3 inhibits assembly of the AIM2 inflammasome and its subsequent production of IL-1β and IL-18 but does not affect other inflammasome complexes, including NLRP1, NLRP3 and NLRC4.

Since POPs are evolutionarily conserved in higher order vertebrates such as primates but are absent from rodents, studying their role during infection *in vivo* has been challenging. Khare *et al.* capitalize on the high degree of sequence conservation between the PYRIN domains of human ALRs and those of mouse ALRs and generate mice that transgenically express human POP3 in their macrophages[1]. This approach allows the authors to assess the function of POP3 in the context of an intact immune system during infection with a live virus. Indeed, the phenotype of the transgenic mouse mirrors that of AIM2-deficient mice, with low concentrations of IL-18 in the serum, impaired activation of natural killer cells and high titers of mouse cytomegalovirus in the spleen. To investigate the function of the IFI16 inflammasome, they infect cells with Kaposi sarcoma–associated herpesvirus and again observe impaired release of IL-1β, which suggests that IFI16 is also a target of POP3.

The interferon responsiveness of *POP3* distinguishes it from genes encoding the other members of the POP family. Type I interferons, including IFN-α and IFN-β, are rapidly produced during viral infection and induce multiple antiviral defense pathways. Although it is not identified in this study, the early interferon needed for transcription of the genes encoding ALRs and POP3 could come from the activation of other PRRs that sense dsDNA in the cytosol such as DDX41, DAI or others[2]; alternatively, cells that have ingested viral particles by phagocytosis and have endosome-restricted sensors of DNA could produce early interferon. On the basis of findings from the present study[1], we propose that a positive feedback loop might then be initiated: POP3-mediated inhibition of AIM2 modestly enhances the cellular production of IFN-β, a phenotype that has also been reported for *Aim2*^{−/−} macrophages[10]. Although
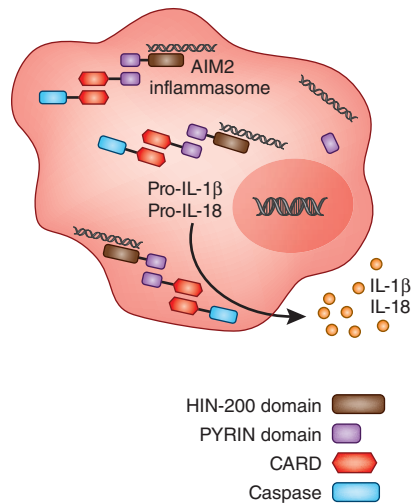

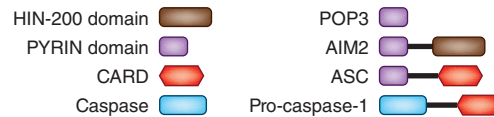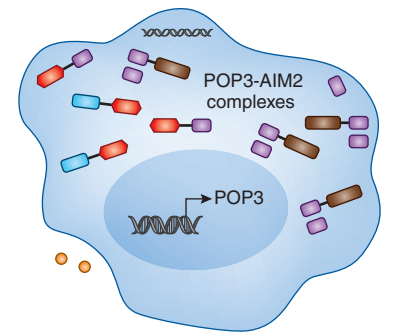**a** Recognition of virus and induction of type I interferons
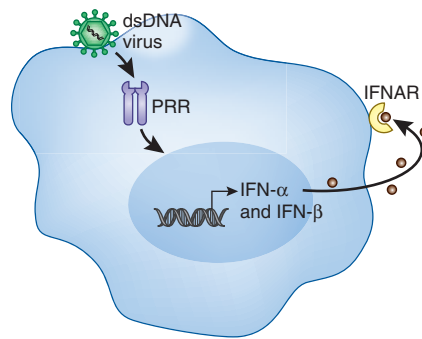
b Induction of ALRs and POP3
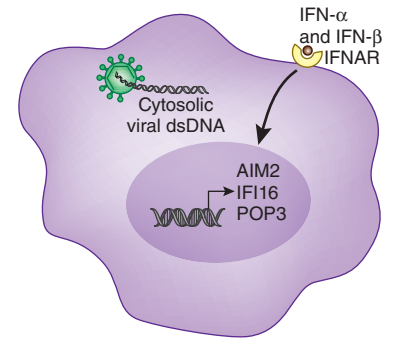
c Inflammasome activation

d POP3 resolution

HIN-200 domain
PYRIN domain
CARD
Caspase

POP3
AIM2
ASC
Pro-caspase-1

Katie Vicari

Figure 1 POP3 regulates excessive ALR inflammasome activity. (**a**) Macrophages in the steady state (blue) are primed to respond to viral invasion through multiple intracellular PRRs; the triggering of those PRRs induces a type I interferon response (IFN-α and IFN-β). (**b**) Autocrine or paracrine IFN-α and IFN-β induce, via the IFN-α receptor IFNAR, transcription of the gene region containing *AIM2*, *IFI16* and *POP3*. That primes the cell (purple) for responsiveness to cytosolic viral dsDNA. (**c**) Ligation of AIM2 in the cytosol nucleates an inflammasome once a threshold of dsDNA is present to overcome the initial low expression of POP3, with the subsequent inflammatory response (red), including release of IL-1β and IL-18. (**d**) The second wave of POP3 expression quenches inflammasome activity and allows the cell to return to the basal state (blue). Nuclear IFI16, which the authors propose is also regulated by POP3 (ref. 1), is not included here. CARD, caspase-recruitment domain.

the underlying mechanism for the augmented IFN-β production is not clearly understood, it might act to amplify ALR 'readiness' in nearby cells in anticipation of viral spread.

Given that AIM2 recognizes pathogen DNA and mammalian DNA equally well because length, rather than sequence, dictates specificity, inappropriate triggering of the AIM2 inflammasome seems a real and likely threat. Another finding of the present study suggests a potential mechanism by which promiscuous activation of the AIM2 inflammasome is prevented[1]. Despite the fact that *POP3, AIM2* and *IFI16* are all upregulated by IFN-β, the kinetics of the expression of *POP3* are distinct from that of neighboring genes encoding

ALRs: after stimulation with cytokines, *POP3* is immediately expressed (<2 hours) and then is not expressed again until later time points (48 hours), whereas expression of *AIM2* and *IFI16* peaks at around 8 hours. Why would the expression of *POP3* precede that of *AIM2* and *IFI16*? One possibility is that early expression of *POP3* might set an initial threshold of dsDNA required for the induction of inflammasome activity. Once such a threshold is reached, the delayed second wave of *POP3* expression acts as a safeguard to ensure resolution as the pathogen is contained. We might also speculate that in cases in which AIM2 is engaged erroneously by host DNA, there would be a limited window of opportunity for inflammasome function.

Despite having AIM2 and other NLRs that form inflammasomes, mice do not have POPs or COPs. However, mice also do not demonstrate evidence of uncontrolled and persistent inflammasome activity. That puzzling observation indicates that either mice have alternative strategies to restrain excessive activity of the ALR inflammasome or they do not need such regulatory mechanisms. The authors[1] point out that mice (but not humans) have a potential alternative AIM2 antagonist, p202, which has a HIN-200 domain[11]. Other potential molecules that could fulfill the function of POP3 are proposed, but the

possibility remains that humans evolved a more stringent control system for ALR inflammasomes than that required in mice. Disease states such as systemic lupus erythematous (an autoimmune disease that most commonly targets host dsDNA) and Aicardi-Goutieres (a congenital neurological disease that stems from hyperactivation of inflammatory DNA-response pathways)[2] might exemplify why a dedicated system of DNA-response safeguards is indeed needed in humans.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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ILCs in the zone

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Innate lymphoid cells, marginal reticular cells and B cell–helper neutrophils interact to promote antibody secretion by B cells in the marginal zone of the spleen in humans and mice.

Innate lymphoid cells (ILCs) are a diverse population of cells that have lymphoid characteristics but lack rearranged antigen receptors. ILCs have been 'distilled' into three groups: ILC1 cells, which include classical natural killer cells, express the transcription factor T-bet and produce the T helper type 1 cytokine interferon-γ; ILC2 cells, which include nuocytes and natural helper cells, express the transcription factor GATA-3 and produce typical T helper type 2 cytokines; and ILC3 cells, which include lymphoid tissue–inducer cells, express the transcription factor RORγt and are associated with the gastrointestinal mucosa[1]. Reflective of their phenotypic variability, ILCs have important roles in several aspects of the immune system, from killing transformed cells to inducing the formation of lymphoid tissue during embryogenesis. In this issue of *Nature Immunology*, Magri *et al.* propose yet another function of ILCs in the immune response: enhancing the production of T cell–independent (TI) antibodies by B cells in the marginal zone (MZ) of the spleen[2].

MZ B cells are a subset of splenic B lymphocytes defined mainly by their anatomical location, peripheral to the B cell follicle where traditional follicular B cells reside. The phenotypes and features of these cells differ in mice versus humans. In mice, MZ B cells are a well-defined population of non-recirculating

$IgM^+IgD^{lo}CD21^{hi}CD23^{lo}$ B cells that are thought to be a lineage separate from follicular B cells. Although they share certain phenotypic characteristics with their mouse counterparts, human MZ B cells recirculate freely and are somatically hypermutated, which suggests a memory B cell origin[3]. Despite such differences, MZ B cells are believed to have a conserved role in both species. Their localization in the MZ, the macrophage-rich border of the splenic white pulp through which blood drains from fenestrated arterioles into venous sinuses, ensures that these B cells are promptly exposed to any antigens entering the bloodstream. MZ B cells thus function as first responders to blood-borne pathogens, producing most of

the low-affinity TI type 2 antibody response that bridges the gap between infection and the production of T cell–dependent antibodies of higher affinity.

Unlike follicular B cells, which after receiving the first signal delivered by the recognition of antigen by the B cell antigen receptor require a second signal from T cells to become fully activated, MZ B cells are thought to rely on other sources of additional stimulation. Various infectious non-self signatures can serve that role. Those include Toll-like receptor (TLR) ligands and the periodic spacing of epitopes characteristic of certain pathogen-derived molecules and of the prototypical TI type 2 model antigen, haptenated Ficoll. Likewise,
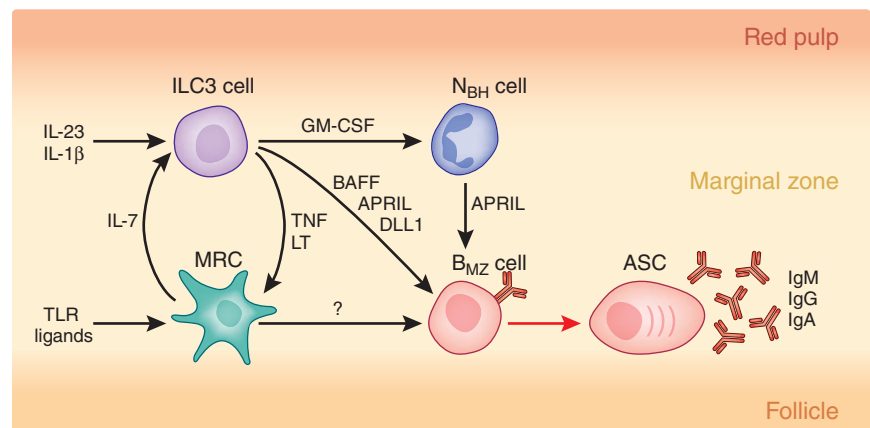
Figure 1 ILCs stimulate antibody production by MZ B cells. A three-way interaction among ILC3 cells, MRCs and marginal zone B cells (B_{MZ} cell) enhances TI antibody production in the spleen, a process further aided by ILC-driven activation of B cell–helper neutrophils (N_{BH}). BAFF, B cell–activation factor; APRIL, proliferation-inducing ligand; DLL1, Notch ligand; TNF, tumor-necrosis factor; Lt, lymphotoxin; ASC, antibody-secreting cell.

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