Illuminating inflammasome activity *in vivo*

Heather D Hickman

Sentinel macrophages in the lymph node provide a first line of defense against invading viruses. A new study visualizes inflammasome activation in virally infected nodal macrophages in mice and shows that this activation augments both innate and adaptive immunity.

Lymph nodes are specialized battlegrounds in which immune cells encounter a diverse array of pathogens actively conveyed from peripheral tissues. Although the node is a crucial location of the initiation of adaptive immunity, it also functions as a barrier against systemic pathogen dissemination. Just inside the node's confines, a continuous layer of subcapsular sinus (SCS) macrophages forms the tissue-lymph interface and immediately encounters lymph-borne pathogens that reach the node[1,2]. SCS macrophages sequester incoming virions, such as those of modified vaccinia Ankara (MVA) virus, a replication-deficient poxvirus that is commonly used as an experimental vaccine vector. The macrophages respond to viral infection in an inflammation-dependent manner[3,4].

Inflammasomes are large, cytosolic multiprotein complexes that sense intracellular pathogens or danger signals and that initiate inflammatory processes. Poxviruses and other DNA viruses activate inflammasomes after triggering cytoplasmic DNA sensors, such as AIM2 (refs. 5,6), which leads to the rapid polymerization of the common adaptor protein apoptosis-associated speck-like protein containing a caspase-recruitment domain (ASC, also known as PYCARD). ASC forms large (micrometer) filamentous scaffolds, which are referred to as ASC specks, that recruit and activate caspase-1, resulting in the maturation of interleukin (IL)-1β. Although inflammasome activation occurs during infections with many different viruses and is an important aspect of immune control, the precise spatiotemporal kinetics with which this occurs *in vivo* are unknown. In this issue of *Nature Medicine*, Sagoo *et al.*[7] visualize this inflammasome activation in MVA-infected SCS macrophages *in vivo* and describe important downstream effects on both innate and adaptive antiviral immunity (**Fig. 1**).

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The authors first visualized inflammasome activation by microscopy in MVA-infected, bone marrow–derived macrophages *in vitro*. To visualize inflammasome activity, Sagoo *et al.*[7] generated a fusion protein of ASC and GFP in these cells (ASC-GFP), and they analyzed the redistribution of diffuse, cytoplasmic GFP signal to concentrated GFP fluorescence in large perinuclear ASC specks. After this *in vitro* proof of principle, the authors generated mice with myeloid cells expressing ASC-GFP, including the SCS macrophages that MVA infects *in vivo*. Within an hour of footpad MVA infection of the mice, specks appeared in the SCS macrophages present in the draining lymph node. Specks peaked within the next few hours and gradually disappeared over the first 12 h after infection. Thus, for the first time, the authors demonstrate a rapid, spatially restricted surge of inflammasome activation upon viral infection *in vivo*[7].

Why was ASC speck formation confined to the period up to 12 h after infection? Lymph node infection with MVA rapidly depletes SCS macrophages; this clearly precludes sustained inflammasome activation[4]. Sagoo *et al.*[7] thus examined macrophage death via pyroptosis—cell death that is initiated by inflammasome activation and characterized by pore formation, chromatin condensation, cellular swelling and rupture[8]. MVA-infected, bone marrow–derived macrophages exhibited inflammasome-dependent pyroptotic death *in vitro* that was not transmitted to neighboring cells, which suggests that direct infection is required for the macrophage death observed *in vivo*. To characterize macrophage death *in vivo*, the authors injected a cell-permeant DNA dye to label lymph node cells, and they concurrently imaged nuclear condensation (representing pyroptosis) and ASC speck formation in SCS macrophages. Indeed, macrophages with activated inflammasomes progressed rapidly to cell death through pyroptosis.

Bursting pyroptotic cells release their cytosolic contents, including oligomerized ASC, which the authors found remained visible for hours *in vivo*. These extracellular ASC specks are known to be catalytically active and capable of propagating inflammation upon reaching the cytoplasm of phagocytosing innate immune cells[9]. Sagoo *et al.*[7] found that innate immune effector cells in MVA-infected nodes recognized specks within minutes of the specks' release, clustering in speck-rich areas of the node and even phagocytosing the specks. This led to a rapid, inflammasome-dependent burst of chemokines and the recruitment of neutrophils, monocytes and natural killer cells to the inflamed node. SCS macrophage inflammasome activation also

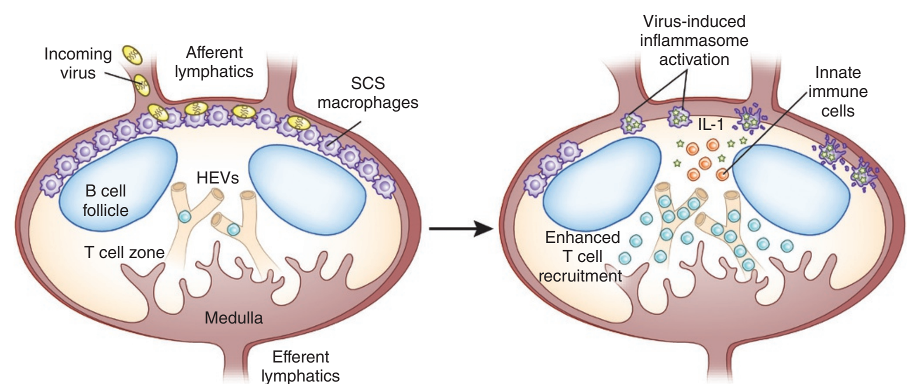

Figure 1 Inflammasome activation in SCS macrophages enhances innate and adaptive immunity. SCS macrophages trap incoming virions and become infected. Sagoo *et al.*[7] develop a method for visualizing inflammasome activation in mice by tagging the inflammasome protein ASC with GFP and analyzing the generation and release of ASC specks (as shown by stars). Macrophages with activated inflammasomes undergo pyroptosis, which liberates specks and mobilizes innate immune effectors (orange). Inflammatory cytokine (such as IL-1) and chemokine production further augments immunity, and increased numbers of $CD8^+$ T cells (teal) are recruited into the lymph node through the high endothelial venules (HEVs).

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dramatically enhanced the adaptive immune response, with higher recruitment of naïve MVA-specific CD8$^+$ T cells to the lymph, leading to greater numbers of virus-specific effectors and a broadened T cell repertoire.

Although other groups have reported the importance of the inflammasome in SCS macrophages after viral infection[3,9], the visualization by Sagoo *et al.*[7] of inflammasome activation *in vivo* as a spatially defined wave extends our understanding of SCS macrophages in viral infections. SCS macrophages have been dubbed immune 'flypaper' on the basis of their efficient acquisition of lymph-borne particulates[1]. The programmed pyroptosis of macrophages to disseminate ASC specks, however, makes them more similar to a network of land mines positioned at the entrance of lymph nodes, exploding upon infection rather than acting as a simple filter for incoming pathogens. It will be important to determine whether this occurs in other cells that also become infected, such as dendritic cells (DCs)[10]. As DCs (and not macrophages) prime T cell responses in the node, DC death could be costly to the host, although it may be necessary to prevent intracellular pathogen replication. It will be of great interest to examine both the contribution of inflammasome-driven pyroptosis to the control of viruses that replicate and spread in lymph nodes, and the consequences of programmed macrophage deletion.

The inflammatory amplification of adaptive immunity also raises possibilities for rational vaccine design. Strategies to deliberately trigger nodal macrophage inflammasomes could be used as an adjuvant for many types of vaccines, including simple protein-based vaccines. As a testament to the importance of inflammasome activation for controlling virus infection, MVA (and other large DNA viruses) encode proteins that block inflammasome activation. Indeed, the genomic deletion of just one of these immunomodulatory proteins dramatically reduces virulence in replicating poxviruses, and it enhances T cell responses in an MVA-based vaccine candidate[11,12]. Shedding light on inflammasomes *in vivo* could further enhance our ability to manipulate activation at appropriate times during the immune response.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

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Depleting senescent cells to combat aging

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A new study in mice suggests that pharmacologically targeting the apoptosis proteins BCL-2 and BCL-xL can clear senescent cells from bone marrow and ameliorate stem cell function during aging, bringing us a step closer to preventing senescence-associated tissue attrition in the clinic.

Senescent cells, now identifiable by a validated set of independent markers, accumulate in tissues during aging or upon tissue damage induced by stimuli such as irradiation[1]. Multiple distinct roles for senescent cells in tissues have been suggested. However, it is now commonly accepted that senescent cells contribute to tissue attrition and to aging-associated initiation of cancer, in part via the factors they secrete, collectively known as the senescence-associated secretory phenotype (SASP). Senescent cells, both *in vivo* and *in vitro*, often express p16(Ink4a), a cyclin-dependent kinase inhibitor that is also known as CDKN2A. Expression of p16(Ink4a) normally renders the growth arrest associated with senescence irreversible[2]. Previous studies have suggested that inducible deletion of cells expressing p16 (Ink4a) at a young age in a genetic model of premature aging delayed the onset of premature-aging initiated diseases in adipose tissue, skeletal muscle and eye tissue[3]. In the same study, late-in-life deletion of cells expressing p16(Ink4a) at least delayed the progression of these diseases. Accordingly, the removal of senescent cells may prevent or delay tissue dysfunction and extend healthspan upon 'normal aging'. However, pharmacological approaches to targeting senescent cells have not been very successful so far, and thus senolytic drugs (small-molecule compounds that selectively remove senescent cells) have only recently been seen as a viable method of delaying senescence. By comparing the transcriptome of senescent and non-senescent cells, two cell type–specific senolytic drugs were recently discovered[4] that improved tissue function in response to cellular senescence induced *in vivo* by ionizing irradiation. Whether the drugs directly acted on senescent cells *in vivo* was not determined. An article in this issue of *Nature Medicine* now demonstrates that pharmacological depletion of senescent cells *in vivo* indeed results in a functional rejuvenation of stem cells in naturally aged mice[5].

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Chang *et al.*[5] first tested a panel of small-molecule compounds known to target pathways that were predicted to be important for the maintenance of senescence *in vitro*. They titrated these compounds *in vitro* into cells after exposing them to ionizing irradiation. This revealed that the small-molecule compound ABT263, which is a specific inhibitor of the apoptosis genes *BCL2* and *BCL2L1* (encoding BCL-2 and BCL-xL, respectively), selectively induced apoptosis of senescent cells in culture in a cell type– and species-independent (mouse as well as human).

The authors then carried out experiments in a mouse model known as p16-3MR-these mice carry a trimodal reporter protein (called 3MR) under control of the p16INK4a (p16) promoter[6], and they can be used to identify, track (via bioluminescence) and selectively kill p16-positive senescent cells *in vivo*[5]. When ATB-263 was given to p16-3MR mice, ABT263 effectively depleted senescent cells in bone marrow, lung and muscle tissue, both in naturally aged animals as well as in those treated with a minimal dose of ionizing irradiation, which induces cellular senescence and

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