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Previews

Mx1-ing it up—Mitochondrial relay for interferon-dependent, unconventional IL-1β release in SLE monocytes

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The role of type I interferon (IFN-I) in systemic lupus erythematosus (SLE) is well documented, but the role of interleukin (IL)-1β remains elusive. In this issue of *Immunity*, Caielli et al. identified an SLE monocyte population coproducing IL-1β and IFN-I and described how mitochondrial nucleic-acid-containing RBCs engage cGAS/STING, RIG-I, MDA5, and NLRP3 for unconventional IL-1β release.

Release of mature interleukin (IL)-1β and IL-18 in response to inflammasome activation are hallmarks of host response to infection, but they also mediate detrimental, sterile inflammation responsible for the pathologies associated with many inflammatory diseases. In contrast, the excessive and pathological activation of the type I interferon (IFN-I) pathway in response to nuclear and mitochondrial self-nucleic acids and the presence of an IFN-stimulated gene (ISG) signature are characteristic of systemic lupus erythematosus (SLE). Chronic IFN-I production in autoimmune pathologies is driven by the release of mitochondrial RNA (mtRNA) and mitochondrial DNA (mtDNA) in response to mitochondrial stress or metabolic reprogramming and activation of retinoic acid-inducible gene I (RIG-I)-like receptor and cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) signaling pathways, respectively. However, mtDNA release also activates the nucleotide-binding domain and leucine-rich repeat-containing (NLR) family pyrin domain-containing 3 (NLRP3) inflammasome and IL-1β production.[1] In another twist, red blood cells (RBCs) of SLE patients uniquely retain mitochondria (Mito$^+$ RBCs) and are potent drivers of IFN-I.[2] Increased expression of the inflammasome components NLRP3 and caspase-1 have been reported in kidney biopsies from lupus nephritis patients, in addition to increased IL-1β production from circulating monocytes in some studies. However, what triggers IL-1β release from SLE monocytes has not been described.

The established release mechanism for mature IL-1β and IL-18 proceeds through pyroptotic and non-pyroptotic Gasdermin D (GSDMD) pores, following proteolytic processing by caspase-1. Increasing evidence also supports the release of the mature cytokines from living cells in the absence of pyroptotic cell death, but the molecular mechanisms are largely elusive.[3] In the current study, Caielli and colleagues identified a mechanism for secretion of mature IL-1β and IL-18 from a subset of SLE monocytes, which required the collaboration of the NLRP3 inflammasome with the IFN-I response induced by mitochondrial nucleic acids triggered by internalized Mito$^+$ RBCs.[4] Importantly, the authors described a non-canonical pathway for IL-1β and IL-18 secretion, which required oligomerization of the ISG IFN-induced guanosine triphosphate (GTP)-binding protein Mx1, which is also known as MxA.

In the present study, Caeilli et al. reported that while classical monocytes from healthy donors expressed neither ISGs nor IL-1β, SLE monocytes with internalized Mito$^+$ RBCs expressed both. This was found to be SLE disease activity index (SLEDAI) dependent and unique to SLE, as monocytes from another interferonopathy, juvenile dermatomyositis, expressed only ISGs but not IL-1β. The authors were able to replicate this response by feeding IgG-opsonized Mito$^+$ RBCs to classical monocytes from healthy donors, which not only upregulated ISG expression but also *IL1B*, coinciding with the release of secreted ISGs and IL-1β. Mitochondrial nucleic acids have an established role in activating cGAS-STING for IFN-I production but also in activating NLRP3.[1] RBCs devoid of mitochondrial nucleic acids failed to produce IFN-I and IL-1β, emphasizing the requirement for mitochondrial nucleic acids to initiate this response. CRISPR-Cas9-based genetic analysis demonstrated the involvement of cGAS-STING in IFN-β production and NLRP3, apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), and caspase-1 (but not absent in melanoma 2 [AIM2]) in the release of IL-1β. Leakage of RBC mitochondrial nucleic acids from the phagolysosome is a necessity for the activation of cytosolic nucleic acid sensors. Indeed, the authors showed the presence of lysosomal-associated membrane protein 1 negative (LAMP1$^-$) mitochondrial nucleic acid$^+$ aggregates by immunofluorescence, suggesting that mitochondrial nucleic acids from RBCs were released into the cytosol. Further imaging and binding analyses demonstrated that RBC mtDNA was bound by cGAS, but surprisingly not by NLRP3, although mtDNA has

been identified earlier as a NLRP3 inflammasome activator.[1] STING-mediated cell death can also activate NLRP3 but was ruled out by the authors. However, mtDNA was released from monocytes that internalized Mito$^+$ RBCs and was detected bound to NLRP3. Nevertheless, mitochondrial nucleic acids from RBCs were required for activating NLRP3, as demonstrated using ethidium bromide to generate RBCs devoid of mitochondrial nucleic acids. Because ethidium bromide removes both mtRNA and mtDNA, the authors tested Mito$^+$ RBCs treated with Actinomycin D to inhibit mtRNA polymerase. mtRNA-deficient Mito$^+$ RBCs failed to elicit IL-1β release. Moreover, mtRNA was present in the cytosol of monocytes after internalization of Mito$^+$ RBCs. Hence, the authors concluded that cytoplasmic release of mtRNA from Mito$^+$ RBCs represented the upstream signal to promote monocyte-derived mtDNA-dependent NLRP3-inflammasome-mediated release of IL-1β. Additional genetic studies identified a redundant requirement of RIG-I and MDA5 in IL-1β release induced by Mito$^+$ RBCs, both of which can recognize cytoplasmic double-stranded RNA (dsRNA) (including mtRNA) upstream of NLRP3 activation.[5] RIG-I and melanoma differentiation-associated gene 5 (MDA5) also contributed to the Mito$^+$ RBC-induced ISG production in parallel to mtDNA-induced activation of cGAS-STING.

Mature IL-1β and IL-18 are leaderless proteins, which are released by unconventional protein secretion, primarily through polymerized GSDMD pores in pyroptotic and living cells. However, the authors did not detect reduced viability of Mito$^+$ RBC-internalized monocytes nor proteolytic cleavage of GSDMD. Not surprisingly, genetic loss of function or pharmacological inhibition of GSDMD had no impact on Mito$^+$ RBC-induced IL-1β release. Using Mx1 as a readout for ISG expression, the authors noticed that Mx1 changed its localization and co-localized with mature IL-1β in intracellular foci. This behavior was unique to Mx1 and was not observed for other ISGs. Furthermore, *MX1* genetic loss of function resulted in significantly reduced IL-1β and IL-18 release, while elevated Mx1 expression enhanced their release, implicating Mx1 in this unconventional cytokine release. Unconventional protein secretion requires translocation of mature IL-1β into organelles or vesicles that ultimately fuse with the plasma membrane for cargo release.[6] Mx1 localized to intracellular membranes and has previously been implicated in the nuclear translocation of viral proteins.[7] The authors detected Mx1 and mature IL-1β in the cytosolic as well as the microsomal fractions, and proteinase K protection assays demonstrated that Mx1 localized to the outside and IL-1β to the lumen of subcellular membranes. Accordingly, genetic deletion of *MX1* resulted in the loss of luminal mature IL-1β. Proteoliposome-based transport assays subsequently showed that Mx1 integrated into membranes and protected mature IL-1β from proteinase K digestion, hence directly implicating Mx1 in the translocation of mature IL-1β across membranes. Importantly, restoration of *MX1*-deleted cells with a mutant form of Mx1 that was unable to form polymers demonstrated the requirement for Mx1 polymerization in IL-1β translocation across membranes. Subcellular fractionation finally identified the *trans*-Golgi network (TGN) as the site of Mx1 integration and mature IL-1β translocation, a site also implicated in NLRP3 activation.[8]

Unconventional protein secretion involves either direct protein penetration across the plasma membrane (type I unconventional secretion), vesicular trafficking (type III unconventional secretion), or GSDMD pores in the plasma membrane.[3,6] GSDMD pores and type III unconventional secretion triggered by transmembrane p24 trafficking protein 10 (TMED10) transmembrane pores at the ER-Golgi intermediate compartment (ERGIC), as well as a slow progressing, potentially type I unconventional secretion at the plasma membrane, have all been described for mature IL-1β release.[3,9,10] GSDMD and TMED10 depend on oligomeric proteins to assemble some sort of pore or channel. Intriguingly, a consensus sequence facilitating interaction of several leaderless proteins with TMED10 has been identified, suggesting that comparable motifs may exist for interaction with Mx1 and potentially other pores/channels involved in type III unconventional secretion that may enable protein translocation at distinct intracellular membrane sites.[10] The data presented here identified Mx1 oligomeric complexes in the TGN as yet another mechanism for type III unconventional secretion of IL-1β and IL-18. Although IFN-I has been shown to inhibit inflammasome activity and release of mature IL-1β through a number of mechanisms, several pathological conditions have now been identified where this counter regulation is compromised. IL-1β$^+$ISG$^+$ monocytes have been detected in the central nervous system of aged mice and in experimental autoimmune encephalomyelitis (EAE), suggesting that this scenario may not be restricted to SLE monocytes. Also, although IL-1β$^+$ISG$^+$ monocytes were detected in pediatric SLE samples and were associated with disease activity, whether this IL-1β and ISG co-expressing monocyte subset represents a pathological subset in adult SLE or contributes to overall disease remains to be established. Additionally, whether activation of RIG-I or MDA5 by other viral triggers can initiate similar pathways, such as in the case of SARS-CoV-2 virus infection, remains to be seen.

Although reciprocal relationships between IL-1β and cGAS-induced ISG expression have previously been shown, the current study reveals that SLE monocytes have developed a special mechanism to utilize internalized unique Mito$^+$ RBCs as the initiating signal in a mitochondrial nucleic acid "relay," involving mtRNA from Mito$^+$ RBCs activating RIG-I and MDA5 to trigger host mtDNA release, cGAS activation, and mature IL-1β translocation into the TGN via IFN-I-inducible Mx1 polymerization to enable its unconventional protein secretion (Figure 1). The lack of mitochondria in RBCs from healthy individuals and their internalization in a quiescent non-inflammatory manner explain why this dual IL-1β$^+$ISG$^+$-expressing population is not observed in the non-inflamed context. Interestingly, RBCs even from healthy individuals are known to scavenge nucleic acids in the circulation, including Toll-like receptor 9 (TLR9)-bound nuclear or mtDNA, suggesting this mechanism of IL-1β release may not be limited to SLE alone. Thus, it will be important to consider this mechanism in different contexts, particularly in situations where clearance of apoptotic or necrotic debris is reduced or defective. These separate and distinct mechanisms to release IL-1β may facilitate the release of this critical cytokine

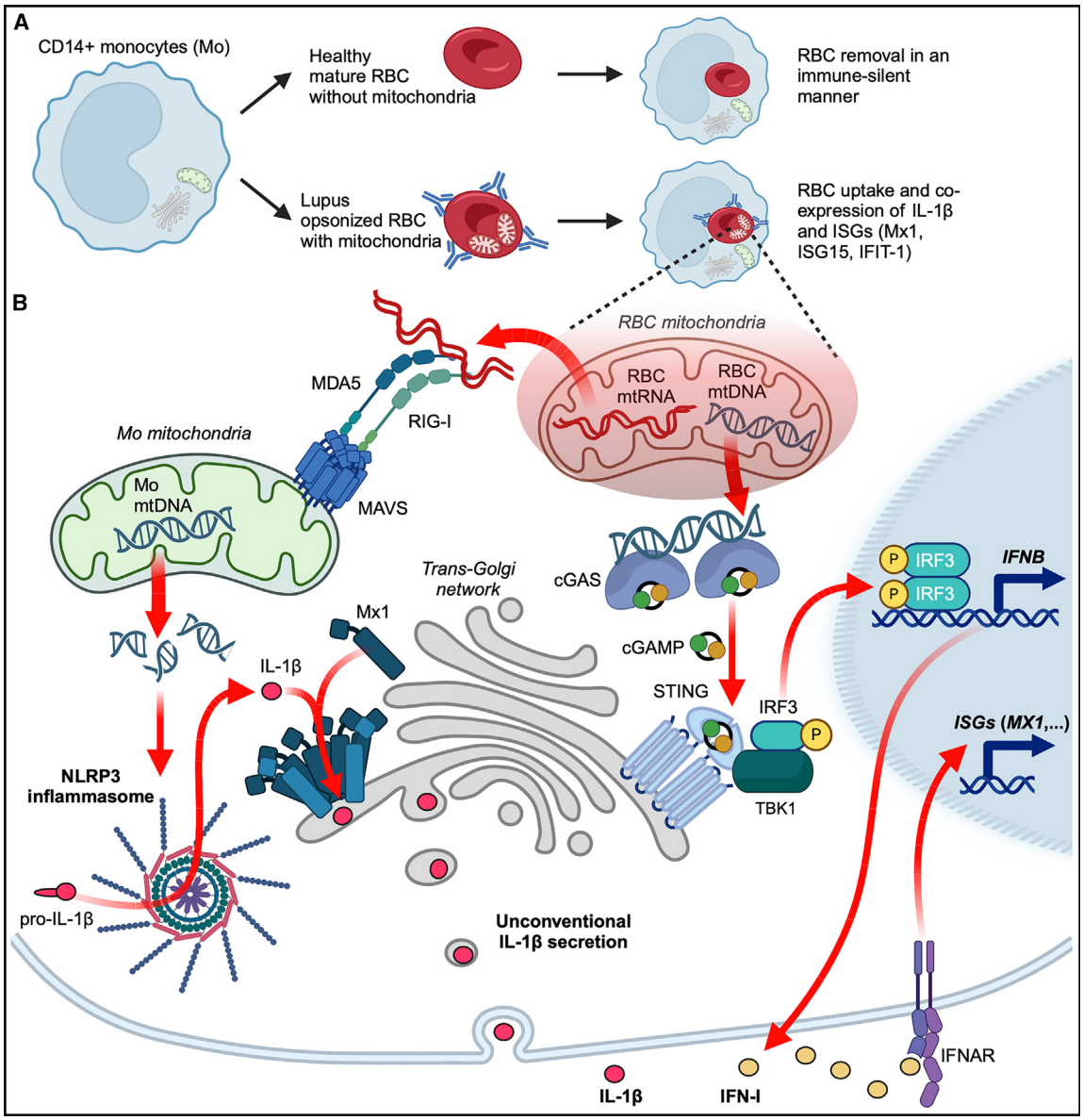

Figure 1. Mito$^+$ RBCs trigger unconventional IL-1β release in a process requiring the ISG Mx1

(A) Red blood cells (RBCs) in the circulation of SLE patients uniquely retain their mitochondria (termed Mito$^+$ RBCs), including mitochondrial RNA (mtRNA) and mitochondrial DNA (mtDNA). Once opsonized, they are internalized by CD14$^+$ classical monocytes to drive IL-1β and ISG expression, whereas healthy individuals remove RBCs from the circulation in an immunologically silent manner.

(B) mtDNA from internalized Mito$^+$ RBCs can activate cGAS-STING to induce IFN-I expression and ISG upregulation (including *mx1*), whereas mtRNA from Mito$^+$ RBCs activates MDA5 or RIG-I and triggers release of mtDNA fragments from host monocytes to trigger NLRP3 activation and IL-1β processing. Mx1 oligomerization at the *trans*-Golgi network (TGN) facilitates IL-1β transport across the membrane into the lumen and subsequent release. Created in BioRender. Dorfleutner, A. (2024) BioRender.com/p13p702.

to promote inflammatory responses under various conditions and in distinct cell types.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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Home at last: Mixed signals guide memory T cells to residency

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Tissue-resident memory T (T_{RM}) cells adapt to diverse environments, providing local long-term protection. In this issue of *Immunity*, Obers et al. and Raynor et al. demonstrate how diet, commensals, and host factors determine T_{RM} cell development, maintenance, and function across tissues.

Tissue-resident memory T (T_{RM}) cells establish long-term regional immunity across organs. Strategically positioned, they are essential for mounting rapid responses to secondary infections and malignancies, making them crucial components of tissue immunity.[1] However, the precise combination of signals that govern T_{RM} cell differentiation, maintenance, and function and how these signals vary between tissues—despite a shared core residency program[1]—remain incompletely understood. In this issue of *Immunity,* Obers et al.[2] and Raynor et al.[3] identify nutrient and cytokine signaling pathways that differentially regulate T_{RM} cells across tissues.

Using a lentiviral CRISPR library targeting mitochondrial and lysosomal genes, Raynor et al. identified negative regulators of small intestine intraepithelial lymphocyte (siIEL) T_{RM} cells: the Flcn, Ragulator, and Rag GTPase complexes. To accomplish this, they transduced $CD8^+$ ovalbumin (OVA)-specific OT-I T cells expressing Cas9 with the aforementioned guide RNA library and transferred the transduced cells into wild-type (WT) mice. The mice were then infected with *Listeria monocytogenes* expressing OVA (LM-OVA), and OT-I cells were analyzed at the effector (7.5 days post-infection, d.p.i.) and memory (30 d.p.i.) phases. Deletion of Flcn, Ragulator, and Rag GTPase complexes resulted in increased siIEL OT-I cells, including those expressing CD69 and CD103, two markers of tissue retention.[1] Flcn deletion also decreased OT-I cells in the spleen, liver, and lung. Consistent with these observations, the authors detected increased Flcn-suppressed signatures and decreased Flcn-activated signatures in intestinal T_{RM} cells versus their counterparts in other tissues in both mice and humans. These findings not only reveal Flcn, Ragulator, and Rag GTPases as suppressors of siIEL T_{RM} cells but also highlight a discrete mechanism (e.g., Flcn dependency) differentially regulating T_{RM} cells across tissues.

Flcn, Ragulator, and Rag GTPases all play important roles in lysosomal nutrient sensing and amino acid-induced intracellular signaling.[4] Raynor et al. found that *in vivo* deficiencies in Flcn, Ragulator, or Rag GTPases, as well as *in vitro* amino acid deprivation, resulted in increased activity of nutrient stress-responsive genes, such as the transcription factor Tfeb, corresponding with increased expression of its downstream lysosomal protein-encoding gene LAMP-1. When activated $CD8^+$ T cells were cultured in arginine-free or glutamine-free media, the proportion of $CD69^+$ $CD103^+$ $CD8^+$ T cells increased, and arginine starvation enriched both core T_{RM} cell and siIEL T_{RM} cell gene signatures. Similarly, low-protein diet increased $CD69^+$ $CD103^+$ OT-I T_{RM} cells in the siIEL while reducing their numbers in the salivary gland, liver, and spleen, with little impact in the lung. Notably,