including TNF, by macrophages[12]. To address whether ChAT^{+} B cells modulate HSC activity and thus limit cardiac damage in the setting of myocardial infarction, the authors used experimental models of acute myocardial infarction and atherosclerosis. Interestingly, the BM of myocardial infarction model mice showed a rapid increase in acetylcholine levels. This increase tied back to acetylcholine production by B cells, as B-cell-specific *Chat*^{cKO} mice had a more severe myocardial infarction phenotype, characterized by increased fibrosis, collagen accumulation and ventricular remodeling and reduced survival, compared to wild-type controls. Similarly, the authors also observed increased myelopoiesis in *Chat*^{cKO} mice after induction of atherosclerosis, with a concordant exacerbation of lesion size and plaque formation. Thus, these data demonstrate an active role for B cell production of acetylcholine as a mechanism that limits 'emergency' hematopoiesis. Going forward, it will be important to identify the physiological trigger(s) that activate B cell acetylcholine production in this setting.

Together, these findings identify a previously unknown circuit through which acetylcholine regulates HSC activity and responses to inflammatory stress. These data complement results from previous studies implicating acetylcholine as a regulator of HSC homing and proliferation[4] and showing that acetylcholine signaling maintains HSC quiescence and self-renewal during blood system regeneration following chemotherapy[2]. Chrna7 and downstream expression of Cxcl12 by MSCs appear to be key components of this regulatory circuitry[2,4]. Here, Schloss et al.[5] show that B cells are an important source of acetylcholine in the BM, thereby underscoring the emerging importance of lymphoid cells as regulators of hematopoiesis. Furthermore, this study supports evolving models that define the HSC niche as the result of combinatorial input of many cell types in the BM, including mature immune cells. Notably, B lymphopoiesis is compromised by chronic inflammatory conditions, including autoimmune disease and aging, and the numbers of B cells in the BM can decline in response to disease- and aging-associated pro-inflammatory cytokines, such as interleukin-1. These settings are defined by aberrant hematopoietic phenotypes reminiscent of reduced cholinergic activity, including myeloid lineage bias and expansion of phenotypic HSCs. The extent to which these changes are related to altered abundance and/or function of acetylcholine-producing B cells remains an open area of investigation with significant clinical implications. Of note, the authors underscore the importance of B-cell-derived acetylcholine in limiting cardiovascular damage in the settings of myocardial infarction and atherosclerosis. Interestingly, aging-associated premalignant blood phenotypes, such as clonal hematopoiesis of indeterminate potential, are associated with myeloid bias, aberrant inflammation and increased risk of cardiovascular disease, including myocardial and atherosclerosis. Understanding the interplay between the hematopoietic system and aging-related changes to the cholinergic circuitry can provide needed insights related to the management of clonal hematopoiesis of indeterminate potential and its associated comorbidities. Hence, the work from Schloss et al.[5] identifies a novel regulatory circuit that maintains HSC function and could represent an important target in the clinical management of numerous inflammatory conditions. ❐

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Competing interests

The authors declare no competing interests.

Check for updates

COVID-19

STINGing type I IFN-mediated immunopathology in COVID-19

The molecular basis for type I interferon (IFN)-mediated immunopathology is unclear. New data now identify the cGAS–STING pathway as a major driver of pathological type I IFN responses in COVID-19.

Evangelos Andreakos

The recently emerged severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus causing coronavirus disease 2019 (COVID-19) has resulted in unprecedented rates of pneumonia, acute respiratory distress syndrome and death, and an unprecedented spectrum of disease manifestations in various organs and tissues beyond the lung. Central to disease vulnerability is the type I IFN system, which is pivotal for antiviral immunity but can also drive excessive inflammation and immunopathology. Now, two studies by Domizio et al.[1] and Neufeldt et al.[2] suggest that the cGAS–STING pathway is crucially involved in mediating detrimental type I IFN responses in COVID-19.

Defective type I IFN responses have been shown to characterize severe or critical COVID-19 cases[3–5] in contrast to other respiratory infections such as flu[5]. This

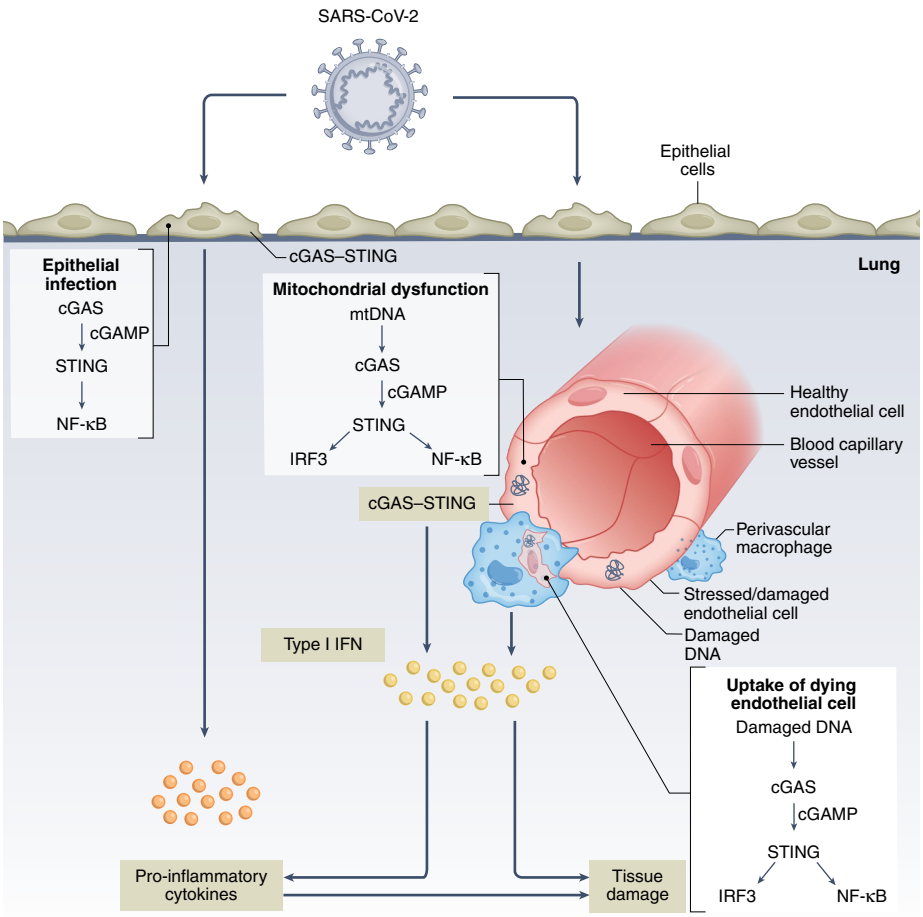

Fig. 1 | Mechanism by which cGAS–STING drives type I IFN-mediated immunopathology in the lung. SARS-CoV-2 infection of epithelial cells induces the activation of cGAS–STING and triggers NF-κB-dependent pro-inflammatory responses. At the same time, SARS-CoV-2 infection of epithelial cells disrupts mitochondrial homeostasis of nearby vascular endothelial cells, causing accumulation of mtDNA, activation of the cGAS–STING pathway and production of type I IFNs. Dying endothelial cells are then taken up by perivascular macrophages, where cGAS recognizes their damaged DNA, leading to the stimulation of STING, IRF3 and NF-κB, and the induction of type I IFNs and pro-inflammatory cytokines. This promotes hyperinflammation and tissue damage.

vulnerability is partly due to genetic 'inborn errors of immunity' or loss-of-function mutations in genes involved in viral RNA sensing and IFN induction or responses including *TLR3*, *TLR7*, *MYD88*, *TBK1*, *IRF7* and *IFNAR1*, and autoantibodies to type I IFNs, most prominently to IFNα2 and IFNω, which increase with age and appear to account for up to 20% of all COVID-19 deaths[6]. Although type I IFNs are essential for antiviral protection against SARS-CoV-2 infection, there is also a paradox. Some patients with critical COVID-19 exhibit high levels of type I IFN, at least at later stages of the disease process. In addition, clinical administration of type I IFNs has been only moderately effective (if at all) in the treatment of COVID-19. In large, randomized trials of hospitalized patients with COVID-19, IFNβ1a has not shown any clinical benefit when compared with the use of remdesivir alone or in patients receiving corticosteroids[7,8]. Instead, potentially harmful effects of type I IFNs were revealed, especially when administered late, in patients with severe disease that were on high-flow oxygen, noninvasive ventilation or mechanical ventilation[7,8]. Other clinical studies have also reported worsened disease and increased mortality after the late administration of type I IFN, highlighting the dual role of type I IFNs in both host protection and immunopathology.

It is well known that type I IFNs can cause immunopathology. In healthy individuals or individuals with chronic conditions, the addition of type I IFN induces flu-like disease and fever, whereas in chronic viral infections and some autoimmune or autoinflammatory diseases, type I IFNs are key drivers of inflammation and tissue damage[9]. Moreover, in acute infections, early administration of type I IFNs in experimental animals induces protective antiviral responses, whereas late administration of IFNs enhances pro-inflammatory cytokine responses and host tissue damage[10,11]. This response is consistent with the idea that type I IFNs constitute a second line of antiviral defense in the respiratory tract, after type III IFNs, which comes into play to enhance antiviral immunity at the expense, however, of collateral damage[11]. More recently, the possibility that type I IFNs inhibit epithelial repair has also been suggested[12]. The production of type I IFNs therefore has to be tightly regulated, and any factors that can shift this balance can lead to aberrant inflammation with devastating consequences for health. However, the underlying mechanisms that can increase or sustain IFN expression are unknown.

The two new studies implicate the cyclic GMP-AMP synthase (cGAS)–stimulator of interferon genes (STING) pathway for this process[1,2]. cGAS is a cytoplasmic DNA receptor that controls immunity to cytosolic DNA by catalyzing the synthesis of an unusual second messenger molecule, the cyclic dinucleotide cGAMP, which binds to and activates STING. STING, in turn, drives the gene expression of type I IFNs and pro-inflammatory cytokines through the induction of the transcription factors interferon regulatory factor 3 (IRF3) and nuclear factor-κB (NF-κB). Domizio et al.[1] now show in *Nature* that a cGAS–STING–IFN signature is prominent in severely damaged lungs of patients with COVID-19. Using autopsy specimens, they found that phosphorylated STING, a marker of STING activation, and increased expression of IFN-stimulated genes (ISGs) characterize patients with histological hallmarks of acute respiratory distress syndrome, such as early diffuse alveolar damage and extensive formation of hyaline membranes, and is linked to a rapidly lethal disease course. This finding extends beyond the lung, as skin lesions that develop in patients with moderate-to-severe COVID-19 also exhibit phosphorylated STING, and high levels of ISGs and pro-inflammatory cytokines (such as tumor necrosis factor and the interleukins IL-1 and IL-6) compared with healthy controls. In both tissues, phosphorylated STING is found in perivascular macrophages and endothelial

cells. Moreover, Neufeldt et al.[2] report in *Communications Biology* that SARS-CoV-2 infection of epithelial cell lines, such as Calu-3 and A549-ACE2, also triggers the cGAS–STING pathway, driving the production of pro-inflammatory cytokines in an NF-κB-dependent manner. Interestingly, in this case, STING activation is non-canonical, as the transcription factor IRF3 is not activated and type I IFNs are not induced, which suggests that STING may shift the balance toward an aberrant pro-inflammatory response[2].

Although these two studies come from a different angle, they also complement each other. Domizio et al.[1] found that in a lung-on-chip model, which mimics the alveolar–capillary interface and enables the study of epithelial–endothelial cell interactions, SARS-CoV-2 infection does not trigger the production of type I IFN in alveolar epithelial cells, consistent with the findings by Neufeldt et al.[2]. By contrast, adjacent endothelial cells and macrophages in this model exhibit high levels of IFNβ. This expression is due to direct engagement of cGAS–STING as endothelial cells contained perinuclear foci of phosphorylated STING after infection, and type I IFN induction was independent of RNA recognition[1].

As to the mechanism involved, Domizio et al.[1] made an important discovery. They found that in skin lesions from patients with COVID-19, endothelial cells showed characteristics of damage including loss of endothelial cell integrity, disruption of mitochondrial cristae, release of mitochondrial DNA (mtDNA) into the cytosol, and nuclear accumulation of cleaved caspase-3, which is indicative of cell death. This was also observed in endothelial cells from the lung-on-chip model after SARS-CoV-2 infection, in which damaged mitochondria and enrichment of mitochondrial proteins in the cytosol were detected. This mitochondrial damage triggered the activation of the cGAS–STING pathway, as depletion of mtDNA could substantially reduce the production of type I IFNs. Notably, dying endothelial cells together with intracellular DNA foci and cleaved caspase-3 fragments were also seen in IFNβ-producing macrophages, which suggests a common mechanism triggers the cGAS–STING pathway in both endothelial cells and macrophages in COVID-19 lesions. This finding is in agreement with a previous report that SARS-CoV-2 causes mitochondrial dysfunction in cells[13]. It is also in line with a previous study demonstrating that gain-of-function mutations of *STING1* in humans trigger type I IFN and pro-inflammatory cytokine responses, which cause cutaneous vasculopathy and pulmonary inflammation[14].

Therefore, the following picture emerges (Fig. 1): SARS-CoV-2 infection of respiratory epithelial cells disrupts mitochondrial homeostasis in nearby vascular endothelial cells, resulting in the accumulation of mtDNA, activation of the cGAS–STING pathway and production of type I IFNs, while endothelial cells eventually die. This initial damage triggers activation of perivascular macrophages through the engulfment of dying endothelial cells and the recognition of their damaged DNA by cGAS, leading to the induction of type I IFNs and pro-inflammatory cytokines, which mediate immunopathology. cGAS–STING-dependent NF-κB-driven pro-inflammatory responses from SARS-CoV-2-infected respiratory epithelial cells further contribute to this process, supporting the targeting of the cGAS–STING pathway for the treatment of severe COVID-19. Indeed, in K18-hACE2 transgenic mice, which are highly susceptible to SARS-CoV-2 infection and develop severe COVID-19-like disease, daily administration of a selective STING inhibitor before or early after SARS-CoV-2 infection led to a significant reduction in cell death, inflammatory cell infiltration, production of pro-inflammatory cytokines, NF-κB activity and type I IFN signaling in the lung, without affecting viral replication[1]. This STING intervention prevented weight loss and improved the survival of experimental mice, confirming the central role that the cGAS–STING pathway has in disease severity. Interestingly, the STING inhibitor suppressed inflammation only at its later stages but not earlier on, which suggests a crucial function for STING in eliciting type I IFN and inflammatory responses in the late phase of infection, which also coincides with excessive tissue damage.

The implications of these findings in terms of therapy are major, as for the first time there is a molecular basis for discriminating early beneficial from late detrimental type I IFN responses in the lung, at least for an RNA virus. Beneficial responses require viral RNA recognition through TLR3, TLR7 and RIG-I-like receptors, whereas detrimental responses are induced by the recognition of damaged DNA and activation of the cGAS–STING pathway. Still, these findings will need to be replicated in a broader context in patients with COVID-19 and at different stages of the disease process. Moreover, important questions remain as to where cGAS–STING activation takes place across the respiratory tract, what triggers mitochondrial damage and endothelial cell death and whether that affects the gas-exchange function and ultimately leads to hypoxemic pneumonia and acute respiratory distress syndrome. It is also unclear whether this process applies to severe or critically ill patients from other respiratory infections. Nevertheless, these studies constitute an important conceptual advance towards our current understanding of the innate immune mechanisms that underlie immunopathology of COVID-19, opening avenues for the development of new therapeutic approaches that deserve urgent attention. ❐

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Competing interests

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