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ILC1: guardians of the oral mucosa against enemy viruses

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Type 1 innate lymphoid cells (ILC1s) regulate inflammation in the tissues; however, their role in anti-viral immunity remains largely unknown. In this issue of *Immunity*, Shannon et al. report that ILC1s invoke an anti-viral effect by producing interferon (IFN)γ at homeostasis, thereby limiting viral replication in the oral mucosa.

Several viruses, including poxviruses, papillomaviruses, herpesviruses, and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; Huang et al., 2020), invade via and replicate in the oral epithelium. Thus, it is of medical importance to better understand tissue surveillance within the oral mucosa. However, little is known about immunological tissue surveillance against viral infections in the oral mucosa, partly because of limited mouse models of oral mucosal viral infections. In this issue of *Immunity*, Shannon et al. (2020) unveiled the spatiotemporal role of type 1 innate lymphoid cells (ILC1s) in the defense against viral infection in the oral mucosa. They have demonstrated that ILC1s are located closely at the basement membrane in the epithelia and slowly move through the mucosal epithelium at steady state. They have also demonstrated that not only do ILC1s produce interferon-γ (IFNγ) to generate an anti-viral state in the oral mucosa, but they also do so prior to a viral infection at homeostasis, thereby limiting its replication after the infection.

ILC1s and natural killer (NK) cells are categorized into group 1 ILCs with the potent ability to produce IFNγ (Spits et al., 2016). ILC1s not only are involved in the regulation of tissue inflammation, pathogenesis, homeostasis, metabolism, and protection, but also host protection against pathogens in their resident environment (Nabekura and Shibuya, 2020). ILC1s are predominantly tissue resident and have no or little cytotoxic activity, whereas NK cells are circulating and cytotoxic innate lymphocytes. However, these group 1 ILC subsets share many biological features other than IFNγ production, such as the requirement of interleukin (IL)-15 for development, dependence on the transcription factor T-bet for functional maturation, overlapping transcriptomic profiles, and expression patterns of surface molecules, including cytokine receptors and NK receptors (Robinette et al., 2015; Seillet et al., 2016; Spits et al., 2016). Despite extensive literature on NK-cell-mediated control of viral infections, as well as on the significance of IFNγ in anti-viral immunity (Novelli and Casanova, 2004; Vivier et al., 2018), only a handful of studies have addressed the role of ILC1s in anti-viral immune responses. One previous study has demonstrated that ILC1s promptly

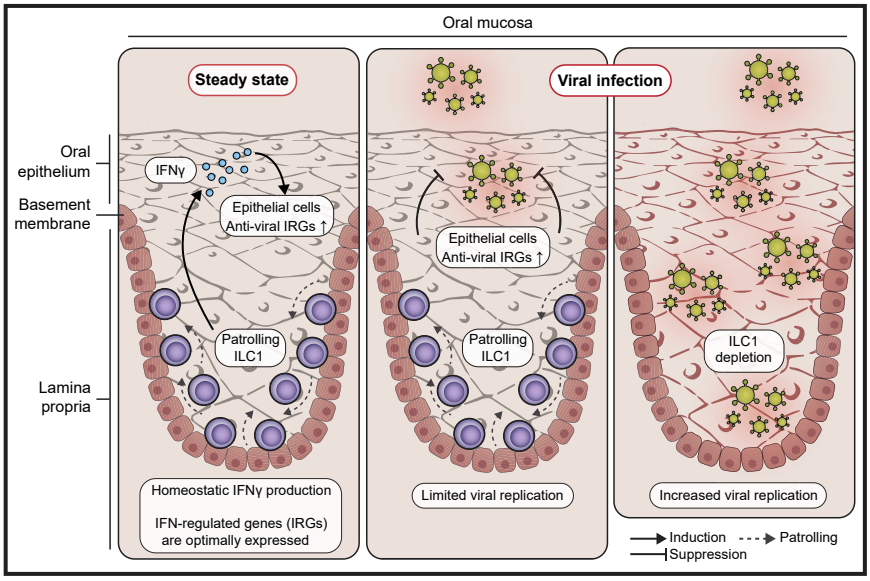

Figure 1. ILC1s maintain a homeostatic anti-viral state in the oral mucosa

ILC1s locate within the basal layers of the oral mucosal epithelium contacting the basement membrane and slowly move through the mucosal epithelium by contacting and squeezing between mucosal epithelial cells at steady state. They produce IFNγ during homeostasis, resulting in the establishment of an anti-viral state of the mucosal epithelium via anti-viral IFNγ-regulated genes (IRGs), such as *Irf3*, *Irf7*, *Mx1*, and *Ifnb1* (left). Upon invasion by a virus, IRGs suppress the replication of virus (middle). Figure illustrated by Mariana Silva Almeida.

produce IFNγ prior to the initiation of NK-cell-mediated anti-viral immune responses in the liver during mouse cytomegalovirus (MCMV) infection, and the rapid IFNγ production by liver ILC1s is essential in limiting MCMV replication during the early course of the viral infection (Weizman et al., 2017). However, previous studies have not clarified the spatiotemporal role of ILC1 prior to viral infection at steady state in their resident tissues, including liver.

Shannon et al. (2020) developed a mouse model of infection with poxvirus vaccinia virus (VACV) in the oral mucosa; they investigated the kinetics of VACV titers in genetically modified mice that lacked particular immune cell subsets and in mice depleted of specific types of immune cells. *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice (lacking all ILC subsets) and NK1.1-treated mice (depleted of NK cells and ILC1s) exhibited high viral burdens, suggesting that either NK cells or ILC1s, or both, are critical for anti-viral immune responses against VACV in the oral mucosa. Furthermore, pretreatment with a neutralizing antibody against IFNγ increased viral replication, which is consistent with the importance of IFNγ in anti-viral immunity (Novelli and Casanova, 2004). Intriguingly, the preferential depletion of NK cells by administration of anti-asialo GM1 antibody prior to the infection did not affect the viral titers; however, partial reduction in the number of ILC1s by intravenous injection of β-nicotinamide adenine dinucleotide hydrate (NAD$^+$), as previously described for the depletion of tissue-resident memory CD8$^+$ T cells, prior to infection significantly increased viral titers. Together, these results support the view that oral mucosa-resident ILC1s, rather than NK cells, produce IFNγ prior to viral infection while serving to restrict viral replication upon infection.

The spatiotemporal location of ILC1s in their resident environment was further elucidated. Using sophisticated intravital microscopy, the authors addressed the spatial locations and movement of ILC1s in the oral mucosa. At steady state (prior to infection), only ILC1s were abundantly present in the oral mucosal tissue, whereas few NK cells were found to reside in the uninfected tissue. By utilizing high-end microscopies and T-bet reporter mice to visualize oral-mucosa-resident ILC1s, the authors revealed that in uninfected tissues, ILC1s were located within the basal layers of the oral mucosal epithelium in contact with the basement membrane, particularly in the downward undulations of rete ridges. Intraviral multiphoton microscopy revealed that these ILC1s slowly moved through the mucosal epithelium by contacting and squeezing between mucosal epithelial cells at steady state. Even after VACV infection, ILC1s primarily remained distributed close to the basement membrane in the mucosal epithelium and did not actively infiltrate surrounding epithelial cells infected with virus; this indicates that oral-mucosal-resident ILC1s distally control viral infection through IFNγ production rather than via direct cell-cell contact required for the death of virus-infected cells, as occurs with NK cells.

The authors also revealed that NK cells rarely infiltrate the oral mucosa by 9 h post infection. Unexpectedly, both depletion of ILC1s and neutralization of IFNγ at 9 h post infection had little impact on the viral burden. By contrast, depletion of ILC1s and neutralization of IFNγ "before" VACV infection yielded a striking increase in viral titers, indicating that the ILC1-mediated anti-viral effect can be attributed to their IFNγ production at steady state or very early during VACV infection. These results support a scenario in which during homeostasis prior to infection and or in the very narrow window of the early course of viral infection, ILC1-derived IFNγ contributes to the restriction of viral replication. Consistent with this scenario and a previous finding that ILC1s constitutively transcribe *Ifng* mRNA (Weizman et al., 2017), the authors found that ILC1s homeostatically produce IFNγ in the uninfected oral mucosa, as demonstrated by flow cytometry and histological analysis. The authors investigated the mechanisms by which expression patterns of IFN-regulated genes (IRGs) promote an anti-viral state in the tissue. After VACV infection, the oral mucosal tissues in *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice showed impaired upregulation of IRGs, such as *Cxcl9*, *Cxcl10*, *Ifnb1*, *Stat1*, *Isg15*, *Irf3*, *Irf7*, and *Mx1* compared with those in *Rag1*$^{-/-}$ mice, suggesting that ILC1-derived IFNγ is required for the optimal upregulation of IRGs and the resultant anti-viral state. Along with the requirement of ILC1s for the upregulation of IRGs after VACV infection, the uninfected oral mucosal tissue in *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice also showed lower

expression of these IRGs than those in *Rag1*$^{-/-}$ mice. More importantly, neutralization of IFNγ in uninfected mice abolished the majority of these anti-viral gene signatures in the oral mucosa. Furthermore, although IRF7, an anti-viral signaling transcription factor that is directly activated by IFNγ, was expressed throughout the uninfected mucosal epithelium prior to viral infection, it was most concentrated in the areas that were in close proximity to ILC1s. As motile ILC1s move through the oral mucosal epithelial cells and homeostatically produce IFNγ in steady state, it is most likely that ILC1s facilitate the expression of IRGs throughout the epithelium by producing IFNγ. Collectively, these findings indicate that oral mucosa-resident ILC1s function prospectively to establish an anti-viral state through IFNγ production prior to viral infection, thereby building a firewall against future viral replication and dissemination (Figure 1).

The authors' findings raise three intriguing questions that remain to be explored. First, what are the stimuli for IFNγ production by these ILC1s in the oral mucosa before and during VACV infection? There are several possibilities: IL-12 from dendritic cells, damage-associated molecular patterns released from damaged oral mucosal epithelial cells, pathogen-associated molecular patterns encoded by VACV, or some combination of these could potentially provide that stimulus. Second, do ILC1s have the ability to establish an anti-viral state in other mucosal tissues, especially in humans—e.g., in the genital mucosa in HIV infection? Third, does homeostatic IFNγ production by ILC1s regulate basal levels of inflammation and the activity of other types of immune cells, such as tissue-resident macrophage subsets, in various other barrier tissues, not just mucosal tissues?

This study is reminiscent of another study reporting an impaired IFNγ production by deficient plasticity from group 2 ILCs to ILC1s in patients with Mendelian susceptibility to mycobacterial disease (MSMD) (Lim et al., 2016). MSMD patients develop opportunistic infections with mycobacterium, such as Bacillus Calmette-Guerin or environmental mycobacteria. In summary, ILC1-derived IFNγ might potently contribute to host protection against a broad range of pathogens, including viruses and intracellular bacteria. Although further studies are needed to test these possibilities, the findings of Shannon et al. shed new light on the unique role of ILC1s as tissue guardians against enemy invading pathogens.

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