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RESEARCH HIGHLIGHT

Mucosal epithelial cells: the initial sentinels and responders controlling and regulating immune responses to viral infections

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In the context of host defense, immune protection is mostly provided by hematopoietic immune cells, including neutrophils, macrophages, T cells, B cells, etc. However, in recent decades, emerging evidence has identified many additional diverse roles of nonhematopoietic cells in mammalian immunity, although most of these studied focused on a single-cell type. In July 2020, Krausgruber et al. in the laboratory of Christoph Bock published the paper "Structural cells are key regulators of organ-specific immune responses" in *Nature*. The authors used multiomics and systematically investigated the regulation of immune-associated genes in three major types of structural cells (epithelial cells, endothelial cells, and fibroblasts) in 12 different organs in mice.[1] The results revealed widespread activities and the regulation of immune-associated genes in these structural cells. Structural cells exhibit organ-specific patterns of immune activity and seem to modulate many interactions with hematopoietic immune cells. Moreover, epigenetically encoded immune programs in structural cells have been identified, which can be triggered in response to lymphocytic choriomeningitis virus (LCMV) infection and cytokine stimulation. Their study highlights an underappreciated component of the immune system, which breaks new ground and sets up a milestone in a new field of structural immunity. Some other nonhematopoietic cells, such as hepatocytes, have also been reported to modulate hematopoietic immune cells.[2] Hence, it is expected that nonhematopoietic cells can also sense infection and other stimuli to extensively modulate hematopoietic immune cells. The study by Krausgruber et al. and related findings open a new area of immunology research, which may help us obtain additional insight into immune responses beyond those of hematopoietic immune cells and will provide novel targets for developing innovative therapies for diseases (Fig. 1).

Among the three structural cell types (epithelial cells, endothelial cells, and fibroblasts), epithelial cells constitute the external and internal surfaces of the body, creating physical barriers between our body and the external environment. Notably, mucosal epithelia are large internal mucosal interfaces in the respiratory, gastrointestinal, and genital tracts that endow the mucosa with vital physiological functions. The study by Krausgruber et al.[1] revealed that mucosal epithelia within specific organs such as the lung or intestine mediate epithelial-specific interactions with hematopoietic immune cells. In the context of infection, mucosal interfaces are often entry portals for many pathogens, especially viruses. Mucosal epithelial cells are usually the initial target cells for viral replication and the establishment of viral infections. Frequent episodes of enteric and respiratory infections can cause severe enteropathy and respiratory diseases, such as the current COVID-19 pandemic caused by SARS-CoV-2 infection. The SARS-CoV-2 entry receptor ACE2 and viral entry-associated protease TMPRSS2 are highly expressed in nasal goblet and ciliated epithelial cells. Thus, SARS-CoV-2 can efficiently infect upper respiratory epithelial cells and then spread to the submucosa and low respiratory tract.[3]

Mammalian innate immune responses against invading microbes are usually stimulated by a wide variety of pathogen-associated molecule patterns (PAMPs) through interactions with germline-encoded pattern recognition receptors (PRRs), including the Toll-like receptor (TLR) family, NOD-like receptor (NLR) family, and AIM2-like receptors (ALRs). The recognition of PAMPs by TLRs is a priming signal (signal 1) that activates the NF-κB pathway to produce proinflammatory cytokines as the first tier of immune defense. The recognition of PAMPs by cytosolic NLRs or ALRs is an activating signal (signal 2) that usually cooperates with TLRs to respond appropriately to pathogenic triggers (PAMPs) or metabolic and genotoxic stresses and induces the inflammasome pathway as the second tier of immune defense. This cascade mechanism has been implicated in innate immune recognition and signaling, immune response and regulation, and immune pathology. Similar to immune cells, mucosal epithelial cells also express a combination of PRRs, which can recognize viral PAMPs and activate the NF-κB pathway to produce proinflammatory cytokines and type I and III interferons for antiviral defense (Fig. 1A). A single-cell analysis of the small intestinal epithelium revealed that Salmonella-induced genes in all infected intestinal epithelial cells were enriched in pathways involved in the defense response to bacteria, including many genes that protect against Salmonella infection.[4] Most recently, a study published in PNAS demonstrated that controlling the intestinal parasite Cryptosporidium relies on epithelial cell-intrinsic NLRP6/caspase-1-mediated inflammasome activation.[5] A study demonstrated the role of an epithelium-intrinsic inflammasome that drives the expulsion of Salmonella-infected cells to restrict intraepithelial proliferation of the pathogen.[6] These studies strongly suggest an intrinsic recognition mechanism in epithelial cells that is crucial for early host perception of pathogens and early and appropriate alert signaling to immune cells to mount an appropriate inflammatory response. During viral entry and replication in the mucosa, mucosal epithelial cells may sense viral glycoproteins, viral DNA, ssRNA, or dsRNA via a diverse range of PRRs, including TLRs, NLRs, and ALRs, and detect viral invasion to initiate the innate immune response. This process is especially important for the host response to primary viral infections in which no virus-specific immunity is available for host protection.

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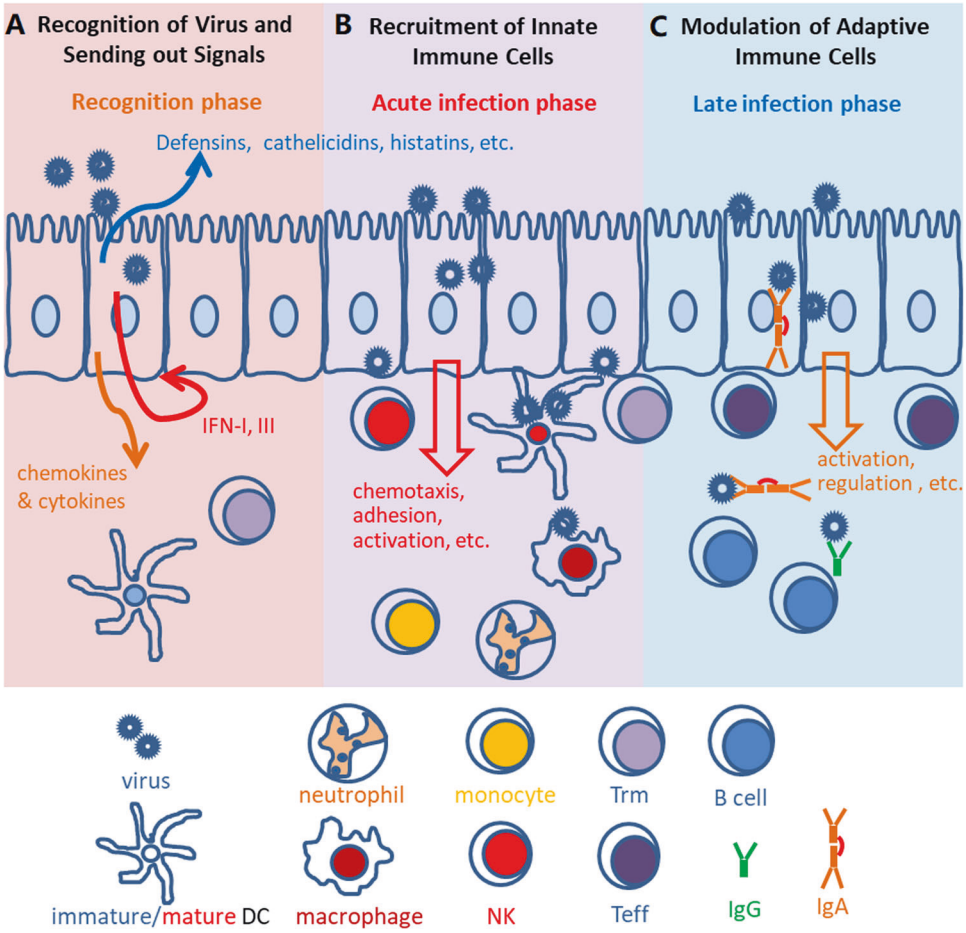

Fig. 1 Model of epithelial cells as initial responders that control and regulate immune responses to viral infections. **A** Upon viral infection, mucosal epithelial cells recognize viral PAMPs, activate various immune-associated genes, produce proteins such as defensins, cathelicidins, histatins, and type I and III interferons for antiviral defense, and secrete proinflammatory cytokines and chemokines as signals to attract and modulate hematopoietic immune cells (left). **B** Epithelial cells interact with interepithelial and subepithelial DCs to activate DCs, recruit innate immune cells such as natural killer (NK) cells, monocytes, and macrophages, and modulate the adhesion, activity, and function of these cells and immune responses to infection during acute inflammation (middle). **C** Finally, epithelial cells recruit and regulate adaptive immune cells by expressing soluble and cell-surface molecules that alter the function of DCs and further modulate the adhesion, cytokine secretion, cytolytic activity, and differentiation of T cells in the late state of infection. Furthermore, the differentiation and antibody secretion of B cells may also be modulated (right)

Krausgruber et al. tested the epigenetic potential for immune gene activation in all three structural cell types upon viral infection.[1] On day 8 after intravenous injection of LCMV, up to 57.9% of genes with unrealized potential were activated in structural cells, with notably high responses in fibroblasts and endothelial cells in the liver, spleen, lungs, and large intestine. Surprisingly, structural cells expressed a variety of chemokines and cytokines in response to viral infection (Fig. 1B). This finding extends our view beyond typical immunological knowledge, in which the secretion of chemokines and especially cytokines has been thought to be mainly associated with immune cells. The results strongly suggested the presence of an interaction network between immune cells and structural cells and that the network could be altered during viral infection. LCMV is a well-studied virus that affects most organs, which allowed the authors to investigate global defense responses. However, the systemic viral infection model is much different from mucosal-initiating viral infections. This notion also explains the notably high responses in fibroblasts and endothelial cells but not in epithelial cells in the liver, spleen, lungs, and large intestine after LCMV challenge. To evaluate the structural cell responses at the early stage of viral challenge, a mucosal viral infection model should be utilized. The characteristics of early epithelial cell responses to mucosal viral infection could give us more concise and straightforward clues to understand the early viral trigger(s) and early activated genes in mucosal epithelial cells stimulated by natural mucosal viral infection. Furthermore, a mucosal viral infection model may provide an opportunity to perform longitudinal studies on the responses of epithelial cells, which would provide data on the early response of epithelial cells to infection at different time points. This system would help to identify the early genes that play key roles in downstream immune regulation. In addition, to avoid overactivation or allergic reactions in the mucosa in response to the initial viral challenge, as well as to other stimuli from the external environment, it is hypothesized that genes with unrealized potential are activated under stringent regulation, such as more robust negative feedback in epithelial cells than in fibroblasts or endothelial cells. The exact mechanism by which structural cells exert their regulatory responses to viral infection also needs further investigation.

In the context of structural immunity, epithelial cells are not only the sentinels that detect viruses via PRRs but also the direct responders to viral invasion via activation of the intrinsic inflammasome pathway. More importantly, epithelial cells play a

key role as frontline responders that send early and appropriate signals to instruct hematopoietic immune cells to mount innate and specific immune responses to pathogen invasions. The study by Krausgruber et al. highlighted the immunoregulatory potential of structural cells,[1] which is an important step toward future systematic, organism-scale elucidation of the immune functions of additional structural cell types. It should be noted that mucosal epithelial cells are also a platform for secretory IgA antibodies to exert multiple functions against viral infection, and the detailed mechanism and function of IgA transepithelial transport and interactions with cellular components during viral replication in the epithelium are worth studying in depth (Fig. 1C).[7,8]

With powerful single-cell approaches, epithelial cells in different organs or tissues, including the eye, mouth, nose, lung, small intestine, large intestine, stomach, and genital tract, should be investigated comprehensively in both steady-state and viral infection scenarios. This approach will facilitate our understanding of epithelial cell recognition and immune regulation and provide information about the immune-associated gene expression status of these cells, thus elucidating the cellular chain of events, from the detection of viral infection to the defense response and immune-cell recruitment and ultimately to the removal of the infectious virus. This epithelial platform is also potentially useful for the development of novel mucosal adjuvants derived from PAMPs[9,10] and the design of safe, convenient, and efficient mucosal vaccines and early therapeutic treatments.

In conclusion, mucosal epithelia are the initial responders that control and regulate immune responses to viral infections. The local mucosal tissues themselves can play key roles in governing downstream immune activity following pathogen invasion via an intrinsic structural mechanism.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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