Cell

A New Shield for a Cytokine Storm

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DOI 10.1016/j.cell.2011.08.027

Highly virulent influenza virus infection results in excessive cytokine production, recruitment of leukocytes, and immune-mediated pulmonary injury. Teijaro et al. (2011) now demonstrate that sphingosine-1-phosphate receptor 1 ligands suppress all features of flu-inflicted pathological inflammation and place the endothelium at the center of this regulatory network.

Recognition and rapid clearance of pathogens by the innate immune system provide the first line of defense in metazoan organisms. However, excessive activation of the innate immune system in response to pathogens can lead to pathological inflammatory consequences. In the case of highly virulent 1918 and avian H5N1 influenza virus infections, early recruitment of inflammatory leukocytes to the lung, followed by excessive early cytokine responses (known as a cytokine storm), is considered to be the key contributor to morbidity and mortality of the infection (Tscherne and García-Sastre, 2011). Likewise, the virulence of the pandemic 2009 H1N1 "swine flu" is associated with viral replication in the lower respiratory tract and more severe pulmonary damage compared to seasonal flu (Tscherne and García-Sastre, 2011). However, the cell types that are responsible for the initiation and amplification of the cytokine storms that follow virulent influenza infection remain unclear. In this issue, Teijaro et al. (2011) have uncovered an unexpected role of endothelial cells in coordinating the inflammatory sequelae via sphingosine-1-phosphate signaling.

Sphingosine-1-phosphate is a metabolite of sphingolipid and is a ligand for a family of five G protein-coupled receptors, $S1P_{1-5}$ (Rosen et al., 2009). Differential expression of these receptors enables control of angiogenesis, heart development, and immunity in a highly specific manner. The sphingosine analog FTY720, a well-known immunomodulator that has recently been approved for the treatment of multiple sclerosis, is phosphorylated in vivo by sphingosine kinase 2 to produce a ligand for the S1P receptors $S1P_{1,\ 3-5}$. Its mode of action is through sequestration of lymphocytes in lymph nodes away from peripheral sites of inflammation. Prolonged exposure to FTY720 causes $S1P_1$ endocytosis and degradation, impairing the ability of lymphocytes to respond to endogenous S1P to egress out of lymph nodes (Cyster, 2005).

The study by Teijaro et al. (2011) followed up on previous findings by the same group that local intratracheal instillation of S1P ligands reduces cytokine responses following respiratory infections with a mouse-adapted influenza virus (Marsolais et al., 2008, 2009) or human 2009 H1N1 influenza isolate (Walsh et al., 2011). These studies also revealed that the $S1P_1$-specific agonists do not affect the generation of adaptive immune responses (CD8 T cells and neutralizing antibodies) and do not alter viral replication in vivo (Marsolais et al., 2008). Using $S1P_1$-specific agonists, the current study shows that stimulation of $S1P_1$ alone recapitulates much of the suppressive phenotype of AAL-R, which is a broad-spectrum agonist of S1P receptors. Notably, intratracheal instillation of CYM-5442 (an $S1P_1$ agonist) on hours 1, 13, 25, and 37 of influenza virus challenge significantly dampened type I interferon (IFN), cytokine, and chemokine release into the bronchoalveolar lavage (BAL) and resulted in a significant decrease in infiltration of monocytes, macrophages, neutrophils, and natural killer (NK) cells into the lung. In addition, oral administration of another $S1P_1$ agonist, RP-002, at 1 and 25 hr after infection resulted in increased survival of mice challenged with 2009 H1N1 human isolate (Figure 1).

To address the mechanism of action, the authors examined the expression of $S1P_1$ using $S1P_1$-eGFP knockin mice (Cahalan et al., 2011). As reported previously, in addition to lymphocytes, a high level of $S1P_1$ expression was found on lymphatic and vascular endothelial cells isolated from the lung (Cahalan et al., 2011). However, influenza-induced cytokine responses and cellular recruitment to the lung were blocked by CYM-5442 in RAG2-deficient mice (devoid of T and B lymphocytes), suggesting that $S1P_1$ stimulation dampens innate immune responses in a manner that is independent of its well-known function in lymphocyte sequestration.

Next, the authors demonstrated that production of chemokines CCL2, CCL5, and CXCL10 from vascular and lymphatic endothelium following influenza infection was significantly reduced by CYM-5442 treatment in vivo. Intratracheal instillation of recombinant CCL2 restored monocyte, macrophage, and NK cell recruitment to the lung of mice treated with CYM-5442. Surprisingly, CCL2 was not sufficient to restore IFN or cytokine responses in the lung. Further, depletion of $CD11b^+$ cells (including monocytes, macrophages, neutrophils, and NK cells) using antibody treatment only resulted in reduction in IFN-γ secretion but did not affect the levels of IFN-α, CCL2, IL-6, or TNF-α in the lung. These data indicate that inflammatory leukocyte recruitment is not sufficient for cytokine storm in the face of CYM-5442 treatment and is not required for the production of the majority of the cytokines in response to influenza infection. Finally, type I IFNs were placed upstream of the cytokine storm

during influenza infection, as IFNαβR-deficient mice failed to secrete type I IFN, chemokines, or cytokines despite the normal recruitment of inflammatory leukocytes.

The results of this study reveal an important role of $S1P_1$ as a regulator of inflammation but also raise a host of questions. First, how does $S1P_1$ agonism result in global suppression of cytokines? This may occur both at the cell-intrinsic level within the lung endothelial cells and at the cell-extrinsic level in the recruited leukocytes. $S1P_1$ receptor is coupled to Gαi, and receptor engagement triggers a multitude of downstream signaling pathways, including PI3K and Rac activation and promoting cell survival, motility, and barrier functions. $S1P_1$ also activates the MAP kinase and phospholipase C pathways and intracellular mobilization of calcium signaling, resulting in cell proliferation and cytokine secretion (Rosen et al., 2009). However, it remains unclear whether any of these pathways directly inhibit cytokine and chemokine production by endothelial cells or, alternatively, whether $S1P_1$-activated endothelial cells produce anti-inflammatory mediators. It is also possible that the anti-inflammatory effect of $S1P_1$ engagement is an indirect consequence of its effect on vascular integrity.

Second, why doesn't the endogenous S1P in the blood or lymph, which is maintained at high concentration (100–1,000 nM in blood and 30–300 nM in lymph), trigger a similar response in the endothelial cells? Unlike S1P, the synthetic agonists of $S1P_1$, including CYM-5442, induce prolonged signaling, polyubiquitination of $S1P_1$ followed by degradation in the lysosomes (Rosen et al., 2009). Whether endocytosis and degradation of $S1P_1$ are requisite for immunosuppression by CYM-5442 and other $S1P_1$ agonists remains to be determined. In this regard, it is interesting to speculate whether similar blockade of inflammation is caused by FTY720 and whether the clinical effects of this compound may, in part, depend on mechanisms that extend beyond lymphocyte sequestration.

Following seasonal influenza infection, the virus replicates primarily in the lung epithelium, followed by infection of alveolar macrophages, dendritic cells, NK cells, and B cells (Manicassamy et al., 2010) (Figure 1). In contrast, H5N1 avian flu targets specifically endothelial cells for replication in birds (Feldmann et al., 2000). Thus, it would be interesting to examine whether $S1P_1$ agonists will be effective in the case of H5N1 influenza. Finally, the possible role of endothelial $S1P_1$ in other conditions associated with excessive cytokine production would be an exciting question for future studies.

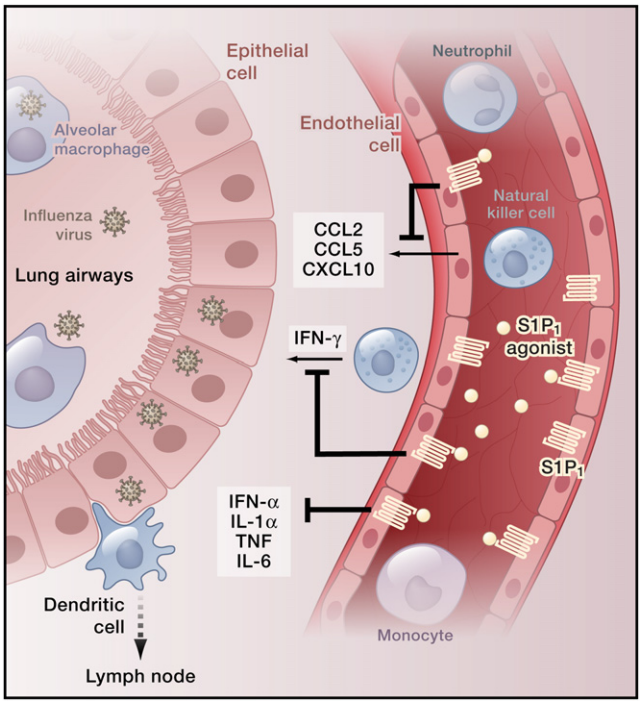

Figure 1. Suppression of Inflammation by $S1P_1$ Agonists
Influenza virus infects the lung epithelial cells and subsequently alveolar macrophages and other leukocytes. This results in type I IFN, cytokine, and chemokine secretion by various cell types both resident and recruited. $S1P_1$ ligands, CYM05442 and RP-002, engage $S1P_1$ on vascular and lymphatic (not shown) endothelial cells and block chemokine secretion from endothelial cells, which, in turn, block recruitment of leukocytes to the lung. In addition, $S1P_1$ signaling suppresses type I IFN, cytokine, and chemokine secretion from other cells, resulting in reduced immunopathology. However, $S1P_1$ agonists do not impair dendritic cell activation, thereby enabling T cell and B cell responses to be initiated in the draining lymph node.

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