**Mini Review**

**Notch system in influenza A/H1N1 virus infection**

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Influenza viruses cause annual epidemics and occasional pandemics that have claimed the lives of millions. Both innate and acquired immunity are essential for protection against influenza virus. A viral infection is initially sensed by the host innate system, triggering a rapid antiviral response that involves the release of proinflammatory cytokines, and eventually leads to the activation of the adaptive immune response. The innate immune response is initiated when cellular pathogen recognition receptors (PRRs) recognize pathogen-associated molecular patterns (PAMPs). The innate immune response by antigen presenting cells (APCs), including dendritic cells (DCs) and macrophages, is initiated quickly to protect from overwhelming infectious organisms, but with time, also can activate the adaptive immune response to the invading pathogens. The adaptive immune response is essential for purging a diverse repertoire of invading pathogens, and CD4+ and CD8+ T cells are required for successful eradication of pathogens. Initial studies have indicated that the interaction of Notch and Notch ligands plays a critical role during development, and further, the Notch system is an important bridge between APCs and T-cell communication circuits. However, the role of Notch system during influenza virus infection is still unknown. Here we review our recent study which shows that Notch signaling through macrophage-dependent Delta-like 1 (Dll1) is critical in providing an anti-viral response during influenza virus infection by linking innate and acquired immunity.

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**A short overview of Notch system**

Notch is a heterodimeric cell-surface receptor that is involved in a broad range of differentiation processes\(^1\). It is composed of an extracellular ligand-binding domain that is non-covalently associated with a single-pass transmembrane domain\(^2\). The notch signaling pathway regulates many aspects of embryonic development, as well as differentiation processes and tissue homeostasis in multiple
Notch ligand Delta-like 1 (Dll1) up-regulation in influenza virus challenged macrophages

Many studies have shown that notch ligand regulation on APCs is a key event for T cell differentiation. Using BM-derived DCs and macrophages, we assessed the gene expression profile of Notch ligands on APCs following influenza virus stimulation. Of the five Notch ligands, Dll1 is the only Notch ligand specifically up-regulated on macrophages following influenza stimulation, but it is not expressed on DCs. Also in murine influenza virus infectious model, the peak expression of Dll1 on lung macrophages in mice coincides with the period of peak inflammation after H1N1 infection. Among the cytokines induced during the innate immune response, activation of type-I IFNs is the most powerful defense mechanism against in-
Role of DLL1-expressed macrophage against influenza virus infection

Macrophages play an important role in regulating IFN-γ production from both CD4+ and CD8+ T cells through RIG-I-induced type-I IFN-dependent pathway (including JAK-STAT pathway) that upregulates the Notch ligand DLL1. Our in vivo influenza H1N1 infectious model demonstrates higher mortality and impaired viral clearance in anti-Dll1Ab treated or GSI treated mice with decreased IFN-γ production when compared with control treated mice.

Influenza viral replication and spread20. Moreover, macrophages, but not DCs, showed enhanced Notch ligand DLL1 expression in response to influenza virus and to type-I IFN cytokines, which suggested that DLL1 induction is dependent on type-I IFNs. We also confirmed this by showing that IFNαR-/- derived macrophages completely failed to induce DLL1. Influenza virus amplifies the type-I IFN response via a positive-feedback loop that activates JAK-1 and Tyk-2 kinases, which leads to the phosphorylation and dimerization of STAT1 and STAT2 protein21, 22. Our studies also showed impaired DLL1 induction on macrophages from STAT1-/- mice and macrophages treated with a JAK-1 inhibitor. PRRs that recognize influenza virus RNA, have been shown to be a key initiator of type-I IFN response in infected cells. These PRRs rely on the RIG-I-like signaling pathway, composed of RIG-I and MDA5. RIG-I-knocked down macrophages, not MDA-5-knocked down macrophages, expressed decreased Notch ligand DLL1 with significantly decreased type-I IFN cytokine production following influenza virus stimulation. Thus, Our results show that influenza virus-induced type-I IFNs are exclusively RIG-I dependent and that their production is essential for the induction of DLL1 through the IFNαR and the JAK-1/STAT1/2 signaling pathway19 (Fig.1).

Closing remarks

Our studies have revealed a critical role of Notch signaling in infectious model using influenza virus. In this model DLL1 influences the development of anti-viral immunity, and may provide mechanistic approaches for modifying and controlling the immune response against influenza virus infection. New type of influenza vaccine to induce DLL1 on macrophages might be one of candidate. For example, intranasal pre-treatment of poly(I:C), a double-stranded RNA (dsRNA) ligand which could induce type-I IFN and increase DLL1 expression on macrophages18, provided high level of protection against lethal challenge with influenza virus25. However, Notch system seems more complicated system, because Notch signaling pathways contribute to immune systems including a role in multiple lineage decisions of developing lymphoid and myeloid cells. A better understanding of the regulation of the notch system might contribute a novel therapeutic approach for influenza virus infection.
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References
