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# Immunity to seasonal and pandemic influenza A viruses

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**Abstract**

The introduction of a new influenza strain into human circulation leads to rapid global spread. This review summarizes innate and adaptive immunity to influenza viruses, with an emphasis on T-cell responses that provide cross-protection between distinct subtypes and strains. We discuss antigenic variation within T-cell immunogenic peptides and our understanding of pre-existing immunity towards the pandemic A(H1N1) 2009 strain.


*Keywords:* Influenza virus; Adaptive immunity; Innate immunity; H1N1 2009; Antigenic variation

## 1. Introduction

Influenza is a rapidly spreading acute respiratory disease that causes profound morbidity and mortality. Annual seasonal influenza epidemics result in ~500,000 deaths worldwide and cause huge losses to global economies. Influenza viruses grow rapidly in the human respiratory mucosa, with respiratory epithelial cells being the primary target, producing large amounts of virus that then infects alveolar macrophages and local dendritic cell (DC) populations. The first line of defence towards influenza infection is mediated by the innate immune system [1]. Infiltration of neutrophils and monocytes/macrophages into the lung is needed for host protection during the initial stages of infection as well as the recruitment of the adaptive arm of immunity, influenza-specific B and T cells.

Immune B cells secrete antibodies to effectively prevent infection by neutralising the virus and are involved in the resolution of the disease process. Antibody-based vaccines towards the variable surface glycoproteins, the haemagglutinin (HA) and neuraminidase (NA) are the most effective way to combat seasonal infections. However, vaccines need to be updated annually due to antigenic changes within the HA and NA. Importantly, antibody-based vaccines fail in the event of an influenza pandemic caused by the emergence of a novel influenza strain or when the vaccine strain does not match the circulating strain, while T cells elicit broader immunity against several influenza strains (both seasonal and pandemic) as they recognise more conserved internal components of the virus. Pre-existing $CD8^+$ T cells directed towards conserved viral regions promote more rapid recovery via the production of pro-inflammatory cytokines and the direct killing of virus-infected cells [2]. Such $CD8^+$ T cell-mediated cross-protection might, in the face of a pandemic, function to decrease disease severity and lead to better clinical outcomes, though the development of any T cell-based vaccination strategy would need to be approached with caution to rule out the possibility of immunopathology. Also, $CD8^+$ T cell responses can exert selective immune pressure, leading to escape mutations in the viral peptide (p) component of the antigenic pMHC-I complex. To date, the extent of viral escape within influenza T cell epitopes (mutations occur in 71.4% of human T cell

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peptides across 23 influenza strains tested [3]) has not received much attention due to the acute nature of influenza infection.

This review summarizes innate and adaptive immunity to influenza A virus infection, with a particular emphasis on $CD8^+$ T cell responses that (at least in animal models) can provide a substantial level of cross-protection between distinct influenza subtypes and strains. We discuss the antigenic variation within T cell immunogenic peptides and review our current knowledge on pre-existing immunity towards the newly emerged pandemic A(H1N1)-2009 strain.

## 2. Innate immune responses to influenza infection

Our understanding of innate immune involvement during influenza virus infection has greatly expanded in the last several years [4]. Influenza virus infection triggers mechanisms mediated via all three major families of innate receptors, namely the Toll like receptors (TLRs), Nod-like receptors (NLRs) and RIG-I like receptors (RLRs). TLR7 recognises influenza ssRNA, activating a transcriptional program that leads to the induction of Type I IFN, IL-12, and IL-6 [5]. The NOD-like receptor NLRP3 is reported to become activated in response to influenza RNA [6–8], in a process that requires the viral M2 protein ion channel [8]. After NLRP3 activation, the cytoplasmic signalling platform called the inflammasome forms, generating active caspase-1, which can then cleave pro-IL-1β, IL-18 and other reported ligands. In a different class of NLRs, NOD2 has been shown to mediate recognition of viral ssRNA in RSV and influenza virus model systems, leading to Type I interferon production [9]. Finally, the prototyptical RLR, RIG-I, recognises influenza virus RNA, a process which appears to induce significant IFN-α production by the infected cell. Knockouts of RIG-I (or a downstream pathway, MAVS), TLR7 or NLRP3 all lead to increased mortality, with RIG-I/MAVS and TLR7 being somewhat redundant for generating a Type I IFN response. A double knockout mouse deficient in both pathways experiences dramatically increased lethality after infection. These results highlight the central importance of the Type I IFN response in early influenza protection [10]. NLRP3-deficiency also results in reduced survival, though from increased early lung damage rather than major defects in Type I IFN production.

These early responses are generally mediated by resident alveolar macrophages, DCs and respiratory epithelial cells. A key feature of any immediate inflammatory responses is the secretion of chemokines to recruit additional populations of potential effector cells [11]. Resident leukocytes and infiltrating cell populations are activated and involved in multiple protective functions, though this area still remains relatively unexplored. Inflammatory monocytes populations have been described using various nomenclatures and phenotypes, but there is general agreement that a $CCR2^+$, bone marrow-derived $CD11b^+$ monocyte is a key constituent of the innate response during the first week to ten days of infection [7,12–14]. The proposed functions of these cells include modulating inflammatory microenvironments, direct killing of infected epithelial cells via TRAIL, and regulation of CD8 T cell proliferation and survival. Similarly, migrating dendritic cells from the lungs and recruited inflammatory DCs from the blood play critical roles in lymph node antigen presentation. While many of these DCs originate in the lung, they generally appear to be phylogenetically distinct from the set that extravasated during the course of infection [15–17]. Neutrophils also invade the lung in the early stages of infection, but their function remains ambiguous, with evidence of both disease control and exacerbation being reported variously for different infection protocols [18,19].

Defining the specific antiviral effector mechanisms of the innate immune response and their contribution to lung pathology and remodelling remains an important area of investigation. The rapid onset of morbidity and even death in some individuals argues strongly for early clinical intervention (either "professional" cells or the host epithelium), but any therapy must not limit the protective effects resulting from the activation of the innate response, including the determining influence on the subsequent generation of adaptive immunity. Clearly, we need a better understanding of the early phase of influenza virus infection.

## 3. The antibody response to influenza virus

Antibody molecules recognise conformational epitopes in the context of whole protein, both on free virus particles and on the surface of infected cells. Following primary influenza virus infection, B cells in the draining mediastinal lymph node (MLN) encounter antigens transported from the site of infection by presenting DCs by day 3 after infection [20]. Specific recognition of viral antigens and co-stimulation signals such as CD40-CD40L from "licensed" DCs results in the rapid division and production of antibody by B cells. Such B cell responses can be either $T_H$-dependent or independent, though any $T_H$-independent responses are likely to be small in magnitude and of reduced longevity. Extra-follicular $T_H$-independent short-lived plasma B cells are generated at the edge of T/B cell zones [21], whereas intra-follicular CD4 $T_H$-B cell interactions occur within the germinal centres. The $T_H$-dependent germinal centre reaction induces long-lived humoural immunity [22]. Both intra- and extra-follicular B cell responses have been shown for influenza virus infection [20].

A striking feature of influenza virus infection is the speed with which the virus replicates once established in the respiratory epithelium. Preformed virus-specific antibodies in the serum or, preferably, at the airway mucosal surface, can block virus entry and the subsequent establishment of infection [23]. As a result, all licensed, inactivated influenza vaccines to date are designed primarily to generate an antibody response [24] against the major haemagglutinin (HA) and neuraminidase (NA) virus surface molecules. A single infection with any strain of influenza viruses elicits lifelong antibody-mediated protection against the exact same virus strain [25], as evidenced by neutralising antibodies found in donors infected with the 1918 "Spanish" influenza virus 90 years previously [26]. This protection is primarily directed at the HA