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# Influenza virus Induced Cytokine Responses: An Evaluation of Host-Pathogen Association

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## Abstract

Influenza is an acute viral infection of the respiratory tract and there are many reports suggesting that many of the clinical and pathological manifestations of influenza virus are due to various cytokines. These proteins act as chemical messengers and aid in viral clearance and cell death, such as, interferon-a (IFN-α), tumour necrosis factor-a (TNF-α), interleukin-1 (IL-1) α and β, interleukin-6 (IL-6), interleukin-8 (IL-8) and monocyte-attracting chemokines. Cytokine gene expression leads to activation of NF-κB, AP-1, STAT and IRF signal transducing molecules in influenza A virus-infected cells. Anti-viral defence mediated by influenza A virus-induced IFN-α/β have proved to be very essential. IFN-α/β is known to prolong T cell survival, upregulate IL-12 and IL-18 receptor gene expression and together with IL-18 stimulate NK and T cell IFN-γ production and the development of Th1-type immune response. It has been observed, though, not completely understood, that the cytokine responses differ depending on the type of host. This review aims to give a composed account of the cytokine/ chemokine responses, with special reference to the differences observed in various host-virus combinations.

**Keywords:** Cytokine; Host; Influenza; Viral infection; IFN; Immune response

## Introduction

Influenza is highly infectious disease and the influenza viruses are a considerable threat to human health all around the world. The prevention and treatment of the disease can be done by vaccines and antiviral substances, but only with certain limitations. The virus is a highly notorious one, and rapidly mutates via genetic drift (characterized by point mutations in antigenically important positions caused by selective pressure from host immune response) and genetic shift (characterized by a substitution of a whole gene from one subtype to another) [1-4], re-surfacing as an uncontrolled pathogen for massive destruction. Thus, further understanding of the immune-pathological mechanisms of influenza virus infection and host immune responses is required. Cytokines/ chemokines are one such group of significance, which demands attention of immunologists and virologists, owing to their various roles in disease pathogenesis. Further, there have been studies which indicate that the cytokine expression, to some extent, is affected by the type of host. In this review, we attempt to compile the current information available on the cytokines/ chemokines induced during an influenza virus infection and also their differential expression in different hosts.

## Cytokines and Chemokines during Influenza Virus Infection

In response to influenza A virus infection, epithelial cells and leukocytes, produce chemotactic (chemokines), proinflammatory, and other immunoregulatory cytokines. It is well-known that cytokines act as local hormones that activate cells of the immune system. The pro-inflammatory cytokine families, tumour necrosis factor (TNF) and interleukin (IL)-1, induce fever while others, viz. IL-2, IL-12 and IL-15, upregulate expression of cells involved in innate immunity [5,6]. Chemokines are a subset of cytokines, which, by their chemoattractant property promote the stable binding of leukocytes to vascular endothelial cells, thereby, directing their migration towards the infection site [7].

### *In vitro* studies

Influenza virus induced cytokine responses have been studied *in vitro* in monocyte/macrophage cell lines, *in vivo* in mice [8-10], ferrets [11], and pigs [12,13]. The key cytokines, type I interferons (IFN-α/β) are produced by influenza A virus-infected epithelial cells and monocytes/macrophages [14-16]. Studies done using IFN-α/β receptor or STAT1 gene knock-out mice have shown the significance of IFN system in antiviral defense against influenza A virus [17]. Relatively poor production of IFN-α/β and proinflammatory cytokines (IL-1, IL-6, TNF-α) has been observed in human lung epithelial cell lines [15] during influenza A virus infection. Instead, influenza A virus infection in monocytes/macrophages have been reported to induce huge levels of IFN-α/β, IL-1β, IL-6 and TNF-α (Figure 1) [14,16,18-21]. Influenza A virus infected macrophages are known to produce IL-18 also, but not IL-12 [16]. However, it has been reported that dendritic cells generate relatively elevated levels of IL-12 following an influenza A virus infection or dsRNA stimulation [22,23]. Production of IL-15 has been documented in influenza A virus infected human PBMCs [24]. Human monocytes, rat alveolar or murine macrophages have been used for analysis on cytokines IFN-α, TNF- α, IL-1, IL-6 and chemokines MIP-1α, MIP-1β, MCP-1, MCP-3, IP-10 and RANTES in cell culture [25-28].

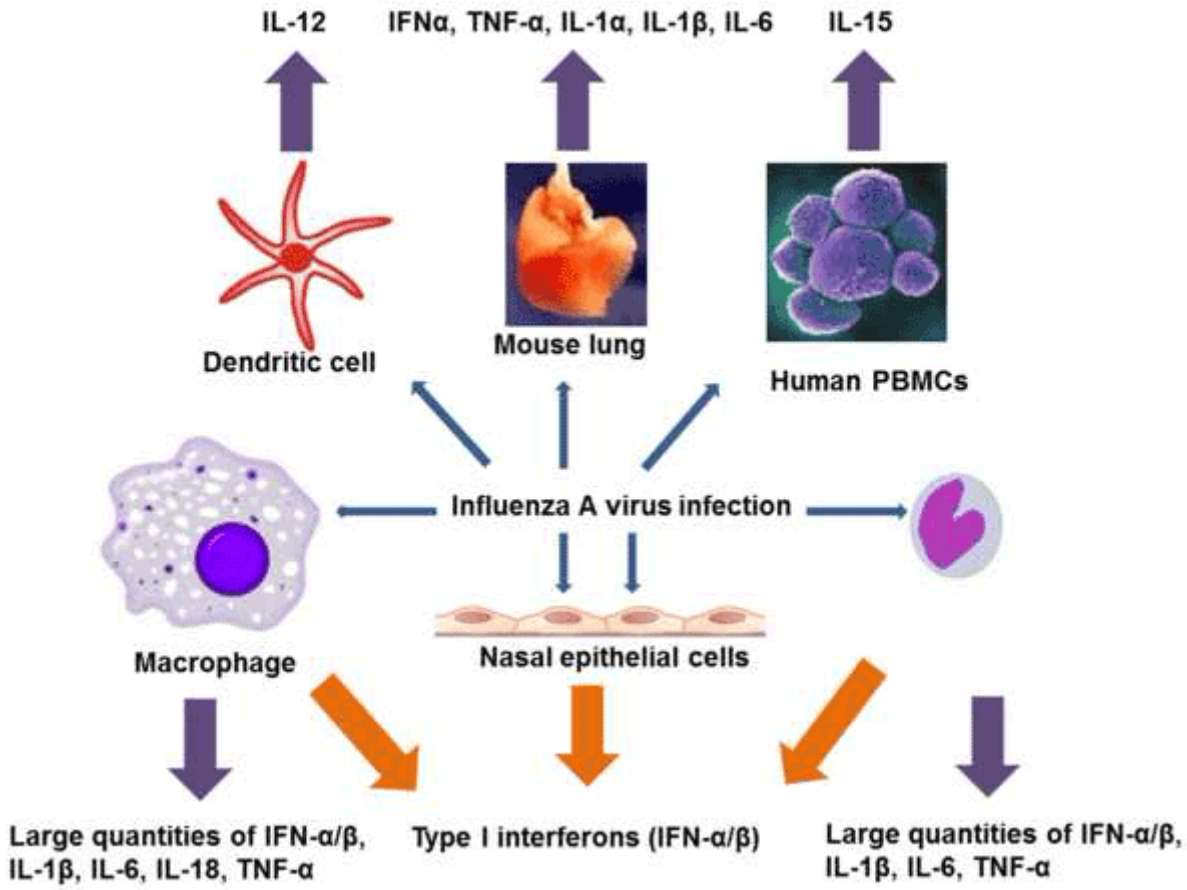


**Figure 1:** Influenza A virus induced cytokine responses in different cell types.
**Abbreviations:** IL: Interleukin; IFN: Interferon; TNF: Tumor Necrosis Factor

## *In vivo* models

The *in vivo* studies of influenza virus infection have majorly been performed on mice, then ferrets and pigs. Various independent research groups have reported increase in IFNα, TNF-α, IL-1 α and β and IL-6 levels in Bronchoalveolar Lavage (BAL) fluid or lung homogenates (Figure 1) after intranasal infection of mice with A/PR/8/34 [29-32]. Treatment of virus infected mice with antibodies to the cytokine have been reportedly associated with IFN-α and IL-1α. Anti-IFN antibody treatment lowers IL-1 levels [29]. It was reported by Kozak et al. [33] that the IL-1β knock-out mice demonstrated higher mortalities on influenza virus infection, than the wild-type mice, correlating with the findings using IL-1 receptor agonist [34]. IL-18 has also proved to be significant for survival during influenza virus infection. A study by Liu and co-researchers found that the IL-18 knock-out mice died at a greater rate than wild-type mice and pathologically massive inflammatory cell infiltrate to the lungs were observed [35]. This suggests an important role of IL-18 in early innate immunity for controlling degree of inflammation and virus propagation. Influenza virus, being an unnatural pathogen of mice, generally requires several passages to become mouse-adapted and cause disease. Further, the clinical manifestations also differ from the natural hosts [36]. Therefore, certain researchers choose ferrets as model for studying influenza- induced cytokine responses, as these animals can be infected with human strains of the virus and also they display same clinical symptoms as humans [11].

A strong sequential association has been reported between virus titres, neutrophil infiltration, disease, and IFN-a, TNF-a, and IL-1 levels in BAL fluids in pigs infected with influenza virus [12,13].

## Human volunteers

Infection of human volunteers with wild-type H1N1 or H3N2 strains has been performed to study the cytokine response in the population. Nasal lavage fluids of infected humans reportedly contain increased levels of proinflammatory cytokines, such as IFN-α, TNF-α, IL-6, IL-8, MIP-1α and β, and MCP-1 [37-40]. It has been stated that most of the early symptoms are due to IFN-α and IL-6 [24–26], produced by non-immune cells at the site of infection causing local inflammatory reactions and certain systemic effects. IFN-α, TNF-α and IL-1α and β are supposedly the most proximal in early cytokine cascade [41] followed by IL-6 and a number of chemokines, viz. neutrophil-attracting IL-8, the macrophage inflammatory proteins (MIPs), and monocyte chemoattractant proteins (MCPs) [42]. IFN-α, TNF-α, IL-1 and IL-6 have multifunctional activities and have been related with symptoms such as, fever, excessive sleepiness and

anorexia. The cytokines, like RANTES (regulated on activation, normal T-cell expressed and secreted) and IL-1β have reportedly been found at undetectable levels during influenza virus infection. However, the human volunteer-challenge studies have been unsuccessful in reproducing severe lower respiratory tract disease often observed in natural influenza.

### Viral strain/ host dependent cytokine responses

It has been reported that the pathogenesis of an infectious disease depends on the host genotype. Srivastava et al. [43] observed that influenza A virus infected DBA/2J mice showed a higher viral load in their lungs, elevated expression of cytokines and chemokines, and a more severe and extended lung pathology in comparison to the infected C57BL/6J mice. The research group analyzed in total 22 cytokines and chemokines and observed increased levels of Il5, Il6, Il1a, Il12, and Csf3 (G-CSF) cytokines in lungs of infected mice. All cytokine expression levels were higher in DBA/2J than in C57BL/6J mice. Correspondingly, the expression of chemokines, viz. Ccl2 (MCP-1), Ccl3 (MIP-1a), Ccl5 (RANTES), Cxcl1 (KC), Cxcl2 (MIP-2), Cxcl9 (MIG), and Cxcl10 (IP-10) was higher in DBA/2J mice as compared to C57BL/6J mice. Real-time PCR analysis for Ccl2, Ccl3, Cxcl10 and Il6 further supported the host dependent cytokine expression hypothesis. A recent study by Knepper et al. demonstrated that a virus isolate from influenza A (H7N9) virus outbreak in Eastern China replicated with similar efficiency as human-adapted influenza A virus in explanted human lung tissue. The group observed that low induction of IFN-β had a correlation with prompt replication of the H7N9 virus, due to suppression of the IFN response by the viral NS1 protein [44]. Nelli and co-researchers studied the immune response against two strains of avian influenza, in respiratory epithelial cells and macrophages derived from both human and pig. They found that cells from both the origins showed comparable susceptibility to initial infection with a highly pathogenic avian influenza (HPAI) H5N1 (A/turkey/Turkey/1/05) and a moderately pathogenic human influenza H1N1 (A/USSR/77) virus. However, contrasting differences in host innate immune response were observed in the two cell types. Human cells exhibited strong cytokine (TNF-α and IL6), and chemokine (CXCL9, CXCL10 and CXCL11) responses to H5N1 virus infection; whereas weak or no induction of TNF-α and chemokine occurred in case of pig epithelial cells and macrophages [45].

A research group retrieved sera samples from patients infected with influenza (H1N1)pdm09 virus (n=77), individuals presenting with non-flu influenza like illness (with mild symptoms) (n=59), individuals vaccinated against influenza (H1N1)pdm09 virus (n=26) and healthy individuals (n=26), to interpret the association between cytokine production and disease severity. Sera samples from 9 hospitalized pandemic (H1N1) 2009 patients and 16 non-flu influenza like illness patients with severe symptoms were also tested. The cytokines assayed in the study, included IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IFN-γ and TNF-α, IL-17 and IL-23 and it was found that mild pandemic (H1N1) infected patients generated significantly elevated levels of IL-2, IL-12, IFN-c, IL-6, TNF-a, IL-5, IL-10, IL-17 and IL-23 as compared to the healthy subjects. A higher IL-6 and IL-10 production was observed in severe pandemic (H1N1) patients, while only IL-10 levels were elevated in the pandemic H1N1 (2009) vaccinated individuals [46].

## Discussion

Studies have suggested that different hosts exhibit large differences in their response to an infection with influenza A virus. The available data emphasizes essential role of a variety of cytokines in both innate and adaptive immunity against influenza A viruses [47,48] aiding in viral clearance and host survival. A thorough understanding of the host-virus relationship demands more exploration in terms of types of cytokines induced in particular hosts.

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