Molecular Immunology 48 (2011) 1253–1262

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Involvement of NF-κB in changes of IFN-γ-induced CIITA/MHC-II and iNOS expression by influenza virus in macrophages

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ARTICLE INFO

Article history:
Received 19 January 2011
Received in revised form 10 March 2011
Accepted 14 March 2011
Available online 8 April 2011

Keywords:
Influenza virus
NF-κB
IFN-γ
CIITA/MHC-II
iNOS
Macrophages

ABSTRACT

Type II interferon (IFN-γ) plays an important role in defense against viral infection. Although this cytokine is found during influenza virus infection, it seems to have no protective function against the virus, and the reasons for this are not clear. To determine how the influenza virus overcomes the antiviral effects of IFN-γ, we examined the effect of A/Puerto-Rico/8/34 (H1N1) (PR8) infection on the expression of various IFN-γ inducible genes involved in defense against virus infection. The results showed that PR8 selectively affects IFN-γ induced MHC-II and iNOS expression in both the murine macrophage-like cell line, Raw264.7, and in primary alveolar macrophages. Infection of IFN-γ treated macrophages with PR8 resulted in decreased expression of CIITA/MHC-II and increased production of iNOS/NO. These changes correlate with activation of NF-κB but not with JAK/STAT signaling. The data indicate one possible mechanism underlying the ineffectiveness of IFN-γ against influenza virus, and suggest that NF-κB may be a promising target for anti-influenza drugs.

1. Introduction

Influenza A virus is one of the main causes of respiratory infections. This virus is still a major public health concern because of its annual death toll and its potential to cause devastating pandemics. Due to the increasing frequency of viral resistance to the four US Food and Drug Administration (FDA)-approved anti-influenza drugs (Ludwig, 2009; Moss et al., 2010), novel antiviral therapies are needed to effectively treat future influenza epidemics or pandemics. Therefore, understanding the pathogenesis of influenza virus infection is essential for safe and effective drug design.

IFN-γ, mainly produced by T cells or NK cells, is one of the key regulatory cytokines in the host immune system responsible for defense against viral infections (Boehm et al., 1997). During influenza virus infection, CD4+ and CD8+ T cells are the major pulmonary cell types that produce IFN-γ (Swain et al., 2004). However, this cytokine seems to have no protective function against the virus (Doherty et al., 2006; Graham et al., 1993; Price et al., 2000). Instead, it may contribute to secondary bacterial infections by inhibiting the initial clearance of bacteria from the lung by alveolar macrophages (Sun and Metzger, 2008). Nguyen et al. (2000) suggest that IFN-γ is not required for mucosal cytotoxic T-lymphocyte responses or for heterosubtypic immunity to influenza A virus infection in mice, and Uetani et al. (2008) recently reported that A/Aichi/2/68 (H3N2) (Aichi) inhibits IFN-γ induced HLA-DRα and CIITA expression in A549 cells. This inhibition may be due to viral inhibition/disruption of the JAK/STAT pathway, although another study suggests that PR8 inhibits only type I (not type II) IFN signaling in A549 cells (Pauli et al., 2008). So far, viral suppression of IFN-γ signaling in macrophages has not been studied, although this cell type is one of the main targets for IFN-γ.

Macrophages are present in various tissues, including the lung, and have important functions in both innate and adaptive immune responses against viruses and other intracellular pathogens. In addition to phagocytosis of virions and virus-infected cells, macrophages are also important antigen-presenting cells, which prime the adaptive immune system. Alveolar macrophages, located at the interphase between air and lung tissue, are critical for modulating disease severity in mice and pigs infected by the influenza virus (Kim et al., 2008; Tate et al., 2010). Besides type II epithelial cells within the respiratory tract, influenza virus also infects dendritic cells and macrophages (Horimoto and Kawaoka, 2005). Infection of macrophages by influenza virus results in the production of viral proteins; however, viral replication is abortive and infectious progeny is not released (Rodgers and Mims, 1981;

Abbreviations: IFN-γ, interferon-gamma; MHC, major histocompatibility complex; iNOS, inducible nitric oxide synthase; NF-κB, nuclear factor-κB; CIITA, class II, major histocompatibility complex, transactivator; NO, nitric oxide; JAK, janus kinase; STAT, signal transducer and activator of transcription; IRF-1, interferon regulatory factor 1; ISG, interferon-stimulated gene; ADAR, RNA-specific adenosine deaminase; PKR, protein kinase; OAS, oligoadenylate synthetase.

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doi:10.1016/j.molimm.2011.03.010

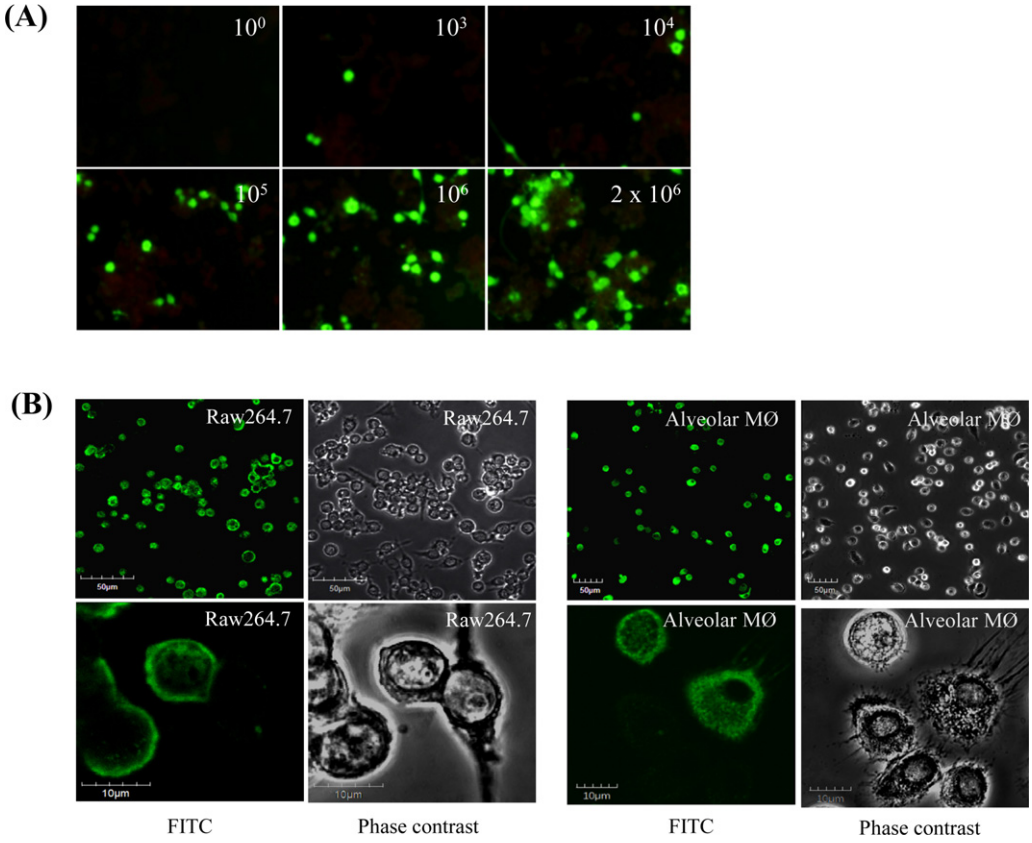

Fig. 1. Infection of macrophages with influenza virus. (A) One day before infection, Raw264.7 macrophages were seeded into 96-well plates so that the cells were about 70% confluent at the time of infection. The next day, increasing doses of PR8 (MOI = 0, 0.001, 0.01, 0.1, 1, and 2, respectively) were used to infect cells. After 1 h of absorption and 24 h of culture, cells were washed, fixed, and immunostained with anti-SIV mouse Ab as described in Section 4. Immunofluorescence was detected using a Nikon Eclipse TE2000-U. The data shown are representative of three independent experiments, all with similar results. (B) One day before infection, mouse alveolar macrophages and Raw264.7 macrophages were seeded onto 8 well-chambered cover glass so that the cell density was about 70% confluent at the time of infection. The next day, cells were infected with PR8 (MOI = 2). After absorption for 1 h at 37 °C, the cells were washed and cultured for further 8 h. The cells were then washed again, fixed, and immunostained with anti-SIV mouse Ab as described in Section 4. Immunofluorescence was detected with an Olympus FV10C-CU. Bar, 10 mm. The data shown are representative of three independent experiments, all with similar results.

Tate et al., 2010). Infected macrophages synthesize and release proinflammatory cytokines and α/β interferon (Herold et al., 2006; Hofmann et al., 1997; Hui et al., 2009), which further limit viral replication and spread within the respiratory tract. Although infection with influenza virus usually results in apoptosis of the host cells, influenza virus-infected macrophages avoid this via CCL5-CCR5 interactions, thereby effectively controlling the infection by removing other infected host cells (Tyner et al., 2005).

In this study, macrophages were infected with the PR8 virus and the expression of various IFN-γ inducible genes, which are important for defense against virus infection, was examined. The results showed that, although influenza virus did not interfere with JAK/STAT signaling, it affected IFN-γ inducible CIITA/MHC-II and iNOS expression via an increase in the level of NF-κB activation.

2. Results

2.1. Effect of influenza virus infection on IFN-γ inducible gene expression in macrophages

To examine the influence of influenza virus on IFN-γ inducible gene expression in macrophages, we first confirmed the susceptibility of these cells to virus infection. As shown in Fig. 1, both Raw264.7 and mouse primary alveolar macrophages were susceptibility to infection with PR8. The infection was in dose-dependent manner. About 63% of Raw264.7 cells and 37% of alveolar macrophages were infected with the virus (MOI = 2). Cell viability was not significantly affected after 2 days of infection with 2×10^6 pfu/ml of the virus, as confirmed by MTT assay (data not shown).

To test whether influenza virus neutralized the antiviral effects of IFN-γ by inhibiting the expression of IFN-γ inducible antiviral genes, we infected Raw264.7 with PR8 (MOI = 2) for 8 h, followed by IFN-γ treatment for 16 h. The expression levels of various genes (MHC-I, MHC-II, ADAR110, ADAR150, PKR, OAS, and iNOS) were then examined by RT-PCR and real-time RT-PCR. IFN-γ strongly induced the expression of iNOS, MHC-II, and MHC-I (Fig. 2A and B). Other antiviral genes, including OAS, PKR, ADAR110, and ADAR150, were only lightly up-regulated by IFN-γ, and changes in their expression levels could only be detected by real-time PCR (Fig. 2B). Most notably, PR8 significantly increased IFN-γ induced iNOS expression, but decreased IFN-γ induced MHC-II expression in Raw264.7. Down-regulation of IFN-γ induced MHC-II expression and up-regulation of IFN-γ induced iNOS expression by PR8 were also obtained in primary alveolar macrophages (Fig. 2C)

2.2. Inhibition of IFN-γ induced MHC-II expression by influenza virus might be mediated by CIITA

CIITA is the master regulator of MHC-II expression and acts as an essential co-activator, attracting and bridging transcription factors onto MHC-II promoters. To ascertain whether the effect of PR8 on MHC-II expression levels may result from changes in CIITA levels, we examined CIITA expression in cells infected with PR8 (MOI = 2)