Antiviral Research 88 (2010) 64–71

Contents lists available at ScienceDirect

Antiviral Research

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

Interferon-β modulates type 1 immunity during influenza virus infection

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ARTICLE INFO

Article history:
Received 13 May 2010
Received in revised form 9 July 2010
Accepted 20 July 2010

Keywords:
Interferon
Influenza virus
Antiviral immunity

ABSTRACT

Influenza viruses are important human pathogens, associated throughout history with worldwide outbreaks and pandemics. The antiviral effects of interferon (IFN)-αs/β against influenza virus infections are well recognized, yet the mechanisms whereby IFNs exert their immunomodulatory effects on an anti-influenza response remain ill-defined. Here, we describe the effects of IFN-β treatment on the immune response during a respiratory influenza (A/WSN/33) A virus infection of mice. A single dose of IFN-β (1×10^5 U) enhanced DC migration into the draining lymph node (DLN) on day 3 post-intranasal infection, and subsequently inhibited the migration from the lungs into the DLN of a newly identified late activator antigen-presenting cell population associated with type 2 immunity, LAPC. IFN-β treatment polarized the immune response towards a type 1 immune response, eliciting enhanced T_H1 effector and cytolytic T cell responses, but diminished T_H2 effector T cell responses in both the DLN and lung tissues of influenza virus-infected mice. Associated with the polarization towards a type 1 immune response, IFN-β treatment of mice resulted in accelerated viral clearance and diminished pulmonary eosinophilia in infected lung tissues.

1. Introduction

Influenza viruses are well characterized pulmonary pathogens that have had and continue to have an impact on global health (Peiris et al., 2009). Influenza viruses replicate in respiratory epithelial cells and produce large numbers of progeny virus which can then infect alveolar macrophages (AMs). Shortly after infection, AMs produce pro-inflammatory molecules, leading to the activation of both innate and adaptive immune cells (La Gruta et al., 2007). The innate immune response to pulmonary influenza virus infection involves the production of IFN-αs/β. IFN-αs/β function by modulating cell growth, establishing an antiviral state and influencing the activation of various immune cells (Katze et al., 2002; Theofilopoulos et al., 2005). Influenza viruses have evolved strategies to evade or block the IFN response as a means to increase their replication efficiency (Bonjardim et al., 2009; Hengel et al., 2005). Specifically, influenza viruses can inhibit IFN production, IFN-inducible signaling and IFN-mediated effector functions (Ehrhardt et al., 2007; Guo et al., 2007; Hale et al., 2008; Qiu et al., 1995; Shin et al., 2007; Zhirnov and Klenk, 2007).

Despite these evasion mechanisms, there is mounting evidence for the therapeutic benefit of IFN treatment for pulmonary influenza virus infection (Beilharz et al., 2007; Kugel et al., 2009; Osterlund et al., 2010; Szretter et al., 2009; Van Hoeven et al., 2009). However, the underlying mechanisms of action of IFNs, beyond their direct antiviral effects in the context of IFN-inducible factors that directly inhibit viral replication, are not clearly defined.

Both Type 1 (T1) and Type 2 (T2) immune responses are induced following influenza virus infections (Doherty et al., 2006; La Gruta et al., 2007). T1 immunity involves various effector cells, including T_H1 T cells and cytotoxic T lymphocytes (CTL) and is critical for viral clearance (Doherty et al., 2006; La Gruta et al., 2007). T2 immune responses contribute to recovery from influenza virus infection, by modulating the anti-influenza humoral response, protecting the host from re-infection (Palladino et al., 1995; Renegar et al., 2004). Notably, influenza virus-induced T2 immune responses are also linked to immunopathology, effecting pulmonary eosinophilia and inducing the production of cytokines that are associated with severe post-infectious encephalitis (Graham et al., 1994; Kaji et al., 2000).

Recently, we identified a novel murine antigen-presenting cell (APC), designated LAPC, that is activated in response to virus infections including vaccinia virus (VACV), coxsackievirus B3 (CVB3) and

Abbreviations: Abs, antibodies; Ags, antigens; AMs, alveolar macrophages; BAL, bronchoalveolar lavage; DCs, dendritic cells; cDCs, conventional DCs; CTL, cytotoxic T lymphocytes; DLN, draining lymph node; IFN, interferon; IL, interleukin; IFNAR, IFN-α/β receptor; MFI, mean fluorescence intensity; mPDCA-1, mouse pDC antigen 1; NK, natural killer; pDCs, plasmacytoid DCs; T1, type 1; T2, type 2; APC, antigen-presenting cell; LAPC, late activator APC.

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doi:10.1016/j.antiviral.2010.07.006

influenza A virus (Yoo et al., 2010). During pulmonary influenza virus infection, LAPCs transport viral antigens (Ags) from infected lung tissues to the DLN and spleen with delayed kinetics of migration compared to DCs. In the DLN, influenza virus-activated LAPCs induce T_H2 effector T cell generation by cell-to-cell contact mediated modulation of GATA-3 up-regulation. *In vivo* LAPC adoptive transfer studies identified that influenza virus-activated LAPCs selectively augment anti-influenza T2 immune responses by increasing (i) the number of T_H2 effector T cells in the DLN, (ii) the amount of circulating anti-influenza immunoglobulin (Ig) and (iii) the production of T2 cytokines in the bronchal alveolar lavage, in influenza virus-infected recipient mice. LAPC recipient mice exhibited exacerbated pulmonary pathology, with delayed viral clearance and enhanced pulmonary eosinophilia. Viewed together, the data indicated that anti-influenza T1 and T2 immune responses are modulated by DCs and LAPCs, respectively.

Here, we provide evidence that IFN-β can polarize the immune response towards T1 immunity, by selectively modulating the migration of DCs and LAPCs into the DLN. Infected mice treated with IFN-β exhibited accelerated viral clearance in lung tissues and diminished pulmonary pathology, reflected by decreased pulmonary eosinophilia.

Cognizant that influenza viruses, including the highly pathogenic avian H5N1 strain and the circulating swine origin H1N1 pandemic 2009 strain (S-OIV, H1N1pdm), develop resistance to the antiviral agents adamantine and/oseltamivir (Bright et al., 2005, 2006; Cheng et al., 2009; Vicente et al., 2009; Wang et al., 2009), new effective antiviral therapies are urgently needed. Our data suggest that IFN-β may have therapeutic potential against influenza A virus infections.

2. Materials and methods

2.1. Animals

C57BL/6J mice were bred and housed in the Toronto General Hospital animal facility. All mice were housed in a specific pathogen-free environment and all experiments were approved by the Animal Care Committee (ACC) of the Toronto General Research Institute.

2.2. Virus infection and IFN-β treatment

Mice (8–12 weeks of age) were anesthetized with Ketamine and Xylazine and infected by intranasal instillation with 50 μl of PBS containing 500 PFU of A/WSN/33 (H1N1) influenza virus (a gift from Dr. Gary Whittaker, Cornell University, Ithaca, NY). At 24 h post-infection, mice received with either mIFN-β (1×10^5 U/mouse, BiogenIdec, Cambridge, MA) or sterile PBS by intra-peritoneal (i.p.) injection. The body weight of mice was monitored on a daily basis and at the indicated times post-infection, mice were sacrificed by cervical dislocation and the mediastinal LNs and lungs were harvested and processed.

2.3. Cell fractionation

Tissues (mediastinal LNs and lungs) were harvested, mechanically disrupted, followed by enzymatic digestion with collagenase D and DNase I (Roche, Mannheim, Germany). In brief, tissues were placed in cold PBS supplemented with 1 mM $MgCl_2$ and 1.8 mM $CaCl_2$ and then compressed between two glass slides. These tissues were then incubated at 37 °C for 30 min with 1 mg/ml collagenase and 0.3 mg/ml DNase I. After incubation, DCs were dissociated from T cells by incubating with 1 mM EDTA for 10 min at room temperature. The cell suspension was filtered through a 70 μm mesh. RBCs (Red blood cells) were removed using ACK-lysis buffer.

2.4. Antibody staining and flow cytometry

Fluorochrome-labelled monoclonal antibodies (mAbs) specific for CD4 (GK1.5), CD8α (53-6.7), CD11c (N418), CD40 (1C10), B220/CD45R (RA3-6B2), CD80 (16-10A1), CD86 (GL1), TcR-β (H57–597), Thy1.2 (53-2.1), IL-4 (11B11), IFN-γ (XMG1.2) and MHC-II (I-A/E) (M5/114.15.2) were obtained from eBioscience (San Diego, CA). FITC-conjugated mAb to mPDCA-1 (JF05-1C2.4.1) was purchased from Miltenyi Biotec (Bergisch Gladbach, Germany). Flow cytometry was performed on a FACS-Calibur (BD Biosciences) and the data were analyzed using Flowjo software (Tree Star, San Carlos, CA).

To examine effector T cell responses, cells were re-stimulated with 50 ng/ml PMA, 500 ng/ml ionomycin (Sigma Aldrich, St. Louis, MO) and Golgi plug (BD Biosciences) for 4 h, washed with FACS buffer (PBS supplemented with 2% FBS) and surface stained with the appropriate identifier fluorochrome-conjugated mAbs. After fixation and permeabilization with Cytofix/Cytoperm buffer (BD Biosciences), intracellular staining was performed using mAbs for each cytokine followed by FACS-analysis.

2.5. Influenza virus titration

Viral titers in lung tissue were determined using an MDCK cell plaque assay. At days 3, 6 and 8 post-infection, mice were euthanized and whole lung tissues were harvested. Lungs were homogenized in serum-free MEM and were frozen and thawed three times. MDCK cells were seeded in MEM in individual wells of a 6-well plate and grown until confluent. Ten-fold serial dilutions of homogenized lung tissues were prepared in serum-free MEM. A total of 200 μl of each dilution was added to individual wells (in duplicate) for 30 min at 37 °C. Cells were then overlaid with 3 ml of 1× MEM containing 0.65% agarose, antibiotics, L-glutamine and 1 μg/ml trypsin. 40 h after incubation at 37 °C, cells were fixed with 2 ml Carnoy's fixative (3:1, methanol:glacial acetic acid) for 30 min. The agarose overlay was then removed and fixed monolayers were stained with crystal violet in 20% ethanol to visualize viral plaques.

2.6. Determination of pulmonary eosinophilia in BAL fluid

Mice were sacrificed at the indicated times post-infection. Lungs and trachea were excised and flushed with 1 ml of PBS using a blunted 23-gauge needle. Cells were collected by centrifugation at 2000 rpm for 5 min. The cells were re-suspended and RBCs were lysed with ACK-lysis buffer. Cells were then washed twice with PBS and re-suspended in FACS buffer. Cell numbers were counted using a hemocytometer. Cells were then stained with fluorochrome-conjugated mAbs specific for SiglecF and CD11c, and analyzed using a FACS-Calibur as previously described (Stevens et al., 2007).

2.7. Statistical analysis

Data were analyzed by Student's *t*-test (two tails, unpaired) unless otherwise noted. A *p*-value of <0.05 was considered to be significant. Data are expressed as mean ± S.E.M.

3. Results

3.1. IFN-β treatment accelerates lung viral clearance

The therapeutic potential of IFN-β treatment against influenza virus infection was examined in a murine respiratory infection model. Briefly, C57BL/6J mice were infected by intranasal inhalation (i.n.) with a sub-lethal dose of A/WSN/33 virus (500 PFU, H1N1). At 24 h post-infection, mice received either 1×10^5 U of IFN-β or an equivalent volume of sterile PBS by intra-peritoneal