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

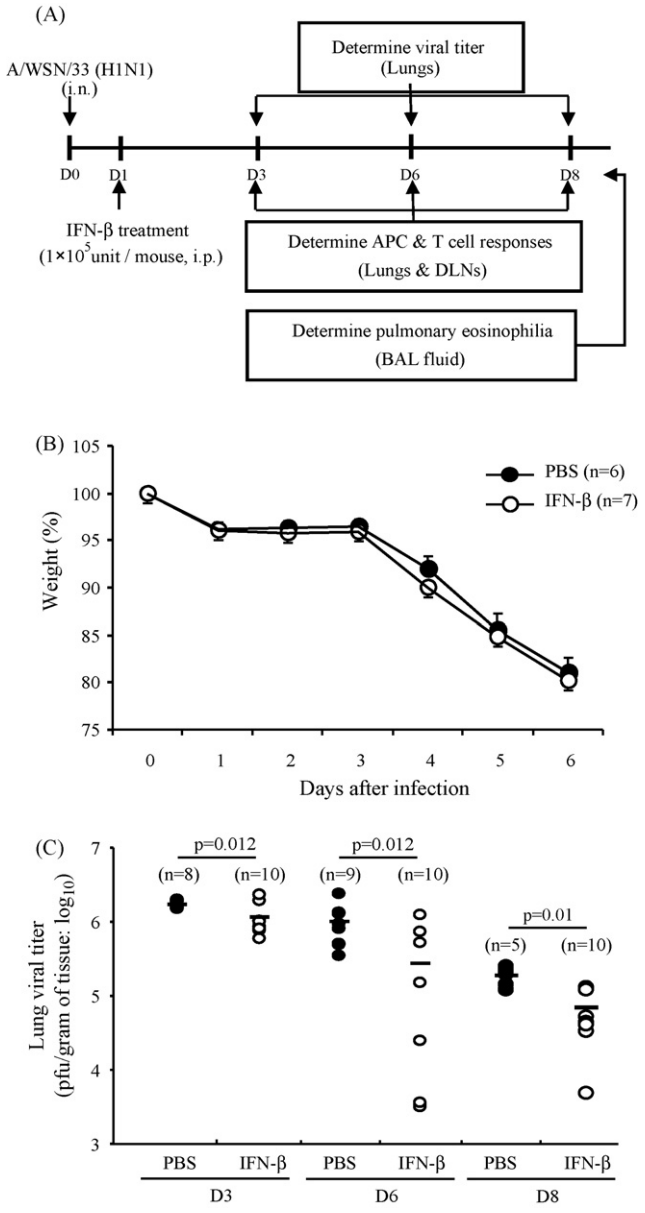

Fig. 1. IFN-β treatment induces accelerated viral clearance in influenza virus-infected mice. (A) Mice ($n = 60$) were infected by intranasal (i.n.) instillation with 500 PFU of influenza A/WSN/33 virus. At 24 h post-infection, mice received either IFN-β (1×10^5 U/mouse) or sterile PBS by i.p. injection. (B) Weight loss was monitored daily and is shown as percent weight loss relative to uninfected controls (mean ± S.E.M.). Mice were sacrificed on days 3, 6 and 8 post-infection and the mediastinal LNs (DLN), lungs and BAL fluid were harvested. (C) Lung viral titers were determined at the indicated time points. Data are representative of two independent experiments and were analyzed using Student's *t*-test.

injection (i.p.). To monitor clinical symptoms of influenza virus infection, mouse weight loss was evaluated daily for 6 days. The infecting dose of A/WSN/33 virus used in these studies results in a non-lethal influenza infection, from which mice recover and eliminate virus. Thus, weight loss over the time period examined, as anticipated, was modest. IFN-β treatment did not affect the weight loss profile of infected mice (Fig. 1B).

On days 3, 6, and 8 post-infection, mice were euthanized, and their lungs, DLNs and BAL fluid were harvested for analysis (Fig. 1A). Viral titers were determined in lung tissues at each time point post-infection, using a standard MDCK plaque assay. Both untreated and IFN-β treated mice exhibited viral clearance from their lungs over the time course examined. Consistent with previous reports (Beilharz et al., 2007; Kugel et al., 2009; Osterlund et al., 2010; Szretter et al., 2009; Van Hoeven et al., 2009), IFN-β treatment accelerated viral clearance from lung tissues compared to control, PBS-treated mice (Fig. 1C). Notably, a single treatment dose, 24 h after virus infection, elicited this response.

3.2. IFN-β modulates APC migration during a pulmonary influenza virus infection

Next, we characterized the immune response following IFN-β treatment. Since IFN-αs/β exert both direct and indirect effects on an immune response, including specific effects on antigen-presenting DCs (Theofilopoulos et al., 2005), we examined the consequence of a single dose of IFN-β on APC responses during influenza virus infection in both lungs and DLNs. Recently, we reported that there are two distinct APCs that play a role in pulmonary influenza virus infection: DCs (cDCs: $CD11c^{high}B220^{-}$ and pDCs: $mPDCA\text{-}1^{+}CD11c^{int}$) and LAPCs ($mPDCA\text{-}1^{+}CD11c^{-}B220^{-}TcR\beta^{-}$) (Yoo et al., 2010). Following pulmonary influenza virus infection, a significant accumulation of both DCs and LAPCs was observed in lung tissues during the early phase post-infection (Fig. 2A). A single treatment dose of IFN-β enhanced cDC accumulation in lung tissue on days 6 and 8 post-infection, both in terms of absolute cell numbers (Fig. 2A) and percent of total cell population (data not shown). However, IFN-β treatment did not significantly affect pDC or LAPC accumulation in the lungs.

By contrast, IFN-β exerted opposing effects on cDC and LAPC accumulation in the DLN of influenza virus-infected mice: cDC accumulation increased on days 3 and 6 post-infection, but LAPC accumulation was diminished, most notably on day 8 post-infection (Fig. 2B). In an earlier report we provided evidence that IFN-β modulates pDC accumulation in LNs through CD69-mediated down-regulation of S1P4 expression on pDCs (Gao et al., 2009). In the present study we observed only a modest increase in the pDC population in the DLN of influenza virus-infected mice on day 6 post-infection, following IFN-β treatment, both in terms of absolute cell numbers (Fig. 2B) and percent of total cell population (data not shown).

During the acute phase of a pulmonary influenza virus infection, the majority of cDCs and LAPCs that accumulate in the DLN originate from infected lung tissues (GeurtsvanKessel et al., 2008; Yoo et al., 2010). cDCs and LAPCs migrate into the DLN from the lungs with distinct migration kinetics. DC migration into the DLN occurs early, with maximal infiltration on day 3 post-infection (Kim and Braciale, 2009; Yoo et al., 2010). By contrast, LAPC migration occurs later, with maximal infiltration on day 8 post-infection (Yoo et al., 2010). We provide evidence that IFN-β treatment augments cDC migration but diminishes LAPC migration from infected lung tissues into the DLN.

IFN-β treatment did not alter the activation levels of DCs and LAPCs, as the surface expression of MHC-II (I-A/E), CD40, CD80 and CD86 on DCs (day 3 post-infection) and LAPCs (day 8 post-infection) in the DLN of IFN-β treated mice was comparable to that observed for control mice (Fig. 3).

3.3. IFN-β skews the immune balance towards a protective T1 immunity

DCs and LAPCs play distinct roles in pulmonary influenza virus infection. Our previous data suggest that DCs modulate anti-influenza T1 immunity in the DLN during the early phase post-infection and that LAPCs induce anti-influenza T2 immunity at later stages post-infection (Yoo et al., 2010). Accordingly, in the next series of experiments, we examined anti-influenza effector T cell responses in the DLN and lung tissue of IFN-β-treated, influenza virus-infected mice.

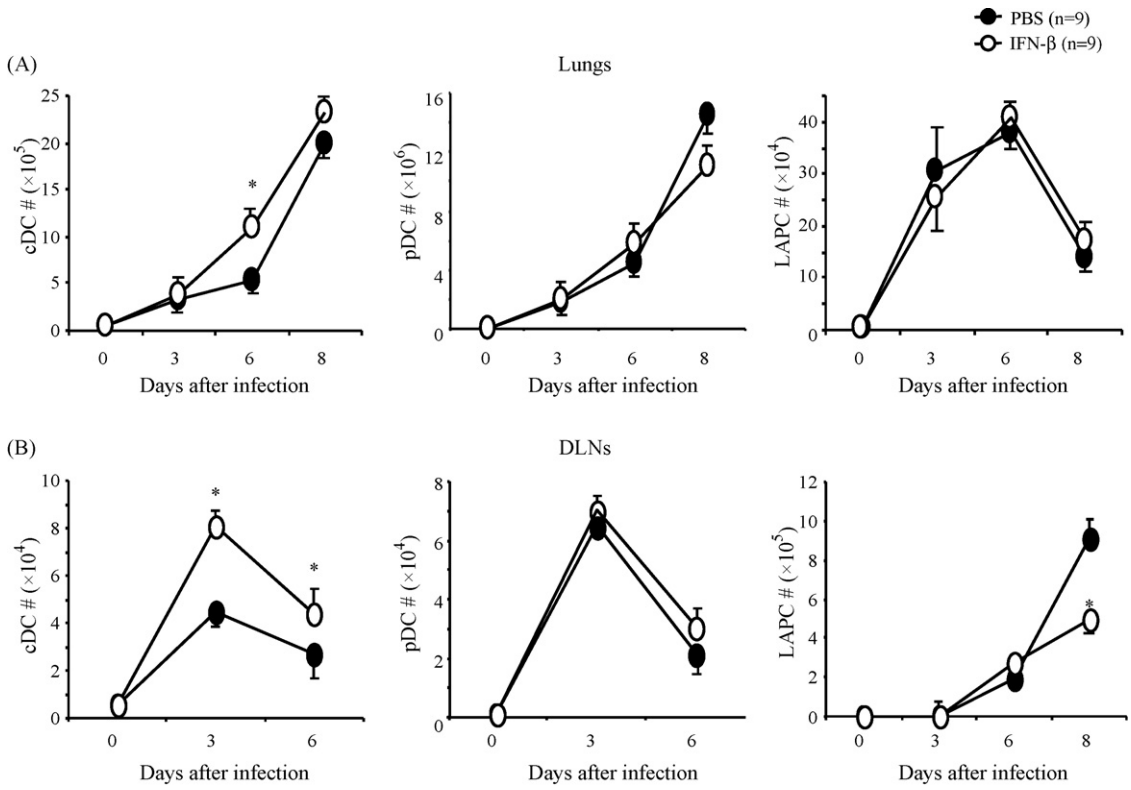

Fig. 2. IFN-β treatment modulates the migratory activity of APCs during pulmonary influenza virus infection. C57BL/6J mice ($n = 24$) were infected i.n. with 500 PFU of A/WSN/33 influenza virus. At 24 h post-infection, mice received either IFN-β (1×10^5 U/mouse) or sterile PBS by i.p. injection. At the indicated times post-infection (days 0, 3, 6 and 8), mice were sacrificed and their DLNs (mediastinal LNs) and lung tissues were harvested, the cells collected and stained with appropriate fluorochrome-conjugated mAbs to monitor DC (cDCs: CD11c^{high}B220$^-$TcRβ$^-$ and pDCs: mPDCA-1$^+$CD11c^{int}) and LAPC (mPDCA-1$^+$CD11c$^-$TcRβ$^-$B220$^-$) infiltration into (A) lungs and (B) DLNs by FACS-analysis. The absolute number of DCs and LAPCs for the indicated time points post-infection is shown. Data represent pooled results from three independent experiments ($n = 75$ total) and are shown as mean ± S.E.M. *Compared to PBS-treated mice, $p < 0.05$.

Consistent with published data (Carding et al., 1993), we observed induction of T1 effector T cell responses (T_H1:Thy1.2$^+$CD4$^+$IFN-γ$^+$ and CTL:Thy1.2$^+$CD8α$^+$IFN-γ$^+$) early post-infection (from day 3 post-infection) in the DLN, which coincided temporally with DC infiltration into the DLN. The anti-influenza T2 effector T cell response (T_H2: Thy1.2$^+$CD4$^+$IL-4$^+$) was induced after 6 days post-infection, coinciding with LAPC infiltration into the DLN (Fig. 4A). Interestingly, a single dose of IFN-β selectively augmented anti-influenza T1 effector T cell responses: in the DLN the T_H1 response was augmented on days 6 and 8 post-infection and CTL responses were enhanced on days 3 and 6 post-infection, both in terms of absolute numbers of T_H1

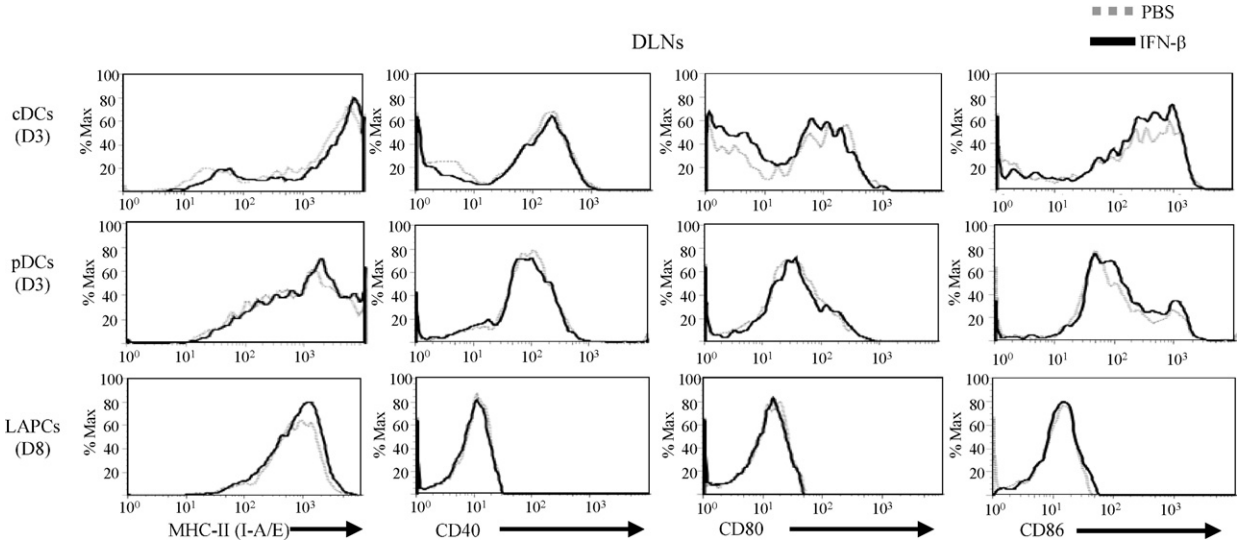

Fig. 3. IFN-β treatment does not enhance APC activation. C57BL/6J mice ($n = 24$) were infected i.n. with 500 PFU of A/WSN/33 influenza virus. At 24 h post-infection, mice received either IFN-β (1×10^5 U/mouse) or sterile PBS by i.p. injection. At the indicated times post-infection (D3 and 8), mice were sacrificed and their DLNs were harvested, the cells collected and stained with appropriate fluorochrome-conjugated mAbs to monitor by FACS the surface expression of MHC-II (I-A/E), CD40, CD80 and CD86 molecules, for the indicated populations. Representative staining from three independent experiments is shown: PBS-treated mice (dashed line); IFN-β treated mice (bold line).

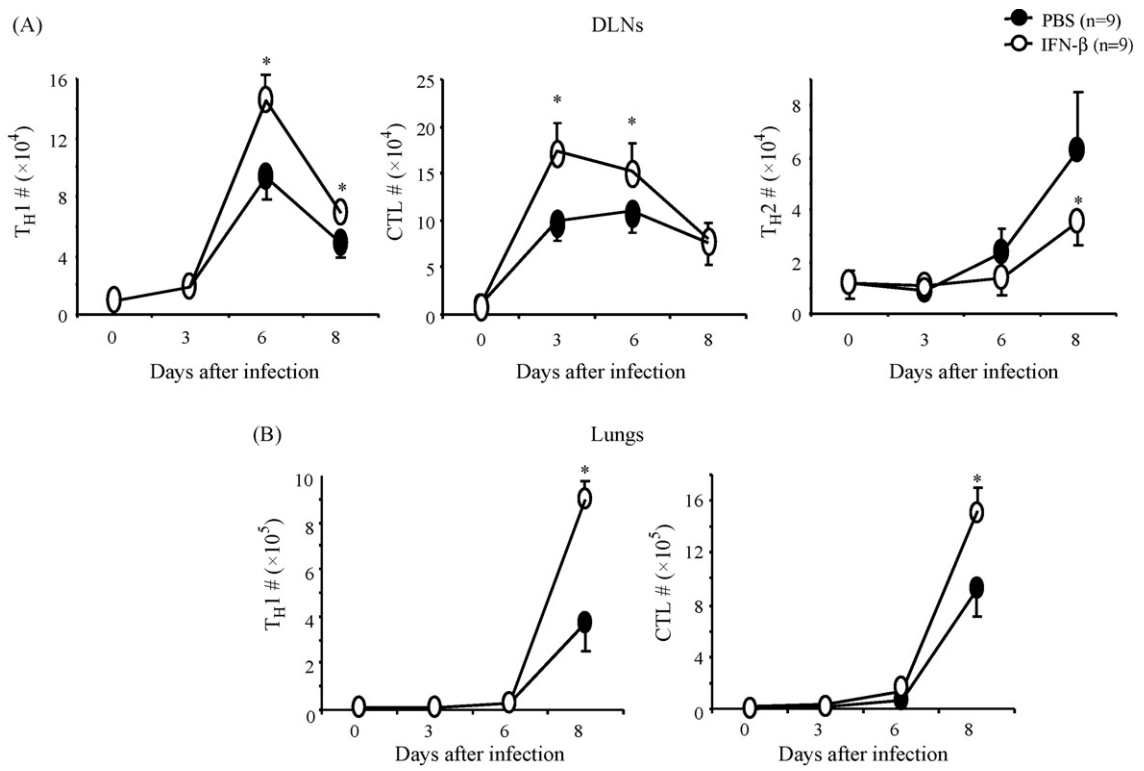

Fig. 4. IFN-β treatment polarizes the immune response towards T1 immunity. C57BL/6J mice ($n = 24$) were infected i.n. with 500 PFU of A/WSN/33 influenza virus. At 24 h post-infection, mice received either IFN-β (1×10^5 U/mouse) or sterile PBS by i.p. injection. At the indicated times post-infection (day 0, 3, 6 and 8), mice were sacrificed and their. (A) DLNs (mediastinal LNs) and (B) lung tissues were harvested, the cells collected and stained with appropriate fluorochrome-conjugated mAbs to monitor T1 effector (T_H1: Thy1.2$^+$CD4$^+$IFN-γ$^+$ and CTL: Thy1.2$^+$CD8α$^+$IFN-γ$^+$) and T2 effector (T_H2: Thy1.2$^+$CD4$^+$IL-4$^+$) T cell responses. The absolute number of T1 and T2 effector cells for the indicated time points post-infection is shown. Data represent pooled results from three independent experiments ($n = 75$ total) and are shown as mean ± S.E.M. *Compared to PBS-treated mice, $p < 0.05$.

and CTLs (Fig. 4A) and their percent of the total cell population (data not shown). Moreover, we observed enhanced T1 effector T cell responses (both T_H1 and CTL) in influenza virus-infected lung tissues from IFN-β treated mice, attaining statistical significance on day 8 post-infection (Fig. 4B). This may be a consequence of increased numbers of T1 effector T cells migrating into the lung tissue from the DLN. However, IFN-β treatment diminished the T2 effector T cell response in the DLN, apparent on day 6 post-infection and reaching statistical significance on day 8 post-infection (Fig. 4A).

3.4. IFN-β treatment inhibits pulmonary eosinophilia

Anti-influenza T2 immunity is implicated in the immunopathology associated with primary influenza virus infection (Graham et al., 1994; Kaji et al., 2000). Pulmonary eosinophilia, a classical T2 inflammatory response, is associated with influenza virus infection (Buchweitz et al., 2007; van der Klooster et al., 2004). Eosinophilia can be induced by T2 pro-inflammatory cytokines in the lungs and is exacerbated following adoptive transfer of anti-influenza T_H2 T cell clones (Fort et al., 2001; Graham et al., 1994; Hurst et al., 2002). Previsouly, we have shown that influenza virus-activated LAPCs modulate pulmonary eosinophilia by inducing T_H2 effector T cell generation and the production of IL-5 and eotaxin in lung tissue (Yoo et al., 2010).

IFN-β selectively inhibited LAPC-mediated anti-influenza T2 effector T cell responses in the DLN. Accordingly, we next examined whether IFN-β treatment affects T2-mediated pulmonary pathology in response to influenza virus infection. We examined pulmonary eosinophilia in BAL fluid on day 8 post-influenza

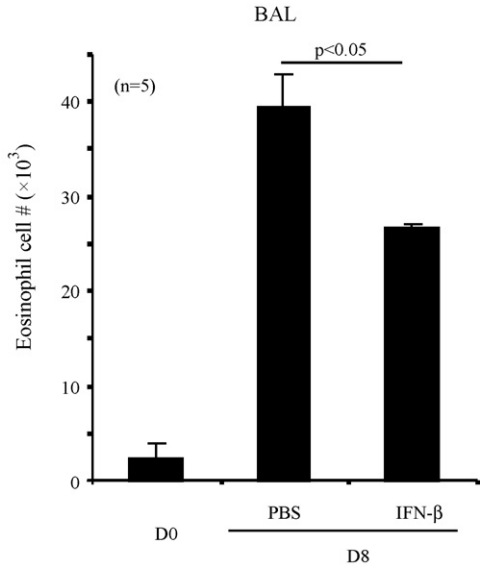

Fig. 5. IFN-β treatment decreases T2-mediated pulmonary eosinophilia. C57BL/6J mice ($n = 10$) were infected i.n. with 500 PFU of A/WSN/33 influenza virus. At 24 h post-infection, mice received either IFN-β (1×10^5 U/mouse) or sterile PBS by i.p. injection. On day 8 post-infection, mice were sacrificed, their BAL fluid harvested, the cells collected and stained with appropriate fluorochrome-conjugated mAbs to monitor eosinophil (SiglecF$^+$CD11c$^-$) infiltration by FACS-analysis. The absolute number of eosinophils is shown. Data representative of two independent experiments are shown as mean ± S.E.M. and were analyzed using Student's *t*-test.

virus infection, comparing PBS-treated and IFN-β treated mice. As shown in Fig. 5, pulmonary influenza virus infection induced a significant infiltration of eosinophils into the lungs at day 8 post-infection. A single dose of IFN-β inhibited pulmonary eosinophilia, approximately two-fold. Notably, this correlates with the selective inhibition of LAPC migration and diminished T_H2 effector T cell responses in the DLN.

4. Discussion

IFN-αs/β are critical effectors of the innate immune response to viral infections (Isaacs and Lindenmann, 1957; Isaacs et al., 1957). Neutralizing antibodies to IFNs exacerbate many viral diseases (Jiang et al., 2004) and many viruses, including influenza viruses, have developed complicated evasion mechanisms to counteract the production and/or antiviral activities of IFNs (Grandvaux et al., 2002).

The type I IFNs constitute a family of multi-member cytokines (IFN-αs, -β, -ε, -κ, -ω, -δ, -τ, and -ζ). From an immunological perspective, the IFN-αs and IFN-β are critical. IFN-β is encoded by a single gene, while both the human and mouse genomes contain 13 functional *Ifn*-α genes (Baig and Fish, 2008). IFNs-α/β bind with high affinity to a cell surface receptor complex comprised of IFNAR1 and IFNAR2 transmembrane subunits, thereby invoking a cascade of signaling events that lead to the transcriptional activation of numerous IFN-stimulated genes (ISGs) (Platanias, 2005). Many ISGs encode proteins with antiviral activities, such as PKR, 2′,5′-oligoadenylate synthetase, RNase L, and Mx proteins. Since Friedman and Ramseur (1974) reported that IFN treatment inhibited murine leukemia virus infection, the antiviral activities of type I IFNs, especially the IFN-αs, have been confirmed *in vivo* for many different viral infections, including HBV, HCV, herpes virus-8, HIV and influenza, in both humans and mouse models (Antman and Chang, 2000; Beilharz et al., 2007; Greenberg et al., 1976; Hoofnagle and Seeff, 2006; Kugel et al., 2009; Osterlund et al., 2010; Szretter et al., 2009; Van Hoeven et al., 2009). Despite the fact that these studies have identified the potent antiviral activity of IFNs, their immunomodulatory effects that contribute to viral clearance have not been extensively addressed.

There is accumulating evidence for immunomodulatory roles for IFNs-α/β: inducing the maturation and activation of DCs, promoting B cell differentiation to plasma cells, influencing NK cell activity, contributing to T cell development and functionality and having a critical role in myelopoiesis (Deonarain et al., 2003; Theofilopoulos et al., 2005). Here we provide evidence of IFN-ß treatment enhancing cDC accumulation in lung tissues (Fig. 2A). This may either be a consequence of increased recruitment of cDC precursors into inflamed lung tissue, mediated by IFN-augmented CCL3 production (Salazar-Mather et al., 2002; Yoneyama et al., 2005), or IFN-inducible enhanced cDC maturation of lung-infiltrated cDC precursors (Luft et al., 1998; Pogue et al., 2004). IFNs have been implicated in modulating cellular migration from inflamed tissues into secondary lymphoid organs (Asselin-Paturel et al., 2005; Luft et al., 1998; Parlato et al., 2001). During inflammation, mature cDCs migrate from inflamed tissue into the DLN *via* the lymphatics, this chemotaxis is mediated by CCR7-CCL21 interactions. pDCs migrate through high endothelial venules (HEVs) *via* CXCR3-CXCL9 mediated chemotaxis (Yoneyama et al., 2005). Given the evidence for IFN increasing DC responsiveness to CCL21 but not to CXCL9 (Asselin-Paturel et al., 2005; Lande et al., 2003; Parlato et al., 2001), this might account for the selective enhanced cDC migration that we observed (Fig. 2B).

In a recent publication we provided evidence that IFN-β regulates the accumulation of pDCs in LNs, mediated by a SIP4-dependent process (Gao et al., 2009). Comparing the retention/accumulation of pDCs in LNs in wildtype and mice null for IFN-β, we proposed that the constitutive basal levels of IFN-β in wildtype mice that mediate the retention of pDCs in the LNs, required for immune surveillance, likely effect a chronic down-regulation of IFN receptors on pDCs, thereby affecting their subsequent responsiveness to IFN-β. Notably, treatment with IFN-β had a modest effect on the accumulation of pDCs in the DLNs of wildtype mice at 24 h post-infection with influenza virus, in keeping with the notion that at this time point, wildtype pDCs make abundant endogenous IFN-β in response to viral infection. In agreement with this, IFN-β treatment of IFN-β null mice had quite a dramatic effect on pDC retention in the DLN, suggesting that in the first 24 h post-infection, IFN-β is important for the retention of pDCs in DLNs (Gao et al., 2009). In the present study, IFN-β treatment did not affect wildtype pDC accumulation in the DLNs of infected mice at days 3 and 6 post-infection. We hypothesize that at these later times any virus-inducible IFN-β that has been produced may also contribute to IFNAR down-regulation on the pDCs, and/or be masked by the effects of endogenous IFN-β production by wildtype pDCs, further limiting their responsiveness in the context of LN accumulation.

Interestingly, in contrast to the enhanced IFN-β inducible cDC migration into the DLN, our data suggest an opposite effect on LAPCs. IFN-β treated mice showed diminished LAPC migration into the DLNs at later times phase post-infection (Fig. 2B). These results suggest that cDCs and LAPCs may use distinct migratory mechanisms that are differentially modulated by IFN-β. The underlying mechanism for LAPC migration into the DLN and IFN-β mediated selective modulation remains to be addressed, and is the subject of our ongoing investigations.

Previously, we have shown that as APCs, DCs and LAPCs have distinctive roles in pulmonary influenza virus infection: adoptive transfer of influenza virus-activated DCs or LAPCs by intravenous injection augmented type 1 or type 2 effector T cell responses, respectively, in the DLN of influenza virus-infected recipient mice (Yoo et al., 2010). Influenza virus-activated DCs induce T1 effector T cell responses (T_H1 and CTL) early post-infection, which are implicated in viral clearance and immunoprotection. LAPCs modulate anti-influenza T2 immunity later during the course of infection, associated with eosinophilia. Here, we provide evidence that IFN-β polarizes the immune balance towards T1 immunity by selective modulation of DC and LAPC migration into the DLN. IFN-β treated mice exhibited augmented T1 but diminished T2 effector T cell responses (Fig. 4). Accordingly, accelerated viral clearance in lung tissues and diminished pulmonary pathology, reflected by decreased pulmonary eosinophilia, were observed in IFN-β-treated mice compared to control mice (Figs. 1C and 5). In these studies, the infecting virus (A/WSN/33) and the virus infective dose used result in a non-lethal influenza infection, from which mice recover and eliminate virus. Thus, weight loss over the time period examined, was modest. Influenza virus infection causes lung tissue damage mediated by local inflammatory responses including infiltrating T1 effector T cells and eosinophils (Graham et al., 1994; Sun et al., 2009), which affect pulmonary function, thereby contributing to disease symptoms such as weight loss. In this study, IFN-β treatment augmented anti-influenza T1 effector T cell responses but diminished eosinophilia in lung tissue (Figs. 4 and 5). Therefore, cognizant that weight loss serves to reflect lung tissue damage – decreased pulmonary function – we infer that the IFN-inducible enhanced T1 effector responses yet diminished eosinophilia, effectively counteract one another in the context of pulmonary function, reflected as overall modest weight loss, a consequence of the infection. Notably, we did not identify either diminished or aggravated symptoms of compromised lung function – weakness, lethargy – in the mice treated with IFN-β. In the absence of any histological evidence for IFN-inducible T_H1 and CD8-driven tissue damage, we infer that there is no IFN-inducible tissue damage. Indeed, results

from a study in STAT-1$^{-/-}$ mice, deficient in type I IFN signaling, show a T_H2 biased host immune response against influenza A virus (A/PR/8/34) infection, with exacerbated immunopathology characterized by granulocytic pulmonary inflammatory infilitrates (Durbin et al., 2000). In other studies, we have obtained evidence for the therapeutic effects of IFN-α treatment in respiratory influenza A virus infection (unpublished data). The associated immunomodulatory effects of IFN-α treatment are the subject of ongoing investigations.

Presently, there are a limited number of clinically approved antiviral agents for influenza virus infections: the M2 ion channel-blockers (adamantanes) – amantadine and rimantadine, and the neuraminidase inhibitors – zanamavir and oseltamivir. Although adamantanes, which block the function of the M2 protein, can reduce the severity and duration of influenza A infection in healthy adults, their use has been limited due to rapid induction of resistant viruses during treatment. In recent years, a high percentage of influenza A (H3N2) viruses circulating in Asia, the USA and eastern Europe and all isolates of the pandemic H1N1 2009 exhibit resistance to adamantanes. Moreover, among influenza A (H1N1) viruses, resistance to oseltamivir has been reported in southern Europe, Hong Kong, Denmark, Japan and Canada (Deyde et al., 2007; Harper et al., 2005; Lackenby et al., 2008), which raises serious concerns relating to the future efficacy of these agents as antivirals.

In animal models of influenza virus infection, IFNs-α/β effectively inhibit both H1N1 and H5N1 viral replication (Kugel et al., 2009; Szretter et al., 2009; Van Hoeven et al., 2009). Herein we show that IFN-β treatment has beneficial effects on the pulmonary immune response to primary influenza A virus infection. Viewed altogether, these results demonstrate that IFN-β is an effective antiviral controlling both viral replication and immunopathology. In ongoing studies we are evaluating the safety and efficacy of IFN treatment in hospitalized individuals with flu-like illness.

Disclosure Statement

Darren P. Baker is an employee of Biogen Idec, Inc.

Acknowledgements

These studies were supported by a Canadian Institutes of Health Research grant MOP-15094 to E.N.F. ENF is the recipient of a Tier 1 Canada Research Chair. J.K.Y. was supported by a Connaught Scholarship and an Ontario Graduate Scholarship in Science and Technology.

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