Oral type 1 interferon protection from lethal influenza virus

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Abstract. The persistence of highly pathogenic avian influenza virus within wild bird populations has forged interest in control measures to limit a possible human pandemic. We have identified an optimal low oral dose of IFN-α that when delivered daily as prophylactic therapy protects C57BL/6J mice from a lethal challenge with mouse adapted human influenza virus A/PR/8/34 H1N1. These results provide strong support for the application of low dose type 1 IFN pretreatment to human influenza control. Additionally, recent data suggests that low dose oral IFN-αA treatment of influenza causes the recruitment of Regulatory T cells from the spleen to the site of inflammation; this response may form the basis of the observed protection from lethal infection.

Keywords: Interferon (IFN); Influenza virus; Innate immunity.

INTRODUCTION

The innate immune response to respiratory viruses initially involves the nasal production of type 1 interferons (IFNs) (Uthaisangsook et al., 2002), the binding of these IFNs to their cognate receptors on oromucosal surfaces (Bosio et al., 1999), followed by the generation of a systemic immune response to the virus (Durbin et al., 2001). Lethal influenza infections are the result of extensive tissue damage caused by viral replication and to a larger extent the induction of an overly aggressive host immune response (Bruder et al., 2006). Studies investigating influenza infection in mice deficient in multiple IFN signalling pathways established that the loss of a functional type 1 IFN system led to exacerbated disease due to excessive cellular inflammatory infiltrate. NS1, a non structural influenza protein, antagonises the interferon mediated antiviral response and has been strongly associated with the virulence of highly pathogenic strains affecting the level of viral replication, inflammation and the rate of infection resolution (Cauthen et al., 2006). These findings demonstrate the important role type 1 IFN plays in the regulation of the immune response to influenza and suggests a more regulated response as part of the underlying mechanism behind successful IFN treatment of influenza. Regulatory T cells (Tregs) are now recognized as key mediators in the control of the immune system. They are a normal component of mucosal surface immunology and have been identified as central players in the regulation of immune responses within the gastrointestinal (Asseman and Powrie, 1998 and respiratory tracts (van Oosterhout and Bloksma, 2005). These characteristics prompted our investigation into the relative frequency of Treg populations in lung and spleen tissue following influenza infection and low dose oral type 1 IFN treatment in mice.

MATERIALS AND METHODS

Mice. Female, 6-8 week old C57BL/6J mice were obtained from the Animal Resource Centre (Perth, Western Australia) and housed in specified pathogen free conditions (Discipline of Microbiology and Immunology, University of Western Australia). All animal work was carried out in accordance with the guidelines of The National Health and Medical Research Council of Australia and the approval of The University of Western Australia’s Animal Ethics Committee.

Interferon. Recombinant mouse IFN-αA (PBL Biomedical Laboratories, USA) was diluted to experimental concentrations with 0.1% bovine serum albumin (BSA) (JRH Biosciences, USA) in phosphate buffered saline (PBS). For delivery of IFN mice were held in the intraperitoneal (ip) injection position and administered 10 µl of solution containing IFN or 0.1% BSA directly into the mouth using a 10 µl micropipette.

Virus stock and inoculation. Mouse adapted human influenza A/PR/8/34 (PR8) virus stocks were propagated in the allantoic fluid of 10 to 12 day old embronated hen eggs. Mice were anaesthetized by methoxyfluorane (MDA, Australia)
inhalation before intranasal inoculation with 30 μl mouse adapted human influenza A/PR/8/34 (PR8) at a titre of 10^2.4 TCID50 as determined in Madin-Darby canine kidney cells. Analysis of lung and spleen tissue by Flow cytometry Single cell suspensions were prepared and analysed as previously described (Beilahrz, et al., 2007).

RESULTS

Figure 1: U-shaped dose response to treatment with IFN-αA at day 7 pi. The efficacy of a range of IFN-αA doses for the treatment of influenza was tested. Weight loss at day 7 post infection is shown. Optimal inhibition of weight loss was observed following treatment with 100 IU IFN-αA or 0.1% BSA directly into the mouth (day -7 to day 0). On day 0 mice were inoculated. Daily treatment with oral doses of IFN-αA or 0.1% BSA continued throughout the experiment and body weight and rectal temperatures were monitored as reliable indicators of disease progression. All oral IFN-αA treatment groups demonstrated weight and temperature readings analogous to untreated group data from day -7 to post infection (pi) demonstrating that IFN prophylaxis had no adverse effects on these parameters. From day 5 pi onwards all groups demonstrated weight and temperature loss associated with the influenza respiratory infection. On day 7 and 8 pi, mice within the control, 1 IU, 10 IU, 1000 IU and 10 000 IU treatment groups were culled due to dramatic temperature and weight loss together with behavioural alterations associated with morbidity. Culling of the severely distressed animals was in accordance with Australian State and Federal animal welfare requirements. In contrast to control animals fed 0.1% BSA, mice treated daily with 100 IU IFN-αA presented with relatively healthy body weight and temperatures. Mice in the 100 IU IFN-αA treatment group were continued on daily oral IFN-αA until day 12 pi. Steady weight gain, normal body temperature and behaviour over this final period show that C57BL/6j mice can be protected from a lethal intranasal PR8 challenge through prophylactic administration of murine IFN-αA given orally at the low dosage of 100 IU (Figure 2). These remarkably clear and repeatable results are in themselves worthy of note in relation to influenza epidemic control measures.

Oral IFN-αA therapy reduced influenza virus replication. Type 1 IFNs are known antiviral agents capable of limiting viral replication through the induction of protein kinase R, the 2’5’ OAS/RNase L system and MxA along with other IFN stimulated genes. We examined lung tissue harvested from day 7 pi mice treated with 100 IU and 10 000 IU to determine whether successful oral IFN-αA treatment correlated with a decreased viral load. RNA was extracted and analysed by real time PCR using primers specific for the influenza matrix gene M1 as previously described (Beilharz et al., 2007). Our results demonstrate that oral administration of both 100 IU (p< 0.05) and 10 000 IU (p< 0.15) IFN-αA reduced viral replication following intranasal inoculation with PR8. The reduction in viral replication does not however correlate completely with improved clinical outcome, as seen in the weight and temperature loss observed in animals treated with 10 000 IU. These findings indicate that the positive effects observed following 100 IU IFN-αA treatment are not solely due to the inhibition of viral replication.

A reduction in splenic regulatory T cells is associated with successful oral type 1 IFN treatment of influenza. Regulatory T cells func-
tion in the maintenance of immune homeostasis, induction of tolerance and potentially play a role in the resolution of infections. These characteristics prompted our investigation into the relative frequency of Treg populations in lung and spleen tissue following PR8 infection and successful low dose oral type 1 IFN treatment. Regulatory T cells were identified as CD4+ cells co-expressing the transcription factor Foxp3. Lung and spleen tissue was harvested at day 7 or day 8 pi as mice were culled due to significant weight and temperature loss associated with infection. Mice successfully treated with 100 IU IFN-αA were also sampled at this time point for comparative analysis. A significant (p<0.002) reduction in splenic Tregs at a dose of 100 IU IFN-αA was observed (Figure 3). This response correlates with increased survival following treatment with 100 IU IFN-αA and may represent the migration of cells to the site of inflammation. In lung tissue, a 2.5% (p<0.03) increase in the frequency of Tregs was observed following treatment with 100 IU IFN-αA. This increase in Treg frequency at the site of infection may form the basis of protection seen following successful IFN treatment.

DISCUSSION

In the late 1960’s and early 1970’s leukocyte IFN was available as nasal spray preparations in Moscow pharmacies for use as both prophylaxis and treatment of influenza [9]. The reported success of these trials prompted the interest of Western scientists who subsequently tested these claims using human volunteers, stronger type 1 IFN preparations and standardised influenza challenges [10]. The reported effects on influenza were minimal, and side effects from intranasal administration of high dose type 1 IFNs (>106 International Units, IU) were considerable. Current “bird flu” concerns prompted us to re-examine the earlier Soviet reports that had used a natural infection protocol, the flu season in Moscow, rather than a fixed large viral challenge, and relatively crude cell supernatant interferon of low concentration. More recently “bell” shaped dose response curves to oral type 1 IFN have been reported in a variety of animal studies (Bosio et al., 1999 and Satoh et al., 1999) suggesting that either the large viral challenge or the high dose of IFN administered in the western trials could account for the contradictory results reported. It is likely optimal low doses of type 1 IFN mimic a natural response that is able to counteract viral virulence factors. Considering the growing concern that pathogenic avian influenza strains endemic in wild bird populations of Cambodia, China, Indonesia and Malaysia may adapt and become transmissible between humans, these results generated in a murine model suggest an immediate re-evaluation of low dose oral type 1 IFN both as a prophylactic and therapeutic intervention for influenza infection in humans.

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REFERENCES


