

SHORT COMMUNICATION

Innate interferons inhibit allergen and microbial specific T_H2 responses

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Several studies provided evidence of innate interferons (IFNs) regulating T_H2 cytokine production using purified CD4⁺ memory cells and T_H2 polarisation via interleukin-4 (IL-4). Vitally, none of these previous studies examined IFN attenuation of T_H2 responses to allergen or antigen. This study therefore sought to investigate the abrogation of specific allergen- and antigen-stimulated T_H2 response in peripheral blood mononuclear cells (PBMC) derived from 12 sensitised individuals by IFN- β and IFN- λ . PBMC were cultured in the presence of house dust mite (HDM) allergen, rhinovirus (RV), influenza vaccine and tetanus toxoid (TT) $\pm$ either IFN- β or IFN- λ for 3 and 5 days. IFN- γ , IL-5 and IL-13 protein levels were measured by ELISA. Quantitative PCR (qPCR) was used to investigate induction of genes involved in control of T_H2 cytokines. No alteration in T_H1 IFN- γ allergen/antigen response was observed with addition of IFN- β or IFN- λ . Consistent abrogation of T_H2 response to HDM and influenza was observed with IFN- β at both time points; attenuation was observed by day 5 with RV and TT. IFN- λ had no consistent effect on T_H2 production except in the presence of RV (multiplicity of infection = 5); a decrease in IL-5 alone was observed in the presence of trivalent inactivated influenza vaccine. GATA binding protein 3 (GATA3) and suppressors of cytokine signalling3 mRNA were differentially regulated in HDM and influenza-stimulated cultures $\pm$ IFN- β . We concluded that IFN- β produced a strong and consistent abrogation of T_H2 cytokine production in the presence of a range of allergen and antigen stimulants.

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Respiratory viruses are associated with the majority of severe asthma exacerbations, with human rhinovirus (RV) infections being the most common viruses identified in children and adults.^{1–3} Atopy and asthma are associated with a reduced capacity to synthesise antiviral cytokines such as the type-I interferons (IFN- α /- β)⁴ and type-III IFN (IFN- λ)⁵ in response to respiratory infections.^{6,7} Administration of these recombinant IFNs has been proposed as a novel treatment for asthma exacerbations precipitated by respiratory virus infections.⁸

The role of the innate type-I IFN- α /- β in promoting T_H1 cytokine production, characterised by IFN- γ , have been thoroughly studied and shown not to be sufficient to drive T_H1 commitment (as reviewed by Huber and Farrar⁹); the role of IFN- λ in T_H1 stimulation has been examined in one study, which showed inconsistent modest elevations of IFN- λ .¹⁰ Atopy and asthma are characterised by a propensity to make T_H2 polarised immune responses to innocuous environmental antigens (allergens). There is substantial suggestive experimental evidence that innate IFN can inhibit T_H2 cytokine secretion, derived from somewhat artificial methodological conditions using polyclonal activation and/or cytokine-induced T_H2 polarisation.^{10–15} Our recent study investigated this further, using RV at a multiplicity of infection

(MOI) of 5 to stimulate peripheral blood mononuclear cells (PBMC) derived from healthy individuals. RV induced substantial T_H2 cytokine release, which could be inhibited by addition of exogenous recombinant type-I IFN.¹⁶ We then demonstrated that B18R recombinant protein blockade of type-I IFN signalling through interferon- α /- β receptor 1 (IFNAR), and depletion of the main source of type-I IFN in response to RV, plasmacytoid dendritic cells, resulted in an increase in T_H2 cytokine release from memory T-cells. Our findings thus supported a direct link between the innate and T_H2 adaptive immune response to RV in healthy individuals.¹⁶

The aim of the current short communication was therefore to determine whether innate IFNs can also inhibit T_H2 recall/memory responses to clinically relevant allergens and microbial antigens in PBMC derived from sensitised individuals. Although we did not detect type-III IFN production by PBMC in response to RV in our previous study,¹⁶ other groups have suggested an important role for these innate effectors in atopy and asthma.¹⁷ We therefore investigated the roles of IFN- β (type-I IFN) and IFN- λ_1 (type-III IFN) in the control of T_H2 cytokine production. Additionally, key molecules that might orchestrate these responses were examined, including IL-4R, GATA3, STAT6 and suppressors of cytokine signalling3 (SOCS3).

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RESULTS AND DISCUSSION

Unstimulated cultures, cultures stimulated with IFN- β alone and with IFN- λ_1 alone were all below the limit of detection for all ELISA assays (data not shown). The limit of detection was 3.91 pg ml⁻¹ for each target; undetectable protein was given a value of 1.95 pg ml⁻¹, which was half the lower limit of detection. The IFN- γ , IL-5 and IL-13 cytokine response patterns varied in response to the different antigens. The viral antigens trivalent inactivated influenza vaccine (TIV) and RV16 induced stronger T_H1 cytokine responses than house dust mite (HDM) and tetanus toxoid (TT). In accordance with the previous findings,^{9,16} IFN- β and IFN- λ had no effect on IFN- γ synthesis at day 3 or day 5, irrespective of the stimulus used (Table 1).

HDM and TIV induce substantially higher synthesis of the T_H2 cytokines IL-13 and IL-5 than RV16 and TT (Table 1). IFN- β significantly inhibited HDM- and TIV-induced IL-13 and IL-5 synthesis, with the effects most consistent at day 5 (Table 1). IFN- λ_1 had no effect on HDM-induced IL-13 and IL-5 synthesis or TIV-induced IL-13 production, but did induce a minor reduction in TIV-induced IL-5 synthesis (Table 1).

Investigating the effects of RV16 at an MOI of 1 revealed minimal induction of IL-13 synthesis at both day 3 and day 5 (Table 1); we therefore used an MOI of 5, as used in our recent study,¹⁶ to investigate the effect of IFN- β and IFN- λ_1 on T_H2 responses in atopic individuals. At an MOI of 5, RV16 induced greater IL-13 synthesis than an MOI of 1 at day 5 (median = 69 pg ml⁻¹ compared with 2.92 pg ml⁻¹, respectively), which was significantly inhibited by IFN- β (median = 45.2 pg ml⁻¹; $P = 0.008$) and by IFN- λ_1 (median = 43.7 pg ml⁻¹; $P = 0.007$; data not shown). TT elicited relatively weak T_H2 responses compared with the other stimuli, however, there was a suggestion that this production was also inhibited by IFN- β at both day 3 and day 5 (Table 1).

As IL-4 is difficult to detect in cell culture supernatants, expression levels of the mRNA encoding the IL-4 receptor, IL-4R, can be used as a marker of IL-4 activation. We examined expression levels in HDM- and TIV- stimulated cultures at day 3, as these conditions induced the strongest IL-5 and IL-13 responses; however, expression levels were not altered from unstimulated cultures at this time point (data not shown). As IL-5 and IL-13 were already upregulated by 3 days, it is possible this was not the optimal time point for detection of changes in *IL-4R* expression.

In order to investigate the potential pathways by which IFN- β and IFN- λ might be exerting suppressive effects on IL-13 and IL-5 production, we examined expression of *GATA3* and *STAT6*, which are transcription factors closely linked to T_H2 differentiation.¹⁸ Previous reports suggest that innate IFNs inhibit *GATA3* expression in T-cells at day 3, which correlated with decreased TH2 cytokine production.^{12,13,15} Expression levels of *STAT6* and *GATA3* were therefore measured by quantitative PCR in mRNA from PBMC stimulated by HDM and TIV, which induced the strongest T_H2 responses at day 3. Cells stimulated with HDM or TIV expressed the levels of *STAT6* and *GATA3* comparable with that of unstimulated cultures and no alteration in expression was observed in the presence of innate IFNs (data not shown). The previous studies showing differences in *GATA3* expression used high concentrations of IL-4 to stimulate naive cells to differentiate towards a TH2 phenotype.^{12,13} When Dai *et al.*¹³ investigated *GATA3* in memory T-cell responses to stimulant + IFN- λ , no difference in *GATA3* expression was observed. Together, these data suggest that *GATA3* is important in naive T cells that have undergone differentiation, but either it does not have a major role in changes in T_H2 cytokine responses in memory cells or there are differences in the timing of gene expression changes between the cell types.^{12,13}

Table 1 T_H1 and T_H2 cytokine production by PBMC stimulated with RV16, HDM, influenza vaccine or TT treated with IFN- β or IFN- λ_1

	IFN- γ Day 3		IL-13 Day 3		IL-5 Day 3		IFN- γ Day 5		IL-13 Day 5		IL-5 Day 5		P
HDM	82.29	(1.95-363.7)	—	72.88	(1.95-912)	—	199.3	(0-1101)	—	418.5	(1.95-4687)	—	—
HDM + IFN- β	137.9	(1.95-660.8)	NS	12.4	(0-377.9)	0.008	456.4	(1.95-3062)	NS	186.6	(1.95-2278)	0.004	0.004
HDM + IFN- λ_1	78.91	(22.73-233)	NS	39.66	(1.95-738.3)	NS	209.5	(1.95-1850)	NS	578.8	(1.95-5122)	NS	NS
TIV	1212	(14.29-2632)	—	24.51	(1.95-134.2)	—	4855	(35.81-26694)	—	177.9	(1.95-2397)	—	—
TIV + IFN- β	1261	(68.75-4927)	NS	8.63	(1.95-122.3)	0.039	4526	(220.2-9480)	NS	96.99	(1.95-1340)	0.023	0.008
TIV + IFN- λ_1	858.7	(46.69-2106)	NS	16.16	(1.95-114.6)	NS	3601	(74.59-15376)	NS	134.2	(1.95-514)	NS	0.003
RV16	204.1	(32.75-3433)	—	9.011	(1.95-177.6)	—	1301	(1.95-5278)	—	2.942	(1.95-45.37)	—	—
RV16 + IFN- β	310.3	(42.21-1186)	NS	1.95	(1.95-15.84)	NS	1038	(1.95-5379)	NS	1.95	(1.95-35.56)	NS	NS
RV16 + IFN- λ_1	220.2	(34.82-2032)	NS	11.07	(1.95-71.71)	0.016	671.2	(1.95-6393)	NS	1.95	(1.95-37.15)	NS	NS
TT	84.48	(1.95-240.8)	—	23.99	(1.95-103.8)	—	68.98	(1.95-1125)	—	28.86	(1.95-672.2)	—	—
TT + IFN- β	50.87	(1.95-423.4)	NS	1.95	(0-24.94)	0.031	205.1	(1.95-770.9)	NS	1.95	(1.95-270.7)	NS	0.031
TT + IFN- λ_1	30.95	(16.78-237.4)	NS	19.3	(1.95-31.51)	NS	195.5	(1.95-1406)	NS	1.95	(1.95-323.5)	NS	NS

Abbreviations: HDM, house dust mite; IFN, interferon; IL, interleukin; NS, non-significant ($P > 0.05$); PBMC, peripheral blood mononuclear cells; RV, rhinovirus; TIV, trivalent inactivated influenza vaccine (split virion); TT, tetanus toxoid. Data are shown as median protein concentration (minimum-maximum) in pg ml⁻¹. Statistical analyses were the comparison between stimulant alone and stimulant + IFN- β or stimulant + IFN- λ_1 , using the Wilcoxon matched pairs signed-rank test. Statistical significance is considered to be $P < 0.05$, indicated in bold. Data were not corrected for multiple statistical testing. IFN- γ representative of T_H1 and IL-13 and IL-5 (indicative of T_H2) protein production was measured in PBMC culture supernatant in response to stimulant (RV16, HDM, TIV and TT) and to stimulant in conjunction with IFN- β or IFN- λ_1 .

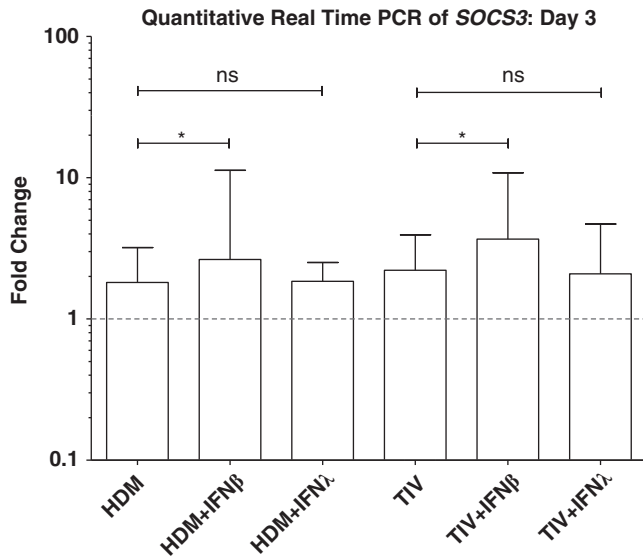


Figure 1 Quantitative PCR of SOCS3 at day 3 poststimulation. Quantification of *SOCS3* in the presence of HDM or TIV ± innate IFNs; qPCR data is normalised such that a fold change of 1 (indicated by the dotted line) is unchanged from unstimulated cultures. Data is shown as median fold change (interquartile range) and the *P* value derived from stimulant compared with stimulant + IFN-β or IFN-λ₁, as measured by Wilcoxon matched pairs signed rank test. **P* < 0.05. 'NS' = not statistically significant.

T-cell cytokine production is also influenced by the SOCS family of molecules. SOCS3 was particularly of interest significant owing to its capacity to regulate IL-4 production,¹⁹ and to antagonise the actions of IL-4 and IL-13.²⁰ Interestingly, we found that IFN-β caused significantly increased SOCS3 expression in HDM- and TIV-stimulated cells; IFN-λ₁ had no effect on SOCS3 expression (Figure 1). Interrogation of the SOCS3 promoter sequence using data from the transcription factor prediction programs TRANSFAC²¹ and ConTra,²² which use consensus sequences to predict potential binding sites, as well as data from the ENCODE project²³ revealed several response elements associated with factors stimulated by IFNAR1 activation by IFN-β, including IRF1, IRF4, NF-κB and STAT1. It is therefore plausible that IFN-β acts via these IFN stimulated the genes to upregulate expression of SOCS3.

CONCLUSION

Our current study extended the findings from our previous study into modulation of T_H2 responses to RV in healthy individuals¹⁶ by thoroughly investigating responses in PBMC derived from sensitised individuals to a variety of clinically relevant allergens and microbial antigens. We demonstrated that IFN-β is able to significantly inhibit T_H2 cytokine production and that IFN-λ₁ can also attenuate T_H2 cytokine production, but is less potent than IFN-β at 1000 U ml⁻¹. In addition, we identified a potential role for SOCS3 as part of the mechanism by which IFN-β inhibits T_H2 cytokine production.

There is an increasing interest in the use of topical recombinant IFNs to treat viral infections in patients with allergic diseases such as asthma, severe allergic rhinitis and atopic dermatitis. Our findings suggest that an additional benefit of this therapeutic approach might include the inhibition of T_H2 recall/memory responses elicited by allergens and microbial antigens, and potential improvement in

allergic inflammation. Testing this hypothesis should therefore be an important priority for future clinical studies.

METHODS

We have systematically characterised the effect of IFN-β and IFN-λ₁ on T_H2 cytokine production in PBMC from 12 HDM allergic individuals (50% female; skin test wheal diameter mean ± s.d. = 8.17 ± 2.11 mm). PBMC were stimulated with HDM (*Dermaphagoides pteronyssinus* extract, devoid of detectable lypopolysaccharide-like activity; kind gift from Professor P. Holt, Perth; used at 10 µg ml⁻¹), (TIV; CSL Ltd, Parkville, Victoria, Australia) used at 0.45 µg ml⁻¹, RV-16 (RV16; used at MOI of 1 and MOI of 5) or (TT; used at 0.5 Lf ml⁻¹).

IFN-β and IFN-λ₁ (eBioscience, San Diego, CA, USA) were used at a final concentration of 1000 U ml⁻¹, based on the induction of equivalent secretion of the IFN-induced chemokine CXCL10 to stimulation of PBMC with RV16 (MOI of 1) in preliminary dose-response experiments (data not shown).

IFN-γ, IL-5 and IL-13 were measured by ELISA, using paired antibodies (eBiosciences, San Diego, CA, USA) in cell culture supernatants at 3 and 5 days poststimulation. RNA was extracted using the RNeasy plus mini kit (Qiagen, Hilden, Germany) and cDNA was created using the transcriptor first-strand cDNA synthesis kit (Roche, Indianapolis, IN, USA). The primer sequences used for quantitative PCR analysis were: *IL-4R* (F: 5'-TATGTCAGCATCACCA AGATTAAGAAA G-3', R: 5'-CCACTGTGACCCCTGAGCAT-3'), *GATA3* (F: 5'-ACTACGGAACTCGGTCAGG-3', R: 5'-CAGGGTAGGGATCCATGAAG-3'), *STAT6* (F: 5'-AACGTGTTGTCAGCCTTCCAG-3', R: 5'-GTCAAAGGGCAGGTT CATCTG-3') and *SOCS3* (F: 5'-CCCCAGAAGAGCCTATTACATCTACT-3', R: 5'-GCATCGTACTGGTCCAGGAAG-3').

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