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Interferon-γ increases IL-12 mRNA expression and attentuates allergic late-onset airway responses in the Brown Norway rat

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ABSTRACT: Interferon gamma is a T-helper cell (Th)-1-type cytokine that has been suggested to inhibit the development of an atopic Th2-type profile of cytokine expression. The aim of this study was to investigated the effect of exogenous rat interferon gamma on antigen-induced airway responses, and on Th1 and Th2-type cytokine messenger ribonucleic acid (mRNA) expression in the Brown Norway rat.

Rats were actively sensitized to ovalbumin and 14 days later underwent an aerosolized ovalbumin challenge. Animals were intratracheally administered either interferon gamma (3,000 U) or control solvent 30 min prior to, and 2 and 4 h following, antigen challenge. Lung resistance was monitored over an 8-h time period. Using *in situ* hybridization and immunocytochemistry, the levels of Th1- (interleukin-12) and Th2-type (interleukin-4 and -5) cytokine mRNA, and major basic protein expression in the bronchoalveolar lavage fluid of these rats 8 h after ovalbumin challenge were also determined.

Administration of interferon gamma attenuated the development of the late-onset airways response in ovalbumin-sensitized antigen-challenged rats ($p<0.05$). The expression of interleukin-4 and -5 mRNA in the bronchoalveolar lavage fluid of interferon gamma treated rats was significantly attenuated compared to ovalbumin-challenged saline-treated controls ($p<0.001$). This was accompanied by a significant increase in the expression of interleukin-12 mRNA, and a reduction in eosinophil numbers.

Intratracheal administration of interferon gamma modulates the allergic late-onset airways response in rats, and this is associated with a reduction in the expression of T-helper cell 2-type cytokines and an increase in interleukin-12 messenger ribonucleic acid expression within the airways. The present results support a role for interferon gamma in the pathophysiology of acute allergic airway responses, possibly by virtue of its ability to modulate T-helper cell 1- 2-type cytokine expression within the lungs.
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The pathogenesis of allergic inflammation is characterized by the presence of eosinophils, and the recruitment of CD4 T-lymphocytes expressing T-helper cell (Th) 2-type cytokines such as interleukin(IL)-4 and IL-5 [1, 2]. In bronchial asthma, this inflammatory milieu is believed to underlie the clinical characteristics of the disorder and may contribute towards the development of the late-onset airways response [3–5]. Although the precise mechanisms governing the initiation and perpetuation of an allergic inflammatory response remain to be elucidated, IL-4 is strongly implicated. This cytokine has the capacity to induce the differentiation of uncommitted Th0 cells to favour the production of Th2-type cytokines [6], and the ability to promote immunoglobulin E (IgE) production [7]. IL-4 also promotes eosinophil recruitment by upregulating the expression of vascular cell adhesion molecule-1 on endothelial cells [8]. Under *in vitro* conditions, the actions of IL-4 are subject to inhibition by Th1-type cytokines, such as interferon gamma (IFN-γ) and IL-12, which attenuate the development and expansion of Th2-type lymphocytes [9, 10] and inhibit IgE production [11].

The interest in IFN-γ as a modulatory influence on the allergic response has prompted several experimental studies in animal models of allergic asthma. In transgenic mice, overexpression of IFN-γ within the airways mucosa inhibits the airways hyperresponsiveness and eosinophil accumulation following antigen challenge [12]. The ability of IL-12 to inhibit airways responsiveness and eosinophilia, in a murine model of antigen-induced airways inflammation, is also partially dependent upon IFN-γ [13]. Whether these *in vivo* effects of IFN-γ can be attributed to its ability to prevent the infiltration of CD4+ T-cells into the airways [14] or its capacity to directly alter the cytokine balance within the airways remains to be assessed.

The sensitized Brown Norway (BN) rat model of allergic inflammation within the airways has been extensively used to investigate the pathogenesis of bronchial asthma. This model recapitulates many of the features of allergic asthma, notably the antigen-induced early (EAR) and late airways responses (LAR) [15], acute airways hyperresponsiveness [16], eosinophilic infiltration [17] and

association with CD4+ lymphocytes expressing Th2-type cytokines [18, 19]. The hypothesis underlying the present study was that exogenous administration of IFN-γ, given prior to and following antigen challenge in sensitized BN rats, would result in inhibition of the LAR, airways inflammation and the associated expression of Th2-type cytokines. Therefore, the effect of intratracheally administered rat IFN-γ on the LAR, cellular composition of bronchoalveolar lavage (BAL) fluid and Th2-type cytokine expression in ovalbumin (OVA)- sensitized rats following antigen challenge was examined.

Materials and methods

Animals and sensitization

Inbred male BN rats (6 weeks; 175–225 g) were purchased from Japan Charles River (Yokohama, Japan). The rats were maintained in conventional animal facilities at the Tokyo Medical and Dental University and sensitized using a modification of a previously described protocol [15]. Briefly, the animals were given a single subcutaneous injection of 0.5 mL normal saline, pH 7.4 containing 0.7 mg OVA (Sigma Immunochemicals, St Louis, MO, USA) with 2.5 mg aluminum hydroxide gel (Wako Chemicals, Osaka, Japan) as adjuvant. Simultaneously, 0.5 mL of *BordeTella pertussis* vaccine (IAF, Lavel-Des-Rapides, Montreal, Canada) containing 2×10^{10} heat-killed bacilli were injected intraperitoneally.

Study protocol

Following measurements of baseline pulmonary function, BN rats were challenged with aerosolized OVA (5% weight (w)/volume (v) in sterile saline), bovine scrum albumin (BSA; 5% w/v in sterile saline), Sigma Immunochemicals) or sterile saline. Aerosols were administered using a Hudson nebulizer (Model 1880; Hudson, Temecula, CA, USA) with an airflow ($\dot{V}$) of 10 $L\cdot min^{-1}$ for 5 min. Those animals in the study group (n=10) received intratracheal administration of 3,000 U recombinant rat IFN-γ (Genzyme Corporation, Cambridge, MA, USA) 30 min prior to the antigen challenge, as well as 120 and 240 min after the challenge. Control animals were administered an equivalent volume of the solvent (sterile saline) at these time points (n=10). To assess the influence of the IFN-γ treatment alone, a further group of sensitized rats (n=8) underwent saline aerosol treatment in conjunction with the cytokine administration. Lung resistance (R_L) was measured before, and at 5, 10 and 15 min following aerosol challenge, and at 15-min intervals thereafter for a total period of 8 h. The animals were suctioned when an increase in R_L was noted. On completion of the lung function measurements, BAL was performed and serum samples were taken for the measurement of OVA-specific IgE levels.

Measurement of airway responses to ovalbumin

Fourteen days following sensitization, the BN rats were anaesthetized by means of an intraperitoneal injection of urethane (1 $g\cdot kg$ body $weight^{-1}$) and orotracheal intubation was performed using a 6-cm length of polyethylene tubing (PE-240; Becton Dickinson & Co., Sparks, MD, USA). The rats were placed on a heating blanket and rectal temperature was monitored continuously using an electronic thermometer (Tele-thermometer; Yellow Springs Instrument Co., Yellow Springs, OH, USA). Animals were kept in the lateral decubitus position and alternated between their right and left sides hourly. The tip of the tracheal tube was connected to a plexiglas box (volume 265 mL; Commercial Plastics, Montreal, Canada) for the measurement of airflow ($\dot{V}$) and for the delivery of aerosols as previously described [16]. A Fleisch pneumotachograph (Fleisch 00; Bionetics Limited, Quebec, Canada) coupled to a piezoresistive differential pressure transducer (PX170-12DV; Omega Engineering, Stanford, CT, USA) was attached to the other end of the box to measure $\dot{V}$. Transpulmonary pressure (P_{tp}) was measured using a water-filled polyethylene catheter tube placed in the lower third of the oesophagus and connected to one port of a differential pressure transducer (Transpac II; Abbott, North Chicago, IL, USA), the other port of the transducer was connected to the plexiglas box. The pressure and $\dot{V}$ signals were amplified, passed through eight-pole Bessel filters (Model 902LPF, Frequency Devices, Haverhill, MA, USA) with cut-off frequencies set at 100 Hz, and recorded with a 12-bit analogue-to-digital converter at a rate of 200 Hz. All signals were stored on a computer. A commercial software package (RHT Infodat Inc., Montreal, Canada) was used to determine R_L. This was calculated using multiple linear regression by fitting the equation $P_{tp}=E_L\ V+R_L\ \dot{V}+K$ to a 10-s segment of data, where V is volume, obtained by integration of $\dot{V}$, E_L is lung elastance and K is a constant. The values of R_L reported are those obtained after subtraction of the endotracheal tube resistance (0.11 $cmH_2O\cdot mL^{-1}\cdot s$).

Bronchoalveolar lavage

BAL was performed 8 h after OVA challenge. The lungs were lavaged through the tracheal tube by means of five instillations of 5 mL of sterile saline at room temperature (20°C) followed by gentle aspiration. For the determination of cytokine messenger ribonucleic acid (RNA) (mRNA) expression, cells were prepared on poly-L-lysine-coated slides. The cytospins were subsequently postfixed for 30 min in 4% freshly prepared paraformaldehyde and washed with phosphate-buffered saline (PBS), pH 7.3 prior to processing. To assess the numbers of major basic protein (MBP)-positive eosinophils, cytospin slides of BAL fluid were allowed to dry briefly and then fixed for 7 min in a methanol:acetone [60:40] mixture. The slides were then allowed to dry before being wrapped in foil and stored at -20°C until further use.

Immunocytochemical evaluation of major basic protein expression

Immunocytochemical detection of MBP-positive cells was performed using the alkaline phosphatase/antialkaline phosphatase (APAAP) technique as previously described [20]. MBP-immunoreactivity was evaluated in BAL cytospins using a monoclonal antibody specific for human MBP (BMK-13; a kind gift of R. Moqbel, University of Alberta Edmonton, Alberta, Canada), which has previously been shown to cross-react with rat MBP [21]. For the negative control preparations, the primary antibody was replaced with either a mouse antihuman isotype

control antibody or tris (hydroxymethyl) aminomethane (tris)-buffered saline. Using this procedure, cells expressing MBP-immunoreactivity stained red.

Cytokine messenger ribonucleic acid determination using in situ *hybridization*

Measurement of the expression of mRNA for IL-4, -5 and -12 in BAL fluid was performed according to previously described methodology [22]. Antisense (complementary to the cytokine mRNA) and sense (identical to the cytokine mRNA) riboprobes were prepared from complementary deoxyribonucleic acid (cDNA) coding for rat IL-4, -5 and -12 mRNA. The cDNA sequences were first inserted into a pGEM vector and linearized using the appropriate restriction enzymes. *In vitro* transcription was carried out in the presence of ^{35}S-uridine triphosphate and the T7 or SP6 RNA polymerase. For the detection of cytokine mRNA, cytospin preparations were permeabilized with Triton X-100 and proteinase K (1 $\mu g \cdot mL^{-1}$) in 0.1 M Tris, pH 7.4 containing 50 mM ethylene diameter tetra-acetic acid (EDTA) for 20 min at 37°C. To prevent nonspecific binding of the ^{35}S-labelled probes, the preparations were incubated with 10 mM *N*-ethyl maleimide and 10 mM iodoacetamide for 30 min at 37°C, followed by incubation in 0.5% acetic anhydride and 0.1 M triethanolamine for 10 min at 37°C. Prehybridization was performed with 50% formamide and 2×standard saline citrate (SSC) for 15 min at 40°C. For hybridization, antisense or sense probes (10^6 counts per minute (cpm)·section^{-1}) were diluted in hybridization buffer. Dithiothreitol (100 mM) was present in the hybridization mixture to ensure blocking of any nonspecific binding of the ^{35}S-labelled probes. Posthybridization washing was performed in decreasing concentrations of SSC at 45°C. Unhybridized single-stranded RNA were removed using ribonuclease (RNase) A (20 $\mu g \cdot mL^{-1}$). After dehydration, the slides were immersed in LM-1 hyperemulsion (Amersham Pharmacia Biotech, Oakville, Ontario, CA) and exposed for 10 days. The autoradiographs were developed in Kodak D-19 (Kodak, Rochester, NY, USA), fixed and counterstained with haematoxylin. For negative *in situ* (ISH) controls, cytospins were hybridized with sense probes or pretreated with RNase prior to the application of the probes. To confirm the translation of IL-12 mRNA, immunocytochemistry was performed using anti-IL-12 (Santa Cruz, Ontario, Canada) and the APAAP technique [20].

Simultaneous in situ *hybridization and immunocytochemistry*

In order to determine the phenotype of cells expressing IL-12 mRNA, simultaneous *in situ* hybridization and immunocytochemistry were performed on BAL fluid from three animals that had been treated with IFN-γ. The IL-12 probe was applied simultaneously with anti-CD3 (T-lymphocyte, Pharmingen, Mississauga, Ontario, Canada) and a rat macrophage marker, (anti-ED_1, Serotec, Toronto, ON, CA) as previously described [18, 23].

Quantification

The EAR was determined from the peak in *R*L within 1 h after the antigen or saline challenge. The LAR was determined by calculation of the area under the curve (AUC) of *R*L against time 3–8 h after the antigen or saline challenge. Slides were coded, and positive cells were counted blindly using 100-fold magnification and an eyepiece graticule. The results were expressed as the mean number of positive cells per 1,000 cells counted.

Immunocytochemical evaluation of major basic protein expression

Data are presented as mean±SEM. Statistical comparisons were performed using the Mann-Whitney U-test for non-parametric data. Data were analysed using a commercial statistical package (Systat 7.0. SPSS, Inc., Elvaston, IL, USA). A p-value of <0.05 was considered statistically significant.

Results

Baseline lung resistance

The intratracheal administration of 3,000 U IFN-γ did not influence baseline *R*L which was 0.23±0.01 and 0.23±0.01 $cmH_2O \cdot mL^{-1} \cdot s^{-1}$ in IFN-γ- and vehicle-treated animals respectively (NS). There was also no difference in baseline measurements of *R*L in OVA- and saline-challenged rats receiving IFN-γ (0.23±0.01 and 0.24±0.01 $cmH_2O \cdot mL^{-1} \cdot s$ respectively, NS).

Effect of interferon gamma on airway responses to ovalbumin

The mean effect of IFN-γ or solvent on *R*L measured during the 8-h following period following antigen or saline challenge is shown in figure 1. Following OVA challenge, there was a significant increase in *R*L in five of the 10 animals treated with saline and six of the 10 treated with IFN-γ, There was no significant difference in the time taken to develop an EAR between the saline-administered (24.0±16.4 min) and the IFN-γ treated animals (25.8±9.6

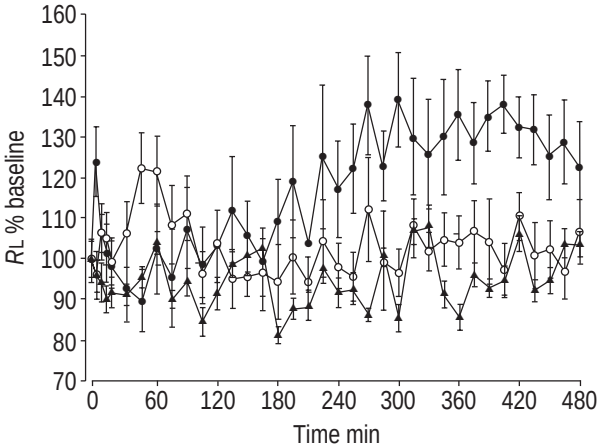

Fig. 1. – Data are presented as mean±SEM. Time course of changes in lung resistance (*R*L) following ovalbumin (OVA) or saline challenge in sensitized Brown Norway (BN) rats. Fourteen days following sensitization, the BN rats were challenged with OVA and were administered either saline (●; n=10) on interferon gamma (IFN-γ) (○; n=10). Control animals received IFN-γ and an equivalent challenge of saline (▲; n=8).

min). The mean peak R_L in the OVA-challenged saline-treated group was 131.6±9.5% of the baseline value compared to 130.6±7.8% baseline in the OVA-challenged IFN-γ treated group (fig. 2a; NS). The mean peak R_L in the saline-treated group was 114.8±14.8% baseline.

Between 3 and 8 h after exposure to the aerosolized antigen, the OVA-challenged/saline-treated rats had a mean AUC of 21.67±4.73 $cmH_2O \cdot mL^{-1} \cdot s \cdot min$ (fig. 2b). This was significantly greater than the change in R_L experienced by the OVA-challenged/IFN-γ-treated rats which had a mean AUC of 6.23±1.66 $cmH_2O \cdot mL^{-1} \cdot s \cdot min$ ($p<0.05$). The mean AUC for the saline-challenged/IFN-γ-treated rats was 4.01±1.07 $cmH_2O \cdot mL^{-1} \cdot s \cdot min$, and this was not significantly different from those animals which underwent OVA challenge while receiving IFN-γ (NS).

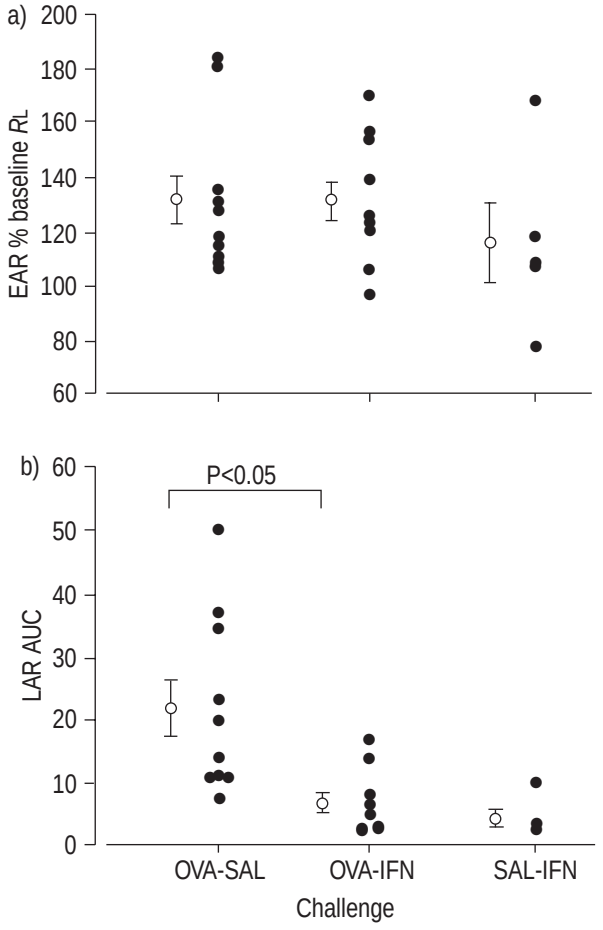

Fig. 2. – Magnitude of: a) the early airway response (EAR) as determined from the peak in lung resistance (R_L) with 1 h of ovalbumin (OVA) or saline challenge; and b) the late airway response (LAR) following OVA or saline challenge in sensitized Brown Norway (BN) rats. Individual data (●) and mean±SEM (○) are presented. Rats receiving the OVA challenge exhibited a significant increase in the peak EAR compared to the saline-challenged interferon gamma (IFN-γ)-treated animals ($p<0.05$). There was no significant difference in peak EAR between the OVA-challenged rats receiving either IFN-γ or control solvent (saline). The increase in R_L 3–8 h after antigen challenge was quantified by calculating the area under the curve (AUC) of R_L against time. Animals that were challenged with OVA and treated with saline (OVA-SAL; n=10) exhibited a significantly greater LAR compared with rats that were administered IFN-γ (OVA-IFN-γ; n=10; $p<0.05$). Control rats received IFN-γ and an equivalent challenge of sterile saline (SAL-IFN-γ; n=8).

Cytokine messenger ribonucleic acid expression and major basic protein-immunoreactivity in bronchoalveolar lavage fluid

Hybridization of the labelled cDNA probes and mRNA encoding IL-4, -5 and -12 was demonstrated by autoradiography (fig. 3). No positive hybridization signals were observed when the sense probe was used, nor when the cells were pretreated with RNase prior to antisense hybridization. Cells exhibiting positive MBP-immunoreactivity were detected by the presence of discrete red cytoplasmic staining.

BAL fluid was obtained 8 h after antigen or saline aerosolization in the rats receiving either IFN-γ or sterile saline and the expression of MBP-immunoreactivity, and IL-4, -5 and -12 mRNA was examined. Sensitized rats that had undergone an OVA challenge exhibited significant increases in the numbers of MBP- and IL-5 mRNA-positive cells compared to BSA-challenged animals (fig. 4; $p<0.001$), confirming the specificity of the allergen challenge. The increase in the numbers of MBP- and IL-5 mRNA-positive cells seen in these saline control animals was significantly attenuated as a result of IFN-γ administration ($p<0.001$).

Similar results were obtained when examining the expression of IL-4 mRNA in OVA-sensitized BN rats (fig. 5a). Numbers of IL-4 mRNA-positive cells were increased in BN rats as a result of OVA exposure compared to BSA-challenged animals ($p<0.001$). IFN-γ also decreased the numbers of cells expressing IL-4 mRNA following OVA-challenge compared to saline controls ($p<0.001$). The numbers of IL-12 mRNA-positive cells were also modified as a result of IFN-γ administration. IL-12 mRNA-positive cell numbers were increased in sensitized OVA-challenged BN rats as a result of IFN-γ administration compared to saline control (fig. 5b; $p<0.001$). Administration of IFN-γ in the absence of OVA challenge also increased IL-12 mRNA-positive cell numbers in BAL fluid; however, this was significantly less than the increase following OVA challenge ($p<0.02$). Immunocytochemistry confirmed the translation of IL-12 mRNA and also showed an increased number of IL-12-immunoreactive cells in the BAL fluid of rats treated with IFN-γ compared to saline ($p<0.001$). Simultaneous ISH and immunocytochemistry demonstrated that 33% of IL-12 mRNA-positive cells in BAL fluid samples obtained after IFN-γ treatment were CD3-positive and 45% were macrophages.

Discussion

The aim of this study was to investigate the influence of exogenous recombinant IFN-γ administration on allergen-induced changes in lung function. The possible underlying molecular mechanisms were also examined by determining the accompanying alterations in cytokine mRNA expression within the BAL fluid. The present results indicate that IFN-γ administration attenuates the development of the allergen-induced LAR in sensitized BN rats without influencing the EAR. This activity was associated with a reduction in the numbers of cells within the lungs expressing IL-4 and -5 mRNA, and MBP-immunoreactivity, and an increase in the numbers of IL-12 mRNA-positive cells.

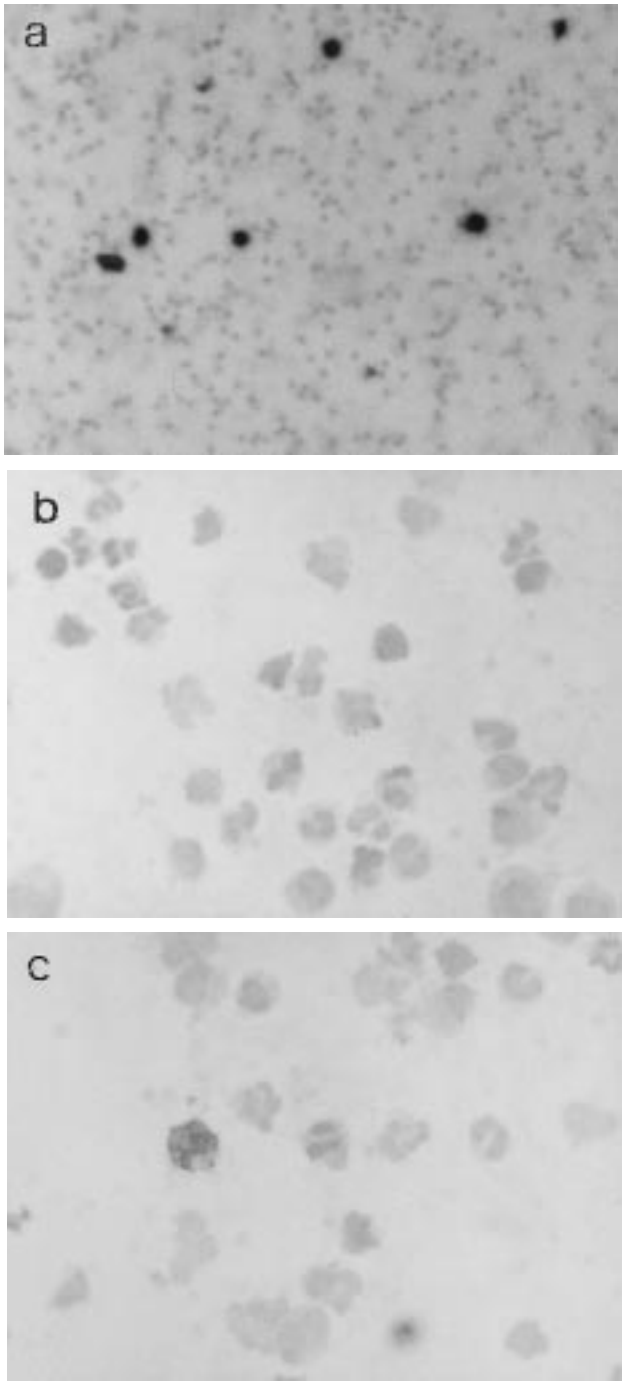

Fig. 3. – a) Representative photomicrograph of *in situ* hydridization to localize interleukin-12 messenger ribonucleic acid in the interferon gamma (IFN-γ)-treated rat. b) Representative example of major basic protein (MBP)-immunoreactivity in bronchoalveolar lavage fluid from rats treated with IFN-γ prior to ovalbumin, compared to controls (c). Note the presence of an MBP-positive cell in the control preparation.

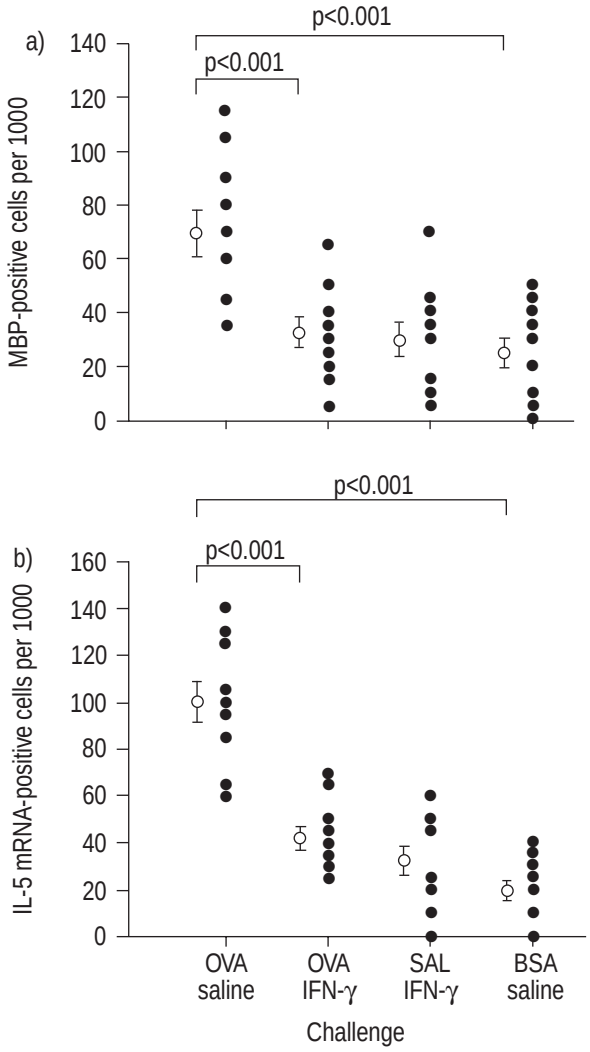

Fig. 4. – Number of: a) major basic protein (MBI); and b) interleukin-5 messenger ribonucleic acid (IL-5 mRNA)-positive cells per 1,000 in the bronchoalveolar lavage fluid of sensitized Brown Norway rats 8 h following ovalbumin (OVA), bovine serum albumin (BSA) or saline challenge. Individual data (●) and mean±SEM (○) are presented. Rats that had received interferon gamma (IFN-γ) with concomitant OVA challenge exhibited significantly decreased numbers of MBP- and IL-5 mRNA-positive cells compared to saline control ($p<0.001$). Those animals challenged with BSA did not exhibit the increase in MBP- and IL-5 mRNA-positive cells number which was evident in OVA-challenged animals ($p<0.001$) confirming the specificity of the allergen challenge.

To date, there have been no studies examining the ability of exogenous IFN-γ to modulate the LAR. The present data demonstrate that this cytokine may inhibit the development of allergen-induced late-onset changes in airways resistance without influencing acute alterations in lung function. These alterations in airway function could not be simply attributed to inhalation of IFN-γ, since this cytokine itself had no influence on R_L. Previous reports in mice have demonstrated that exogenous administration of IFN-γ inhibits allergen-induced eosinophilia and airways hyperresponsiveness [14, 24, 25]. The ability of IFN-γ to attenuate these allergic manifestations is also evident following liposome-mediated gene transfer to the pulmonary epithelium [12] and as a result of inhalation [25], suggesting it is locally mediated. Studies using IFN-γ gene knock-out mice have confirmed the role of this cytokine in reducing allergic manifestations. These animals have a diminished ability to eliminate eosinophils from the airways following allergen challenge, resulting in persistent lung eosinophilla [26]. It should be recognized, however, that with any animal model there are uncertainties as to the precise relationship it has to the clinical condition.

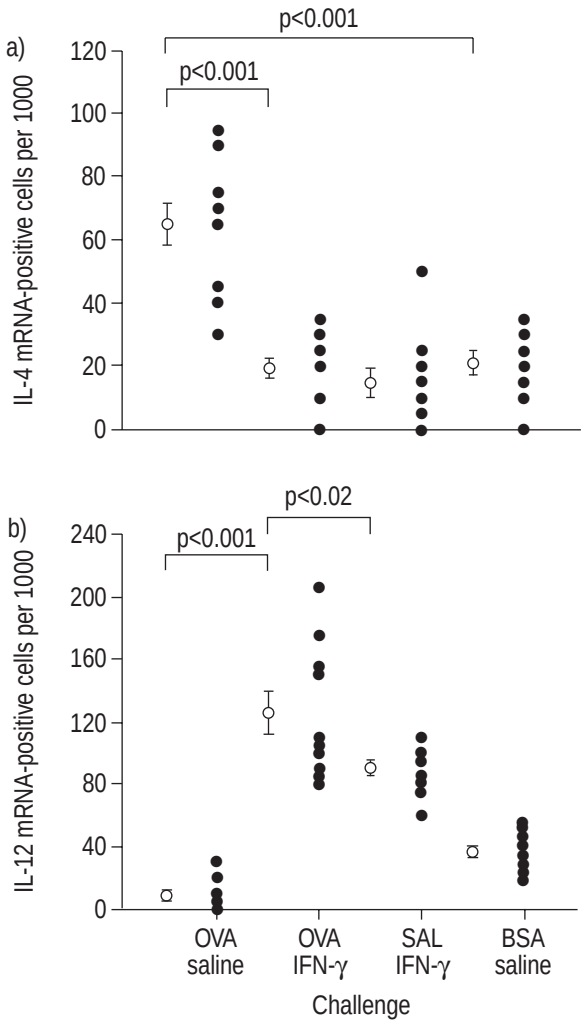

Fig. 5. – Number of; a) interleukin (IL)-4 messenger ribonucleic acid (mRNA)- and b) IL-12 mRNA-positive cells per 1,000 in the bronchoalveolar lavage fluid of sensitized Brown Norway (BN) rats 8 h following ovalbumin (OVA), bovine serum albumin (BSA) or saline challenge. Individual data (●) and mean±SEM (○) are presented. Rats that had received interferon gamma (IFN-γ) with the OVA challenge exhibited significantly decreased numbers of IL-4 mRNA-positive cells compared to saline control ($p<0.001$). IL-4 mRNA-positive cell numbers were increased in OVA-challenged control animals compared to BSA-challenge animals ($p<0.001$). Numbers of IL-12 mRNA-positive cells were increased in sensitized OVA-challenged BN rats as a result of IFN-γ administration compared to saline control ($p<0.001$). Administration of IFN-γ in the absence of OVA challenge increased IL-12 mRNA-positive cell numbers in BAL fluid, although significantly less than following OVA challenge ($p<0.02$).

Although there are no clinical studies examining the influence of IFN-γ in attenuating the late asthmatic response *per se*, prior reports have suggested that exogenous administration of this cytokine does not alter mean symptom scores, airways responsiveness, airway function as determined by the forced expiratory volume in one second or acute allergen-induced bronchoconstriction [27, 28]. Obviously, further studies are required to determine the exact nature of the relationship between the LAR and IFN-γ in asthmatic patients.

The mechanisms underlying the attenuation of the LAR by IFN-γ may include the reduced eosinophil presence within the lungs and/or the alteration in cytokine profile. Both eosinophils and cytokines such as IL-5 have been implicated in the development of the LAR in humans [3, 4] and in the sensitized BN rat [21]. Although *in vitro* studies have suggested that IFN-γ may augment eosinophil recruitment by stimulating the production of eosinophil chemoattractants [29] and the expression of specific adhesion molecules [30], once within the tissues, IFN-γ has a tendency to reduce eosinophil numbers. IFN-γ may enhance the apoptosis of resident eosinophils [31] or inhibit the local differentiation of eosinophil progenitors [32]. In mild atopic asthmatics, exogenous IFN-γ has been shown to reduce eosinophil numbers within the lungs [27], suggesting the inhibition of eosinophil survival and/or local differentiation takes precedence.

In addition to its direct effects on eosinophils, IFN-γ may be inhibiting the LAR *via* an action on the local cytokine profile. The present results would indicate that exogenous administration of IFN-γ, prior to and after the allergen challenge, shifts the T-cell cytokine expression from a predominantly Th2-type to a Th1-type response. A previous report in mice has suggested that the actions of IFN-γ in attenuating the allergen-induced lung eosinophilia and airways hyperresponsiveness could not be attributed to a direct action on cytokine production by T-lymphocytes [25]. However, *in vivo* IFN-γ may influence cytokine production by inhibiting antigen-induced Th2-type cell proliferation [9]. Furthermore, mast cells are a potential source of IL-4 within the airways and IFN-γ has been reported to inhibit the proliferation and development of these cells [33, 34].

IFN-γ may also act indirectly to alter the cytokine balance within the airways *via* the production of IL-12. IL-12 has been shown to stimulate the differentiation of naive T-cells into Th1-type cells, whilst at the same time inhibiting the development of Th2-type cells [35]. In an established antigen-specific response, however, differentiated Th2-type cells lose their ability to transduce membrane signals in the response to IL-12. Nevertheless, in early development of Th2 cells, the presence of IFN-γ is able to maintain IL-12 receptor expression and restore the ability of these cells to functionally respond to IL-12 [36]. Whether pretreatment of the sensitized rats with IFN-γ prior to allergen challenge is critical in this respect remains to be determined.

The increase in the numbers of cells expressing Th2-type cytokines (IL-4 and -5) and the elevated numbers of MBP-positive cells following allergen challenge are consistent with previous reports in the sensitized BN rat [19, 21, 37]. This, however, is the first report of IL-12 mRNA expression in this model of allergic airways constriction in the rat. Consistent with the preferential expression of Th2-type cytokines, there is a relative paucity of BAL fluid cells expressing IL-12 mRNA as a result of allergen challenge. This may be attributed to the allergen-induced cytokine milieu. Alveolar macrophages are the major source of IL-12 mRNA within the airways [23] and potent inhibitors of macrophage activation (IL-4 and -10 have been shown to be released following allergen challenge [38, 39]). The increase in IL-12 mRNA expression as a result of IFN-γ treatment is a either consequence of a direct action of this cytokine in stimulating macrophage activation [40] or a result of the reduction in IL-4 and possibly IL-10 expression.

In conclusion, the present results indicate that intratracheal administration of interferon gamma, prior to and after allergen challenge, attenuates the late airways response and associated bronchoalveolar lavage fluid eosinophilia. These actions of interferon gamma are associated with a concomitant decrease in T-helper cell 2-type (interleukin-4 and -5) cytokine messenger ribonucleic acid expression and an increase in interleukin-12 messenger ribonucleic acid expression. Further studies are required to elucidate the role of interferon gamma as an immunomodulatory cytokine in the development of allergic airways disease.

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