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Review Article

Modulation of pulmonary allergic responses by mucosal cytokine–gene transfer*

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ABSTRACT

Recent clinical and experimental animal studies have provided evidence for a pivotal role of T lymphocytes and Th2 cytokines in the development of allergic inflammatory responses and airway hyperreactivity. These studies suggest also that the Th2 cytokine-associated inflammatory responses are potential targets of developing novel and effective therapies. Using a novel gene-transfer approach, we investigated the role of a Th2-inhibitory cytokine, IFN-γ, in the regulation of antigen (Ag)-induced lung inflammatory response and airway hyperreactivity by transfer of the *IFN-γ* gene into mouse lung mucosal cells. Our results showed that mice receiving the *IFN-γ* gene demonstrate a lower degree of Ag- and Th2 cell-induced airway hyperresponsiveness and a reduced eosinophilia in the lung. These results provided evidence that the instillation of the *IFN-γ* gene into the lung is effective in modulating the allergic inflammation and bronchial hyperreactivity in an experimental animal model.

Key words: airway hyperreactivity, allergic inflammation, gene transfer, Th2 cytokines.

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INTRODUCTION

Allergen-induced IgE and inflammatory responses have been implicated in the pathogenesis of a variety of allergic diseases, including asthma.[1] The inflammation is characterized by various types of cellular infiltrates, including eosinophils and T cells. Numerous clinical and experimental animal studies have indicated a pivotal role of T lymphocytes and cytokines in the development of allergic inflammatory responses and airway hyperreactivity.[2–9] In particular, a subset (Th2) of $CD4^+$ T cells, which has been distinguished functionally by its pattern of cytokine secretion, is thought to play a key role. Th2 cells are thought to promote pulmonary allergic responses through their secretion of the cytokines IL-4 and IL-5, which promote IgE production and mast cell development, and eosinophilia, respectively.[10–12] Cytokines released by the opposing pathway (Th1), such as IFN-γ, inhibit the development and expansion of Th2 cells and cytokine production.[10] These studies demonstrate that airway inflammation plays a critically important role in mediating bronchial hyperresponsiveness. It is also evident that the Th2 cytokine-associated inflammatory responses are potential targets for developing novel and effective therapeutic regimens.

Recent advances in the genetic manipulation of somatic cells and tissues as a form of 'gene therapy' have provided an important and novel therapeutic approach in a variety of human genetic and acquired diseases. In regard to the lung diseases, the use of gene therapy has been to modify the milieu of the tissue or directly modify cells within the tissue, with the purpose of modulating the progression of disorders; for example, there have recently been several attempts to transfer normal cystic fibrosis

transmembrane conductance regulator (CFTR) genes to airway epithelial cells *in vivo*.[13–15] The objective of a later study by Li *et al.* was, therefore, to explore the potential modulation of antigen-induced allergic responses by using gene-transfer approaches.[16] In this study, we investigated the role of a Th2-inhibitory cytokine, IFN-γ, in the regulation of Ag-induced lung inflammatory response and airway hyperreactivity by transfer of the *IFN-γ* gene into mouse lung mucosal cells.[16] We hypothesized that delivery of the *IFN-γ* gene to the mucosal cells of the lung would decrease local eosinophilic inflammation, thereby limiting tissue destruction and loss of pulmonary function. To test this hypothesis, we utilized a murine model of Ag-induced allergic inflammation and bronchial hyperreactivity, in which Ag exposure or challenge induced pulmonary eosinophilia and airway hyperreactivity, concomitant with increases of Th2 cytokines.[8,9] Significantly, a potent IFN-γ inducer, IL-12, has been shown to be able to reverse the Ag-induced inflammation and airway hyperresponsiveness in our murine model.[8] We present here a brief description and summary of our studies (for detailed information and methodology, see ref. 16).

METHODS

IFN-γ gene construct and mucosal gene transfer

To generate an *IFN-γ* gene construct, the *IFN-γ* coding-region gene was inserted into the polylinker site of an expression vector, pCDNA3, containing a bovine growth hormone poly A tail, and a *neo* gene. The expression of the *IFN-γ* insert is driven by a CMV promoter. The resulting plasmids were propagated in *Escherichia coli* and purified by an additional cesium chloride gradient purification to minimize endotoxin contamination. Table 1 describes the gene construct and route of gene transfer used in this study (see also ref. 16). For liposome-mediated mucosal gene transfer, groups of mice were treated through aspiration, at varying time points, with phosphate-buffered saline (PBS) or lipofectAMINE, or a mixture of plasmid DNA and lipofectAMINE.

Mice, immunization, cell transfer, analysis of airway response and cytokines

For generating Ag- and Th2 cell-induced airway allergic responses, the protocols for Ag immunization, challenge, and Ag-specific Th2 cell transfer have been published elsewhere.[16] Various quantitative parameters of airway inflammation and bronchial responsiveness were also obtained as previously described.[16] Briefly, AKR mice were sensitized intraperitoneally with 100 μg of conalbumin (CA) or PBS absorbed in 2 mg of alum on PBS once a week for two weeks, followed by intratracheal challenge twice (1 week apart) with 100 μg of CA in PBS or PBS alone. A CA-specific T-cell clone, D10.G4.1 derived from AKR/J mice, was used in cell transfer experiments. Prior to cell transfer, resting D10 cells (5×10^6 cells in 0.05 mL with or without the addition of Ag (CA, 100 μg/mL) were transferred intratracheally into naive mouse lungs. Control mice received either Ag alone, PBS or remained untreated. Three days after the last Ag challenge and at various time points after cell transfer, airway responsiveness was determined. The peak airway pressure changes over time following intravenous injection of mice with acetylcholine (Ach; 50 μg/mL) were measured as airway pressure-time index (APTI, cm H_2O–s).[8,9] The lungs were then lavaged, and the bronchoalveolar lavage fluid (BALF) cell differential counts and percentages were determined by Diff-Quik staining of cytospin slides. The levels of cytokines (IL-4, IL-5, IFN-γ) in the BALF were determined by ELISA as previously described.[16]

RESULTS AND DISCUSSION

We first developed a murine model for studying the airway allergic responses[16] (and Li *et al.*, unpubl. data). In this model, male AKR mice (6–8 weeks old) were intraperitoneally sensitized and intratracheally challenged with CA or PBS. Three days after the challenge, mice were anesthesized and bronchoalveolar lavages were performed. The airway reactivity was also determined by measuring airway pressure changes following intravenous injection of mice with Ach. The time-inte-

Table 1. Gene constructs used

Construct	Promoter	Transgene	Target	Gene delivery
pcDNA (vector control)	CMV	—	Epithelium	Plasmid–liposome mix (aspiration)
pcDNAIFN	CMV	*IFN-γ*	Epithelium	Plasmid–liposome mix (aspiration)

grated change in peak airway pressure, referred to as APTI, was calculated and served as the measurement of airway responsiveness.[8,9] We demonstrated that significant increase in airway reactivity is observed in CA-challenged mice as compared with saline challenged mice. Concomitant with increases in airway reactivity, the number of eosinophils in the BALF was significantly increased in Ag-challenged animals as compared with their controls. On the other hand, Ag challenge had no significant effect on the numbers of either macrophages or neutrophils in BAL fluids of sensitized mice. In addition, histological examinations of the challenged lungs demonstrated predominant infiltrating eosinophils in the connective tissues between blood vessels and the airways; results also showed perivascular accumulation of the inflammatory cells including eosinophils and lymphocytes. Furthermore, these allergic responses can be seen in naive mice following adoptive transfer of naive mice with a CA-specific Th2 clone, D10 (Li *et al.* unpubl. data).

Using this murine model and the *IFN-γ* gene construct, we performed a gene transfer study to examine the *in vivo* efficacy of transduced *IFN-γ* genes on the expression of Ag- and Th2-cell-induced inflammatory and bronchial responses in mice. We first analyzed the tissue expression of *IFN-γ* following administration of the plasmid–liposome mixture through aspiration. The level of IFN-γ in the BALF was determined by ELISA assays of BALF collected at various time points (12, 24, 48 and 72 h). Naive mice received either no treatment, vector control (mock; pcDNA, 10 μg/mouse) or *pcDNAIFN* (10 μg/mouse). Results showed that while BALF from untreated or mock-treated mice had no detectable IFN-γ, a time-dependent increase in IFN-γ levels was observed in mice treated with pcDNAIFN, but not with a vector control (pcDNA). In addition, *in situ* hybridization analysis showed that the expression of IFN-γ was localized predominantly in the epithelial cells, and few pneumocytes were also stained positive. The expression of IFN-γ transcripts and the presence of the *pcDNAIFN* gene construct was detected only in the lungs, but not in the spleens or livers, of transduced mice.

Using the plasmid-based construct, the *in vivo* efficacy of transduced IFN-γ in modulating both CA- and Th2-cell-induced airway allergic responses was examined. Our results showed that mice receiving *pcDNAIFN* demonstrate a lower degree ($P<0.001$ *vs* mock-transduced group; Statview, Brain power Inc., Calabasas, CA, USA) of Ag-induced airway responsiveness and a reduced eosinophilia in the lung BALF (Fig. 1). In contrast, lipofectAMINE- or mock-transduced mice showed a similar level of airway hyperreactivity and eosinophilic

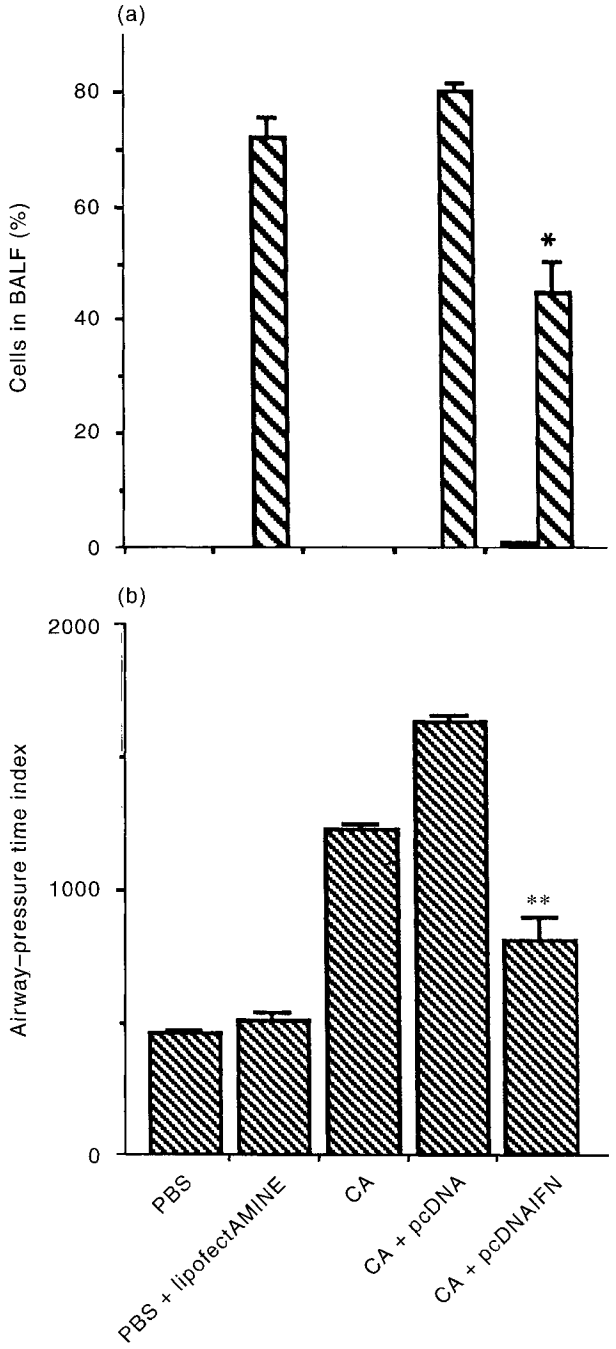

Fig. 1 (a) Male AKR mice were sensitized i.p. with conalbumin (CA, 100 μg) or phosphate-buffered saline (PBS) 14 days prior to intratracheal challenges with either PBS or CA (50 μg). Three days prior to intratracheal challenge, mice aspirated 50 μL of the following: PBS alone; lipofectAMINE alone (25 μL lipofectAMINE + 25 μL PBS); pcDNA + lipofectAMINE (10 μg plasmid in 25 μL PBS + 25 μL of lipfectAMINE); *pcDNAIFN* + lipofectAMINE (10 μg plasmid in 25 μL PBS + 25 μL of lipofectAMINE). Three days after the challenge, BALF were collected for cell differentials. (■) Neutrophil, (▨) eosinophil. (b) Airway reactivity was examined as described in Methods (see also ref. 16). *$P<0.01$ *vs* mock-treated; **$P<0.001$ *vs* mock-treated.

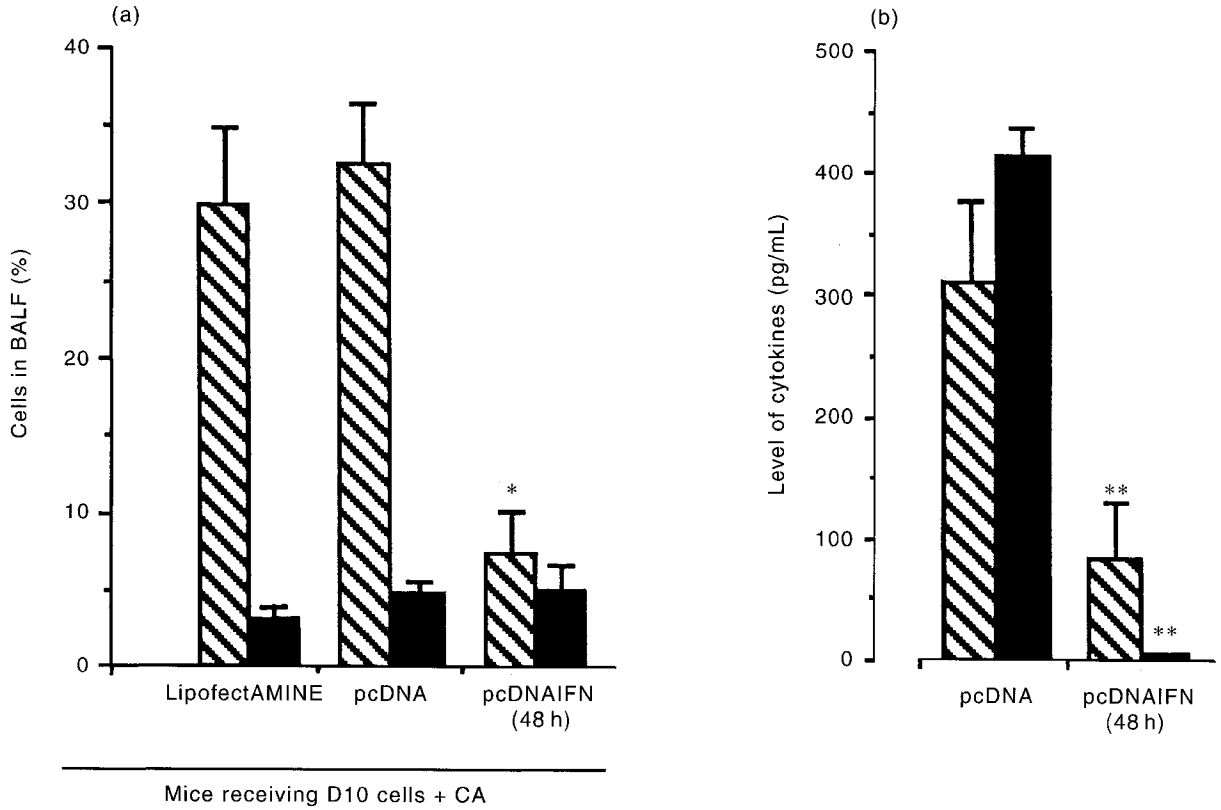

Fig. 2 Forty-eight hours prior to the Th2 (D10) cell transfer, naive mice aspirated 50 μL of the following: lipofectAMINE alone (25 μL lipofectAMINE + 25 μL PBS); pcDNA + lipofectAMINE (10 μg plasmid in 25 μL PBS + 25 μL of lipofectAMINE); *pcDNAIFN* + lipofectAMINE (10 μg plasmid in 25 μL PBS + 25 μL of lipofectAMINE). (a) The BALF cell differential (▨) eosinophils and (■) neutrophils and (b) the levels of cytokines in the BALF were determined 2 days after the cell transfer (▨) IL-5, (■) IL-4. *$P<0.001$ *vs* mock-treated; **$P<0.001$ *vs* mock-treated.

inflammation as that of mice challenged with Ag alone. Also of significance is the finding that a significant decrease in the levels of eosinophils and Th2 cytokines (IL-4 and IL-5) in the BALF was observed in mice receiving the *IFN-γ* gene transfer (Fig. 2; $P<0.001$ *vs* mock-transduced group; Statview) 48-h prior to the cell transfer. These results demonstrated the feasibility of the gene-transfer approach, and provided evidence that instillations of *IFN-γ* gene into the lung are effective in inhibiting the allergic inflammation and bronchial hyperreactivity. Our ongoing studies involve examinations of the level, distribution and stability of the transduced *IFN-γ* gene, and of the inhibitory mechanisms of the transduced *IFN-γ* genes *in vivo*.

Airway inflammation and bronchial hyperreactivity are associated with cellular infiltrations, and are characterized by activation of Th2 cytokines. Our studies were designed to explore the potential therapeutic approach using cytokine–gene transfer in the regulation of lung inflammatory response and airway hyperreactivity. Furthermore, similar gene-transfer approaches using different cytokine genes either individually or in combination can potentially be studied to gain further understanding of the molecular mechanisms of cytokine involvement in the regulation of airway inflammation and bronchial hyperreactivity. Also of interest are the recent findings that intramuscular allergen–gene transfer results in the expression of allergen in the muscle cells, and in the development of the immune response through a distinct antigen-presentation pathway. Such an immune response is inhibitory to the subsequent induction of specific IgE and airway hyperreactivity by the allergen proteins.[17,18] These molecular and functional studies provide the experimental basis needed to investigate further the general applicability of this approach.

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