Inhibition of Chronic Airway Inflammation and Remodeling by Galectin-3 Gene Therapy in a Murine Model

Esther López, Victoria del Pozo, Teresa Miguel, Beatriz Sastre, Carmen Seoane, Esther Civantos, Elena Llanes, M. Luisa Baeza, Pilar Palomino, Blanca Cárdaba, Soledad Gallardo, Félix Manzarbeitia, José M. Zubeldia and Carlos Lahoz

*J Immunol* 2006; 176:1943-1950; 
doi: 10.4049/jimmunol.176.3.1943
http://www.jimmunol.org/content/176/3/1943

Why *The JI*?

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

*average

References  This article cites 65 articles, 13 of which you can access for free at: http://www.jimmunol.org/content/176/3/1943.full#ref-list-1

Subscription  Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions  Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts  Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Asthma is a chronic disease characterized by airway inflammation, airway hyperresponsiveness (AHR) and reversible airway obstruction. In addition, structural changes causing remodeling occur as a result of an imbalance in tissue regeneration and repair mechanism (1, 2). Infiltration of eosinophils and T lymphocytes, goblet-cell and smooth-muscle-cell hyperplasia, subepithelial deposition of extracellular matrix proteins, and neovascularization predominate in chronic asthma (3, 4).

Increasing evidence suggests that eosinophils are associated with development of lung dysfunction and subsequent immunopathological changes (5–9) through the release of lipid mediators, cytokines, cytotoxic proteins, and oxygen and nitrogen radicals (10, 11). Furthermore, evidence from animal models supports the view that eosinophils play an important role in tissue remodeling. One key cytokine regulating the trafficking of eosinophils from bone marrow to the lung is IL-5, which regulates eosinophil proliferation, differentiation, and release from bone marrow (12, 13), and also acts in activation and tissue survival of eosinophils (14).

The specificity of IL-5 has also raised the possibility that blocking its activity might be a useful therapy for allergic diseases (15, 16). In this respect, several approaches have been made using monoclonal anti-IL-5 Abs, with varying results (17–23). Although these studies point to an important role for the eosinophil in asthma, the eosinophil hypothesis must nevertheless be tested more directly by attempting to generate eosinophil-focused therapies. Different experimental strategies have therefore been developed, such as GATA-1 knockout (KO) (24, 25) or CD49d and α4β1 therapies for eosinophilic gastroenteritis (26), induction of eosinophils apoptosis by ligation of Siglec-8 (27), and anti-IgE therapy (28).

As a novel therapy, this paper evaluates the role of Galectin-3 (Gal-3) in a chronic asthma model. We previously reported that Gal-3 induces down-regulation of IL-5 gene expression (29, 30). Gal-3 is an IgE-binding protein which belongs to a family of proteins that bind β-galactosides. It has a unique N-terminal domain, a highly conserved repetitive sequence rich in proline and glycine, and a globular C-terminal domain containing the carbohydrate recognition site. Gal-3 is expressed in a variety of tissues and cell types (31). In addition, it has been implicated in different processes, including inflammation and allergic pathologies (32, 33), and the effect on the IL-5 gene raises interesting possibilities in the regulation of allergic reactions (29, 30). In a previous work, intratracheal instillation of plasmid-Gal-3 in acute sensitized rats that had been Ag-challenged by inhalation was shown to lead to an improvement, in terms of both cellular inflammatory infiltrate and pulmonary function (34). We therefore wanted to ascertain
whether these beneficial effects would also apply to a chronic model.

Accordingly, this study sought to assess the efficacy of delivery of therapeutic Gal-3 by gene therapy in a well-characterized mouse model of chronic airway inflammation, hyperresponsiveness, and remodeling.

Materials and Methods

Animals

All experimental procedures conformed to international standards of animal welfare and were approved by the Fundación Jiménez Díaz animal committee. Male A/J mice (specific pathogen free; 5 wk old) were purchased from Harlan Iberica.

Induction of chronic asthma by intranasal administration of OVA and Gal-3 gene treatment

Mice were placed in a small box and anesthetized with inhaled Forane. Anesthetized mice were instilled with 1 mg/ml OVA (grade V; Sigma-Aldrich) intranasally. Administration of OVA was performed 3 days/week for 12 wk, using a previously described method (35).

To analyze the minimal dose of plasmid-Gal-3 that induced IL-5 inhibition, we tested several doses ranging from 25 to 100 μg of DNA. Whereas 25 μg had no effect, 50 μg proved to be the minimal dose that brought about complete IL-5 gene inhibition. Above 100 μg, a plateau was reached (data not shown). Fourteen days after the first OVA instillation and once every 15 days thereafter, mice were treated by intranasal instillation with 50 μl of plasmid (1 mg/ml) encoding Gal-3 (pEGFP-Gal-3, n = 39) or with plasmid without insert (pEGFP, n = 20) or with saline instead of plasmid instillation (OVA, n = 35) as positive controls. An additional negative control group was used in which mice were injected with saline and exposed to saline inhalation (SS, n = 20).

Plasmid DNA preparation

The human Gal-3 gene (BlueScript SK-GAL-3 furnished by Dr. F. T. Liu, La Jolla Institute for Allergy and Immunology, San Diego, CA) was cloned into a eukaryotic expression vector pEGFP plasmid (BD Clontech). pDNA was purified using the Wizard DNA purification system (Promega) and stored at −20°C in pyrogen-free saline.

Bronchoalveolar lavage (BAL), cell analysis, and RNA preparation

Twenty-four hours after the last administration of Ag, the mice were anesthetized and the lungs lavaged three times with sterile 0.5 ml of PBS. Cells obtained were counted and used for RNA extraction and for cytometric analysis (34). Total RNA was isolated from lungs by TRIzol Reagent (Invitrogen Life Technologies) and was treated with DNase I (Promega). RNA was measured by spectrophotometry, and 1 μg of RNA was used for first-strand cDNA synthesis with the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen Life Technologies).

Gal-3 detection

Immunohistochemistry was performed with the paraffin-embedded sections. The endogenous peroxidase activity as well as nonspecific protein binding was sequentially blocked using 0.3% hydrogen peroxide and 5% normal goat serum, respectively. The sections were incubated with affinity purified rabbit anti-Gal-3 Ab (BD Biosciences), or normal rabbit IgG Ab (control) at 10 μg/ml for 30 min at room temperature and were then washed five times in PBS. Bound Ab was detected by sequential incubation with biotinylated goat anti-rabbit Ab and streptavidin-HRP followed by 3,3-diaminobenzidine (DakoCytomation). Slides were then washed in water and counterstained with hematoxylin Gill no. 2 (Sigma-Aldrich).

Cytokine expression in lung tissues by real-time quantitative PCR

Quantitative real-time PCR was performed on a 7500 Real-time PCR System (Applied Biosystems). TaqMan PCR was performed using a 20-μl final reaction volume containing 10 μl of TaqMan Universal PCR Master Mix (Applied Biosystems), 1 μl of 20× Assays-on-Demand Gene Expression Assay Mix, and 9 μl of cDNA diluted in RNase-free water. Each assay was performed in triplicate. The PCR conditions used in all reactions were: 2 min at 50°C, 10 min at 95°C, with 40 two-step cycles (95°C for 15 s and 60°C for 60 s). Assays-on-Demand Gene Expression primers to IL-4, IL-5, IL-13, IL-10, and rRNA (used as Endogenous) were obtained from Applied Biosystems (www.appliedbiosystems.com/).

Determination of total serum IgE and OVA-specific IgG1 and IgE

Total serum IgE and OVA-specific IgE were measured using an ELISA kit. OVA-specific IgG1 was determined by ELISA using a biotin anti-mouse IgG1 (BD Biosciences/BD Pharmingen).

Measurement of collagen

The amount of pulmonary fibrosis was determined by measuring total pepsin-soluble lung collagen, using the Sircol Collagen Assay kit (Biocolor) as described previously (36).

Determination of airway responsiveness to methacholine

Mice airway responsiveness was assessed every 15 days until 12 wk. Four individual whole body plethysmograph chambers, obtained from Buxco, were used as described elsewhere (37).

Tissue processing and histological analysis

Right lungs were taken from mice under terminal anesthesia, inflated with 10% formalin, and immersed in the same solution before tissue processing in paraffin-embedded blocks. Five-micrometer sections were stained with H&E to evaluate general morphology. Mucus-secreting goblet cells were visualized on periodic acid-Schiff (PAS), and Masson Trichrome stain was used for assessment of subepithelial fibrosis. Analyses were performed in a blind fashion, and slides were presented in random order for each examination.

The results were analyzed, in blind conditions, using an arbitrary intensity scale: 0, no cellular inflammatory infiltrate, or basal extracellular matrix (ECM) staining or no staining; 1, mild cellular inflammatory infiltrate or mild staining; 2, moderate cellular inflammatory infiltrate or moderate staining; 3, severe cellular inflammatory infiltrate or intense staining) using H&E, Masson, and PAS, respectively. Semi-quantification was done in the animals studied: pEGFP-Gal-3, n = 39; OVA, n = 35; pEGFP, n = 20; SS, n = 20.

Statistical analysis

Results were expressed as mean ± SD with a group size of 20–40 from three different experiments. Variance analysis was used for statistical comparisons between groups, followed by the Tukey test (where applicable) using GraphPad Prism 4 Software. Statistical significance was set at p < 0.05.

Results

Gal-3 expression in lungs

After instillation of 50 μg of plasmid into the lungs, Gal-3 was detected by immunohistochemistry. As shown in Fig. 1, immunohistochemical analysis of Gal-3 expression showed an increase in staining (brown color) in the lungs of mice treated with plasmid-encoding Gal-3 (Fig. 1C) compared with the normal lungs (Fig. 1A) and with inflamed lungs (Fig. 1B). In lungs treated with gene therapy, Gal-3 was expressed mainly in peribronchial epithelial cells. Staining was not observed when normal rabbit IgG was used instead of rabbit anti-Gal-3 Ab (Fig. 1D). These results indicated that mice receiving the Gal-3 gene overexpressed Gal-3 protein.

Effect of plasmid-Gal-3 treatment on the quantitative expression of cytokine genes in the lung tissues

Activated T cells migrate to the lungs and release inflammatory cytokines to orchestrate the allergic response. We therefore assessed the effect of Gal-3 gene therapy on lung cytokine levels after 12 wk of chronic OVA challenge in a situation similar to chronic asthma. The results indicated that IL-5, IL-4, IL-10, and IL-13 levels were increased with respect to negative controls treated with saline instead of Ag (p < 0.05; Fig. 2). When mice were treated during 12 wk with Ag and pEGFP-Gal-3, the relative levels of IL-5 expression was significantly lower than it was among OVA-exposed mice without gene treatment: 0.45 vs 0.86, p <
0.01, representing close to 75.2% inhibition (Fig. 2A). However, treatment with empty plasmid does not significantly modify the IL-5 expression comparing with the OVA group. Also, there were statistically significant differences comparing groups treated with empty plasmid and with plasmid encoding for Gal-3: 0.76 vs 0.45, statistically significant differences comparing groups treated with IL-5 expression comparing with the OVA group. Also, there were no significant differences in the yield of airway dissection. As shown in Fig. 3, the BAL eosinophil count was 4.38 ± 1.05% for treated (pEGFP-Gal 3, n = 39) vs 11.09 ± 2.4 for untreated (OVA, n = 35) animals (p < 0.01). The treatment with empty plasmid do not apparently modify eosinophil count in asthmatic mice (9.8 ± 2.6, n = 20). In peripheral blood, the reduction obtained was significant, albeit to a lesser extent than that obtained in BAL fluid (BALF).

These results indicate that administration of Gal-3 into the lung modulates allergen-induced BALF and peripheral blood eosinophilia.

Determination of total serum IgE and OVA-specific IgG1 and IgE

We wished to ascertain whether treatment with plasmid-encoding Gal-3 might alter total and specific IgE levels. OVA-sensitized mice had high levels of total and specific serum IgE. As can be seen in Table II, there were no detectable changes in IgE from mice treated with plasmid with Gal-3 and OVA and pEGFP groups. Data shown in Table II indicate that Gal-3 does not interfere with total or with specific IgE synthesis. Determination of specific IgE against OVA showed that the response against the Ag was not modified in treated mice.

Airway total collagen determinations

To quantify collagen deposition in the lung, the Sircol collagen assay was used. Collagen content of each airway preparation was normalized with rRNA gene used as Endogen. OVA group (n = 35); pEGFP group (n = 20); pEGFP-Gal-3 group (n = 39); and SS group (n = 20). Significant differences in IL-5 (+, p < 0.01) and IL-10 (+, p < 0.05) expression levels were obtained for pEGFP-Gal-3 vs OVA and pEGFP groups. In the case of empty plasmid, no significant differences were found compared with the OVA group.

Airway hyperresponsiveness

Airway function was assessed using whole body plethysmography. Enhanced pause, a calculated value that correlates with measurement of airway resistance, was performed at 12 wk in chronically OVA-exposed mice. Allergen-treated mice developed a significantly enhanced response to methacholine when compared with
saline-treated animals (SS) after 12 wk (Fig. 4). As expected, Gal-3-treated mice displayed a decreased response to methacholine compared with OVA asthmatic controls at 4 wk with significance being reached at methacholine concentrations of 48 mg/ml (data not shown).

As can be seen in Fig. 4 in the chronic phase (12 wk), animals treated with Gal-3 gene therapy displayed strong and significantly inhibited responses to cholinergic stimulation with different methacholine doses (6, 12, and 24 mg/ml). Asthmatic groups of mice treated with plasmid without insert were tested, and no differences were observed with respect to the OVA-positive group (Fig. 4). Gal-3 gene therapy treatment thus conferred protection against methacholine-induced AHR, during acute and chronic asthma.

**Histopathologic examination**

Chronic allergen challenge resulted in increased inflammatory infiltrates in perivascular and peribronchial regions, consisting mainly of eosinophils (Fig. 5B). In contrast, in plasmid-encoding Gal-3-treated mice (Fig. 5D), inflammatory cell infiltrates were absent, with a pattern similar to that of control mice (saline group, Fig. 5A). Fig. 5C shows that after sensitization and airway challenge with OVA and instillation with empty plasmid, the infiltrate is similar to that obtained in the OVA group. Increased subepithelial deposition of ECM proteins, specifically collagen, is a prominent feature of airway remodeling. We examined connective tissue in lung sections stained with Masson Trichrome. Saline control mice showed a thin uniform layer of matrix in peribronchial subepithelial regions (Fig. 5E), whereas chronic Ag challenge was associated with an increase in matrix in the subepithelial layer of the airways and in perivascular regions, as shown by a dramatic increase in both the extent of collagen deposition and intensity of staining (Fig. 5F), also found in the pEGFP empty plasmid group (Fig. 5G). In marked contrast, matrix deposition in these same regions was consistently abrogated in Gal-3-treated mice (Fig. 5H).

Excessive mucus secretion from hyperplastic goblet cells is also a characteristic feature of the asthmatic airway. To determine the extent of mucus cell metaplasia following prolonged allergen challenge and Gal-3 treatment, paraffin-embedded sections of lung were stained with PAS. During chronic inflammation, an increase in PAS-positive cells was observed in the bronchial epithelium in OVA (Fig. 5J) and pEGFP groups (Fig. 5K) when compared with saline controls (Fig. 5I). In Gal-3 gene-treated mice (Fig. 5L), the PAS-positive cells were significantly reduced at a rate similar to that of negative control mice (Fig. 5I). These data show that intratracheal deposition of a vector with a gene that codifies for Gal-3 in mice causes a specific reduction of inflammation and this inhibition is not due to plasmid because treatment with pEGFP empty does not modify the characteristic pattern of inflammation.

Semi-quantitative analysis of stains from the groups of mice revealed that inflammatory cell infiltrates, subepithelial deposition of collagen, and mucus secretion from goblet cells were significantly different between pEGFP-Gal-3 and OVA and pEGFP mice. In contrast, the grading of inflammation and stain were similar between pEGFP-Gal-3 and SS groups (Table III).

**Discussion**

Assessment of the efficacy of intranasal administration of plasmid carrying Gal-3 in a murine model of chronic allergic asthma

---

### Table I. Eosinophils in peripheral blood and bronchoalveolar lavage in the chronic stage

<table>
<thead>
<tr>
<th>Peripheral Blood</th>
<th>BAL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Eosinophils/mm³</strong></td>
<td><strong>% Eosinophils</strong></td>
</tr>
<tr>
<td>pEGFP-GAL-3</td>
<td>386 ± 150&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>OVA</td>
<td>1584 ± 600</td>
</tr>
<tr>
<td>pEGFP</td>
<td>1624 ± 408</td>
</tr>
<tr>
<td>SS</td>
<td>287 ± 100</td>
</tr>
</tbody>
</table>

<sup>a</sup> Total eosinophils were counted in a Fuchs-Rosenthal chamber using eosin dye.

<sup>b</sup> Percent of eosinophils in peripheral blood and bronchoalveolar lavage by flow cytometry.

<sup>c</sup> Total cells were counted using Trypan Blue vital dye.

<sup>d</sup> Data represents median ± SD of n = 20–40 mice/group.

<sup>e</sup> p < 0.01 vs. OVA and pEGFP groups.

---

### Table II. Total and specific Ig in serum<sup>a</sup>

<table>
<thead>
<tr>
<th>OVA-Specific Ig (OD)</th>
<th>Total IgE Levels (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG&lt;sub&gt;1&lt;/sub&gt;</td>
<td>IgE</td>
</tr>
<tr>
<td>1.92 ± 0.1</td>
<td>0.793 ± 0.06</td>
</tr>
<tr>
<td>2.01 ± 0.1</td>
<td>0.798 ± 0.02</td>
</tr>
<tr>
<td>2.3 ± 0.3</td>
<td>0.791 ± 0.035</td>
</tr>
<tr>
<td>0.1 ± 0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.022 ± 0.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ig levels values represented (mean ± SD) for each group (20–40 mice/group).

<sup>b</sup> p < 0.05, vs other groups.

---

**FIGURE 3.** Collagen levels in lungs. After 12 wk of OVA challenge or SS, lungs were analyzed for total pepsin soluble collagen content. To evaluate baseline collagen content lungs from the saline group (SS), mice were assayed. Results are expressed as mean ± SD of micrograms of total collagen per 100 mg of lung, (n = 6 to 8 for each group). Differences between pEGFP-Gal-3 and OVA groups proved statistically significant (***, p < 0.001).
showed that Gal-3 expression in lungs following intranasal instillation of plasmid-encoding Gal-3 produced a significant therapeutic benefit with considerable improvement in AHR and remodeling.

Gal-3 belongs to a family of soluble proteins with affinity for β-galactose-containing oligosaccharides. In previous in vitro studies, we demonstrated that Gal-3 down-regulates IL-5 gene expression in different cell types, such as Ag-specific T cell lines, human eosinophils, and PBMC from allergic patients (29, 30). The IL-5 down-regulation observed in these cells is specifically due to Gal-3, because this effect is reversed when specific anti-Gal-3 Abs are used (29). Subsequent in vivo studies at our laboratory have shown that intratracheal instillation of plasmid-encoding Gal-3 in acute asthmatic rats leads to improvement in the cellular inflammatory infiltrate and pulmonary function (34).

Human asthma, however, is a chronic disease, with structural changes leading to airway remodeling (38–40). We therefore investigated the possibility of extending our acute rat model to a chronic asthma situation, so as to study the effect of Gal-3 gene therapy and its influence on the remodeling process.

Recently, innovative protocols have been published which involve chronic inhalation of OVA (35, 41) in a murine chronic asthma model. This model has the additional advantage of relying on natural immunization (without the use of adjuvant), which is more like the situation experienced by asthmatic patients. These studies also demonstrated that there is a strain-specific response similar to genetic restrictions observed in humans. A/J mice are more prone to develop the chronic model with the characteristics of remodeling.

Using this experimental model, we have shown that down-regulation of the IL-5 gene in the lung, through nasal instillation of plasmid-encoding Gal-3, brings about a reduction in the eosinophil count in BAL and peripheral blood, and is associated with a significant decrease in bronchial hyperreactivity, ECM protein expression in the airway and mucus, and collagen accumulation in the airways. These data support the role of eosinophils in these processes.

**FIGURE 4.** Effect of pEGFP-Gal-3 on bronchial responsiveness to methacholine. Methacholine dose response at 12 wk after treatment. A significant reduction of bronchial hyperreactivity was observed in the pEGFP-Gal-3-treated group which reached normal values (equal to the control group, SS) on week 12. When asthmatic mice were treated with empty plasmid, bronchial responsiveness to methacholine is similar to that obtained with the OVA-positive group. Values are expressed as mean ± SD; n = 20–40/group for each methacholine dose point. *p ≤ 0.05 and ***, p ≤ 0.001.

**FIGURE 5.** Histopathologic findings of pulmonary tissues in chronic asthma and effect of Gal-3 gene therapy. Comparison of structural changes in airways of negative control (SS), untreated positive control (OVA), empty plasmid (pEGFP), and Gal-3-treated (pEGFP-Gal-3) mice shown with H&E (row A–D), Masson Trichrome (row E–H) and PAS (row I–L) staining of lung sections. Photomicrographs representative of lungs removed from the saline group (negative control) are in the first column, from the OVA-positive group are represented in the second column, from the pEGFP empty plasmid in the third column and finally, the pEGFP-Gal-3 group is represented in the fourth column. The SS group (A) and Gal-3-treated mice (D) show no inflammatory cell infiltrates, unlike the OVA-positive group (B) and pEGFP (C) in which peribronchial and perivascular inflammatory infiltrate, predominantly consisting of eosinophils and lymphocytes, is present. In the absence of OVA challenge, SS (E) and Gal-3 gene-treated mice (H) exhibited minimal peribronchial Trichrome staining (blue color) due to a reduction in collagen deposition. In contrast, repetitive OVA challenge for 12 wk induced circumferential peribronchial Trichrome staining (F), which was significantly reduced in Gal-3-treated mice (H). No reduction was obtained in mice treated with pEGFP (G). Control preparation from mice not challenged with OVA (SS) exhibited minimal epithelial PAS staining (purple color, I). In contrast, repetitive OVA challenge for 12 wk induced epithelial PAS staining (arrow in J), which was significantly inhibited by pEGFP-Gal-3 treatment (L). Hence, inhibited mucus secretion and conserved airway epithelium structure was observable in treated mice. pEGFP alone treatment does not produce a reduction in PAS staining (K).
Table III. Semiquantitative measurement of the lung histology

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Grading Scale</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OVA</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>27</td>
<td>p &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>pEGFP</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>18</td>
<td>p &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>pEGFP-GAL-3</td>
<td>25</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive Masson stain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OVA</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>30</td>
<td>p &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>pEGFP</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>17</td>
<td>p &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>pEGFP-GAL-3</td>
<td>23</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive PAS stain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OVA</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>31</td>
<td>p &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>pEGFP</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>19</td>
<td>p &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>pEGFP-GAL-3</td>
<td>28</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Semiquantitative analysis using an arbitrary intensity scale.
* Fisher’s exact test: comparisons between groups were made vs the basal saline group.
* Inflammation: 0, No cellular inflammatory infiltrate. 1, Mild cellular inflammatory infiltrate. 2, Moderate cellular inflammatory infiltrate. 3, Severe cellular inflammatory infiltrate.
* n. Number of animals studied.
* Positive Masson stain: 0, Basal ECM staining. 1, Mild staining. 2, Moderate staining. 3, Intense staining.
* Positive PAS stain: 0, No staining. 1, Mild staining. 2, Moderate staining. 3, Intense staining.

Processes and suggest a link between eosinophilic infiltration and airway remodeling, as reported elsewhere (25, 42, 43). We contend that IL-5 down-regulation reduces the number of eosinophils in BAL, pulmonary tissues, and peripheral blood, and that this, in turn, leads to a reduction in eosinophilic inflammation during the acute period and a clear improvement in chronicity and remodeling.

IL-5 is one of the main regulatory cytokines that modulates eosinophils, the major inflammatory effector cells in allergic disorders (44, 45). Furthermore, IL-5 plays an important role in airway remodeling observed in experimental asthma (46). These characteristics make IL-5 an excellent target for treatment of eosinophilic airway inflammation. The role of IL-5 in eosinophil biology has been extensively studied, though the precise mechanisms that enhance eosinophilopoiesis in bone marrow of asthmatics remain to be fully elucidated. Inhalation of allergen is known to increase the number of IL-5-responsive eosinophil progenitors in bone marrow (47). In vivo animal models indicate that IL-5 production restricted to the airways is insufficient to explain this phenomenon, and that systemic IL-5 is implicated (48). There have been several recent attempts to dissect the role of the eosinophil in asthma by using humanized mAbs against IL-5, with variable results (17, 18). Despite the fact that anti-IL-5 almost totally ablated eosinophils in the blood and sputum (17), tissue eosinophils were nevertheless reduced rather than depleted (18), possibly as a result of down-regulated IL-5 expression of airway eosinophils (49).

In our chronic experimental model, the use of Gal-3 gene therapy led to a 72–92% inhibition in airway and blood eosinophils in 12-wk-treated mice. Gene therapy has some advantages over conventional pharmacologic treatments. It avoids parenteral administration with its possible adverse effects. Delivery of therapeutic proteins by gene therapy has the additional advantage of enhancing the efficacy, convenience, and cost-effectiveness of chronic disease treatment (50). It has been shown that direct gene transfer results in rapid DNA uptake and that 1–3 days after administration, genes express a biologically active protein in lung (51), which remains there for as long as 28 days (52).

As pointed out, AHR is not dependent on IgE production in this model, a finding consistent with the report that allergic AHR develops normally in IgE-deficient mice (53). The marked improvement in airway hyperreactivity in Gal-3-treated mice plus the inhibition of airway remodeling (measured as diminished expression of ECM proteins in the airways and mucus accumulation) are in line with other published data, which postulate a new role for eosinophils and suggest a link between eosinophilic infiltration and airway remodeling (9, 42, 43, 46). Pulmonary function analysis thus shows that Gal-3 treatment brought about a marked inhibition in AHR.

Eosinophils produce fibrogenic factors and induce fibroblast proliferation. There is increasing evidence to show that eosinophils may be important in the pathophysiology of airway remodeling (54, 8). Our data suggest that Gal-3-induced eosinophil depletion leads to inhibition of airway alteration (or remodeling, as it is known) i.e., cellular infiltration, goblet cell hyperplasia, subepithelial fibrosis, etc. In the gene therapy treated mice, histologic analysis of the airways after chronic allergen challenge showed that there were fewer or no peribronchial and peribronchial cellular infiltrates and that the structure of the airway epithelium was conserved. In light of these results, Gal-3 therapy might be thought useful for the purpose of preventing airway remodeling.

We also tested variations in other cytokines, such as IL-4, IL-10, and IL-13, which are important in allergic reactions. IL-10 is a pleiotropic cytokine released from various cells, including T cells. The role of IL-10 in asthma remains controversial: whereas some studies report IL-10 expression to be higher in subjects with asthma than in control subjects (55, 56), others report lower IL-10 levels (57, 58). We observed an increase in IL-10 levels in the lungs of nontreated asthmatic mice. High IL-10 levels in the OVA group of mice suggest that IL-10 may serve as an endogenous feedback, an inhibitory mechanism to modulate the inflammatory response.

With respect to the results yielded for other cytokines, such as IL-4 and IL-13, no significant changes were found as a result of the treatment. These data confirm our previous studies, in which Gal-3 was observed to specifically inhibit IL-5 transcription, with no collateral effects on other cytokines. The explanation for this may lie in the finding that IL genes are differentially regulated (59, 60).

In contrast with our results, a recent paper has demonstrated in a model of OVA-induced asthma, that Gal-3-deficient mice developed fewer eosinophils than did positive control asthmatic mice (61). Several differences may have contributed to this divergence. In the first place, the mice in our experiment received the Gal-3 gene by nasal instillation directly into the bronchial tree, with the ensuing increase in this protein in the respiratory tract, which is precisely the site where the inflammatory reaction takes place. Second, the strain used in the above experiment is not as prone to asthmatic allergic reaction as the A/J mouse (35), and finally, and most remarkably, is the consideration that the effects of exogously administered substances (either by using a recombinant protein or a gene delivery vector) are not necessarily the same as the endogenous ones as has been widely documented (62, 63). Furthermore, we have demonstrated that in mice treated with gene therapy, there is an induction of this protein in peribronchial epithelial cells when compared with lungs of normal noninduced mice.

Interestingly, the inhibitory effects of Gal-3 on the inflammatory cytokines and chemokines in guinea pig asthma models with special mention to selective inhibition of IL-5 gene transcription have been recently reported (64).
The beneficial effect of Gal-3-gene therapy is due to IL-5 down-regulation and the ensuing improvement in eosinophilic inflammation. The possibility of another complementary effect induced by DNA itself, in a manner akin to that reported for DNA vaccination (65, 66), has been ruled out, because experiments conducted using plasmid without insert failed to interfere with the asthmatic inflammation.

Interventions that regulate Th2 cytokine effector pathways are attractive as potential therapeutic targets, and we assume that concomitant treatment throughout the period of chronic allergen exposure might result in abrogation of Th2 immune-mediated airway inflammation, with subsequent attenuation of aspects of airway remodeling and AHR.

Our results thus confirm that, through inhibition of IL-5 gene transcription, Gal-3 gene therapy in experimental chronic asthma leads not only to the abrogation of acute asthma, but also, most importantly, to an improvement in chronic airway inflammation and changes in the remodeling process.

Disclosures

The authors have no financial conflict of interest.

References


