

Treatment of Experimental Asthma by Decoy-mediated Local Inhibition of Activator Protein-1

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Rationale: Asthma is associated with increased expression of a typical array of genes involved in immune and inflammatory responses, including those encoding the prototypic Th2 cytokines interleukin (IL) 4, IL-5, and IL-13. Most of these genes contain binding sites for activator protein-1 (AP-1) within their promoter and are therefore believed to depend on AP-1 for their expression, suggesting that this transcription factor could be of particular importance in asthma pathophysiology. **Objective:** To clarify the role of AP-1 in the effector phase of pulmonary allergy. **Methods:** Ovalbumin (OVA)-sensitized mice were intratracheally given decoy oligodeoxyribonucleotides (ODNs) specifically directed to AP-1 or scrambled control ODNs before challenge with aerosolized OVA. Twenty-four hours after the last OVA challenge, airway hyperresponsiveness was measured and allergic airway inflammation was evaluated quantitatively. AP-1 decoys were localized using flow cytometry and immunohistochemistry. AP-1 activity in the lung was assessed using electrophoretic mobility shift assay. **Measurements and Main Results:** Intratracheally delivered AP-1 decoys efficiently targeted airway immune cells, thus precluding AP-1 activation on OVA challenge. Decoy-mediated local inhibition of AP-1 resulted in significant attenuation of all the pathophysiologic features of experimental asthma—namely, eosinophilic airway inflammation, airway hyperresponsiveness, mucous cell hyperplasia, production of allergen-specific immunoglobulins, and synthesis of IL-4, IL-5, and IL-13. Scrambled control ODNs had no detectable effects. **Conclusions:** Our results reveal a key role for AP-1 in the effector phase of pulmonary allergy and indicate that specific AP-1 inhibition in the airways may have therapeutic value in the control of established asthma.

Keywords: allergy; eosinophils; gene therapy; lung; transcription factors

The prevalence, morbidity, and mortality of asthma have increased worldwide over the last decades (1). Allergic asthma is recognized as a chronic inflammatory disease of the airways that results from aberrant CD4⁺ Th2 lymphocyte responses to common environmental stimuli (2–5). The hallmarks of allergic asthma are as follows: infiltration of eosinophils into the bronchial wall and lumen, exaggerated mucus production in the airways, elevated serum IgE levels, and airway hyperresponsiveness (AHR) to specific and nonspecific stimuli (2–5).

From a molecular point of view, the pathology in asthma occurs as a consequence of abnormal overexpression of a large number of genes involved in immune and inflammatory responses, such as those encoding the prototypic Th2 cytokines interleukin (IL) 4, IL-5, and IL-13 (2–5). Most of these genes have been demonstrated to contain binding sites for nuclear factor- κ B (NF- κ B) and/or activator protein-1 (AP-1) within their promoter or enhancer and are therefore believed to depend on NF- κ B and/or AP-1 for their expression (6, 7), suggesting that these transcription factors could be of particular importance in the pathophysiology of asthma. This hypothesis is further supported by the finding that glucocorticosteroids (GCs), the most effective treatment for asthma, exert their antiinflammatory effects at least partly through inhibition of NF- κ B and AP-1 (8–11).

Studies using knockout mice have shown that some NF- κ B (p50 and c-Rel) and AP-1 (JunB and JunD) family members are required for full differentiation of Th2 cells and are thus essential for induction of allergic airway inflammation (12–15). However, to develop new therapeutic strategies, it is essential to define the role of these transcription factors in established allergic reactions. Recently, we have clarified the role of NF- κ B in the effector phase of experimental asthma using decoy oligodeoxyribonucleotides (ODNs) specifically directed to this transcription factor (16). Decoys are synthetic double-stranded ODNs bearing the consensus binding sequence of a specific transcription factor (17–19). When introduced into cells, decoys impair the authentic interaction between the target transcription factor and genomic DNA, with subsequent inhibition of gene expression (17–19). Intratracheal delivery of NF- κ B decoy ODNs to ovalbumin (OVA)-sensitized wild-type mice led to selective abrogation of NF- κ B activity in the airways on OVA provocation. Local NF- κ B inhibition was associated with strong reduction of eosinophilic inflammation, mucus production, and AHR, but not allergen-specific IgE synthesis, thus validating NF- κ B as a target for asthma therapy (16).

The present study analyzed the role of AP-1 in experimental asthma using the decoy ODN approach. Our aim was to definitively address the following three questions: (1) does AP-1 also play a key role in the effector phase of allergic airway inflammation, (2) might selective AP-1 antagonization be used for the treatment of established asthma, and (3) is AP-1 inhibition more or less efficient at preventing the effector Th2 responses in the lung than NF- κ B inhibition? Some of the results of this study have been previously reported in the form of an abstract (20).

METHODS

AP-1 Decoy ODNs

AP-1 decoy ODNs (sense strand: 5'-GCT TGA TGA GTC AGC CGGA-3') were generated as described (16). Scrambled ODNs were used as controls (sense strand: 5'-GCA GTT AGG CAG CTG TGCA-3').

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Induction of Allergic Airway Disease and Treatment with AP-1 Decoys

Female BALB/c mice, 6 to 8 weeks old (Harlan Nederland, Horst, The Netherlands), were sensitized on Days 0 and 14 by intraperitoneal injection of 10 μ g OVA (grade III; Sigma-Aldrich, St. Louis, MO) mixed with Inject Alum (Pierce, Rockford, IL). Sham-immunized mice received alum alone. On Days 28 through 30, mice were challenged for 1 hour by exposure to an aerosol of 1% (wt/vol) OVA. Twenty-four hours after the last challenge, AHR was measured and allergic airway inflammation was characterized.

AP-1 decoys and scrambled ODNs were given by intratracheal instillation (15 nmol in 30 μ l Tris-EDTA buffer/mouse) on Days 28 and 30 (6 hours before OVA inhalation) to OVA-sensitized mice. In experiments aimed at localizing AP-1 decoys or scrambled ODNs, the last intratracheal administration was performed with fluorescein isothiocyanate-(FITC) labeled or biotinylated ODNs. The different ODN treatments had no obvious detrimental effect. The protocol was approved by the Animal Ethics Committee of the University of Liège.

Measurement of AHR

Responsiveness to β -methacholine (MCh) was assessed in conscious mice using single- or double-chamber whole-body plethysmography (Buxco Europe Ltd., Winchester, UK) and increases in enhanced pause (Penh) or specific airway resistance (sRaw), respectively, as indexes of airway obstruction (21, 22). Penh measurements were taken as previously described (16). For double-chamber plethysmography, baseline measurements were taken and averaged for 3 minutes after acclimation of the animals to the boxes. Afterward, phosphate-buffered saline (PBS) or increasing doses of MCh (ranging from 3 to 30 mg/ml saline) were nebulized into the nasal chamber for 1.5 minutes, and sRaw measurements were taken and averaged for 1.5 minutes after each nebulization. Airway reactivity was expressed as a fold-increase in Penh or sRaw for each concentration of MCh compared with Penh or sRaw value after PBS challenge.

Bronchoalveolar Lavage, Cytology, and Cytokine Assays

Cell density in bronchoalveolar lavage fluid (BALF) was assessed by the use of a hemocytometer. Cell differentials were performed on cyto-spin preparations stained with Diff-Quick (Dade Behring, Dudingen, Germany). BALF levels of IL-4 (Endogen, Rockford, IL), IL-5 (Endogen), IL-13 (R&D Systems, Minneapolis, MN), IFN- γ (Endogen), and eotaxin (R&D Systems) were determined by ELISA.

Lung Histology and Immunohistochemistry

Lungs sections were stained with hematoxylin and eosin. Mucin was assessed by periodic acid-Schiff stains. Biotinylated decoys and biotinylated scrambled ODNs were localized by immunohistochemistry using the Immunohistowax processing method (A Phase; Gosselies, Belgium) (23). Briefly, after histowax processing, sections were dewaxed in acetone for 5 minutes and blocked with blocking reagent (Roche Diagnostics, Basel, Switzerland) for 12 hours. Endogenous peroxidase activity was then blocked with 3% H₂O₂ for 1 to 3 hours, and biotinylated decoys were detected using the ABC kit from Vector Laboratories (Burlingame, CA; 1/50 in blocking reagent) for 30 minutes at room temperature followed by 30 to 40 minutes' incubation with Sigma Fast 3,3'-diaminobenzidine (Sigma-Aldrich Co.).

Flow Cytometry

To localize FITC-labeled decoys and scrambled ODNs, immune cells from BALF, lung tissue and thoracic lymph nodes were examined for FITC positivity by using flow cytometry (16). Lungs and thoracic lymph nodes were minced and filtered through nylon mesh before flow cytometry analyses. Cells were incubated (for 30 minutes on ice in PBS containing 1% normal mouse serum) with either biotin-conjugated antimouse CD11c (HL3; PharMingen, San Diego, CA) and phycoerythrin-conjugated antimouse I-A^d (AMS-32.1; PharMingen) Abs, phycoerythrin-conjugated F4/80 (CI:A3-1; Serotec, Oxford, UK), Cy-chrome-labeled antimouse CD3 ϵ (145-2C11; PharMingen) and allophycocyanin-conjugated antimouse CD19 (1D3; PharMingen) Abs, phycoerythrin-conjugated antimouse CCR3 Abs (83101; PharMingen), or isotype controls (PharMingen). Biotin labeling was revealed by addition of allophycocyanin-

conjugated streptavidin (PharMingen). Cells were washed, fixed with paraformaldehyde (0.25%), and analyzed using a FACScalibur (Becton Dickinson, San Jose, CA).

Apoptosis Assays

Immune cells from BALF and lung tissue were assayed for apoptosis using annexin-V-FITC staining (Roche Diagnostics) and flow cytometry analyses.

Electrophoretic Mobility Shift Assays

Electrophoretic mobility shift assays were performed as previously described (16). The sequence of the wild-type AP-1 probe was 5'-GGT TCG CTT GAT GAG TCA GCC GGA A-3'; the sequence of the mutated AP-1 probe was 5'-GGT TCG CTT GAT GCT ATC GCC GGA A-3'. The Abs specific for c-Jun (sc-1694 X), JunB (sc-8051 X), JunD (sc-74 X), c-Fos (sc-52 X), and FosB (sc-7203 X) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

Determination of Serum Levels of Total and OVA-specific Immunoglobulins

Serum levels of total IgE and OVA-specific IgE, IgG1, and IgG2a were measured by ELISA (16).

Statistical Analysis

Data are presented as means $\pm$ SD. Normality of the values was confirmed using a Kolmogorov-Smirnov test. The differences between mean values were estimated using an analysis of variance test followed by a Fisher's protected least standard deviation (PLSD) test. A value of $p < 0.05$ was considered significant.

RESULTS

Intratracheal Delivery of AP-1 Decoy ODNs Prevents AP-1 Activation in the Airways on Allergen (OVA) Challenge

To investigate the effects of allergenic provocation on AP-1 activity in the lung, sham-immunized and OVA-sensitized mice were challenged with OVA for 3 consecutive days (these mice are hereafter referred to as sham/OVA and OVA/OVA mice, respectively). Twenty-four hours after the last OVA challenge, mice were killed, and nuclear extracts were prepared from the whole lung and BALF cells. The nuclear extracts were further assessed for AP-1 DNA-binding activity using electrophoretic mobility shift assays. Only a faint basal AP-1 DNA-binding activity was observed in nuclear extracts from sham/OVA mice, presumably reflecting weak AP-1 activity, whereas nuclear extracts prepared from OVA/OVA mice displayed a much higher AP-1 DNA-binding activity, indicating stronger AP-1 activity (Figure 1A). Oct-1 DNA-binding activity, used as a loading control, was similar in all samples (Figure 1A). DNA-binding competition experiments using a 50-fold excess of unlabeled wild-type and mutated AP-1 probes confirmed specificity of AP-1 binding (data not shown).

AP-1 is not a single protein but an array of dimeric basic region-leucine zipper proteins that belong to the Jun (c-Jun, JunB, and JunD) and Fos (c-Fos, FosB, Fra1, and Fra2) subfamilies (24). To analyze the composition of the AP-1 complexes present in lung extracts, electrophoretic mobility shift assays were performed in the presence of antibodies directed against the different AP-1 proteins. These supershift experiments showed that the retarded AP-1 complexes observed in lung extracts from sham/OVA and OVA/OVA mice contain c-Jun, JunD, and c-Fos subunits (Figure 1B). Indeed, the anti-c-Jun, anti-JunD, and anti-c-Fos antibodies were all able to partly remove the AP-1 complexes, whereas the anti-JunB and anti-FosB antibodies did not react with these complexes (Figure 1B).

In an attempt to specifically inhibit AP-1 *in vivo*, we first designed a double-stranded ODN containing the consensus AP-1

binding site (this ODN is hereafter referred to as AP-1 decoy). Then, we tested the ability of AP-1 decoys to selectively inhibit AP-1 activity in the airways of OVA/OVA mice. Decoy and control scrambled ODNs were intratracheally administered to OVA-sensitized mice 6 hours before the first (Day 28) and the last (Day 30) OVA challenge. We found that local delivery of AP-1 decoys reduced AP-1 DNA-binding activity in the whole lung and totally abrogated AP-1 DNA-binding activity in BALF cells (Figure 1A). AP-1 decoys therefore have the ability to inhibit the increase in AP-1 activity in the lungs of OVA/OVA mice by preventing AP-1 from binding to its genomic response elements. In contrast, scrambled ODNs had no detectable effects (Figure 1A). Neither AP-1 decoys nor scrambled ODNs affected Oct-1 activity (Figure 1A), demonstrating the specificity of our decoy ODN approach.

These results show that AP-1 is activated in the lungs of OVA-sensitized mice after OVA challenge and that AP-1 activity may be efficiently and specifically blocked by intratracheal administration of AP-1 decoys.

AP-1 Decoys Target Airway Immune Cells and Reduce the Survival Rate of Eosinophils, but Not of the Other Immune Cell Types

Recently, we have shown that intratracheal administration of NF- κ B decoys to OVA-sensitized mice results in efficient nuclear transfection of airway immune cells, associated with suppression of NF- κ B activation on OVA challenge (16). In this previous study, no decoys were detectable in lung structural cells (i.e., bronchial epithelial cells, endothelial cells, and fibroblasts) and draining lymph node cells. We sought to determine whether distribution of intratracheally delivered AP-1 decoys was similar

to that of NF- κ B decoys. Accordingly, the last intratracheal administration of AP-1 decoys (Day 30) was performed with FITC-labeled ODNs. Intratracheal delivery of FITC-labeled AP-1 decoy ODNs to OVA/OVA mice led to DNA uptake by pulmonary and BALF macrophages, dendritic cells, T cells, and eosinophils (Figure 2). Only few B cells (CD19⁺) were found in the whole lung and BALF, but nearly all had incorporated AP-1 decoys (data not shown). Macrophages, dendritic cells, and T and B cells obtained from thoracic lymph nodes were all FITC-negative (data not shown). Pulmonary distribution of AP-1 decoys was further studied by immunohistochemistry after local administration of biotinylated ODNs. Biotinylated AP-1 decoys were principally found in the nucleus of peribronchial and perivascular immune cells (Figure 3, *upper right* and *lower panels*). By contrast, lung structural cells, including bronchial epithelial cells, endothelial cells, and fibroblasts, did not incorporate AP-1 decoys (Figure 3, *upper right panel*). Localization of intratracheally delivered scrambled ODNs, as evaluated by flow cytometry and immunohistochemistry, was identical to that of AP-1 decoys (data not shown).

To assess whether AP-1 decoys affected the survival of target cells, immune cells from lung tissue of untreated OVA/OVA mice and OVA/OVA mice treated with either AP-1 decoys or scrambled ODNs were assayed for apoptosis by staining with

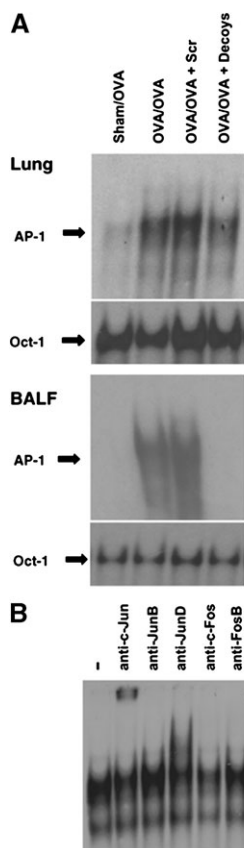


Figure 1. Local administration of decoy oligodeoxynucleotides (ODNs) attenuates activator protein-1 (AP-1) activation in the airways on allergen challenge. Mice were either sensitized with ovalbumin (OVA) + alum (Days 0 and 14) and challenged by repeated exposure to an aerosol of OVA (Days 28–30) (OVA/OVA mice) or sham-sensitized with alum only and OVA-challenged (sham/OVA mice). AP-1 decoys or scrambled (Scr) ODNs were given by intratracheal instillation on Days 28 and 30 (6 hours before OVA inhalation) to OVA/OVA mice. (A) Twenty-four hours after the last OVA challenge (Day 31), nuclear protein extracts were prepared from the whole lung or from bronchoalveolar lavage fluid (BALF) cells. These extracts were assessed for AP-1 and Oct-1 DNA-binding activity by electrophoretic mobility shift assays. (B) To characterize specific AP-1 complexes, supershift analysis was conducted with Abs directed against c-Jun, JunB, JunD, c-Fos, and FosB. Visible supershifts are indicated by solid arrowheads. These results are representative of at least six comparable experiments.

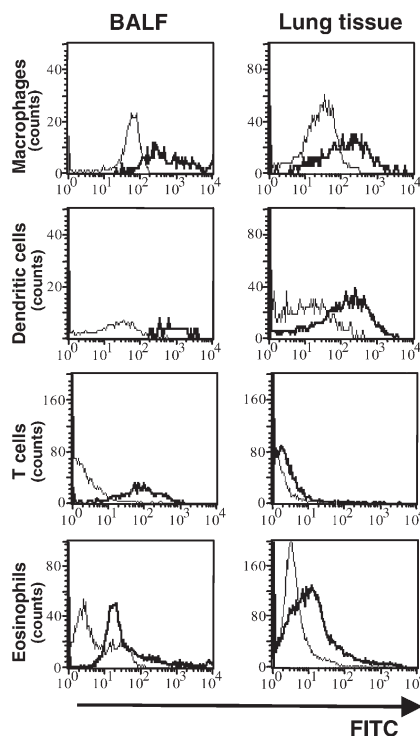


Figure 2. AP-1 decoys target lung immune cells. Mice sensitized (Days 0 and 14) and challenged (Days 28–30) with OVA received AP-1 decoy ODNs intratracheally on Days 28 and 30 (6 hours before OVA challenge). The last instillation of AP-1 decoys (Day 30) was performed with fluorescein isothiocyanate (FITC)-labeled ODNs. Twenty-four hours later (Day 31), macrophages (F4/80⁺), dendritic cells (I-A^{dh}iCD11c^{med-hi}), T cells (CD3e⁺), and eosinophils (large and granular CCR3⁺, CD3⁺ cells) from BALF and lung tissue were assessed for FITC positivity using flow cytometry (*bold lines*). Cells from mice that received unlabeled decoys were used as controls (*thin lines*). These results represent three comparable experiments.

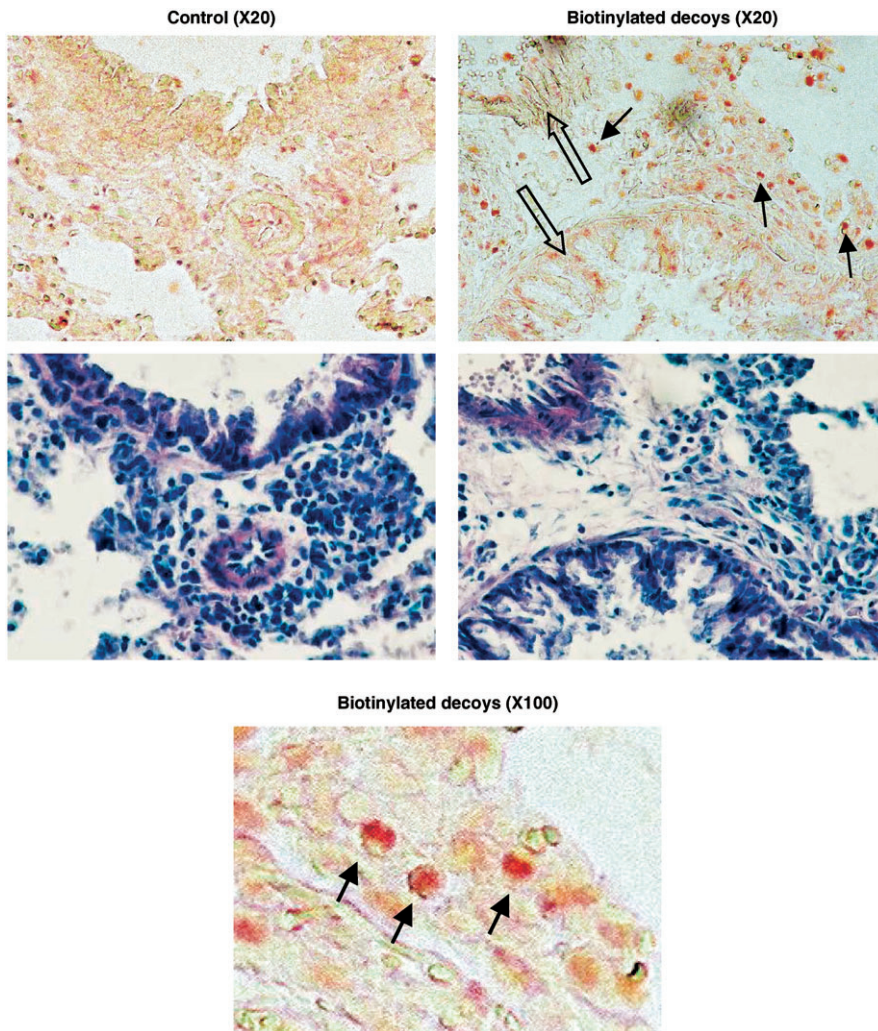


Figure 3. Intratracheally administered AP-1 decoys target the nucleus of airway immune cells. Mice sensitized (Days 0 and 14) and challenged (Days 28–30) with OVA received AP-1 decoy ODNs intratracheally on Days 28 and 30 (6 hours before OVA challenge). The last ODN administration was performed on Day 30 with biotinylated AP-1 decoys, which were localized 24 hours later using immunohistochemistry. Biotinylated AP-1 decoys targeted peribronchial and perivascular immune cells (solid arrows, upper right panel) and were mainly located in the nucleus of these cells (solid arrows, bottom panel). Lung structural cells did not incorporate biotinylated ODNs (open arrows, upper right panel). Lung sections from mice that received unlabeled decoys were used as controls (upper left panel). The red staining on these sections is due to the presence of debris. Hematoxylin–eosin staining of adjacent sections has been performed (middle left and right panels). These results represent three comparable experiments.

annexin-V-FITC. As shown in Table 1, macrophages, dendritic cells, and T cells from the lung tissue of decoy-treated OVA/OVA mice did not demonstrate any significant changes in apoptotic rates compared with the corresponding cells obtained from untreated OVA/OVA mice or OVA/OVA mice treated with scrambled ODNs. Conversely, the percentage of annexin-V-FITC-positive eosinophils was significantly higher in decoy-treated OVA/OVA mice compared with untreated OVA/OVA mice or OVA/OVA mice treated with scrambled ODNs. Similar results were obtained in the BALF (data not shown).

Taken together, these data indicate that intratracheally delivered AP-1 decoys target airway immune cells and increase the apoptotic rate of eosinophils, but do not affect the survival of macrophages, dendritic cells, or T cells.

Local Treatment with AP-1 Decoys Suppresses All the Pathophysiologic Features of Experimental Asthma

Peribronchial and perivascular eosinophilic infiltrates, epithelial cell hypertrophy, and mucus overproduction are all characteristic features of allergic airway inflammation (25). To assess the effects of local administration of AP-1 decoys on these parameters, the lungs of sham/OVA mice, untreated OVA/OVA mice, and OVA/OVA mice treated with either AP-1 decoys or scrambled ODNs were examined histologically. Inflammatory cell infiltrate, epithelial cell size, and mucus production were all significantly increased in untreated and scrambled ODN-treated OVA/OVA mice compared with sham/OVA counterparts (Figures 4A and 4B). In contrast, all these inflammatory signs were attenuated

TABLE 1. PERCENTAGE OF APOPTOTIC (ANNEXIN-V-FITC-POSITIVE) IMMUNE CELLS IN LUNG TISSUE OF UNTREATED OVA/OVA MICE AND OVA/OVA MICE TREATED WITH EITHER AP-1 DECOYS OR SCRAMBLED OLIGODEOXYNUCLEOTIDES

	Dendritic Cells (%)	Macrophages (%)	Eosinophils (%)	T cells (%)
OVA/OVA	25.3 ± 5.6	13 ± 1.5	23.1 ± 4.5	18.2 ± 0.9
OVA/OVA + Scr	33 ± 3.7	17.7 ± 6.4	25 ± 2.1	12.4 ± 2.3
OVA/OVA + decoys	25.8 ± 12.7	16.1 ± 4.5	41.1 ± 3.2*	15.5 ± 0.6

Definition of abbreviations: AP-1 = activator protein-1; FITC = fluorescein isothiocyanate; OVA = ovalbumin; Scr = scrambled.

* Significantly different from the values obtained in OVA/OVA mice ($p < 0.05$).

in OVA/OVA mice treated with AP-1 decoys (Figures 4A and 4B). These decoy-mediated modifications were congruently reflected in the decreased total cell and eosinophil counts measured in the BALF of decoy-treated OVA/OVA mice as compared with untreated and scrambled ODN-treated OVA/OVA animals (Figure 4C).

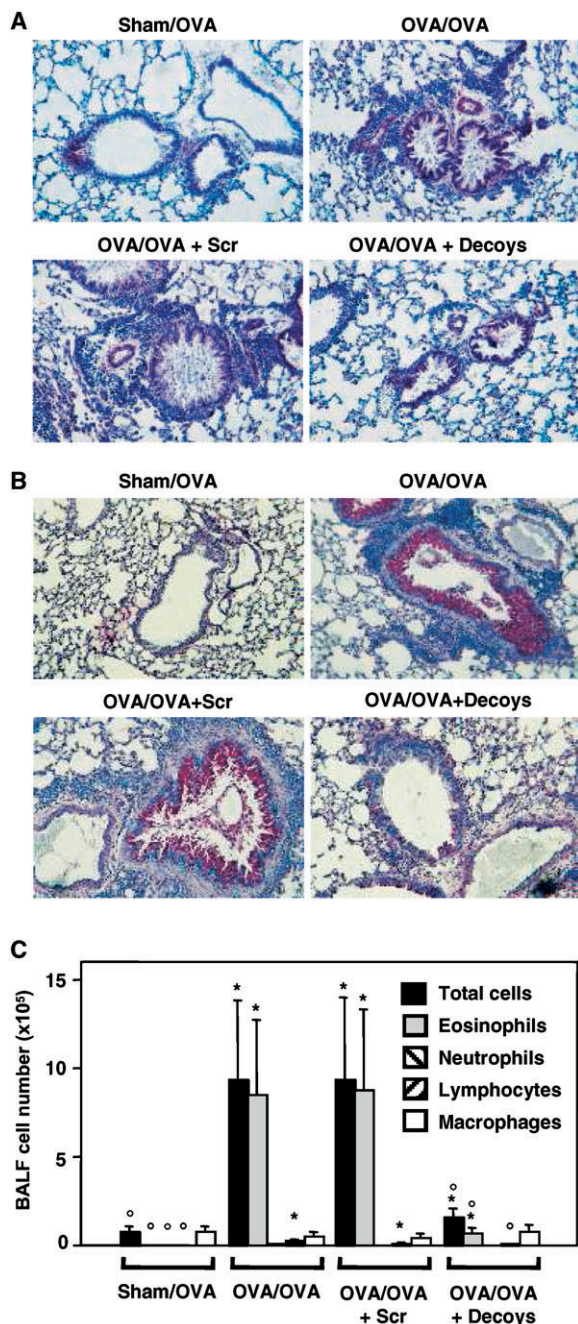


Figure 4. Intratracheal administration of AP-1 decoys reduces eosinophilic inflammation and mucus production in the airways of OVA/OVA mice. Lung sections of sham/OVA mice, untreated OVA/OVA mice, and OVA/OVA mice treated with either AP-1 decoys or Scr ODNs were stained with either hematoxylin and eosin (A) or periodic acid-Schiff (B). (C) The BALF recovered from the animals was subjected to total and differential cell counts. Data are presented as means $\pm$ SD ($n = 6$ in each mice group). *Significantly different from the results obtained in sham/OVA mice; °significantly different from the results obtained in OVA/OVA mice. Comparable results were obtained in six experiments.

We next analyzed whether treatment with AP-1 decoys would affect AHR in OVA/OVA mice. For that, responsiveness to MCh was assessed by using single- or double-chamber whole-body plethysmography and increases in Penh or sRaw as an index of airway obstruction, respectively. As shown in Figure 5, OVA/OVA mice showed a considerable increase in both Penh (Figure 5A) and sRaw (Figure 5B) compared with sham/OVA counterparts, revealing increased AHR. Furthermore, it was found that treatment of OVA/OVA mice with AP-1 decoys leads to a significant reduction of AHR to levels comparable to those recorded in sham/OVA mice (Figures 5A and 5B). Finally, we observed that scrambled ODNs had no effect on AHR in OVA/OVA mice (Figures 5A and 5B).

As expected in this model, serum levels of total IgE and OVA-specific IgE and IgG1 were markedly increased in OVA/OVA mice compared with sham/OVA control animals (Figure 6). Administration of AP-1 decoys but not scrambled ODNs resulted in a significant decrease in serum IgE and IgG1 levels in OVA/OVA mice (Figure 6). OVA-specific IgG2a levels were undetectable in the samples.

We finally examined cytokine levels in the airways of the mice. IFN- γ was present at lower levels in the BALF of untreated and scrambled ODN-treated OVA/OVA mice compared with sham/OVA animals (Figure 7). Interestingly, AP-1 decoy treatment restored IFN- γ concentrations in the airways of OVA/OVA mice (Figure 7). As shown in Figure 7, Th2 cytokines (IL-4, IL-5, and IL-13) and eotaxin, a chemoattractant for eosinophils and Th2 cells (26, 27), were produced at high levels in the airways of OVA/OVA mice, whereas these cytokines were barely detectable in the BALF of sham/OVA counterparts. Eotaxin and Th2 cytokine production in the airways of OVA/OVA mice was significantly suppressed after local administration of

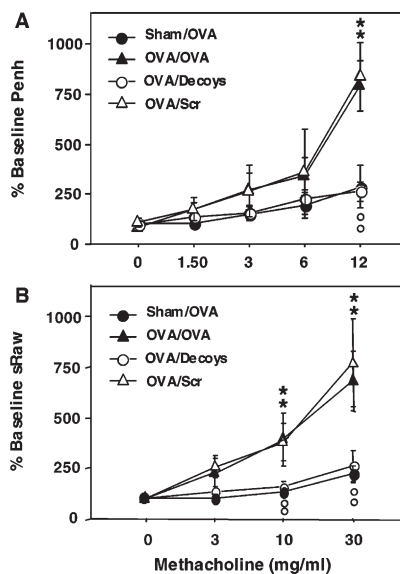


Figure 5. Treatment with AP-1 decoys abrogates airway hyperresponsiveness (AHR) in OVA-challenged mice. AHR was assessed in sham/OVA mice, untreated OVA/OVA mice, and OVA/OVA mice treated with either AP-1 decoys or Scr ODNs using single-chamber (A) or double-chamber (B) plethysmography, and by analyzing Penh and sRaw responses, respectively. (A) Phosphate-buffered saline (PBS) or increasing concentrations of methacholine (MCh; 1.5–12 mg/ml) were nebulized for 3 minutes, and Penh measurements were taken and averaged for 3 minutes after each nebulization. (B) PBS or increasing concentrations of MCh (3–30 mg/ml) were nebulized for 1.5 minutes, and sRaw measurements were taken and averaged for 1.5 minutes after each nebulization. Data are expressed as the fold-increase above PBS challenge values. Data are presented as means $\pm$ SD ($n = 6$ in each mice group). *Significantly different from the results obtained in sham/OVA mice; °significantly different from the results obtained in OVA/OVA mice. These results represent three comparable experiments.

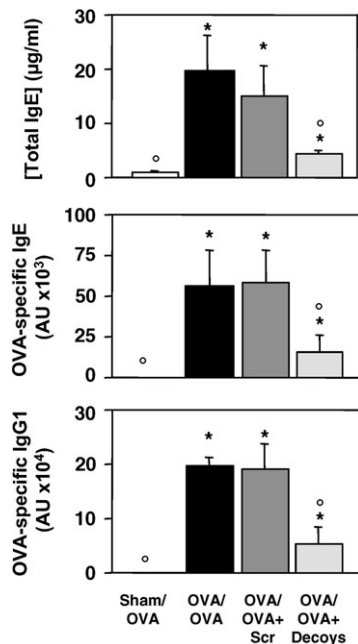


Figure 6. Local delivery of AP-1 decoys inhibits specific immunoglobulin production in OVA/OVA mice. Serum samples were taken from sham/OVA mice, untreated OVA/OVA mice, and OVA/OVA mice treated with either AP-1 decoys or Scr ODNs. Levels of total IgE and OVA-specific IgE and IgG1 were measured in these samples by ELISA. OVA-specific IgE and IgG1 levels are expressed using arbitrary units (AU). Data are presented as means $\pm$ SD ($n = 6$ in each mice group). *Significantly different from the results obtained in sham/OVA mice; °significantly different from the results obtained in OVA/OVA mice. These results are representative of three similar experiments.

AP-1 decoys (Figure 7). Scrambled ODNs did not cause measurable changes in eotaxin and Th2 cytokine production (Figure 7).

Together, these results demonstrate that local treatment with AP-1 decoys suppresses all the pathophysiologic features of experimental asthma—namely, eosinophilic airway inflammation, AHR, and increased production of mucus, allergen-specific IgE and IgG1, and Th2 cytokines.

DISCUSSION

JunB and JunD, two AP-1 subunits, have been shown to be required for full differentiation of Th2 cells (14, 15). Several lines of evidence suggest that AP-1 also plays a role in the effector phase of allergic responses. First, AP-1 binding sites are present in the 5'-flanking regions of many immune and inflammatory response genes that are overexpressed during this phase, including those encoding the prototypic Th2 cytokines IL-4 and IL-5 (6, 28, 29). Second, GCs and two oxidoreductase inhibitors—namely, MOL 294 and PNRI-299—are believed to exert their antiinflammatory effects in pulmonary allergy at least partly through AP-1 inhibition (8, 11, 30, 31). Recently, we have shown

that intratracheal delivery of “naked” decoy ODNs to OVA-sensitized mice allows specific transcription factor inhibition in the airways on OVA challenge (16). In the present study, we have taken advantage of the decoy approach we have developed to clearly demonstrate a key role for AP-1 in the effector phase of airway allergy.

Our study demonstrates for the first time the presence of activated AP-1 in airways with allergic inflammation. AP-1 is a dimer composed of proteins that belong to the Jun (c-Jun, JunB, and JunD) and Fos (c-Fos, FosB, Fra-1, and Fra-2) subfamilies (24). The Fos proteins, which are unable to dimerize with each other, form stable heterodimers with Jun proteins and thereby enhance their DNA binding activity (24). Among the Jun subfamily, c-Jun and JunB can activate IL-4 promoter activity, whereas it is suspected that JunD might repress IL-4 transcription (15, 28, 32). Supershift analysis revealed the presence of c-Fos, c-Jun, and JunD subunits in the activated AP-1 complexes observed in lung extracts from OVA/OVA mice. Surprisingly, JunB, which is selectively expressed by Th2 cells (32), was not found. It is expected that JunD repression by the AP-1 decoys would increase Th2 cytokine expression (15). However, in the case of AP-1 decoy treatment, the activity of c-Jun, which promotes Th2 cytokine expression (28), is also significantly repressed. The observed reduction in Th2 cytokine expression on AP-1 decoy treatment is therefore the result of the inhibition of AP-1 complexes containing both activators (c-Jun) and potential repressors (JunD) of Th2 cytokine transcription. Consistent with our previous report that lung structural cells are refractory to double-stranded ODN transfection (16), intratracheally delivered AP-1 decoys only targeted airway immune cells (i.e., macrophages, dendritic cells, eosinophils, and T and B cells) of OVA/OVA mice. This finding could account for the observed different effects of AP-1 decoys in whole lung and BALF cells. Indeed, decoy-induced AP-1 inhibition was total in BALF, which only contains immune cells, whereas it was only partial in the whole lung, which contains both structural and immune cells. Interestingly, treatment with AP-1 decoys selectively induced eosinophil apoptosis, suggesting that AP-1 is a central regulator of eosinophil survival. However, although AP-1 regulates a large array of genes involved in regulation of cell survival (e.g., *Bcl2*, *Bcl-XL*, *Bcl3*, *BIM*) (33), a role for AP-1 in the control of eosinophil apoptosis has never been reported to our knowledge.

The decoy-mediated local inhibition of AP-1 was accompanied by a suppression of IL-4, IL-5, IL-13, and eotaxin synthesis in the airways of OVA/OVA mice. AP-1 binding sites are found within the promoter of the genes encoding these cytokines

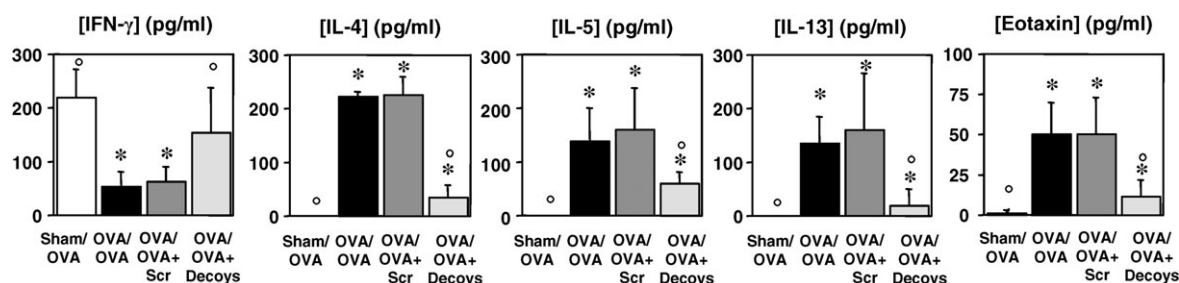


Figure 7. Treatment with AP-1 decoys suppresses IL-4, IL-5, IL-13, and eotaxin, but not IFN- γ , production in the airways on allergen challenge. IFN- γ , IL-4, IL-5, IL-13, and eotaxin concentrations were measured in the BALF of sham/OVA mice, OVA/OVA mice, and OVA/OVA mice treated with either AP-1 decoys or Scr ODNs. Cytokine levels were determined by specific ELISA and are presented as means $\pm$ SD ($n = 6$ in each mice group). *Significantly different from the results obtained in OVA/OVA mice; °significantly different from the results obtained in sham/OVA mice. These results are similar to six comparable experiments.

(28, 29, 34), which supports a direct effect of the AP-1 decoys on their expression in the target immune cells. However, eotaxin is predominantly secreted by structural cells of the lung, which did not incorporate AP-1 decoys. This suggests that there may be an indirect effect of AP-1 inhibition in immune cells on the overall inflammatory status of the lung. Inhibition of AP-1 activity in airway immune cells may suppress their production of proinflammatory mediators essential for the induction and maintenance of the inflammatory response in lung structural cells. This would be in accordance with the observation that the activity of "proinflammatory" transcription factors (e.g., NF- κ B) in airway immune cells is crucial for maintaining the inflammatory response in structural cells of the lung (35).

IL-4 is the key Th2-differentiating cytokine (36, 37). IL-13, which is closely related to IL-4 and binds to the α chain of the IL-4 receptor (IL-4R α) (38, 39), was also shown to play a crucial role in Th2 cell development *in vivo* (40). Moreover, both IL-4 and IL-13 are able to induce B-cell-class switching to IgE production (38, 39, 41, 42), confirming that these cytokines have overlapping functions. However, although eosinophil egression, AHR, and mucus overproduction require signaling through IL-4R α (2, 3, 43), these processes appear to be mediated by IL-13 rather than by IL-4 (2, 3, 44, 45). IL-5 is strictly required for airway eosinophilia (46, 47). Last, eotaxin is a potent chemoattractant for both eosinophils and Th2 cells (26, 27). Given the respective roles of IL-4, IL-5, IL-13, and eotaxin in allergy, the combined deficiency of all these cytokines in the airways of OVA/OVA mice treated with AP-1 decoys could explain the significant attenuation of eosinophilic inflammation, AHR, and mucus and OVA-specific IgE and IgG1 production in these animals. However, it cannot be ruled out that other important mediators in allergic airway inflammation may also be directly or indirectly affected by AP-1 decoy treatment, but their identification requires further investigations.

We have previously shown that decoy-induced blockade of NF- κ B activation in the lungs of OVA/OVA mice is associated with strong attenuation of eosinophilic inflammation, AHR, and mucus, IL-5, and IL-13 production (16). By contrast, IL-4 and OVA-specific IgE and IgG1 synthesis is not reduced after treatment with NF- κ B decoys (16). The present study demonstrates that intratracheally delivered AP-1 decoys have broader effects than NF- κ B decoys. Indeed, AP-1 decoys significantly reduced all the characteristic features of airway allergy, including IL-4 and OVA-specific immunoglobulin production. Three explanations could account for the differences between the effects of AP-1 and NF- κ B decoys. First, AP-1 decoys, but not NF- κ B decoys (16), selectively induced the apoptosis of eosinophils, one of the most important IL-4-producing cells (48). Second, the promoter/enhancer region of the IL-4 gene contains AP-1 but not NF- κ B binding sites (28). Third, it is believed that the p50 homodimer, a NF- κ B complex that is activated on allergen challenge, might indirectly repress IL-4 transcription (16). Accordingly, it is possible that suppression of p50 homodimer activity in the airways of OVA/OVA mice treated with NF- κ B decoys results in exaggerated IL-4 synthesis in local Th2 cells and eosinophils. This increased IL-4 production would therefore compensate for the reduced accumulation of Th2 cells and eosinophils in the lungs of NF- κ B decoy-treated mice.

GCs are the most effective treatment for asthma (49, 50). They act by increasing (transactivation) or by inhibiting (transrepression) gene transcription (50, 51). Transactivation is mediated by binding of the hormone-activated glucocorticoid receptor (GR) to a DNA sequence called glucocorticoid response element (GRE). Genes involved in the control of gluconeogenesis, arterial pressure, and intraocular tension contain GRE (52–55). Thus, transactivation may account for most of the GC side

effects, such as diabetes, arterial hypertension, edema, and glaucoma. By contrast, GCs exert most of their beneficial anti-inflammatory effects through transrepression (50, 51). Transrepression results from inhibitory protein–protein interactions between the hormone-activated GR and AP-1 or NF- κ B (56). Of note, abnormal interactions between GR and AP-1, due to increased c-Fos expression, seem to be the molecular basis of steroid resistance in asthma (57, 58). The present study shows that decoy-mediated local AP-1 inhibition abrogates eosinophilic airway inflammation, AHR, and mucus and allergen-specific IgE production. We thus postulate that specific AP-1 inhibitors could mimic the beneficial effects of GCs (transrepression) without inducing the detrimental side effects that accompany GC treatment (transactivation). Moreover, we hypothesize that direct AP-1 antagonization could be of particular interest in the control of steroid-resistant asthma.

In conclusion, our results (1) reveal a critical role for AP-1 in the effector phase of pulmonary allergy; (2) indicate that specific AP-1 inhibition in the airways may have therapeutic value in the control of established asthma, including steroid resistant asthma; and (3) demonstrate that AP-1 inhibition is more efficient at preventing the effector Th2 responses in the lung than NF- κ B inhibition.

Conflict of Interest Statement: None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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References

- Viegi G, Annesi I, Matteelli G. Epidemiology of asthma. In: Chung F, Fabbri LM, editors. *Asthma*, European Respiratory Monograph No. 23. Sheffield, UK: European Respiratory Journals Ltd.; 2003. p. 1–25.
- Wills-Karp M. Immunologic basis of antigen-induced airway hyper-responsiveness. *Annu Rev Immunol* 1999;17:255–281.
- Ray A, Cohn L. Th2 cells and GATA-3 in asthma: new insights into the regulation of airway inflammation. *J Clin Invest* 1999;104:985–993.
- Busse WW, Lemanske RF. Asthma. *N Engl J Med* 2001;344:350–362.
- Larche M, Robinson DS, Kay AB. The role of T lymphocytes in the pathogenesis of asthma. *J Allergy Clin Immunol* 2003;111:450–463.
- Barnes PJ, Adcock IM. Transcription factors and asthma. *Eur Respir J* 1998;12:221–234.
- Pahl HL. Activators and target genes of Rel/NF-kappaB transcription factors. *Oncogene* 1999;18:6853–6866.
- Schule R, Rangarajan P, Kliewer S, Ransone LJ, Bolado J, Yang N, Verma IM, Evans RM. Functional antagonism between oncoprotein c-Jun and the glucocorticoid receptor. *Cell* 1990;62:1217–1226.
- Ray A, Prefontaine KE. Physical association and functional antagonism between p65 subunit of transcription factor NF- κ B and the glucocorticoid receptor. *Proc Natl Acad Sci USA* 1994;91:752–756.
- Caldenhoven E, Liden J, Wissink S, Van de Stolpe A, Raaijmakers J, Koenderman L, Okret S, Gustafsson JA, Van der Saag PT. Negative cross-talk between RelA and the glucocorticoid receptor: a possible mechanism for the antiinflammatory action of glucocorticoids. *Mol Endocrinol* 1995;9:401–412.
- De Bosscher K, Vanden Bergh W, Haegeman G. The interplay between the glucocorticoid receptor and nuclear factor-kappaB or activator protein-1: molecular mechanisms for gene repression. *Endocr Rev* 2003;24:488–522.
- Yang L, Cohn L, Zhang DH, Homer R, Ray A, Ray P. Essential role of nuclear factor kappaB in the induction of eosinophilia in allergic airway inflammation. *J Exp Med* 1998;188:1739–1750.
- Donovan CE, Mark DA, He HZ, Liou HC, Kobzik L, Wang Y, De Sanctis GT, Perkins DL, Finn PW. NF-kappa B/Rel transcription factors: c-Rel promotes airway hyperresponsiveness and allergic pulmonary inflammation. *J Immunol* 1999;163:6827–6833.
- Hartenstein B, Teurich S, Hess J, Schenkel J, Schorpp-Kistner M, Angel P. Th2 cell-specific cytokine expression and allergen-induced airway inflammation depend on JunB. *EMBO J* 2002;21:6321–6329.

15. Meixner A, Karreth F, Kenner L, Wagner EF. JunD regulates lymphocyte proliferation and T helper cell cytokine expression. *EMBO J* 2004;23:1325-1335.
16. Desmet C, Gosset P, Pajak B, Cataldo D, Bentires-Alj M, Lekeux P, Bureau F. Selective blockade of NF- κ B activity in airway immune cells inhibits the effector phase of experimental asthma. *J Immunol* 2004;173:5766-5775.
17. Bielinska A, Shivdasani RA, Zhang LQ, Nabel GJ. Regulation of gene expression with double-stranded phosphorothioate oligonucleotides. *Science* 1990;250:997-1000.
18. Sullenger BA, Gallardo HF, Ungers GE, Gilboa E. Overexpression of TAR sequences renders cells resistant to human immunodeficiency virus replication. *Cell* 1990;63:601-608.
19. Mann MJ, Dzau VJ. Therapeutic applications of transcription factor decoy oligonucleotides. *J Clin Invest* 2000;106:1071-1075.
20. Desmet C, Gosset P, Garzé V, Pajak B, Fiévez L, Lekeux P, Bureau F. Selective inhibition of AP-1 activity in airway immune cells ameliorates experimental asthma: abstracts of the American Thoracic Society International conference 2005. *Proc Am Thorac Soc* 2005;2:A737.
21. Hamelmann E, Schwarze J, Takeda K, Oshiba A, Larsen GL, Irvin CG, Gelfand. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am J Respir Crit Care Med* 1997;156:766-775.
22. Flandre TD, Leroy PL, Desmecht DJ. Effect of somatic growth, strain, and sex on double-chamber plethysmographic respiratory function values in healthy mice. *J Appl Physiol* 2003;94:1129-1136.
23. Pajak B, De Smedt T, Moulin V, De Trez C, Maldonado-Lopez R, Vansanten G, Briend E, Urbain J, Leo O, Moser M. Immunohistowax processing, a new fixation and embedding method for light microscopy, which preserves antigen immunoreactivity and morphological structures: visualisation of dendritic cells in peripheral organs. *J Clin Pathol* 2000;53:518-524.
24. Shaulian E, Karin M. AP-1 as a regulator of cell life and death. *Nat Cell Biol* 2002;4:E131-E136.
25. Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma: from bronchoconstriction to airways inflammation and remodeling. *Am J Respir Crit Care Med* 2000;161:1720-1745.
26. Jose PJ, Griffiths-Johnson DA, Collins PD, Walsh DT, Moqbel R, Totty NF, Truong O, Hsuan JJ, Williams TJ. Eotaxin: a potent eosinophil chemoattractant cytokine detected in a guinea pig model of allergic airways inflammation. *J Exp Med* 1994;179:881-887.
27. Sallusto F, Mackay CR, Lanzavecchia A. Selective expression of the eotaxin receptor CCR3 by human T helper 2 cells. *Science* 1997;277:2005-2007.
28. Li-Weber M, Giasi M, Krammer PH. Involvement of Jun and Rel proteins in up-regulation of interleukin-4 gene activity by the T cell accessory molecule CD28. *J Biol Chem* 1998;273:32460-32466.
29. Ogawa K, Kaminuma O, Okudaira H, Kikkawa H, Ikezawa K, Sakurai N, Mori A. Transcriptional regulation of the IL-5 gene in peripheral T cells of asthmatic patients. *Clin Exp Immunol* 2002;130:475-483.
30. Henderson WR Jr, Chi EY, Teo JL, Nguyen C, Kahn M. A small molecule inhibitor of redox-regulated NF-kappa B and activator protein-1 transcription blocks allergic airway inflammation in a mouse asthma model. *J Immunol* 2002;169:5294-5299.
31. Nguyen C, Teo JL, Matsuda A, Eguchi M, Chi EY, Henderson WR Jr, Kahn M. Chemogenomic identification of Ref-1/AP-1 as a therapeutic target for asthma. *Proc Natl Acad Sci USA* 2003;100:1169-1173.
32. Li B, Tournier C, Davis RJ, Flavell RA. Regulation of IL-4 expression by the transcription factor JunB during T helper cell differentiation. *EMBO J* 1999;18:420-432.
33. Eferl R, Wagner EF. AP-1: a double-edged sword in tumorigenesis. *Nat Rev Cancer* 2003;3:859-868.
34. Hein H, Schluter C, Kulke R, Christophers E, Schroder JM, Bartels J. Genomic organization, sequence, and transcriptional regulation of the human eotaxin gene. *Biochem Biophys Res Commun* 1997;237:537-542.
35. Bureau F, Delhalle S, Bonizzi G, Fievez L, Dogne S, Kirschvink N, Vanderplasschen A, Merville MP, Bours V, Lekeux P. Mechanisms of persistent NF-kappa B activity in the bronchi of an animal model of asthma. *J Immunol* 2000;165:5822-5830.
36. Swain SL, Weinberg AD, English M, Huston G. IL-4 directs the development of Th2-like helper effectors. *J Immunol* 1990;145:3796-3806.
37. Kopf M, Le Gros G, Bachmann M, Lamers MC, Bluethmann H, Kohler G. Disruption of the murine IL-4 gene blocks Th2 cytokine responses. *Nature* 1993;362:245-248.
38. Minty A, Chalon P, Derocq JM, Dumont X, Guillemot JC, Kaghad M, Labit C, Leplatois P, Liauzun P, Miloux B. Interleukin-13 is a new human lymphokine regulating inflammatory and immune responses. *Nature* 1993;362:248-250.
39. Punnonen J, Aversa G, Cocks BG, McKenzie AN, Menon S, Zurawski G, de Waal Malefyt R, de Vries JE. Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. *Proc Natl Acad Sci USA* 1993;90:3730-3734.
40. McKenzie GJ, Emson CL, Bell SE, Anderson S, Fallon P, Zurawski G, Murray R, Grecis R, McKenzie AN. Impaired development of Th2 cells in IL-13-deficient mice. *Immunity* 1998;9:423-432.
41. Pene J, Rousset F, Briere F, Chretien I, Bonnefoy JY, Spits H, Yokota T, Arai N, Arai K, Banchereau J. IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons gamma and alpha and prostaglandin E2. *Proc Natl Acad Sci USA* 1988;85:6880-6884.
42. Emson CL, Bell SE, Jones A, Wisden W, McKenzie AN. Interleukin (IL)-4-independent induction of immunoglobulin (Ig)E, and perturbation of T cell development in transgenic mice expressing IL-13. *J Exp Med* 1998;188:399-404.
43. Gavett SH, O'Hearn DJ, Karp CL, Patel EA, Schofield BH, Finkelman FD, Wills-Karp M. Interleukin-4 receptor blockade prevents airway responses induced by antigen challenge in mice. *Am J Physiol* 1997;272:L253-L261.
44. Grunig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM, Sheppard D, Mohrs M, Donaldson DD, Locksley RM, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998;282:2261-2263.
45. Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, Donaldson DD. Interleukin-13: central mediator of allergic asthma. *Science* 1998;282:2258-2261.
46. Nakajima H, Iwamoto I, Tomoe S, Matsumura R, Tomioka H, Takatsu K, Yoshida S. CD4+ T-lymphocytes and interleukin-5 mediate antigen-induced eosinophil infiltration into the mouse trachea. *Am Rev Respir Dis* 1992;146:374-377.
47. Foster PS, Hogan SP, Ramsay AJ, Matthaei KI, Young IG. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J Exp Med* 1996;183:195-201.
48. Borish LC, Steinke JW. 2. Cytokines and chemokines. *J Allergy Clin Immunol* 2003;111:S460-S475.
49. Barnes PJ. New directions in allergic diseases: mechanism-based anti-inflammatory therapies. *J Allergy Clin Immunol* 2000;106:5-16.
50. Adcock IM. Glucocorticoids: new mechanisms and future agents. *Curr Allergy Asthma Rep* 2003;3:249-257.
51. Adcock IM. Glucocorticoid-regulated transcription factors. *Pulm Pharmacol Ther* 2001;14:211-219.
52. Jantzen HM, Strahle U, Gloss B, Stewart F, Schmid W, Boshart M, Miksicsek R, Schutz G. Cooperativity of glucocorticoid response elements located far upstream of the tyrosine aminotransferase gene. *Cell* 1987;49:29-38.
53. Imai E, Miner JN, Mitchell JA, Yamamoto KR, Granner DK. Glucocorticoid receptor-cAMP response element-binding protein interaction and the response of the phosphoenolpyruvate carboxykinase gene to glucocorticoids. *J Biol Chem* 1993;268:5353-5356.
54. Ming M, Chan W, Wang TT, Roberts KD, Bouvier M, Lachance S, Carriere S, Chan JS. Beta-adrenoceptors and dexamethasone synergistically stimulate the expression of the angiotensinogen gene in opossum kidney cells. *Kidney Int* 1996;50:94-101.
55. Nguyen TD, Chen P, Huang WD, Chen H, Johnson D, Polansky JR. Gene structure and properties of TIGR, an olfactomedin-related glycoprotein cloned from glucocorticoid-induced trabecular meshwork cells. *J Biol Chem* 1998;273:6341-6350.
56. Cato AC, Wade E. Molecular mechanisms of anti-inflammatory action of glucocorticoids. *Bioessays* 1996;18:371-378.
57. Adcock IM, Lane SJ, Brown CR, Lee TH, Barnes PJ. Abnormal glucocorticoid receptor-activator protein 1 interaction in steroid-resistant asthma. *J Exp Med* 1995;182:1951-1958.
58. Lane SJ, Adcock IM, Richards D, Hawrylowicz C, Barnes PJ, Lee TH. Corticosteroid-resistant bronchial asthma is associated with increased c-fos expression in monocytes and T lymphocytes. *J Clin Invest* 1998;102:2156-2164.