

Molecular mechanisms of decreased steroid responsiveness induced by latent adenoviral infection in allergic lung inflammation

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Background: We recently reported that allergic lung inflammation in guinea pigs became steroid resistant in the presence of latent adenoviral infection.

Objective: We sought to investigate the molecular mechanisms that underlie steroid resistance in adenoviral infection.

Methods: Guinea pigs with a latent adenoviral infection were sensitized and challenged with ovalbumin (OVA) and given daily injections of budesonide (20 mg/kg administered intraperitoneally). Sham-infected animals received either saline challenge without budesonide injection or OVA challenge with or without budesonide. The inflammatory response in the lung was measured by means of quantitative histology. Eotaxin, monocyte chemoattractant protein 1 (MCP-1), and RANTES expression in the lung were analyzed by means of Northern blotting, and the binding activity of activator protein 1 (AP-1) and nuclear factor κ B in nuclear extracts from the lung was analyzed with electrophoretic mobility shift assays. **Results:** OVA challenge increased eosinophil infiltration and eotaxin and MCP-1 mRNA expression in the lungs, and glucocorticoids reduced these increases in the sham-infected, but not the adenovirus-infected, animals. Changes in binding activity of AP-1, but not nuclear factor κ B, paralleled changes in eotaxin and MCP-1 mRNA.

Conclusion: We conclude that latent adenoviral infection inhibits the anti-inflammatory effects of glucocorticoids on allergen-induced eotaxin and MCP-1 expression through AP-1, leading to steroid-resistant allergic lung inflammation. (*J Allergy Clin Immunol* 2002;109:35-42.)

Key words: Transcription factor activator protein 1, chemokines, eosinophils, epithelial cells, nuclear factor κ B

Glucocorticoids are remarkably effective in controlling inflammation in asthmatic airways,^{1,2} but subsets of patients with asthma fail to achieve a satisfactory response, even to high doses of systemic glucocorticoids.³ This steroid resistance poses a major therapeutic problem in asthma, and recent studies^{4,5} have shown that it can be associated with viral respiratory tract infection. Using a guinea pig model, we recently showed that latent adenoviral infection causes ovalbumin (OVA)-induced allergic lung inflammation to become steroid resistant.⁶ Because human lung epithelial cells harbor and express the adenovirus *E1A* gene,⁷ the mechanism of the steroid resistance in this animal model could provide insight into steroid resistance in human asthma.

A key molecular mechanism by which glucocorticoids reduce airway inflammation in asthma is their suppression of the proinflammatory genes activated in allergic inflammation.³ Several studies^{8,9} have shown that chemokines and T_H2-specific cytokines, such as IL-4, IL-5, and IL-13, are important to the pathogenesis of asthma. The chemokines eotaxin; eotaxin 2; monocyte chemoattractant protein (MCP) 1, 3, and 4; and another protein called RANTES have also been identified in the epitheliums of patients with allergic airway inflammation.¹⁰⁻¹² Glucocorticoids suppress the expression of these chemokines by epithelial cells and reduce the eosinophilic infiltrate into airway tissue.^{11,13} The ubiquitously expressed transcription factors activator protein 1 (AP-1) and nuclear factor κ B (NF- κ B) are known to be involved in the upregulation of proinflammatory genes, including those of allergy-related chemokines,¹⁴ and these 2 transcription factors are increased in the epitheliums of patients with asthma.^{15,16} Recent evidence suggests that glucocorticoids exert transrepression through protein-protein interactions with AP-1¹⁷ and NF- κ B.¹⁸ These reports suggest that glucocorticoids reduce allergic inflammation by downregulating the elevated allergen-induced chemokine expression through the interaction of the ligand-activated glucocorticoid receptor (GR) with AP-1 and NF- κ B.

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Abbreviations used

AP-1: Activator protein 1
GR: Glucocorticoid receptor
JNK: Jun N-terminal kinase
MCP: Monocyte chemoattractant protein
NF- κ B: Nuclear factor κ B
OVA: Ovalbumin

The present study was designed to investigate the molecular mechanisms of the steroid resistance reported in our previous study.⁶ It focuses on the expression of the chemokines involved in the eosinophilic response induced by allergen sensitization and challenge and the control of this expression by the transcription factors AP-1 and NF- κ B.

METHODS**Animals**

Female guinea pigs (Cam Hartley strain, *Cavia porcellus*, 250–300 g) were purchased from Charles River Canada and housed in polycarbonate cages fitted with high-efficiency particulate air filter covers. The animals were provided with water and guinea pig chow ad libitum. The studies were approved by the University of British Columbia Animal Care Committee and followed published guidelines of the Canadian Council on Animal Care.

Adenoviral infection

The preparation of wild-type adenovirus 5 and sham inoculum have been described.⁶ Guinea pigs were anesthetized with 4% halothane balanced with oxygen. During anesthesia, 6 guinea pigs were infected with adenovirus 5, and 18 were sham infected, as previously described.⁶

Allergen-induced lung inflammation and steroid treatment

Three weeks after infection or sham infection, all 6 adenovirus 5-infected and 12 of the sham-infected animals were sensitized and challenged with OVA, according to a previous protocol (see Fig 1 in reference 6). The remaining 6 sham-infected animals were sham sensitized with normal saline and served as control animals for allergen sensitization and challenge. Budesonide (10 mg/mL, Astra Draco AB) was prepared as previously described.⁶ The 6 adenovirus 5-infected and 6 sham-infected guinea pigs sensitized and challenged with OVA were administered budesonide (20 mg/kg) intraperitoneally on 12 occasions over 16 days starting 24 hours before the first allergen challenge. The remaining 12 sham-infected guinea pigs were injected with a volume of vehicle alone similar to that used to administer the steroids. Three hours after the last OVA challenge or saline exposure, guinea pigs were killed with an overdose of sodium pentobarbital administered intraperitoneally. Lungs from the animals were processed in groups of 4, one from each of the 4 treatment groups, for a total of 6 groups. Serum samples were obtained to assess the level of steroids the animals received.

Tissue preparation

After the lungs were removed en block with the trachea, the left lung was isolated from the main stem bronchus, and 0.8 and 0.5 g of the left lung was used for RNA and nuclear protein extraction, respectively. The right lung was weighed and then inflated with 50% Optimal Cutting Temperature compound (Tissue Tek, Miles Inc) in PBS (pH 7.4). The inflated right lung was weighed again to

determine the inflation volume. The right upper and middle lobes were frozen in liquid nitrogen and stored at -70°C for DNA extraction and cryosectioning, followed by immunohistochemistry. The right lower lobe was cut into 3 blocks in the transverse plane, fixed in buffered 10% formalin, and processed into paraffin.

Analysis of lung inflammation

Quantitative histology to estimate volumes of inflammatory cells (eosinophils, macrophages, and T-lymphocyte subsets) in the airways and lung parenchyma (airspace and alveolar tissue combined) was performed on the right lung, as described previously.⁶ Immunohistochemical staining for adenovirus E1A protein was also performed as previously reported.⁶

DNA probes used for Southern or Northern blot analysis

E1A DNA probe, an *AluI* fragment from nucleotides 626 to 1367 of adenovirus 5 (GenBank accession No. NC 001406), was used for Southern hybridization. Full-length guinea pig cDNAs of eotaxin,¹⁹ MCP-1,²⁰ and RANTES²¹ were used as probes for Northern blot analysis. Human 18S ribosomal cDNA, a 1.6-kb *EcoRI* fragment cloned into the plasmid pBS KS, crosshybridized with the guinea pig 18S ribosomal RNA and was used as an internal control for RNA loading.

Detection of the adenovirus 5 E1A gene by means of PCR

DNA was extracted from the frozen right lung of 6 sham-infected, OVA-challenged, steroid-treated animals and 6 adenovirus 5-infected, OVA-challenged, steroid-treated animals, as previously described.²² The oligonucleotide primers for the E1A PCR were nucleotides 768 to 789 (5'-TAATGTTGGCGGTGCAGGAAGG-3') and nucleotides 1253 to 1232 (5'-TCAGGCTCAGGTTCAGACACAG-3') of the adenovirus 5 sequence (see above). The expected PCR product is 486 bp. Amplification was performed as described,²² except that the MgCl_2 concentration was 2 mmol/L. All PCR reactions were accompanied by appropriate positive and negative controls. One hundred copies of adenovirus 2 DNA (Bethesda Research Laboratories)²² served as a positive control. DNA extracted from sham-infected guinea pigs and 10 mmol/L TRIS HCl (pH 8.0) with 1 mmol/L EDTA without added DNA were used as negative controls. The PCR product was analyzed by means of 1.5% agarose gel electrophoresis and Southern blotting, as previously described.²²

Measurement of mRNA expression by Northern blot analysis

The 0.8 g of the left lung from each guinea pig was homogenized with a Polytron (Biospec Products) in 20 mL of lysis buffer supplied by using the RNeasy Maxi kit (QIAGEN Inc). The homogenate was frozen in liquid nitrogen and stored at -70°C until RNA extraction with the RNeasy Maxi kit. Twenty micrograms of total RNA was separated on a 1.3% agarose gel containing 2% formaldehyde and transferred to Hybond-N filters (Amersham). The amount, quality, and size of the RNA were assessed by monitoring the 18S and 28S ribosomal RNA bands stained with ethidium bromide. Membranes were probed with DNA labeled with $\alpha^{32}\text{P}$ [dCTP] (3000 Ci/mmol, Amersham), as described above. After autoradiography, the density of each band representing the chemokine mRNA or 18S ribosomal RNA was measured with Eagle Eye II (Stratagene) and NIH Imaging Software. The density of the chemokine band was normalized to that of the 18S ribosomal RNA band.

Electrophoretic mobility shift assay

Nuclear proteins were extracted according to the method of Dignam et al.²³ The 0.5 g of the left lung was minced with a razor blade

in 4 mL of 10 mmol/L HEPES (pH 7.6), 10 mmol/L KCl, 2 mmol/L MgCl₂, 1 mmol/L dithiothreitol, 0.1 mmol/L phenylmethylsulfonyl fluoride, 0.1 mmol/L EDTA, and 50 µg/mL leupeptin. The minced tissue was frozen in liquid nitrogen and stored at -70°C until nuclear proteins were extracted. For extraction, the minced tissue was thawed at 4°C, and 1.0 mL was incubated on ice for 15 minutes before it was mixed vigorously with 25 µL of 10% Nonidet P-40 for 15 seconds and then centrifuged for 20 seconds at 12,000g at 4°C. The nuclear pellet was treated as described,²⁴ and the supernatant containing the nuclear proteins was stored at -70°C.

Double-stranded oligonucleotides used as probes in the electrophoretic mobility shift assay were 5'-CGC TTG ATG AGT CAG CCG GAA-3' (Promega) for AP-1 and 5'-AGT TGA GGG GAC TTT CCC AGG C-3' (Promega) for the NF-κB binding sequence of the gene encoding immunoglobulin κ-chain. The underlined sequences are binding motifs for AP-1 and NF-κB, respectively. The preparation of the phosphorous 32-labeled double-stranded oligonucleotides, the binding of the proteins in the nuclear extracts to the labeled oligonucleotides, and the competition assays with excess unlabeled oligonucleotide (3.5 pmol) were carried out as previously described.²⁴ For supershift assays of NF-κB, 2 µg of rabbit anti-mouse polyclonal p65 or p50 antibodies (Santa Cruz Biotechnology) or control rabbit anti-mouse immunoglobulin (Dako) was added to the nuclear extract with the labeled oligonucleotide. For AP-1 supershift assays, 2 µg of rabbit anti-mouse polyclonal c-Fos or c-Jun antibodies (Santa Cruz Biotechnology) or control rabbit anti-mouse immunoglobulin (Dako) was used. The anti c-Jun antibody broadly reacts with Jun B or Jun D, as well as with c-Jun. Electrophoresis and autoradiography were performed, as previously described.²⁴ Nuclear extracts from the 4 animals in each of the 6 groups were analyzed on the same gel, the density of specific bands for NF-κB or AP-1 was measured with a densitometer as above, and the band density of the transcription factor from each treated animal was expressed as a ratio of that from the saline control.

Statistical analysis

Data from the morphologic and Northern blot analyses were first analyzed by means of 1-way ANOVA. When significant differences were found, then the OVA-sensitized and OVA-challenged group was compared with the saline-exposed group to determine the effect of OVA challenge. Multiple comparisons between the 3 treatment groups were made with *t* tests to determine the effect of adenovirus 5 infection on steroid treatment in OVA-induced inflammation.²⁵ The density ratios from the electrophoretic mobility shift assay were also analyzed by means of 1-way ANOVA, and then multiple comparisons between the 3 treatment groups were made with *t* tests as above. Corrections for multiple comparisons were made with the Bonferroni method,²⁵ and the level of significance was less than .05. Correlation between eotaxin mRNA expression and the total volume of eosinophils in the lung (airways and parenchyma combined) of each guinea pig was performed with linear regression analysis.

RESULTS

Latent adenoviral infection

PCR confirmed a latent infection by demonstrating the presence of E1A DNA in the lungs of adenovirus 5-infected guinea pigs at 6 weeks after infection (Fig 1). No E1A PCR product was detected in the lungs of sham-infected animals. Similarly, E1A protein was localized by means of immunohistochemistry in the nuclei of epithelial cells in the lungs of adenovirus 5-infected guinea pigs but was not detected in sham-infected animals (data not shown).

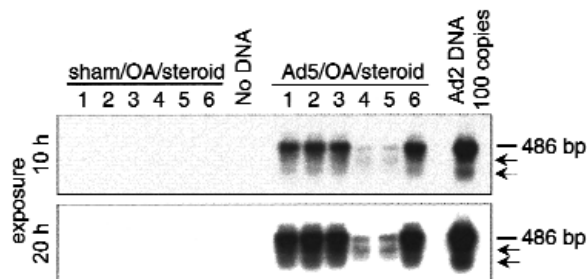


FIG 1. Detection of adenovirus 5 E1A DNA by means of PCR and Southern blot analysis. Representative autoradiograms of 10 and 20 hours' exposure show amplification of an E1A DNA target of 486 bp from DNA extracted 6 weeks after infection from lungs of 6 adenovirus 5-infected guinea pigs and from 100 copies of adenovirus 2 DNA used as a positive control but not from 6 sham-infected guinea pigs. Arrows indicate single-stranded E1A products. OA, Ovalbumin; Ad5, adenovirus 5.

Measurement of steroid in serum

Steroid concentrations in the serum of the sham-infected, OVA-challenged, steroid-treated group and the adenovirus 5-infected, OVA-challenged, steroid-treated group were similar (87.8 ± 24.2 vs 62.5 ± 18.9 nmol/L [mean $\pm$ SEM], $P > .5$) and not detected in those not receiving steroids.

Effect of latent adenoviral infection on steroid treatment of lung inflammation induced by means of OVA sensitization and challenge

In the airway wall OVA sensitization and challenge increased eosinophils ($P < .003$; Fig 2, A and D), as well as macrophages ($P < .02$) and CD4⁺ cells ($P < .005$) but not CD8⁺ cells (data not shown). Similar results were found in the lung parenchyma, where OVA sensitization and challenge increased eosinophils ($P < .001$; Fig 2, B), as well as macrophages ($P < .02$) and CD4⁺ cells ($P < .02$) but not CD8⁺ cells (data not shown).

Steroid treatment markedly reduced the eosinophil response induced by means of OVA sensitization and challenge both in the airways ($P < .02$; Fig 2, A and E) and parenchyma ($P < .01$; Fig 2, B) of sham-infected animals but had no effect on other inflammatory cells analyzed (data not shown). In the adenovirus 5-infected group steroids did not reduce the eosinophilic response to OVA either in the airways ($P < .01$; Fig 2, A and F) or parenchyma ($P < .01$; Fig 2, B).

Chemokine mRNA expression

Chemokine mRNA expression was analyzed 3 hours after the last OVA challenge or saline exposure because this expression has been documented to peak at 3 hours after allergen challenge.²⁶ OVA sensitization and challenge increased the expression of eotaxin and MCP-1 mRNA but not RANTES mRNA (Fig 3, A-C, respective-

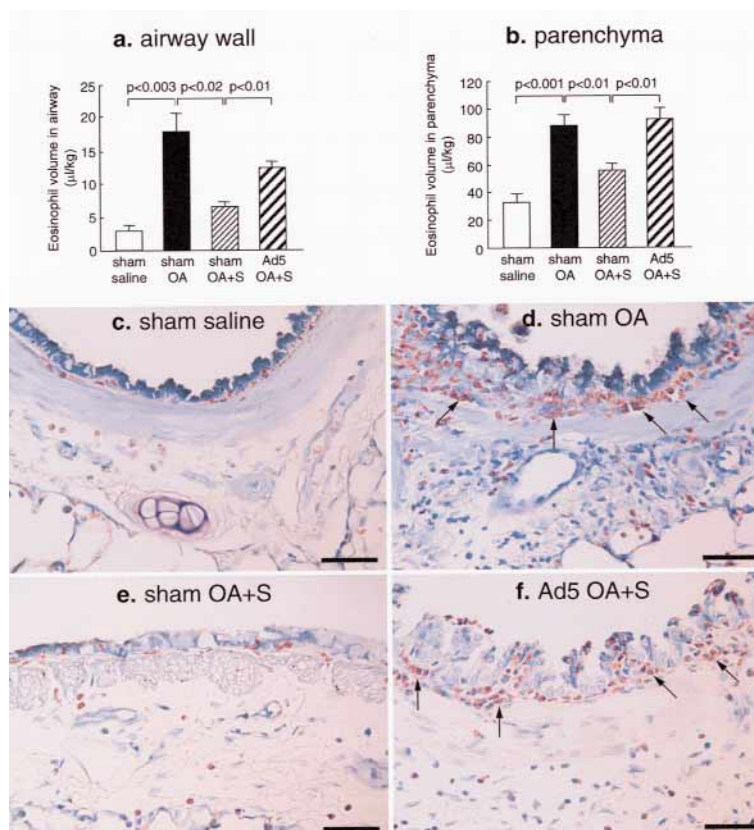


FIG 2. Eosinophil infiltration into the lung. **a** and **b**, Quantification of eosinophils in airways (**a**) and parenchyma (**b**) of guinea pigs without treatment (*sham/saline*), after sensitization and challenge with ovalbumin (*sham/OA*), after sensitization and challenge with ovalbumin and steroid treatment (*sham/OA+S*), and after latent adenovirus 5 infection, sensitization, and challenge with ovalbumin and steroid treatment (*Ad5/OA+S*). Data are shown as means $\pm$ SEM ($n = 6$ per group). **c** through **f**, Micrographs of airways from guinea pigs treated as in **a** stained for eosinophils with Hansel stain. Arrows indicate eosinophilic infiltration. Bar represents 50 μ m.

ly). Steroid treatment downregulated all 3 chemokine mRNAs in sham-infected, but not adenovirus 5-infected, animals (Fig 3). There was a strong correlation between eosinophil infiltration and eotaxin mRNA expression in the lung (Fig 4).

Effect of glucocorticoids on AP-1 and NF- κ B DNA binding activity in OVA-induced lung inflammation

OVA sensitization and challenge increased AP-1 binding activity (Fig 5, A and B). Steroids downregulated this increase in AP-1 binding in sham-infected animals ($P < .05$) but not in those with latent adenoviral infection. In some animals the constitutive NF- κ B binding activity found in the control group varied slightly with each treatment. However, in contrast to AP-1, average binding of NF- κ B for each treatment group was not different from that of the control group (data not shown).

Confirmation of the specificity of AP-1 and NF- κ B binding

Specificity of the bands representing the complexes of

AP-1 bound to oligonucleotides with their binding sequence was confirmed by displacement with excess cold AP-1-specific oligonucleotide, whereas the nonspecific oligonucleotide did not (Fig 5, A). Furthermore, supershift assays with antibodies against specific subunits of AP-1 confirmed the presence of c-Fos in the AP-1 complex (Fig 5, A). Although the intensity of the band representing AP-1 binding was reduced substantially in the presence of the c-Jun antibody, no supershift was observed (Fig 5, A). Similar competition assays and antibody binding studies confirmed the specificity of bands representing complexes of NF- κ B as those of the p50 and p65 homodimers (data not shown).

DISCUSSION

The quantitative histologic data obtained in this study confirmed our previous report⁶ showing that latent adenoviral infection neither induced nor enhanced the eosinophilic lung inflammation caused by OVA sensitization and challenge. It also showed that latent adenoviral infection causes allergen-induced eosinophilic lung inflammation to become resistant to glucocorticoid treat-

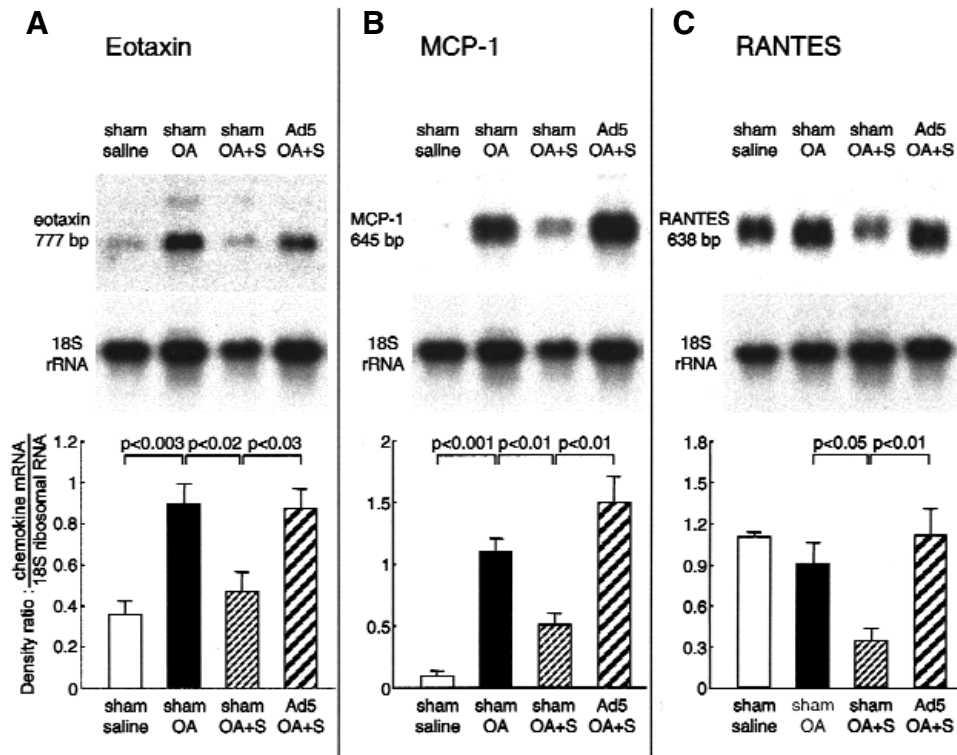


FIG 3. Chemokine mRNA expression in the lung by means of Northern blot analysis. Two top panels, Representative autoradiograms of eotaxin (A), MCP-1 (B), and RANTES (C) mRNAs (upper panel) are compared with autoradiograms of the same blot hybridized with 18S ribosomal RNA (rRNA) probe (middle panel). Bottom panel, Average ratio of the density of each chemokine mRNA band to that of the corresponding 18S ribosomal RNA. The treatment of the guinea pigs is the same as in Fig 2. Data are shown as means $\pm$ SEM (n = 6 per group). OA, Ovalbumin; Ad5, adenovirus 5.

ment. The purpose of this study was to gain a better understanding of the mechanism of this steroid resistance. The epithelial cells of the lung are a likely source of this steroid resistance because they are the cells that express the adenoviral transactivating protein E1A during latent infection both in guinea pigs²⁷ and human patients.⁷ Recent reports^{8,9} show that epithelial cells are one of major sources of these chemokines that influence allergen-induced lung inflammation. Our purpose was to determine whether latent adenoviral infection inhibits the effect of glucocorticoids on allergen-induced mRNA expression of chemokines and whether latent adenoviral infection reverses the effect of glucocorticoids on DNA binding of AP-1 and NF- κ B.

The results show a close correlation between eotaxin mRNA production and the number of eosinophils present in the lung. They also show that OVA sensitization and challenge increased both eosinophilic infiltration and eotaxin mRNA and that glucocorticoid treatment reversed both of these changes in the sham-infected animals. However, the presence of latent adenoviral infection prevented the glucocorticoid-induced reduction of both the eosinophilic infiltrate and the production of eotaxin mRNA. These results strongly suggest that eosinophil infiltration is directed by eotaxin in guinea pig lungs. The importance of eotaxin in causing the

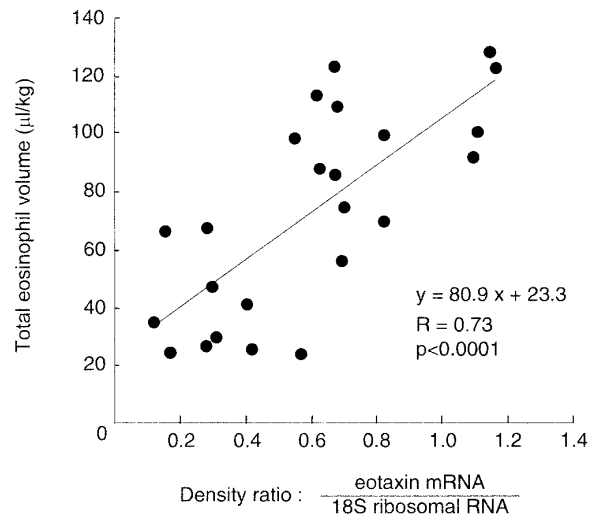


FIG 4. Correlation between eotaxin mRNA expression and eosinophil infiltration in the lung. For each guinea pig, the total eosinophil volume was plotted against the ratio of density of the eotaxin to that of the 18S ribosomal RNA band.

eosinophilic inflammation has also been shown in mice in which a specific neutralizing antibody against eotaxin reduced the eosinophilic component of allergen-induced inflammation.²⁸ However, results from this study²⁸ and

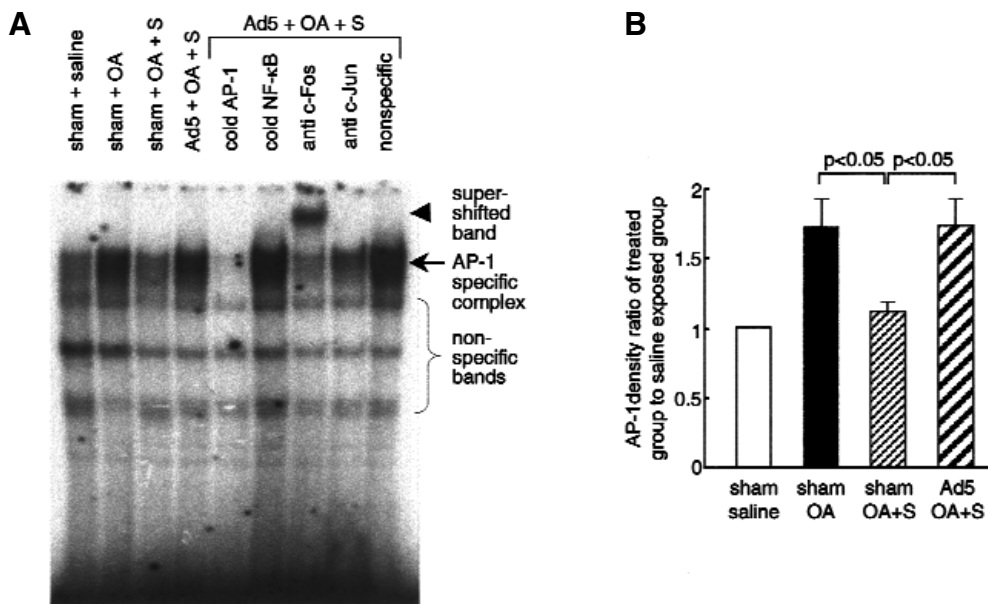


FIG 5. Detection of AP-1 binding activity in nuclei of lung cells by means of electrophoretic mobility shift assay. **A**, A representative autoradiogram of an AP-1 electrophoretic mobility shift assay with nuclear extracts in the first 4 lanes from the left for a sham-infected and saline-treated animal; a sham-infected and OVA-sensitized and challenged animal; a sham-infected, OVA-sensitized and challenged, and steroid-treated animal; and an adenovirus 5-infected, OVA-challenged and sensitized, and steroid-treated animal, respectively. The next 5 lanes represent nuclear extracts from an adenovirus 5-infected, OVA-sensitized and challenged, and steroid-treated animal. Lane 5 from the left represents competition with excess unlabeled AP-1 oligonucleotide (*cold AP-1*), and lane 6 represents competition with excess unlabeled NF-κB oligonucleotide (*cold NF-κB*). In lanes 7 to 9 anti-c-Fos antibody, anti-c-Jun antibody, and rabbit IgG, respectively, were added to the nuclear extracts. **B**, Ratio of the density of the AP-1 band from guinea pigs in the 3 treated groups to that of the saline control group on the same autoradiogram. Data are shown as means $\pm$ SEM (n = 6 per group). OA, Ovalbumin; Ad5, adenovirus 5; S, steroid.

ours contrast with those on eotaxin knockout mice^{29,30} in which the eosinophilic infiltrate was only partially reduced, suggesting that a chemokine other than eotaxin may also participate in the eosinophil response. Studies of human asthmatic airways³¹ have shown that the presence of eotaxin makes a more significant contribution to the recruitment of eosinophils into airway tissue than RANTES and MCP-4. Taken together, these results suggest that eotaxin is an important inducer of eosinophils into the airway tissue, and our results suggest that the presence of a latent adenoviral infection prevents glucocorticoids from reducing its expression and, consequently, the eosinophilic infiltrate.

MCP-1 mRNA expression was also associated with steroid-resistant eosinophilic inflammation in our model. The overexpression of MCP-1 during the sensitization phase has been shown to decrease T_H1 -type and increase T_H2 -type immune responses in vivo.³² Furthermore, development of the T_H2 -type response in the lung is decreased in MCP-1-deficient mice.³³ In murine models of allergic lung inflammation,²⁸ treatment with neutralizing antibody to MCP-1 resulted in decreases in both mononuclear cell influx and airway hyperresponsiveness. Also, when anti-MCP-1 antibody was administered throughout the OVA sensitization and challenge phases,

an almost complete reduction in eosinophils, as well as macrophages and lymphocytes, in the lung was observed.²⁸ These results suggest a central role for MCP-1, both in induction and maintenance of allergic lung inflammation, and our results suggest that one of the mechanisms underlying steroid-resistant allergic lung inflammation is the modulation of MCP-1 expression by latent adenoviral infection.

In contrast to eotaxin and MCP-1, RANTES mRNA expression was not increased by OVA challenge in our model. This result is consistent with a report in murine lungs in which OVA inhalation did not increase the expression of RANTES.³⁴ In guinea pigs RANTES activates macrophages but not eosinophils,²¹ and overexpression of RANTES in murine lungs causes a mononuclear cell-rich inflammation but not eosinophilic infiltrates.³⁵ These data suggest that RANTES plays a minor role in allergen-induced eosinophilic lung inflammation in the guinea pig and mouse. An examination of the role of RANTES in murine models of T_H1 - or T_H2 -type responses of lung inflammation³⁶ demonstrated greater elevations of RANTES in the T_H1 -type response. Anti-RANTES antibody also reduced the T_H1 -type response and enhanced the T_H2 -type response, suggesting that

RANTES contributes more to the T_H1-driven nonallergic response than to T_H2-driven allergic responses.

A novel finding of our study is that changes in the DNA binding activity of AP-1 but not NF- κ B parallel those in eotaxin and MCP-1 mRNA expression. Both AP-1 and NF- κ B are involved in the upregulation of proinflammatory genes, including chemokines,¹⁴ and these 2 transcription factors are known to be expressed and activated in epithelial cells of patients with asthma.^{15,16} The genes encoding eotaxin³⁷ and MCP-1³⁸ have binding sites for both AP-1 and NF- κ B, and our results suggest that latent adenoviral infection results in a reversal of AP-1 repression to inhibit the effects of glucocorticoids on allergen-induced expression of chemokines. If latent adenovirus infection of the cells producing eotaxin or MCP-1 affected NF- κ B binding in these cells, such a result may have been overshadowed by the constitutive NF- κ B activation in other cells of the whole lung extract. Glucocorticoid suppression of inflammation through the GR by means of protein-protein interactions with AP-1 and NF- κ B directly or through the coactivator, CREB-binding protein, has been suggested.^{17,18,39} Adenovirus E1A has been shown to inhibit steroid hormone action by interacting with CREB-binding protein,⁴⁰ and a similar mechanism might interfere with the downregulation of allergen-induced expression of chemokines by glucocorticoids. Such a scenario is supported by findings suggesting a reduced interaction between GR and AP-1 in peripheral mononuclear cells from steroid-resistant compared with steroid-responsive patients with asthma and a concomitant increase in the DNA binding activity of AP-1 in resistant patients.⁴¹ GR antagonizes AP-1 by inhibiting Jun N-terminal kinase (JNK),⁴² and adenovirus E1A can induce glucocorticoid-resistant activation of JNK in lung epithelial cells.⁴³ Therefore an alternative mechanism for adenovirus-induced steroid resistance in allergic lung inflammation is by activation of JNK. This suggests that adenovirus E1A may inhibit the action of GR on AP-1 in several ways and enhance its activation despite the presence of glucocorticoids.

The data reported here show that many of the features of steroid-resistant lung inflammation can be reproduced in guinea pigs with a latent adenoviral infection. They suggest that modulation of host gene transcription by means of latent adenoviral infection is involved in the development of steroid resistance through its effect on AP-1. These findings provide insight and suggest a mechanism for the reported association between adenoviral infection and steroid-resistant asthma.⁴ However, because differences between cytokines and their inflammatory cascades exist between guinea pigs and human patients, application of our findings to human disease should be made with appropriate caution.

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