

Latent adenoviral infection modifies the steroid response in allergic lung inflammation

Katsuhiko Yamada, MD,^a W. Mark Elliott, PhD,^a Shizu Hayashi, PhD,^a Ralph Brattsand, PhD,^b Clive Roberts, PhD,^a Timothy Z. Vitalis, PhD,^a and James C. Hogg, MD^a Vancouver, British Columbia, Canada, and Lund, Sweden

Background: Steroid-resistant asthma develops after adenoviral bronchiolitis.

Objective: We sought to determine the effect of steroids on allergic lung inflammation in the presence of latent adenoviral infection.

Methods: Guinea pigs with latent adenoviral (n = 12) or sham (n = 12) infections were sensitized and challenged with ovalbumin (OA) or sham sensitized and challenged with saline solution. The effect of steroids (20 mg/kg administered intraperitoneally) on OA-induced lung inflammation was examined by using quantitative histology as the outcome measure.

Results: Latent adenoviral infection increased CD8⁺ cells in the airway wall and CD8⁺ cells, macrophages, B cells, and CD4⁺ cells in the lung parenchyma. Ovalbumin challenge, on the other hand, increased eosinophils, macrophages, B cells, and CD4⁺ cells in both the airway wall and lung parenchyma independent of the effect of latent adenoviral infection. In the sham-infected groups steroid treatment caused the expected reduction in the eosinophilic infiltrate induced by OA challenge in the airways without affecting the other cells. In the presence of both latent adenoviral infection and OA challenge, steroid treatment had no effect on allergen-induced eosinophilia but reduced CD8⁺ cells in the airways and CD8⁺ cells, CD4⁺ cells, and B cells in the parenchyma.

Conclusion: Latent adenoviral infection and OA challenge result in different types of lung inflammation, and the presence of latent adenoviral infection causes OA-induced eosinophilic airway inflammation to become steroid resistant. (J Allergy Clin Immunol 2000;106:844-51.)

Key words: Adenovirus, allergic eosinophilic inflammation, epithelial cells, steroid resistance

Corticosteroids are highly effective in the control of asthma,^{1,2} and studies based on bronchial biopsies have confirmed that inhaled corticosteroids reduce the number

Abbreviations used

OA: Ovalbumin
pfu: Plaque-forming units
RSV: Respiratory syncytial virus

and activation of mast cells, eosinophils, and T lymphocytes in asthmatic airways.^{3,4} Subsets of patients with asthma⁵ or atopic dermatitis⁶ fail to respond to corticosteroid therapy, and although the mechanism of this steroid resistance is not completely understood, it has been associated with viral infection.^{7,8}

The present study was designed to test the hypothesis that latent adenoviral infection might cause allergen-induced lung inflammation to become steroid resistant. We used an animal model of latent adenoviral infection previously developed in our laboratory⁹ in which adenovirus 5 E1A DNA and protein persist in the lung at least 7 weeks after the cessation of viral replication. Allergic inflammation was induced by sensitization followed by multiple aerosolized challenges with ovalbumin (OA)¹⁰ in both adenovirus 5-infected and sham-infected animals. The nature of the inflammatory process induced by latent adenoviral infection, OA challenge, and their interaction was assessed with and without steroid treatment by using quantitative histologic methods.

METHODS

Animals

Female guinea pigs (Cam Hartley strain, *Cavia porcellus*, 250-300 g) were purchased from Charles River Canada (St Constant, Quebec, 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 animal experimental committee of the University of British Columbia and followed the published guidelines of the Canadian Council on Animal Care.

Viruses and cell culture

Wild-type adenovirus 5 was obtained from the American Type Culture Collection (Rockville, Md) and propagated in monolayer cultures of A549 cells grown in minimal essential media (Gibco BRL Life Technologies, Inc, Gaithersburg, Md) supplemented with 10% FBS. Two to 5 days after inoculation with adenovirus 5, cells were vortexed with sterile glass beads to break up the monolayer. The cell suspension was frozen-thawed twice, sonicated for 30 seconds 3 times, and spun at 2000g for 10 minutes. The supernatant was collected, and its viral

From ^athe University of British Columbia Pulmonary Research Laboratory, St Paul's Hospital, Vancouver; and ^bAstra Draco AB, Lund.

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Reprint requests: James C. Hogg, MD, The University of British Columbia Pulmonary Research Laboratory, St Paul's Hospital, 1081 Burrard St, Vancouver, British Columbia, V6Z 1Y6, Canada.

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titer was determined by plaque assays on A549 monolayers grown on 6-well plates. Titers ranged from 10^8 to 10^9 plaque-forming units (pfu) per milliliter. The supernatant was layered on top of a CsCl step gradient of 1.45 and 1.33 g/mL and spun for 3 hours at 100,000g at 4°C. An opaque band at the interface between the two CsCl layers was recovered and dialyzed against PBS 7.4 (0.149 mol/L NaCl, 0.012 mol/L Na_2HPO_4 , and 0.004 mol/L KH_2PO_4 , pH 7.4) at 4°C and stored at -70°C in 10% glycerol. The titer of the purified virus was greater than 10^{10} pfu/mL when determined by using plaque assay. Sham inoculum was prepared from uninfected A549 cells treated in an identical manner to the infected cells.

Adenoviral infection

Guinea pigs were anesthetized with 4% Halothane balanced with oxygen. During anesthesia, 10^9 pfu of purified adenovirus 5 in 200 μL of PBS applied to the nares was aspirated into the lungs. Sham inoculum was applied in an identical fashion in the sham-infected groups. Latent infection was established by 3 weeks after infection.⁹

OA sensitization and challenge

As described by Ishida et al,¹⁰ guinea pigs were placed in a covered polycarbonate cage and sensitized to OA by exposure for 10 minutes to an aerosol spray of 1% OA (wt/vol; Sigma Chemical, St Louis, Mo) with 4% (vol/vol) heat-killed pertussis vaccine (Connaught Laboratories, Toronto, Ontario, Canada) in normal saline solution. Challenge consisted of delivering an aerosol spray of 0.5% OA (wt/vol) solution over a 5-minute period. Sham sensitization with normal saline solution containing 4% heat-killed pertussis vaccine and challenge with normal saline solution were performed in a separate group of animals that served as controls for allergen sensitization and challenge. Diphenhydramine (Sigma Chemical; 0.2 mL of 40 mg/mL in normal saline solution) was administered intraperitoneally 1 hour before each OA challenge to prevent anaphylactic shock.

Steroid treatment

A commercially available steroid (budesonide; Astra Draco AB, Lund, Sweden) was suspended (10 mg/mL) in 0.3% (wt/vol) carboxymethylcellulose sodium (medium viscosity; Sigma Chemical), 0.04% (wt/vol) polyoxyethylenesorbitan monooleate (Sigma Chemical), and 0.88% (wt/vol) sodium chloride. A topical steroid dose of 2 to 5 mg/kg is needed to block bronchoconstriction during the immediate or late allergic bronchial response.¹¹ The dose of steroids required to reduce allergic lung inflammation induced by repeated OA challenge in guinea pig models is not known. Therefore a high dose of steroids (20 mg/kg) was administered intraperitoneally to ensure a systemic anti-inflammatory effect.

Study design and experimental protocol

The experimental protocol is shown in Fig 1. Twenty-four guinea pigs were divided into two groups. One group was infected with adenovirus 5, and the other was infected with sham inoculum. Three weeks after inoculation, 8 infected and 8 sham-infected guinea pigs were chosen randomly for sensitization with a single dose of aerosolized 1% OA (wt/vol) solution containing 4% (vol/vol) heat-killed pertussis vaccine. One week later, the sensitized guinea pigs were repeatedly challenged on 5 occasions over 15 days with an aerosol spray generated from the 0.5% OA (wt/vol) solution. To control for the effect of allergen challenge, the remaining 4 infected and 4 sham-infected guinea pigs received an aerosol spray of saline solution during both the sensitization and challenge exposures. Steroids were administered to 4 infected and 4 sham-infected guinea pigs sensitized and challenged with OA on 12 occasions over 16 days, starting 24 hours before the first allergen challenge. To control for the effect of steroid treatment, the remaining 16 guinea pigs received an

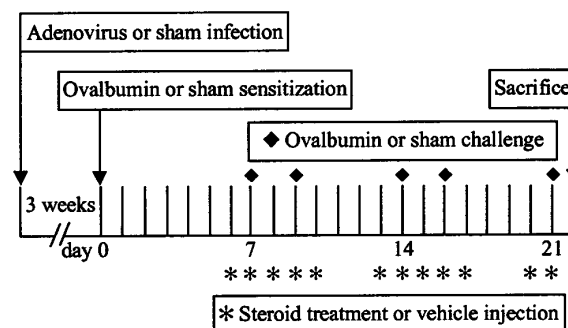


FIG 1. Schematic diagram of the experimental protocol. See text for further explanation.

injection of an equal volume of vehicle used to deliver the steroids. Twenty-four hours after the last OA challenge or saline exposure, guinea pigs were killed with an overdose of sodium pentobarbital administered intraperitoneally. On death, the body weight of each animal was measured. Serum samples were obtained to assess the level of steroid the animals received.

Tissue preparation

Each analysis described below was performed on a predetermined lung lobe on the basis of the assumption that the exposure to any particular lobe would be similar across experimental groups.¹² After the lungs were removed en block with the trachea, the left lung was isolated, weighed, and dried to a constant weight in a 100°C oven. The right upper and lower lobes were weighed, inflated with a 1:1 mixture of Optimal Cutting Temperature (OCT) compound (Tissue Tek; Miles, Inc, Elkhart, Ind) and PBS 7.4, and reweighed to determine the inflation volume. The right upper lobe was frozen in liquid nitrogen and stored at -70°C for cryosectioning and immunohistochemistry. The right lower lobe was cut into 4 blocks in the transverse plane, fixed in buffered 10% formalin, and processed into paraffin.

Total lung collagen

Lung collagen content was estimated by measuring the lung hydroxyproline content of the hydrolysate of the dried left lung,¹³ according to the method of Stegemann and Stalder.¹⁴ The results were compared with a standard curve (0-100 μg of 4-hydroxyproline per assay). The assay was validated by using BSA standards spiked with a range of concentrations of hydroxyproline and also by using purified type I and III collagen, both of which were hydrolyzed in parallel with the lung tissue samples.¹³

Quantitative histology

Quantitative histology was performed on the right lung. Serial 4- μm sections from each of the paraffin-embedded blocks of the right lower lobe were stained with either hematoxylin and eosin or Hansel's stain¹⁵ to identify the lung compartments (airspace, alveolar tissue, airway wall, and blood vessels) or eosinophils, respectively. Serial 8- μm sections from the frozen right upper lobe were analyzed for macrophages, B cells, and T-lymphocyte subsets by using immunohistochemical staining (see below). The volumes of lung compartments and of inflammatory cells in the lung were estimated by point counting according to a multilevel cascade design.¹⁶ A digital imaging system (Bioview; Infrascan, Richmond, British Columbia, Canada) was used to overlay a point-counting grid on the sections, and the density of the grid was adjusted to maintain the coefficient of error below 0.10.¹⁷ The volume fraction was obtained by counting the number of points falling on an object or region of

TABLE I. Mean volume of inflammatory cells in the airway wall of the right lung (n = 4 per group)

								Two-way ANOVA	
	Sham saline vehicle	Adenovirus 5 saline vehicle	Sham OA vehicle	Adenovirus 5 OA vehicle	Sham OA steroid	Adenovirus 5 OA steroid	Viral effect	OA effect	
Eosinophils ($\mu\text{L/kg}$)	4.92 $\pm$ 1.00	4.14 $\pm$ 0.75	29.63 $\pm$ 5.65	26.44 $\pm$ 7.00	9.11 $\pm$ 2.84	27.83 $\pm$ 3.89	.67	.001	
B cells ($\mu\text{L/kg}$)	0.74 $\pm$ 0.47	0.83 $\pm$ 0.26	2.81 $\pm$ 0.27	3.34 $\pm$ 0.38	2.08 $\pm$ 0.41	2.29 $\pm$ 0.77	.40	.001	
Macrophages ($\mu\text{L/kg}$)	1.38 $\pm$ 0.49	2.11 $\pm$ 0.50	8.50 $\pm$ 1.04	4.16 $\pm$ 0.57	8.53 $\pm$ 1.29	9.72 $\pm$ 0.74	.24	.001	
CD4 ⁺ cells ($\mu\text{L/kg}$)	0.58 $\pm$ 0.17	1.43 $\pm$ 0.38	2.86 $\pm$ 0.77	2.55 $\pm$ 0.40	1.80 $\pm$ 0.20	3.80 $\pm$ 0.80	.59	.004	
CD8 ⁺ cells ($\mu\text{L/kg}$)	0.33 $\pm$ 0.22	1.53 $\pm$ 0.37	0.78 $\pm$ 0.17	1.81 $\pm$ 0.23	0.47 $\pm$ 0.10	0.90 $\pm$ 0.10	.001	.19	

Values are given as means $\pm$ SE.

interest and dividing this number by the total number of points in the fields examined. The volume fraction of the airway wall and blood vessels was determined by point counting the whole section at 40 $\times$ magnification. The airway wall volume fraction included both the mucosa and adventitial tissue but excluded the cartilage, which does not change in chronic nonfatal asthma.¹⁸ The volume fraction of the airspace and alveolar tissue was determined by point counting 7 randomly chosen fields of each section at 40 $\times$ magnification. The volume fraction of eosinophils, macrophages, B cells, and T-lymphocyte subsets in the airway wall was determined in the entire wall area at 250 $\times$ magnification. The volume fraction of these cells in the lung parenchyma (airspace and alveolar tissue combined) was determined on 7 randomly chosen fields of each section at the same magnification. The resulting volume fraction of each lung compartment and of each cell type was multiplied by the lung volume to obtain the volume of each lung compartment and each type of inflammatory cell. All of the histologic slides were coded to blind the observers to the experimental groups.

Immunohistochemistry

Eight-micrometer serial sections from the frozen right upper lobe were cut onto silanized slides in a cryostat (FRIGOCUT 2800N), air-dried, and fixed in 10% formalin for 15 minutes. Monoclonal mouse IgG₁ antibodies specific for guinea pig T lymphocyte helper-inducer subset (clone CT7, analogous to human CD4⁺ subset), T lymphocyte cytotoxic-suppressor subset (clone CT6, analogous to human CD8⁺ subset), all guinea pig tissue B cells (clone MsGp9), and a cytoplasmic component of all guinea pig tissue macrophages and monocytes (clone MR-1) were purchased from Serotec Ltd (Kidlington, Oxford, UK). A monoclonal mouse IgG_{2a} antibody to adenovirus 5 E1A protein was purchased from Cedarlane Laboratories (Hornby, Ontario, Canada). Positive controls to localize adenovirus 5 E1A protein were A549 cells infected with adenovirus 5 and negative sham-infected guinea pig lungs, which were used as negative controls. Each of the serial sections was stained with one of these antibodies or with a nonspecific isotype-matched mouse IgG as a negative control. Antibody binding was detected with the alkaline phosphatase antialkaline phosphatase method.¹⁹ The sections were not counterstained to avoid masking of the staining achieved with the primary antibodies.

Statistical analysis

The data were analyzed by using two-way ANOVA to determine the independent effects of adenoviral infection, OA challenge, and steroid treatment. The level of significance for the independent effects was a *P* value of less than .05. To determine an interaction between adenovirus 5 infection and steroid treatment on OA-induced inflammatory changes, multiple comparisons were analyzed by using *t* tests.²⁰ Corrections for multiple comparisons were made by using the Bonferroni method,²⁰ and the level of significance was maintained at a *P* value of less than .05.

RESULTS

Tissue analysis

Mean value of the body weight in each group was about 500 g, with no difference between the groups. OA sensitization and challenge increased the wet weight (2.23 $\pm$ 0.10 vs 2.64 $\pm$ 0.08 g/kg [mean $\pm$ SE], *P* < .005), the dry weight (0.44 $\pm$ 0.02 vs 0.51 $\pm$ 0.02 g/kg, *P* < .01), the weight of hydroxyproline (5.66 $\pm$ 0.18 vs 6.54 $\pm$ 0.27 $\mu\text{g/kg}$, *P* < .02), and the proportion of the lung taken up by airway wall (0.40 $\pm$ 0.03 vs 0.55 $\pm$ 0.04 mL/kg, *P* < .02), whereas latent adenoviral infection did not increase either the lung weights or the volume of the lung compartments. Steroid treatment reduced the increase in the wet weight of the lung (2.64 $\pm$ 0.08 vs 2.42 $\pm$ 0.08 g/kg, *P* < .05) as a result of the OA challenge. The presence of latent adenoviral infection was confirmed by localizing adenovirus 5 E1A protein to the nuclei of epithelial cells in the lungs of adenovirus 5-infected guinea pigs. The E1A protein could not be demonstrated in sham-infected animals (data not shown).

Morphometry

The results concerning the volume per kilogram of body mass (microliters per kilogram) of each cell type in all of the experimental groups are shown for the airway wall in Table I and the lung parenchyma in Table II.

Latent adenoviral infection

The results of the two-way ANOVA showed that latent adenoviral infection independently increased the numbers of CD8⁺ cells (*P* < .001) in the airway wall and the numbers of B cells (*P* < .01), macrophages (*P* < .05), CD4⁺ cells (*P* < .04), and CD8⁺ cells (*P* < .005) in the lung parenchyma (Fig 2, A).

OA sensitization and challenge

The same analyses showed that OA sensitization and challenge independently increased the numbers of eosinophils (*P* < .001), B cells (*P* < .001), macrophages (*P* < .001), and CD4⁺ cells (*P* < .004) but not CD8⁺ cells in the airway wall. Similar results were found in the lung parenchyma (Fig 2, B).

Steroid treatment

The levels of steroid in serum from the sham-infected, OA-challenged, steroid-treated group and the adenovirus 5-infected, OA-challenged, steroid-treated group were

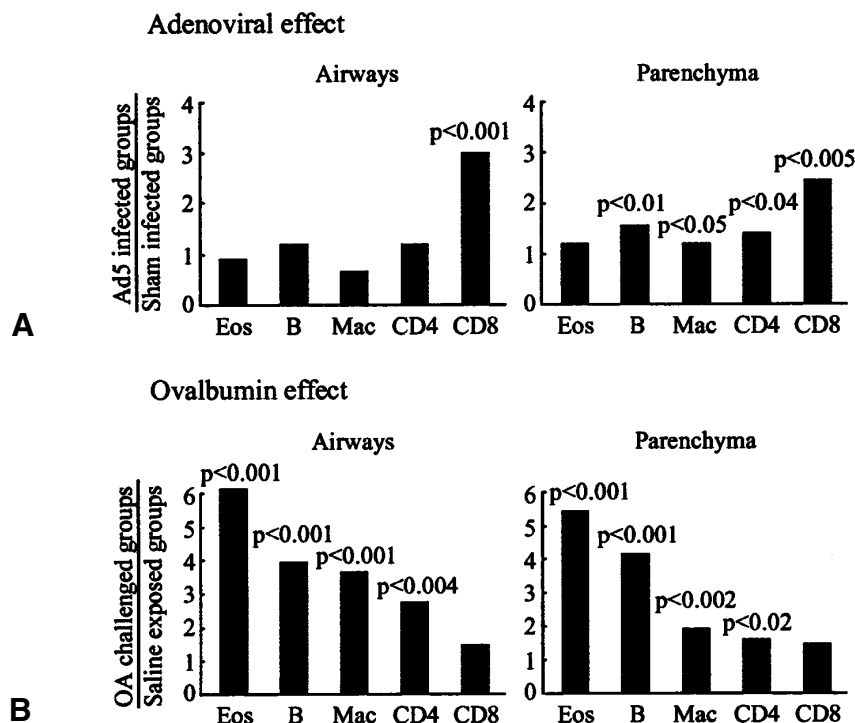


FIG 2. Inflammatory cells in the airways and parenchyma of (A) adenovirus 5 (*Ad5*)-infected animals compared with sham-infected animals and of (B) OA-sensitized and challenged animals compared with sham-sensitized and challenged animals. *P* values shown here are from a two-way ANOVA. Note that latent adenoviral infection increased CD8⁺ cells in the airways and parenchyma and also increased numbers of B cells (*B*), macrophages (*Mac*), and CD4⁺ cells in the parenchyma. OA sensitization and challenge increased numbers of eosinophils (*Eos*), B cells, macrophages, and CD4⁺ cells in both compartments without affecting CD8⁺ cells. ANOVA showed that the effects of latent adenoviral infection and allergen challenge occurred independently without interaction when both were delivered to the same animal. The ratios were prepared from the data shown in Tables I and II.

similar (38.7 ± 4.3 vs 49.9 ± 6.3 nmol/L [mean $\pm$ SE], $P > .1$) and were undetectable in animals that did not receive steroid treatment.

In the airway wall of the sham-infected group, steroid treatment markedly reduced the eosinophil response induced by OA challenge ($P < .03$; Figs 3, A, and 4, A and B) but had no effect on the other cell types. In the adenovirus 5-infected group, on the other hand, steroids reduced the numbers of CD8⁺ cells increased by adenovirus 5 infection in the airway wall ($P < .03$; Figs 3, A, and 4, E and F) but had no effect on eosinophils (Figs 3, A and 4, C and D) or other inflammatory cells. In the parenchyma steroid treatment reduced the numbers of CD8⁺ cells ($P < .05$), B cells ($P < .01$), and CD4⁺ cells ($P < .04$) induced by adenovirus 5 infection but did not affect eosinophils in either sham- or adenovirus 5-infected groups (Fig 3, B). Interestingly, latent adenoviral infection increased the eosinophil response to OA challenge ($P < .05$; Fig 3, B) despite steroid treatment in the parenchyma.

DISCUSSION

A guinea pig model of latent adenoviral infection previously developed in our laboratory⁹ was used to assess

3 specific issues: (1) whether latent adenoviral infection induces eosinophilic lung inflammation; (2) whether latent adenoviral infection is able to amplify allergen-induced lung inflammation; and (3) whether latent adenoviral infection can induce steroid resistance in an allergen-induced inflammatory response. Our analysis was based on a quantitative histologic technique that provides the volume of the lung taken up by each cell type. This type of analysis has distinct advantages because it infers 3-dimensional properties from 2-dimensional histologic images to better reflect the 3-dimensional nature of inflammatory changes within the tissue.¹⁶ It is based on the method of stereology, which converts the 2-dimensional volume fraction to absolute volume by using the total lung volume as the reference volume. This provides the volume of the lung taken up by each cell type expressed in microliters per kilogram of the animal's body mass. Although numerical density (the number of cells/volume of a lung compartment) can also be derived by using the disector method,¹⁶ we chose not to use this method because the extra sections required to make this calculation would have greatly increased the workload of this study as a result of the number of animals and the number of cells being examined.

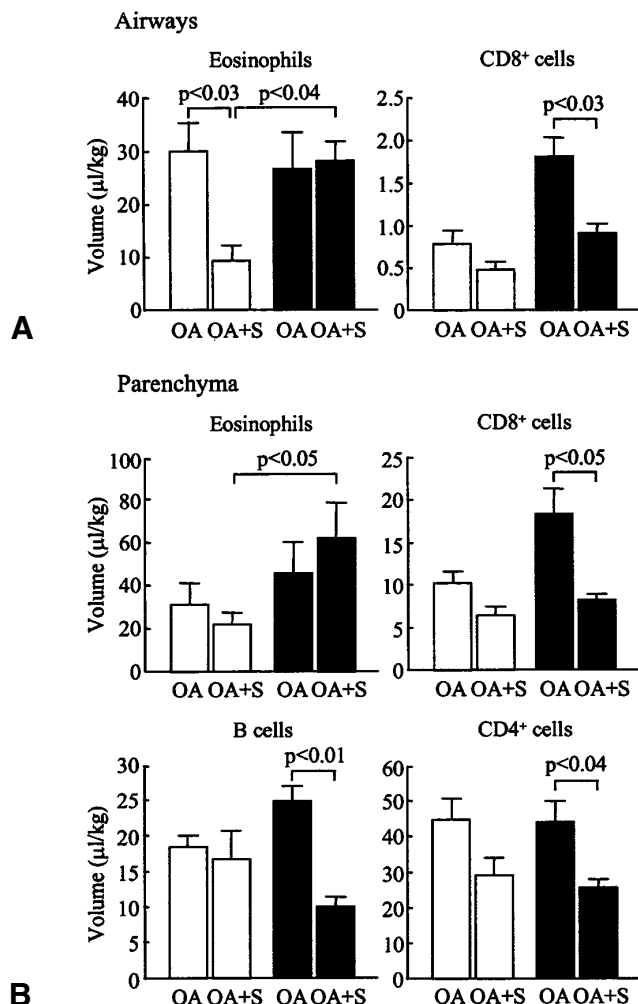


FIG 3. Effects of steroid treatment on inflammatory cells in (A) the airways and (B) the parenchyma of the sham-infected groups (open bars) and adenovirus-infected groups (filled bars). *P* values shown here from unpaired *t* tests were corrected for multiple comparisons by using the Bonferroni method. Note that steroid treatment decreased the eosinophilic response in the airways of the sham-infected but not the adenovirus-infected group. See text for further explanation. OA, Ovalbumin challenge; S, steroid treatment.

The results showed that latent adenoviral infection independently increased the numbers of CD8⁺ cells in both the airways and lung parenchyma, which is consistent with the expected cytotoxic T-cell response to viral infection. Latent adenoviral infection increased the numbers of B cells, macrophages, and CD4⁺ cells, in addition to CD8⁺ cells, only in the parenchyma. The reason for this difference between airway and parenchymal inflammation is not clear but may be caused by the virus targeting both a greater number and a different type of epithelial cells in the parenchyma than in the airways.²¹

ANOVA showed that allergen sensitization and challenge affect the response of inflammatory cells independent of latent adenoviral infection. OA challenge increased the numbers of eosinophils, B cells, macrophages, and CD4⁺ cells in a manner that is consistent with findings from human asthmatic airways.^{22,23} These results show that latent adenoviral infection nei-

ther induced eosinophilic inflammation on its own nor enhanced OA-induced eosinophilia in the lungs. This contrasts with other animal studies^{24,25} using respiratory syncytial virus (RSV), which induced both eosinophilic lung inflammation and airway hyperresponsiveness to methacholine in guinea pigs²⁴ and mice.²⁵ The murine model study also showed that a preceding RSV infection enhanced the eosinophilic response to allergen challenge in the lungs.²⁵ One of the possible explanations for the difference between our results and those based on RSV infection is the difference in cytokine profiles generated by the two viruses. RSV induces allergy-related chemokines, such as RANTES, monocyte chemoattractant protein 1, and macrophage inflammatory protein 1 α ,²⁶ and adenovirus upregulates TNF- α , IL-1, and IL-6.²⁷ Other possibilities included differences in the protocol, where use of adjuvant during sensitization in our study generated a 6-fold increase in eosinophils on subsequent

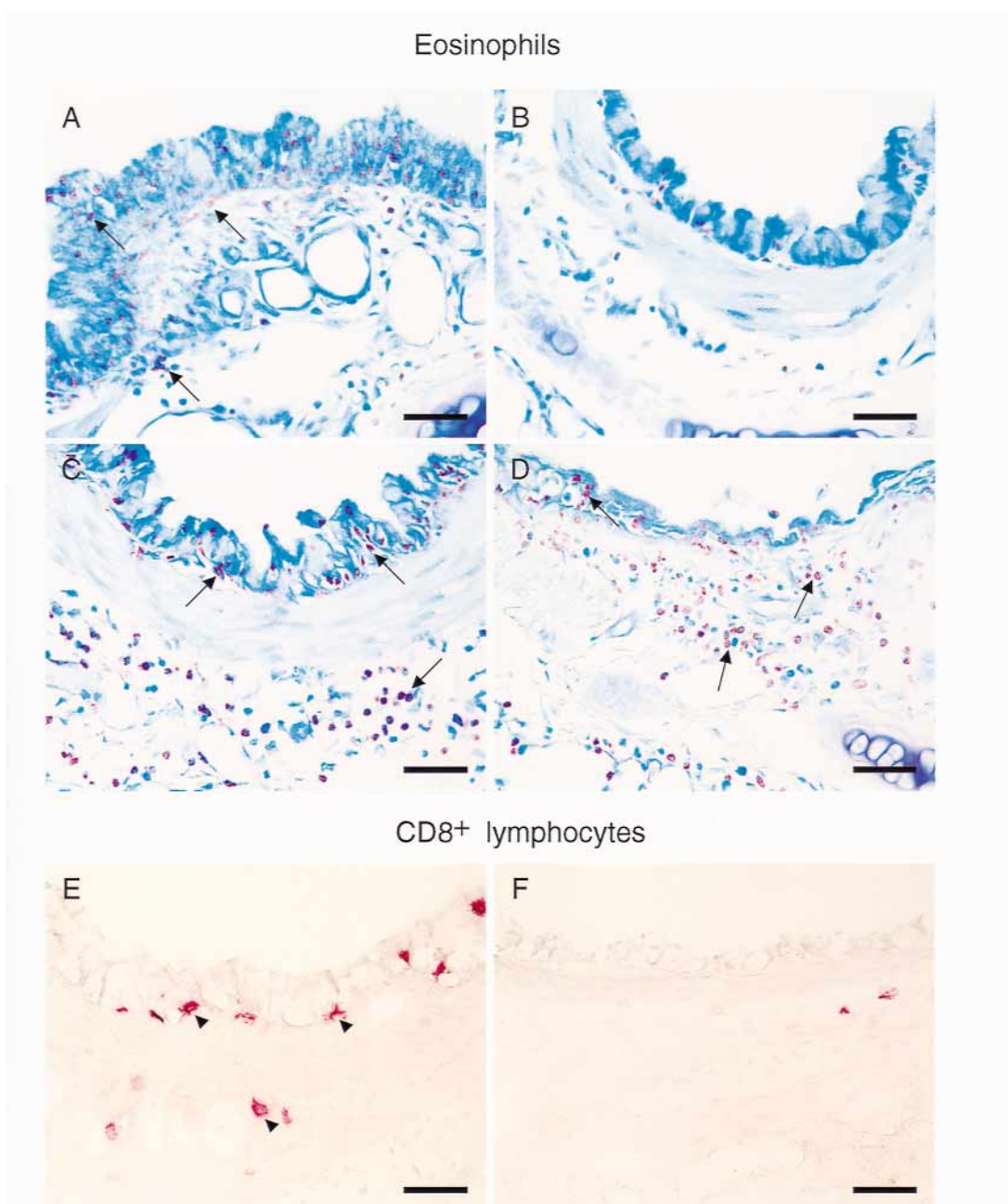


FIG 4. Eosinophil and CD8⁺ cell infiltration in the airways demonstrated with Hansel's stain and immunohistochemistry, respectively. **A**, Airway eosinophilia (arrows) induced by OA sensitization and challenge of a sham-infected guinea pig. **B**, Airway of a sham-infected guinea pig sensitized and challenged with OA and treated with steroids in which the number of eosinophils is reduced. **C**, Airway eosinophilia (arrows) of an adenovirus-infected, OA-sensitized and challenged guinea pig. **D**, Airway of an adenovirus-infected, OA-sensitized and challenged guinea pig showing that steroid treatment failed to reduce airway eosinophilia (arrows). **E**, CD8⁺ cell infiltration (arrowheads) in the airway of an adenovirus-infected guinea pig sensitized and challenged with OA. **F**, Airway of an adenovirus-infected, OA-sensitized and challenged guinea pig showing that steroid treatment reduced CD8⁺ cells. Bar represents 50 μm.

OA challenge in the absence of latent adenoviral infection compared with the result of the murine study²⁵ in which adjuvant was excluded and there was little or no eosinophilic response with repeated allergen challenge in the absence of a preceding RSV infection.

A novel finding of our study is that latent adenoviral infection caused the OA-induced eosinophilic airway inflammation to become steroid resistant. Gern et al²⁸ have shown that IFN-γ produced by T lymphocytes after viral infection prolongs the survival of peripheral blood

TABLE II. Mean volume of inflammatory cells in the parenchyma of the right lung (n = 4 per group)

	Sham saline vehicle	Adenovirus 5 saline vehicle	Sham OA vehicle	Adenovirus 5 OA vehicle	Sham OA steroid	Adenovirus 5 OA steroid	Two-way ANOVA	
							Viral effect	OA effect
Eosinophils ($\mu\text{L/kg}$)	10.57 $\pm$ 4.18	3.69 $\pm$ 1.16	31.47 $\pm$ 10.25	45.77 $\pm$ 15.19	22.72 $\pm$ 5.04	62.71 $\pm$ 17.14	.71	.001
B cells ($\mu\text{L/kg}$)	2.87 $\pm$ 0.94	7.52 $\pm$ 2.21	18.56 $\pm$ 1.51	24.90 $\pm$ 2.09	16.74 $\pm$ 4.00	10.06 $\pm$ 1.26	.01	.001
Macrophages ($\mu\text{L/kg}$)	18.54 $\pm$ 2.31	47.90 $\pm$ 9.44	70.29 $\pm$ 9.30	53.37 $\pm$ 4.19	82.76 $\pm$ 11.94	70.40 $\pm$ 6.44	.05	.002
CD4 ⁺ cells ($\mu\text{L/kg}$)	16.56 $\pm$ 1.44	40.35 $\pm$ 6.60	44.74 $\pm$ 5.93	44.02 $\pm$ 6.04	29.10 $\pm$ 4.76	25.74 $\pm$ 1.97	.04	.02
CD8 ⁺ cells ($\mu\text{L/kg}$)	4.07 $\pm$ 0.52	16.69 $\pm$ 4.26	10.23 $\pm$ 1.38	18.24 $\pm$ 3.03	6.49 $\pm$ 0.92	8.17 $\pm$ 0.68	.005	.19

Values are given as means $\pm$ SE.

eosinophils. We think that this is a remote possibility for the cause of the persistent eosinophilia in our study because the increase in T lymphocytes, especially CD8⁺ cells, induced by adenovirus was reduced by steroids. We suspect that the epithelial cells of the lung are a more likely source of steroid resistance because they express the adenoviral-transactivating protein E1A during latent infection in both guinea pigs⁹ and human subjects.²⁹ Recent evidence³⁰ has shown that epithelial cells are a major source of eosinophil-attracting chemokines, and two separate studies^{31,32} have demonstrated a reduction in the expression of these chemokines in airway epithelial cells and a reduction in the numbers of eosinophils in the airways of patients with allergic airway disease after steroid treatment. Our results showing that latent adenoviral infection does not induce eosinophilic lung inflammation indicate that the expression of eosinophil-attracting chemokines is not upregulated by latent adenoviral infection. On the other hand, adenovirus E1A protein has been shown to inhibit steroid hormone action by interacting with coactivators of transcription.³³ We speculate that E1A expressed by latently infected epithelial cells interferes with the downregulation of eosinophil-attracting chemokines by steroids because we found that latent adenoviral infection reversed the anti-inflammatory effect of steroids. Because human lung epithelial cells are known to harbor and express the gene encoding adenovirus E1A,²⁹ we suspect that this form of latent viral infection may be relevant to the steroid resistance seen in asthma.

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