

Fas deficiency delays the resolution of airway hyperresponsiveness after allergen sensitization and challenge

Catherine Duez, PhD,^a Adrian Tomkinson, PhD,^a Leonard D. Shultz, PhD,^b

Donna L. Bratton, MD,^a and Erwin W. Gelfand, MD^a Denver, Colo, and Bar Harbor, Me

Background: In asthma, persistent inflammation might be the result of (1) an impaired ability to clear inflammatory cells from the airways and/or (2) impaired apoptotic responses. **Objective:** In a mouse model, we investigated the regulatory role of Fas (CD95)-induced apoptosis in the development and resolution of airway inflammation and airway hyperresponsiveness (AHR).

Methods: Mice that were either Fas-sufficient (wild-type; WT) or Fas-deficient (*lpr*) were sensitized by intraperitoneal injections of ovalbumin (OVA) and challenged once intranasally with OVA (IP-IN mice). Control (IN) mice were challenged only.

Results: IP-IN WT mice developed AHR at 48 hours; changes in airway resistance resolved by 96 hours. Airway responsiveness at 48 hours in IP-IN *lpr* mice was similar to that in IP-IN WT mice. However, in contrast to WT mice, IP-IN *lpr* mice sustained significant AHR at 96 hours in comparison with IN *lpr* mice; the AHR resolved by 6 days. Bronchoalveolar lavage fluid cell composition was similar in all of the different groups at 48 hours and 96 hours. Both IP-IN WT mice and *lpr* mice exhibited similar tissue eosinophilia, whereas IP-IN *lpr* mice had significantly lower numbers of TdT-mediated dUTP nick end labeling (TUNEL)-positive cells in comparison with IP-IN WT mice at 48 hours. Anti-IL-5 antibody given to IP-IN *lpr* mice 48 hours and 72 hours after the challenge significantly decreased AHR and eosinophilic inflammation and increased TUNEL-positive cell numbers at 96 hours.

Conclusion: These results suggest that Fas expression can regulate the onset and resolution of AHR through an increase in eosinophil apoptosis. (J Allergy Clin Immunol 2001;108:547-56.)

Key words: Lung, eosinophils, apoptosis, inflammation, in vivo animal models

Abbreviations used

AHR:	Airway hyperresponsiveness
BAL:	Bronchoalveolar lavage
EPO:	Eosinophil peroxidase
MBP:	Major basic protein
MCh:	Methacholine
OVA:	Ovalbumin
RL:	Lung resistance
TBS:	TRIS-buffered saline solution
TUNEL:	Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling
WT:	Wild type

Airway inflammation and airway hyperresponsiveness (AHR) are well-established characteristics of allergic asthma. Accumulating data support the notion of a relationship between the presence of activated airways inflammatory cells and morphologic changes in airway tissues and the development and severity of AHR. Among the inflammatory cells, increased numbers of T cells of the T_H2 subset and eosinophils are found in bronchial biopsies and in the bronchoalveolar lavage (BAL) fluid of asthmatic patients.¹ Eosinophils are thought to play a key role in the development of AHR because they contain and release a number of cytotoxic proteins, including major basic protein (MBP), eosinophil cationic proteins, and eosinophil peroxidase (EPO), whereas T_H2 cells are believed to orchestrate the inflammatory reaction through the release of T_H2 cytokines, such as IL-4, IL-5, and IL-13. IL-5 appears to be the main cytokine involved in the development of eosinophilia in vivo, inasmuch as it promotes differentiation, activation, recruitment, and survival of eosinophils.² In particular, animal models highlight the importance of IL-5 in the development of AHR.³⁻⁶ However, the role of IL-5 in the development of AHR is still controversial; it might depend on sensitization and challenge protocols and route of exposure of the bronchoconstrictor agent.^{7,8} Most studies have focused on factors influencing the development of inflammation and AHR, whereas few have investigated their resolution.

Resolution of inflammation requires not only interruption or termination of the initiating signals but also removal of the inflammatory cells. In asthma, there is increasing evidence that ongoing inflammation is the

From ^athe Division of Cell Biology, Department of Pediatrics, National Jewish Medical and Research Center, Denver; and ^bthe Jackson Laboratory, Bar Harbor.

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Reprint requests: Erwin W. Gelfand, MD, Department of Pediatrics, National Jewish Medical and Research Center, 1400 Jackson Street, Denver, CO 80206.

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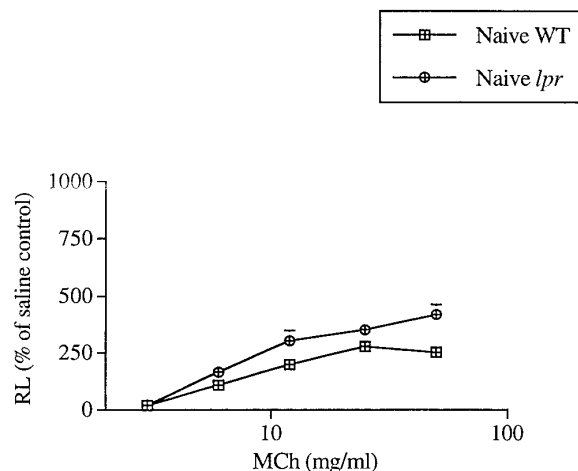


FIG 1. Airway reactivity of naive Fas-sufficient (WT) and Fas-deficient mice (*lpr*). Baseline RL values were 0.51 ± 0.01 in WT mice ($n = 10$) and 0.53 ± 0.02 in *lpr* mice ($n = 10$).

result of an impaired ability to clear inflammatory cells from the airways and/or impaired apoptotic responses. Although the number of eosinophils is increased in sputum, nasal polyps, and bronchial biopsies of asthmatic patients, the percentage of eosinophils undergoing apoptosis appears to be decreased.⁹⁻¹² Steroid-treated patients exhibit decreased airway eosinophilia but increased numbers of apoptotic eosinophils in the tissue.¹² Blood eosinophils from asthmatic patients not taking steroid medication survive longer than those from healthy control subjects, reinforcing the hypothesis that an alteration in apoptotic processes could contribute to the chronic tissue eosinophilia associated with asthma.¹³

Eosinophil survival is regulated not only by the presence or absence of survival factors such as IL-5 and GM-CSF but also by the engagement of surface death receptors.¹⁴ One of the death receptors expressed by human and murine eosinophils is Fas (CD95/APO-1),^{15,16} which belongs to the TNF receptor family. Fas ligand (FasL/CD95L) binds to its receptor and induces a cascade of caspase activation that results in morphologic and biochemical changes characteristic of apoptosis.¹⁷ Administration of anti-Fas antibody to the lungs after the induction of a lung eosinophilia induces a marked reduction in the number of eosinophils in the airways.¹⁶ On the other hand, the frequency of eosinophil apoptosis and the kinetics of AHR development and resolution have not been fully evaluated. In this study, we used mice lacking functional Fas because of the *lpr* (lymphoproliferation) mutation to analyze the onset and resolution of airway inflammation and hyperresponsiveness in a model of ovalbumin (OVA)-sensitized and challenged mice.

MATERIALS AND METHODS

Animals

Fas/*lpr* (Fas-deficient; *lpr*) mice on a C3H/HeJ background and congenic (C3H/HeJ) Fas-sufficient (wild-type; WT) mice were obtained from Jackson Laboratories (Bar Harbor, Me). All experi-

mental procedures were performed according to the protocol approved by the Institutional Animal Care and Use Committee of the National Jewish Medical and Research Center.

Sensitization and airway challenge

Mice were sensitized by intraperitoneal injection of 20 μ g OVA (grade V, Sigma Chemical, St Louis, Mo) emulsified in 2.25 mg aluminum hydroxide (AlumImject; Pierce, Rockford, Ill) on days 1 and 14. Sensitized mice were challenged by a single intranasal challenge with OVA (20 μ L, 5 mg/mL in saline solution; IP-IN mice) on day 28. Forty-eight hours, 96 hours, and 6 days after the last OVA challenge (days 30, 32, and 34), AHR was assessed and tissues were obtained for further analysis. Control mice groups received OVA challenge only (IN mice).

Anti-IL-5 antibody treatment

Some *lpr* mice were treated with anti-IL-5 (TRFK5 clone) or rat IgG control antibody, injected intravenously (50 μ g per mouse) 48 hours and 72 hours after challenge. Analysis was performed 96 hours after challenge.

Determination of airway responsiveness

Airway responsiveness was assessed in terms of change in airway function after challenge with aerosolized methacholine (MCh) administered for 10 seconds (60 breaths/min; tidal volume, 500 μ L) in increasing concentrations (3.125, 6.25, 12.5, 25, and 50 mg/mL). Anesthetized (intraperitoneal pentobarbital sodium, 70 to 90 mg/kg), tracheostomized (18G cannula) mice were mechanically ventilated (160 breaths/min; tidal volume, 150 μ L; positive end-expiratory pressure, 2 to 4 cm H₂O), and lung function was assessed through use of methods described by Takeda et al.¹⁸ Lung resistance (RL) was continuously computed (Labview, National Instruments, Austin, Tex) by fitting flow, volume, and pressure to an equation of motion. Maximum values of RL were taken and expressed in terms of percentage change from baseline after saline aerosol.

Bronchoalveolar lavage

Immediately after assessment of AHR, lungs were lavaged via the tracheal tube with Hank's balanced salt solution (1×1 mL, 37°C). Total leukocyte numbers were counted with a hemocytometer. Differential cell counts were performed by counting at least 300 cells on cytocentrifuged preparations (Cytospin 2; Shandon Ltd., Runcorn, Cheshire, United Kingdom), stained with Hema 3 (Fisher Diagnostics, Pittsburgh, Pa), and differentiated through use of standard hematologic procedures.

TUNEL/MBP staining

Lungs were fixed in 10% formalin. Apoptotic cells were detected through use of the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) method (Boehringer Mannheim, Mannheim, Germany), according to the manufacturer's instructions. Briefly, sections were permeabilized with proteinase K (100 μ g/mL) for 30 minutes at 37°C and then incubated with recombinant terminal deoxynucleotidyl transferase and deoxyribonucleoside triphosphate (dNTP) for 1 hour at 37°C. Negative and positive controls were included in each experiment; a section incubated with dNTP only served as the negative control, and a section treated with 2 U of DNase RQ1 (Promega, Madison, Wis) before the TUNEL reaction served as the positive control. Sections were then incubated with alkaline phosphatase complex (Boehringer Mannheim) for 1 hour at room temperature and developed with Fast Blue substrate (Sigma, St Louis, Mo).

Sections were permeabilized with porcine trypsin (Sigma) at 0.2 mg/mL in CaCl₂ (0.11 mol/L)/TRIS buffer (50 mol/L, pH 7.4) for 30 minutes at 37°C. Tissues were saturated with 10% goat serum in TRIS-buffered saline solution (TBS) for 30 minutes before incuba-

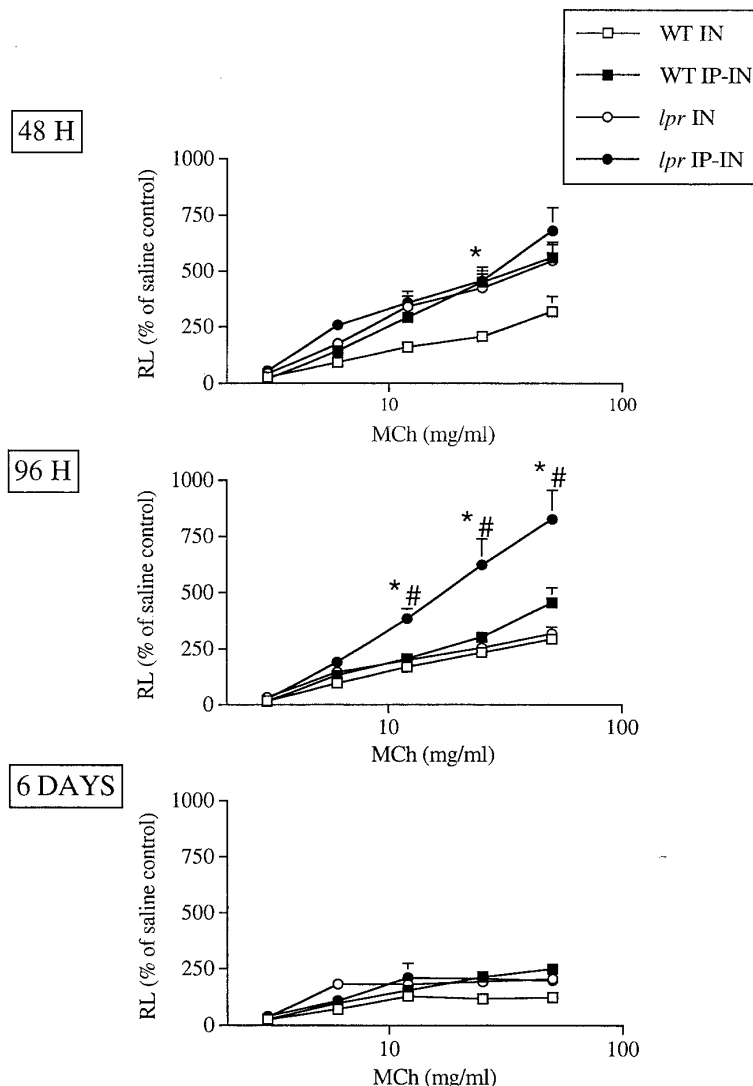


FIG 2. AHR of sensitized and challenged Fas-sufficient (WT) and Fas-deficient mice (*lpr*) at 48 hours, 96 hours, and 6 days after challenge. Baseline RL values were as follows: 0.58 ± 0.05 ($n = 16$), 0.54 ± 0.02 ($n = 16$), and 0.54 ± 0.01 ($n = 8$) in WT challenged-only (IN) mice; 0.55 ± 0.03 ($n = 19$), 0.61 ± 0.02 ($n = 16$), and 0.64 ± 0.06 ($n = 9$) in WT sensitized and challenged (IP-IN) mice; 0.64 ± 0.05 ($n = 14$), 0.70 ± 0.03 ($n = 15$), and 0.69 ± 0.11 ($n = 9$) in *lpr* IN mice; and 0.64 ± 0.04 ($n = 16$), 0.72 ± 0.03 ($n = 15$), and 0.67 ± 0.05 ($n = 10$) in *lpr* IP-IN mice at 48 hours, 96 hours, and 6 days, respectively. * $P < .05$ in comparison with IN groups; # $P < .05$ in comparison with WT mice.

tion with rabbit antimouse MBP antibody (kindly provided by Dr J. J. Lee, Mayo Clinic, Scottsdale, Ariz) or rabbit serum for control sections diluted in TBS/3% goat serum overnight at 4°C. After washing, tissues were incubated with 1/2000 alkaline phosphatase-goat antirabbit antibody (Pharmingen, San Diego, Calif) in TBS/3% goat serum for 1 hour at room temperature. The reaction was developed through use of Fast Red substrate (Sigma). Tissues were counterstained with methyl green (Sigma).

Morphometric analysis

Immunohistochemistry data were quantitatively analyzed, after capture of tissue section images taken with a Kodak MDS 120 digital camera (Eastman Kodak Company, Rochester, NY), through

use of NIH Scion Image analysis software (version 1.62, developed at the US National Institutes of Health). Positive cell numbers were determined by counting positive cells in the airway wall area between the basal membrane of the epithelium and the outer limit of the adventitia. Measurements were normalized to the length of the basement membrane. The measured values were averaged for each animal, and the mean values were determined for each group.

Statistical analysis

Comparisons for all pairs were performed by means of Tukey-Kramer significant difference and Wilcoxon tests. P values for significance were set to .05. The value for every measurement is expressed as the mean $\pm$ SEM.

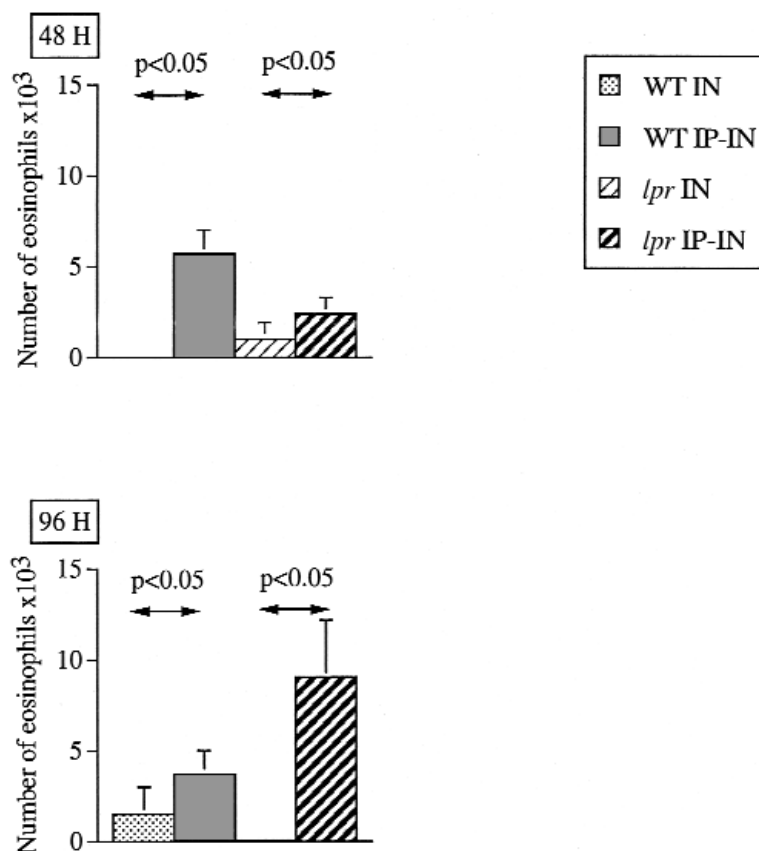


FIG 3. BAL fluid eosinophil numbers of sensitized and challenged Fas-sufficient (WT) and Fas-deficient mice (*lpr*) at 48 hours and 96 hours after the challenge. BAL fluid cell composition was determined in sensitized and challenged (IP-IN) mice ($n = 16$ to 19) and in challenged-only (IN) mice ($n = 16$). Each result is expressed as a number of cells $\times 10^3$.

RESULTS

Maintenance of AHR in Fas-deficient (*lpr*) mice

Airway reactivity was first measured in naive mice to determine whether Fas deficiency affected baseline airway reactivity. Dose-response curves showed no significant differences between Fas-sufficient (WT) and Fas-deficient (*lpr*) mice with respect to airway reactivity (Fig 1). Kinetic analysis of airway reactivity (RL) in WT mice indicated that sensitized and challenged (IP-IN) WT mice developed AHR 48 hours after the challenge, which progressively resolved by 96 hours and 6 days (RL values at an MCh dose of 50 mg/mL: $561\% \pm 68\%$, $453\% \pm 68\%$, and $250\% \pm 12\%$ of saline solution control) in comparison with challenged-only (IN) WT mice ($318\% \pm 68\%$, $293\% \pm 54\%$ and $124\% \pm 14\%$ of saline solution control; Fig 2). Surprisingly, IN *lpr* mice exhibited higher airway reactivity ($548\% \pm 73\%$ of saline solution control) than IN WT mice ($232\% \pm 40\%$ of saline solution control), though only transiently at 48 hours, and sensitized or nonsensitized *lpr* mice given saline solution intranasally showed the same airway responsiveness as *lpr* IN mice (data not shown). Heightened airway reactivity to inhaled MCh at 48 hours in *lpr* IN mice appears to be nonspecific and not associated with an increase in airway

inflammation. In contrast, IP-IN *lpr* mice developed AHR at 48 hours, which remained at the same level at 96 hours and resolved at 6 days (RL values at an MCh dose of 50 mg/mL: $681\% \pm 101\%$, $826\% \pm 129\%$, and $200\% \pm 29\%$ of saline solution control). These data demonstrate that resolution of AHR was delayed in the *lpr* mice.

Airway inflammation in Fas-sufficient (WT) and Fas-deficient (*lpr*) mice

BAL cells were counted and differentiated. No differences were found between the 4 groups in total cell, macrophage, lymphocyte, and neutrophil numbers at all time points analyzed (data not shown). Although lymph nodes were larger in *lpr* mice than in WT mice, this increase in lymphocyte number was not reflected in BAL lymphocyte numbers in *lpr* mice. Overall, eosinophil recruitment into the BAL was modest ($<1\%$ in IN groups, 1.5% to 20.5% in IP-IN groups) but increased in both IP-IN WT and *lpr* mice in comparison with IN WT and *lpr* mice at 48 and 96 hours (Fig 3).

Lung sections were stained to identify eosinophils and apoptotic cells (Fig 4). Both WT and *lpr* IP-IN mice exhibited tissue eosinophilia at 48 hours (2100 ± 500 and 2400 ± 400 positive cells per 100 mm of basement membrane, respectively), which decreased by 96 hours (to 1300 ± 500

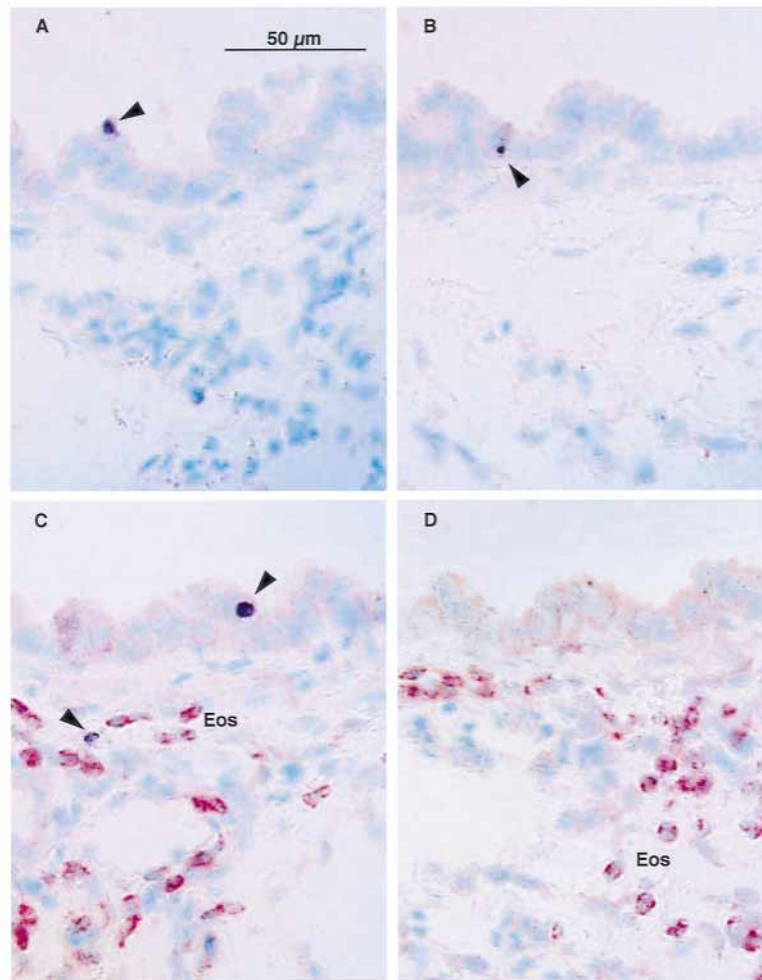


FIG 4. Tissue eosinophil and TUNEL staining in sensitized and challenged Fas-sufficient (WT) and Fas-deficient mice (*lpr*) at 48 hours. **A** and **B**, Control mice were challenged only (**A**, IN WT mice; **B**, IN *lpr* mice). **C** and **D**, Mice were sensitized by OVA intraperitoneal injection and challenged by a single intranasal OVA administration (**C**, IP-IN WT mice; **D**, IP-IN *lpr* mice). Tissues were stained with MBP-specific antibody, and apoptotic cells were detected through use of the TUNEL method. Sections were counterstained with methyl green. TUNEL-positive cells are indicated (arrowheads); eosinophils exhibit a red staining (Eos).

and 1500 ± 500 positive cells per 100 mm of basement membrane, respectively; Fig 5). In contrast, the number of TUNEL-positive cells was significantly lower in *lpr* IP-IN mice (100 ± 100 positive cells per 100 mm of basement membrane) than in WT IP-IN mice (300 ± 100 positive cells per 100 mm of basement membrane) at 48 hours. No differences were observed at 96 hours (Fig 6). Thus, although *lpr* IP-IN mice exhibited similar numbers of BAL and airway tissue eosinophils, a significantly lower number of apoptotic cells was detected by TUNEL staining at 48 hours, a time point preceding the persistence of AHR.

Effect of anti-IL-5 antibody injection on AHR and inflammation in Fas-deficient (*lpr*) mice

To determine whether the prolonged AHR in *lpr* mice was eosinophil-dependent, we administered anti-IL-5 antibody at a time when eosinophilic inflammation was fully established, at 48 hours after the challenge. As illus-

trated in Fig 7, *lpr* IP-IN mice once again had persistent AHR at 96 hours, and anti-IL-5 treatment significantly attenuated AHR in these mice (PC200 [the dose of MCh giving a 200% increase in RL] values were 19 ± 6 and 45 ± 10 , respectively).

Airway eosinophils were significantly decreased in anti-IL-5 treated animals in comparison with the IgG control antibody-treated group (numbers of MBP-positive cells per 100 mm of basement membrane were 733 ± 160 and 1352 ± 271 , respectively) at 96 hours (Fig 8). Moreover, there was a significant trend toward an increase in the number of TUNEL-positive cells in the anti-IL-5-treated *lpr* mice in comparison with the IgG control antibody-treated group (numbers of TUNEL-positive cells per 100 mm of basement membrane were 160 ± 14 and 103 ± 17 , respectively). These data extend the findings that IL-5-dependent eosinophilia in combination with a decrease in apoptosis is associated with sustained AHR in *lpr* mice.

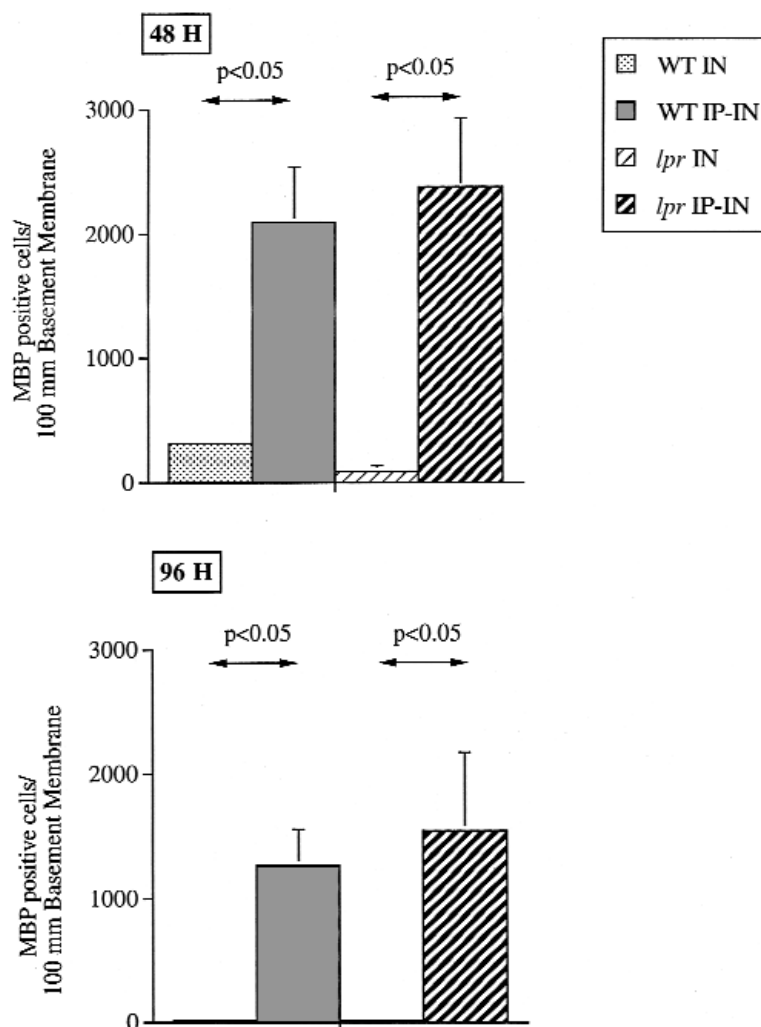


FIG 5. Tissue eosinophils in sensitized and challenged Fas-sufficient (WT) and Fas-deficient mice (*lpr*) at 48 hours and 96 hours after the challenge. Tissues from sensitized and challenged (IP-IN) mice ($n = 8$) and challenged-only (IN) mice ($n = 8$) were stained with MBP-specific antibody. Each result is expressed as the number of positive cells per 100 mm of basement membrane.

DISCUSSION

In this study, we evaluated the influence of Fas, an important factor in activation-induced cell death, in the development and resolution of AHR in OVA-sensitized and challenged mice. The kinetics of AHR were first evaluated in WT C3H/HeJ mice, the strain background carrying the mutated Fas allele. WT C3H/HeJ mice developed AHR to inhaled MCh at 48 hours, and it resolved by 96 hours—a pattern previously seen in BALB/c mice.¹⁹ WT C3H/HeJ mice were found to be less responsive to inhaled MCh than BALB/c mice, inasmuch as higher doses of MCh were used to demonstrate airway reactivity; of note, several studies evaluating the impact of genetic background on the development of AHR had difficulty demonstrating AHR in this strain of mice.²⁰⁻²³ Differences in the protocols used to sensitize and challenge the mice and in the administration of the bronchoconstrictor agent (delivered intravenously vs

aerosolized) likely explain some of the differences in our findings. In parallel to the airway reactivity data, we found fewer eosinophils in the BAL fluid at 48 and 96 hours after sensitization and challenge than was demonstrated in BALB/c mice. This lower BAL eosinophil number was also reflected in lower, virtually undetectable, levels of EPO in BAL (data not shown). Similarly, low to undetectable levels of cytokines were measured in the BAL fluid (data not shown), again in contrast to what was seen in BALB/c mice. However, WT C3H/HeJ mice did exhibit a significant peribronchial eosinophilic infiltrate at 48 hours, which decreased by 96 hours. The kinetics of eosinophil inflammation were similar to the findings in BALB/c mice, which revealed the importance of tissue eosinophilia over BAL eosinophilia,¹⁹ emphasizing the relationship between tissue eosinophilia and the development of AHR.

Naive *lpr* mice and WT mice demonstrated similar RL values at baseline and after exposure to increasing doses

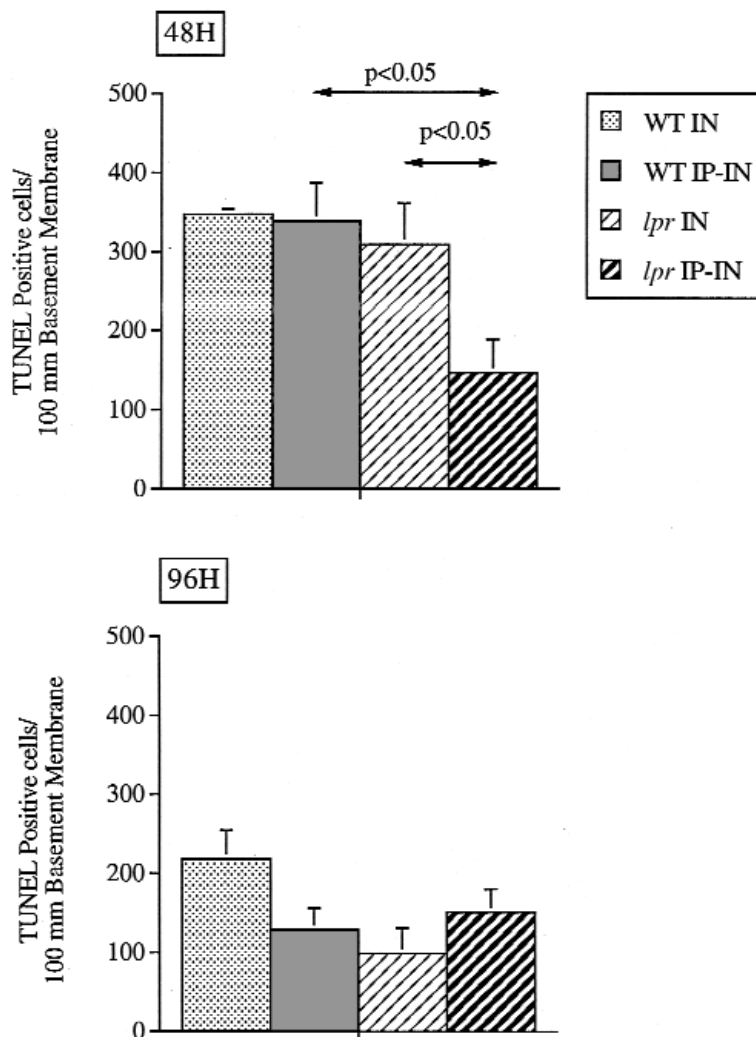


FIG 6. Number of TUNEL-positive cells in the tissue of sensitized and challenged Fas-sufficient (WT) and Fas-deficient mice (*lpr*) at 48 hours and 96 hours after challenge. Tissues from sensitized and challenged (IP-IN) mice ($n = 8$) and challenged-only (IN) mice ($n = 8$) were stained for apoptotic cells through use of the TUNEL method. Each result is expressed as the number of positive cells per 100 mm of basement membrane.

of MCh, indicating that Fas deficiency per se does not affect airway function. After sensitization and challenge, *lpr* IP-IN mice developed AHR and airway eosinophilic inflammation at 48 hours to the same level as WT IP-IN mice, suggesting that Fas deficiency does not prevent the normal development of the response. Nonsensitized but challenged (IN) *lpr* mice had airway responsiveness at 48 hours that was noticeably elevated in comparison with that in WT IN mice and similar to the levels in IP-IN WT and *lpr* mice. However, no tissue eosinophilia was demonstrated, BAL eosinophil numbers were not different in WT IN mice and *lpr* IN mice at this time point, and airway responsiveness normalized by 96 hours. Moreover, sensitized or nonsensitized *lpr* mice given saline solution intranasally showed the same airway responsiveness as *lpr* IN mice (data not shown). This level of airway reactivity in IN *lpr* mice at 48 hours might be nonspecific, as observed at earlier time points in other

strains of mice.¹⁹ Furthermore, the responsiveness could be linked to slower resolution of nonspecific responses because of the Fas deficiency. What is more important, AHR was maintained in the IP-IN *lpr* mice at 96 hours, in contrast to what was seen in the IP-IN WT mice. By 6 days, AHR was abolished in the IP-IN *lpr* mice, suggesting that Fas deficiency delayed but did not ablate the resolution of AHR.

To evaluate the mechanism underlying the delayed resolution of AHR in the IP-IN *lpr* mice, we analyzed the inflammatory response in the lung and BAL fluid. No differences in BAL fluid cell composition and cell numbers, BAL fluid EPO levels, tissue MBP-positive eosinophil numbers, and CD4 and CD8 cell numbers (data not shown) between IP-IN WT and *lpr* mice were seen at any time point. Analysis of apoptotic cells through use of the TUNEL method revealed a low number of positive cells, similar to what has been seen in

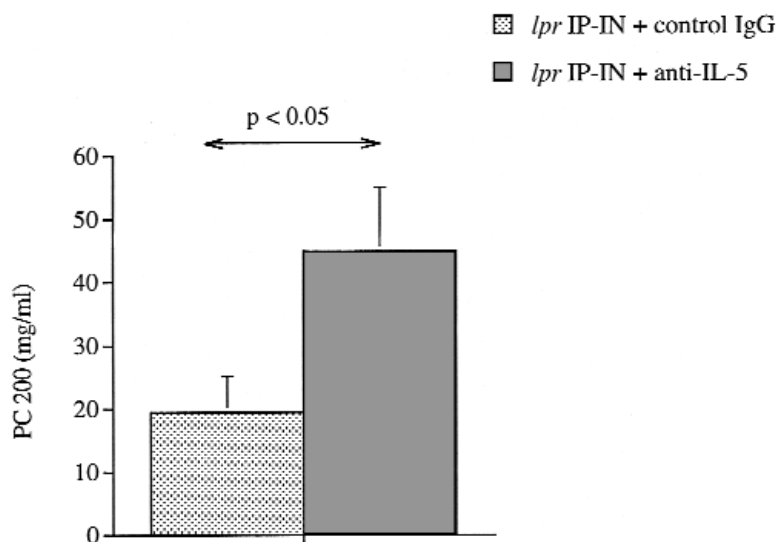


FIG 7. Inhibition of AHR in Fas-deficient mice (*lpr*) at 96 hours after treatment with anti-IL-5 antibody. Forty-eight hours and 72 hours after the challenge, sensitized mice (IP-IN) received an intravenous injection of anti-IL-5 antibody (n = 12) or control IgG (n = 8). Airway resistance was evaluated at 96 hours and expressed as PC200 values. Baseline RL values were 1.00 ± 0.04 in control IgG-treated *lpr* mice and 1.01 ± 0.04 in anti-IL-5-treated *lpr* mice.

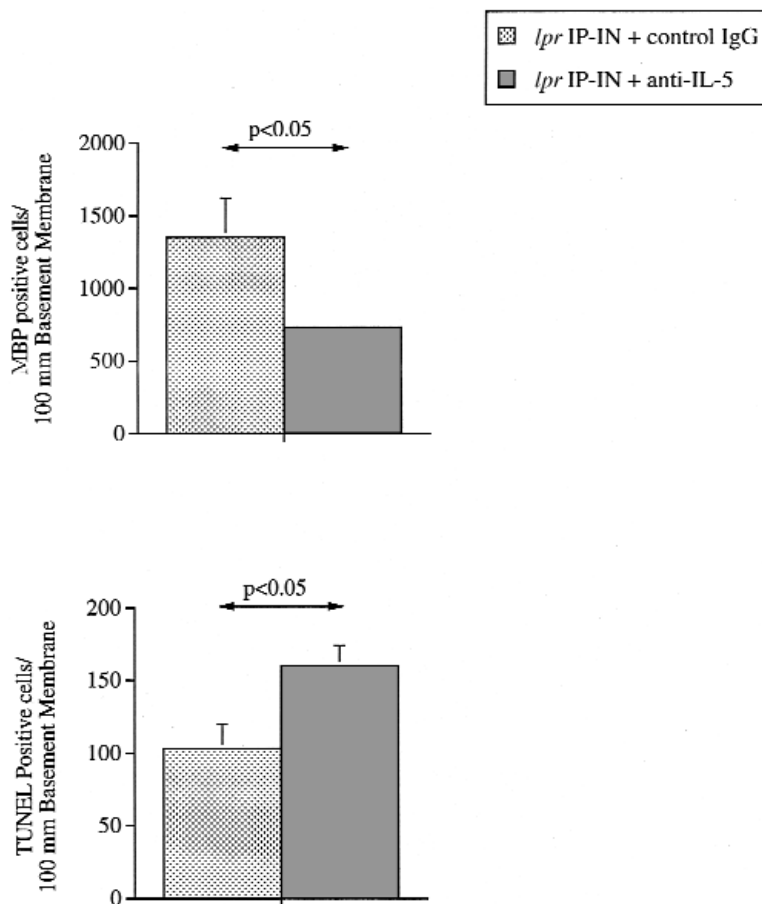


FIG 8. Airway inflammation at 96 hours in Fas-deficient mice (*lpr*) treated with anti-IL-5 antibody. Mice were sensitized by OVA intraperitoneal injection and challenged by a single intranasal OVA administration (IP-IN mice). Forty-eight hours and 72 hours after the challenge, mice received an intravenous injection of anti-IL-5 antibody (n = 12) or control IgG (n = 8). Airway eosinophilia and number of TUNEL-positive cells were evaluated at 96 hours. Each result is expressed as the number of positive cells per 100 mm of basement membrane.

other animal and human studies.^{11,12,24} TUNEL, the only available method that allows detection of apoptotic cells in tissue, might not detect all apoptotic cells because of insensitivity with respect to early stages of apoptosis. Indeed, DNA fragmentation, detected by TUNEL, might occur relatively late in apoptosis and might follow changes at the membrane surface, allowing apoptotic cells to be recognized, phagocytized, and rapidly digested.²⁵⁻²⁷

A relationship between increased Fas-induced eosinophil apoptosis and decreased eosinophilia has been shown in another study, in which intranasal administration of anti-Fas, which induces eosinophil apoptosis in vitro, also reduced eosinophil numbers in the BAL fluid and tissues of antigen-challenged mice in the ensuing 48 hours.¹⁶ More recently, anti-Fas intranasal administration during OVA challenge has been shown to inhibit the development of AHR as measured 24 hours after the last OVA challenge.²⁸ In our study, TUNEL-positive cell numbers in the airways of *lpr* IP-IN mice were significantly decreased in comparison with those in WT mice at 48 hours, and this was associated with maintenance of AHR at 96 hours. Although we were not able to demonstrate a direct link between eosinophil survival and the delay in resolution of AHR in the *lpr* mice, additional observations suggest that eosinophils were critically responsible for AHR. Because many studies show the IL-5 dependency of AHR,^{3,4,6,7,29-31} we used neutralizing antibody to IL-5 to further highlight the role of eosinophils in the prolongation of AHR demonstrated in *lpr* mice. Although anti-IL-5 might have inhibited further recruitment of eosinophils into the airways and thereby inhibited AHR, anti-IL-5 was delivered after the establishment of eosinophilic inflammation and AHR to minimize this effect. Anti-IL-5 treatment was found to significantly decrease AHR and airway tissue eosinophilia and to significantly increase TUNEL-positive cell numbers at 96 hours. Thus in the *lpr* mice, as the number of TUNEL-positive cells increased, airway eosinophilia decreased. Inasmuch as IL-5 is not the sole survival factor for eosinophils (eg, there is GM-CSF), this could explain the partial effect of anti-IL-5 treatment.

In summary, Fas-deficient mice sensitized and challenged with allergen demonstrate a prolongation of AHR. These effects appear to be eosinophil-dependent and secondary to a decrease in apoptosis. Our data support the hypothesis that persistent inflammation and AHR can result from an impairment of normal apoptotic processes in the lung. A defect in Fas signaling results in prolongation of AHR with eosinophilic inflammation and reduced apoptosis in this murine model, thus reinforcing the idea that impaired apoptosis could be involved in the chronic inflammation and persistent AHR in asthma.

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