

V.P. Kurup
H.Y. Choi
P.S. Murali
J.-Q. Xia
R.L. Coffman
J.N. Fink

Immune responses to *Aspergillus* antigen in IL-4-/- mice and the effect of eosinophil ablation

Authors' affiliations:

V.P. Kurup, P.S. Murali, J.-Q. Xia, J.N. Fink,
Allergy and Immunology Division, Department
of Medicine, Medical College of Wisconsin,
Milwaukee, Wisconsin, and Research Service,
VA Medical Center, Milwaukee, Wisconsin
H.Y. Choi, Department of Pathology, Medical
College of Wisconsin, Milwaukee, Wisconsin,
and Laboratory Service, VA Medical Center,
Milwaukee, Wisconsin
R.L. Coffman, DNAX Research Institute of
Molecular and Cellular Biology, Palo Alto,
California, USA

Correspondence to:

Viswanath P. Kurup, PhD
VA Medical Center
Research Service 151-I
5000 West National Avenue
Milwaukee
WI 53295
USA

Key words: *Aspergillus fumigatus*; IL-4 knockout mice;
interferon-gamma; interleukin-4; interleukin-5; Th2 response.

Background: Exposure to *Aspergillus fumigatus* allergens results in enhanced total serum IgE and peripheral blood eosinophils in mice. The associated pulmonary inflammation and immunologic responses are comparable to those detected in human allergic bronchopulmonary aspergillosis. Allergen-induced cytokines are thought to regulate the inflammatory and immune responses in these animals.

Methods: In the present study, we exposed C57BL/6 and BALB/c mice to *A. fumigatus* antigen. Both wild-type and IL-4 knockout phenotypes of animals of both strains were used. Some animals were also treated with anti-IL-5 or anti-IFN- γ . Total serum IgE, *Aspergillus* species IgG subclass, peripheral blood eosinophils, and lung histology were studied.

Results: The results demonstrate similar lung inflammation in all wild-type and IL-4-/- animals exposed to *A. fumigatus* antigen. Similarly, in spite of the diverse immune response produced by the anticytokine treatment, no major differences were detected among any of the animal groups studied.

Conclusions: It can be concluded that *A. fumigatus* exposure in an immunologically unaltered host is predominantly of a Th2 type, and that depletion of the Th2 cytokine leads to a similar lung inflammation but with a characteristic Th1 response, suggesting that the pathogenesis of allergic aspergillosis is the result of multiple induction pathways.

Date:

Accepted for publication 8 October 1998

To cite this article:

Kurup V.P., Choi H.Y., Murali P.S., Xia J.-Q., Coffman R.L. & Fink J.N. Immune responses to *Aspergillus* antigen in IL-4-/- mice and the effect of eosinophil ablation. *Allergy* 1999; **54**, 420–427.

Copyright © Munksgaard 1999

ISSN 0105-4538

Aspergillus-caused allergic diseases, particularly allergic bronchopulmonary aspergillosis (ABPA), have been recognized with increasing frequency in recent years (1–3). ABPA is a disabling disease and has high morbidity and mortality; it must be recognized and treated early before irreversible

pulmonary damage has occurred (2). Understanding the immunopathogenesis is vital in arriving at an early diagnosis and in successful patient management. We, as well as other investigators, have developed murine models of ABPA in order to understand the pathogenesis of the disease (4–15). We have shown that the intranasal administration of *Aspergillus* antigens resulted in elevated total serum IgE, *Aspergillus*-specific IgG, and peripheral blood and lung eosinophilia. A predominantly Th2 type of response was demonstrable in BALB/c mice exposed to *Aspergillus* antigens (6, 7). Depletion of specific cytokines in this model preferentially modulated the Th1/Th2 responses (8, 11). The increase in the levels of IgE was truncated by the administration of anti-IL-4, while anti-IL-5 completely ablated the peripheral blood and lung eosinophilia (11). On the other hand, administration of anti-IFN- γ consistently downregulated the Th1 response, resulting in enhanced blood and lung eosinophils and serum IgE (8). In a previous study, we demonstrated that IL-4 knockout (KO) and wild-type (WT) mice on challenge with *Aspergillus* antigen showed similar lung inflammation (12). However, these studies were carried out with C57BL/6 and 129J mice strains with disrupted IL-4 genes. Most of the animal model studies have been carried out with BALB/c mice because of the closer resemblance of this model to human ABPA. Therefore, we undertook the present study to determine the role played by IL-4 in the immunopathogenesis of ABPA.

In the present study, we used IL-4 KO mice belonging to both BALB/c and C57BL/6 strains and exposed them intranasally to *Aspergillus* antigens. We selectively depleted IL-5 and IFN- γ and evaluated the immune responses following *Aspergillus* antigen challenge in these animals. The WT mice demonstrated enhanced IgE and eosinophil

responses in anti-IFN- γ -treated animals compared to untreated animals, while no eosinophils were demonstrated in mice treated with anti-IL-5. On the other hand, IL-4 KO mice showed strong IgG2a response, while anti-IFN- γ antibody treatment resulted in enhancement of the Th2 response, as evidenced by the eosinophil increase. The inflammatory responses in the lungs of these mice remained the same, suggesting a Th1 role in the induction of lung inflammation and a Th2 role in the IgE and eosinophil responses.

Material and methods

Animals

WT BALB/c and C57BL/6 mice, 10–12 weeks old, were used in the present study and were purchased from Charles Rivers Laboratories, Wilmington, MA, USA. The IL-4^{tm1Cgn} mutation (16) (IL-4 KO) was back-crossed seven generations onto the BALB/cAnN strain at DNAX and nine generations onto the C57BL/6J strain (obtained from Dr R. Schwartz, National Institutes of Health). Both strains were maintained by brother-sister mating as homozygous mutants at DNAX and bred for experiments at the Veterinary Medical Unit of the VA Medical Center. Animal studies were approved by the Institutional Subcommittee on Animal Care and Use. WT and KO animals were sensitized with *Aspergillus fumigatus* (*Af*) antigen by intranasal instillation, as described below.

Aspergillus antigen

The antigen used to immunize the mice was a mixture of culture filtrate antigen and cytosol extract of *Af* (6, 7). The culture filtrate antigen was prepared by growing the fungus in a synthetic broth for 2–3 weeks. The fungus was incubated at 37°C, and the broth was separated from the mycelium by filtration. The filtrate was dialyzed extensively against deionized water at 4°C before freeze-drying. The antigen was evaluated for protein content, polyacrylamide gel electrophoresis profile, and reactivity with patient and control sera. Mycelial antigens were prepared by homogenizing a 4-day-old aerated culture of *Af* grown in synthetic medium, as described before (12).

Homogenization was effected in a French press, and the extract was dialyzed and concentrated to 1 mg/ml. All antigens were compared for their reactivity with in-house standards.

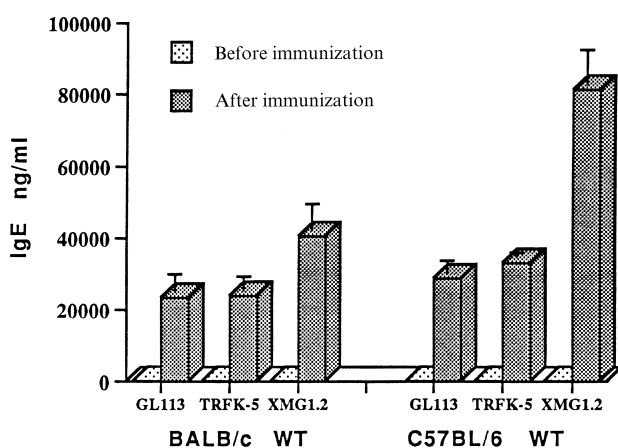


Figure 1. Total serum IgE in various groups of WT BALB/c and C57BL/6 mice before and after exposure to *Aspergillus* antigen. Control (GL-113), anti-IL-5 (TRFK-5), and anti-IFN- γ (XMG1.2).

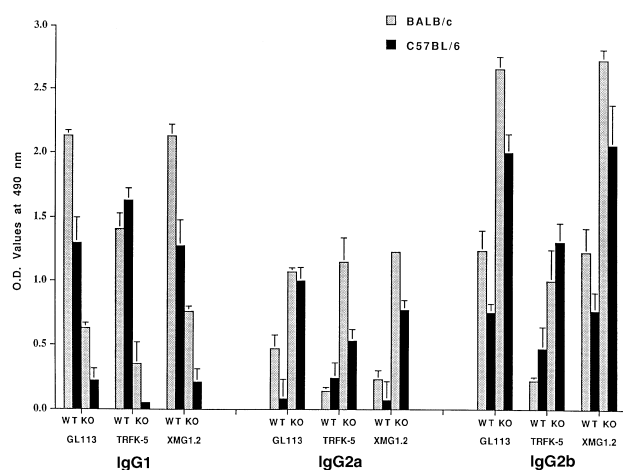


Figure 2. *Aspergillus*-specific IgG subclasses (IgG1, IgG2a and IgG2b) antibodies in sera of various groups of WT and IL-4 KO mice belonging to both BALB/c and C57BL/6 strains. Baseline antibody levels (preimmunization) were subtracted from values obtained with sera on termination of experiments. Control (GL-113), anti-IL-5 (TRFK-5), and anti-IFN- γ (XMG1.2).

Sensitization of mice

Ten milligrams of culture filtrate antigen was dissolved in 1 ml of the mycelial antigen. Animals were lightly anesthetized with Metofane (Methoxyflurane, Pitman-Moore, Mundelein, IL, USA). About 50 μ l of *Af* containing 50 μ g of protein in phosphate-buffered saline (PBS) was instilled through the nostrils of the animals, as previously described (6, 12). The sensitization was carried out for 4 weeks with twice weekly exposures to *Af* antigen. The animals were bled 1 week after the eighth exposure, and the sera were analyzed for anti-*Aspergillus* antibodies. The animals were then boosted with an additional intranasal *Af* antigen when significant *Af*-specific antibodies were detected by enzyme-linked immunosorbent assay (ELISA). The animals were killed 5 days after the last sensitization and their sera studied for *Af*-specific IgG. The lungs were fixed in formalin and subjected to histologic analysis (7, 11, 12). Peripheral blood was studied for eosinophils after staining with Discombe's fluid (6, 12).

Treatment of animals with anticytokine antibodies

Different groups of WT and KO animals were treated with 0.5 mg/ml antimouse IL-5 (TRFK-5), anti-IFN- γ (XMG1.2), or control antibody (GL-113), as previously described (8, 11, 12). The antibodies were injected intraperitoneally into the groups of mice 24 h before the first antigen challenge and thereafter every week for the duration of the experiment.

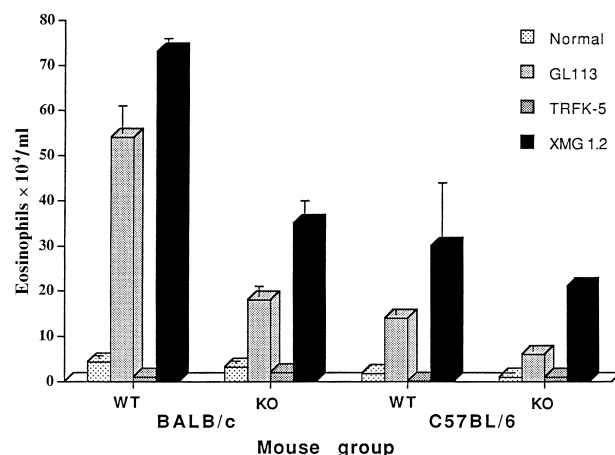


Figure 3. Peripheral blood eosinophils in WT and IL-4 KO mice treated with anticytokine antibodies and exposed to *Aspergillus* antigen. Control (GL-113), anti-IL-5 (TRFK-5), and anti-IFN- γ (XMG1.2).

Serum IgE

Total serum IgE levels were evaluated with a monoclonal antibody-based ELISA, as previously described (6). The IgE concentration in the serum was expressed as nanograms/ml after comparing optical density values of mouse IgE standard.

Af-specific antibodies

Anti-*Af* isotypes in the sera of mice belonging to IgG1, IgG2a, IgG2b, and IgG3 were studied by ELISA, by the method reported previously (6, 12). In brief, *Af* antigen was coated onto wells of microtiter plates (Immulon 2, Fisher Scientific, Itasca, IL, USA) at a concentration of 1 μ g/ml. Serum dilutions in PBS-Tween 20 were added and incubated for 3 h at room temperature. The plates were washed, biotinylated isotype specific anti-mouse antibody was added, and they were again incubated. This was followed by washing and addition of streptavidin-conjugated horseradish peroxidase. After further incubation and washing, the color was developed with orthophenylene diamine (OPD), and the optical density was determined with an ELISA reader. The data are presented as net optical density values after subtraction of the baseline.

Eosinophils in the blood

Blood eosinophils were evaluated in all mice before sensitization and at the time they were killed. Blood from tail vein or heart was collected in 10 units of heparin per ml

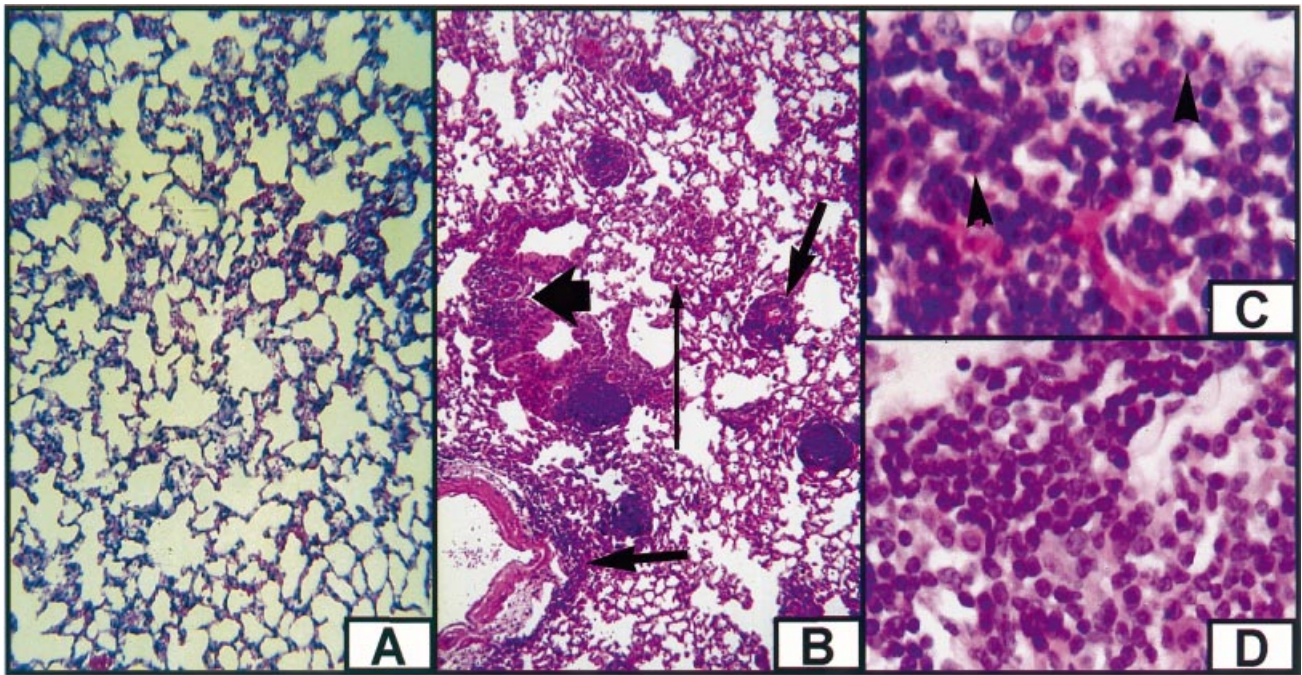


Figure 4. A) Lung of normal control mouse showing normal structure ($\times 63$). B) Lung of WT BALB/c mouse treated with anti-IL-5 (TRFK-5) shows moderate peribronchial chronic inflammation (thick arrow), perivascular chronic inflammation (thin arrows), and intra-alveolar macrophages (long thinner arrow) ($\times 63$). C) Higher magnification of B showing prominent peribronchial or perivascular chronic inflammatory infiltration, consisting primarily of lymphocytes, mixed with small numbers of plasma cells, histiocytes, and eosinophils (arrowheads) ($\times 400$). D) Higher magnification of lung disorder of IL-4 KO BALB/c mice, showing similar degree of prominent peribronchial or perivascular chronic inflammatory infiltration with similar cells as shown in lungs of WT BALB/c mice (B and C), but without eosinophils in infiltration ($\times 400$).

and stained with Discombe's fluid, and eosinophils were counted in a hemocytometer (6, 13).

Histologic studies

Lungs were inflated with 10% neutral buffered formalin to prevent atelectasis, and the specimens were fixed in 10% buffered formalin, processed, and embedded in paraffin. Sections were cut at a 5- μ m thickness and stained with hematoxylin and eosin. The inflammatory response and infiltration of eosinophils and other inflammatory cells in the different groups of animals were graded by microscopic examination as "no", "low", "moderate", or "severe" response. The presence of eosinophils among inflammatory cells was graded as "o" for no eosinophils, "+" for minimal (1–3), "++" for a few (4–10), "+++" for moderate (11–30), and "++++" for numerous (>31) in the high-power field of the lung section. The pathologic reactions were read blinded, and the results were recorded and compared (11, 12).

Statistical analysis

Student's *t*-test (two-tailed, independent samples) was used to compare mean values from different groups of mice with

software (Statworks, Cricket Software, Inc., Philadelphia, PA, USA). The WT and KO mice belonging to the two strains BALB/c and C57BL/6 were compared. A *P* value less than 0.05 was considered statistically significant.

Results

Serum IgE in the various mouse groups

None of the IL-4 KO mice belonging to C57BL/6 or BALB/c showed a detectable amount of total serum IgE before or after antigen exposure. However, all WT mice sensitized with *Af* antigen and treated with control antibody (GL-113) showed enhanced serum IgE levels of over 50-fold from baseline (Fig. 1). Anti-IFN- γ -treated WT mice consistently showed higher levels of total serum IgE than control antibody-treated WT mice, and this increase was more pronounced and statistically significant in C57BL/6 mice ($P=0.03$). Anti-IL-5 antibody treatment failed to show any major differences in the levels of total serum IgE compared to GL-113-treated mice belonging to both strains of WT mice. *Aspergillus*-specific IgE was not detectable or showed only very low levels in the exposed animals.

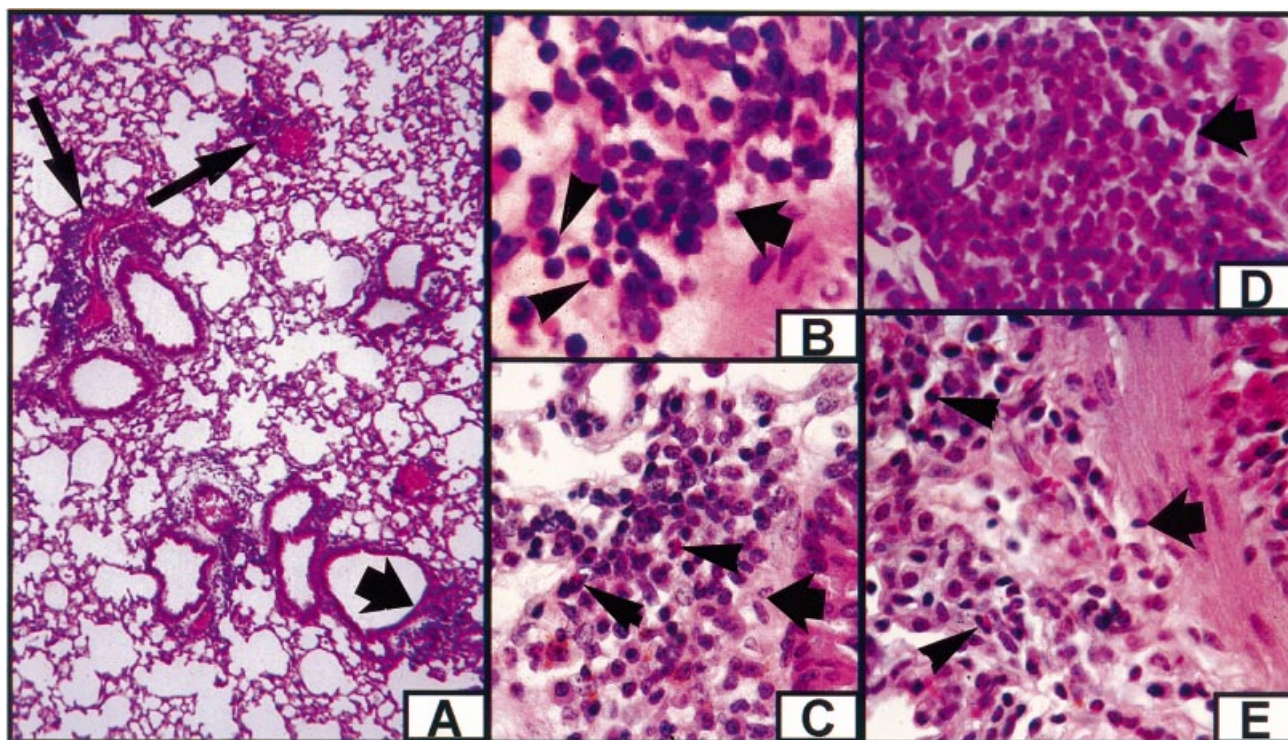


Figure 5. A) Lung of IL-4 KO C57BL/6 mouse treated with anti-IFN- γ (XMG1.2) shows chronic interstitial inflammatory infiltration primarily adjacent to airways (thick arrow) and blood vessels (thin arrows) ($\times 63$). B) Higher magnification of (A) peribronchial chronic inflammation (thick arrow), demonstrating many lymphocytes admixed with plasma cells, histiocytes, and few eosinophils (arrowheads) ($\times 400$). C) Higher magnification of moderate peribronchial chronic inflammation (thick arrow) of lung of IL-4 KO BALB/c mice treated with anti-IFN- γ (XMG1.2), showing similar inflammatory cells with many lymphocytes, intermingled with small numbers of plasma cells, histiocytes, and eosinophils (arrowheads), as shown in IL-4 KO C57BL/6 (B) ($\times 400$). D) Higher magnification of peribronchial inflammatory infiltration of lung of WT BALB/c mouse treated with anti-IFN- γ (XMG1.2) shows large accumulation of eosinophils (arrow) ($\times 400$). E) Higher magnification of severe peribronchial chronic inflammation (thick arrow) of lung of C57BL/6 WT mouse treated with anti-IFN- γ (XMG1.2) shows many lymphocytes, with small numbers of plasma cells and histiocytes including some eosinophils (arrowheads). Bronchial epithelium shown in right upper side is hyperplastic with oncocyctic metaplasia of respiratory epithelium ($\times 400$).

***Aspergillus*-specific IgG subclasses**

Specific serum anti-*Af* IgG1, IgG2a, and IgG2b antibodies of both IL-4 KO and WT C57BL/6 and BALB/c mice exposed to *Af* antigen are shown in Fig. 2. All values presented are those after subtraction of baseline values. IgG3 antibodies to *Af* in these animals showed no significant differences among the different groups. The *Af*-specific IgG1 response was considerably reduced in IL-4 KO animals belonging to both strains compared to WT mice ($P < 0.001$). Anti-IL-5 treatment resulted in a slight reduction of *Af*-specific IgG1 in the sera of WT BALB/c mice, while the specific IgG1 in C57BL/6 mice showed no significant difference (Fig. 2). The *Af*-specific IgG2a antibody showed significant elevation in IL-4 KO animals compared to the WT mice (Fig. 2). No major differences were detected in the BALB/c KO mice treated with anti-IL-5 or IFN- γ . Anti-IFN- γ -treated C57BL/6 IL-4 KO mice showed a decrease in the IgG2a response compared with control antibody-treated mice (Fig. 2).

Significantly enhanced production of *Af*-specific IgG2b antibodies was detected in all IL-4 KO animals compared to the corresponding WT animals. In WT mice, treatment with anti-IL-5 resulted in a marked reduction in IgG2b *Af*-specific antibody levels in both strains of mice, while anti-IFN- γ antibody treatment failed to show any major difference from the controls (Fig. 2).

Eosinophils

There was a marked increase in the eosinophil response in WT BALB/c mice treated with control antibody (GL113) and exposed to *Af* antigen (Fig. 3). A 10-fold increase in numbers of eosinophils compared to pre-exposure levels was noted in the peripheral blood of these animals, while only a sevenfold increase was detected in the C57BL/6 WT mice. Anti-IFN- γ treatment resulted in further enhancement of eosinophils in peripheral blood, while groups of animals treated with anti-IL-5 resulted in complete depletion of eosinophils. A similar

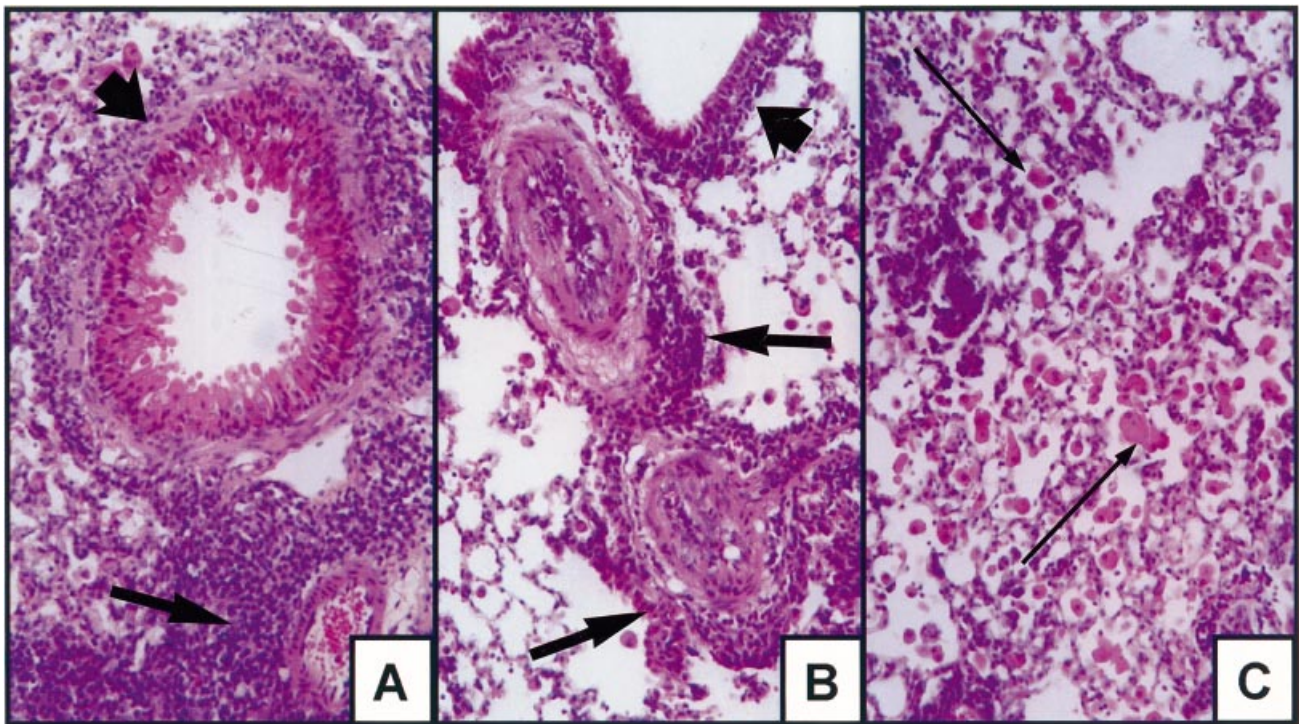


Figure 6. A) Lung of C57BL/6 WT mouse treated with anti-IL-5 (TRFK-5), showing severe peribronchial (thick arrow) and perivascular (thin arrow) chronic inflammation. No eosinophils are seen. Respiratory epithelium of small bronchus is hyperplastic and shows oncocytic metaplasia, with degenerative change of surface epithelium ($\times 160$). B) Lung of same mouse (A) showing severe fibromuscular thickening of vascular wall with perivascular (thin arrows) and intravascular chronic inflammation, causing marked stenosis of lumen. There is no evidence of destructive or necrotizing acute vasculitis. Peribronchiolar chronic inflammation (thick arrow) is noted ($\times 160$). C) Lung of C57BL/6 WT mouse treated with anti-IFN- γ (XMG1.2), showing numerous, large, intra-alveolar macrophages, which are deeply eosinophilic, coarsely granular, and occasionally multinucleated (arrows) ($\times 160$).

pattern was observed with both strains of IL-4 KO mice. Both induced and uninduced eosinophil numbers were lower in IL-4 KO mice than in the corresponding WT strains. But *Af* was still capable of inducing a significant number of IL-5-dependent eosinophils in IL-4 KO mice (Fig. 3).

Histology of the lungs

The histopathologic features of the different groups and strains of mice subjected to *Af* antigen exposure were qualitatively similar, but quantitatively different histologic responses were detected. For comparison, the normal mouse lung is shown in Fig. 4A. The lungs of animals exposed to *Af* antigen (Figs. 4B, 5A–E, 6A–B) showed chronic interstitial infiltration with or without eosinophils, depending on the animals and the various treatments. The infiltration was predominantly in peribronchial (thick arrows) and perivascular (thin arrows) locations. The intervening pulmonary parenchymal areas were only minimally or mildly involved. The inflammatory infiltration expanded to the interstitium and consisted primarily of lymphocytes with small numbers

of plasma cells, epithelioid histiocytes, neutrophils, and varying numbers of eosinophils (arrowheads, Figs. 4C, 5B, C, and E). There were no eosinophils in some of the lesions (Figs. 4B and D) and from few to numerous in others (Figs. 4C and 5A–D). Lung changes were also characterized by intra-alveolar inflammatory infiltration containing macrophages (long thinner arrows, Figs. 4B and 6C), in addition to the other cells mentioned above. Some of these macrophages were large, deeply eosinophilic, coarsely granular, and occasionally multinucleated (Fig. 6C). The bronchioles were surrounded by chronic inflammatory infiltrates and lined by hyperplastic respiratory epithelium containing many goblet cells. The surface of the epithelium showed degenerative changes along with oncocytic metaplasia and intraepithelial inflammatory cells (Figs. 5E and 6A). Chronic inflammatory infiltration, with a few eosinophils adjacent to blood vessels extending to the vascular wall, was also present. Many of these vessels also showed moderate to severe fibromuscular thickening of the walls with marked stenosis or obliteration of the lumens (Fig. 6B). There was no evidence of necrotizing acute vasculitis, nodular granulomas, or giant-cell reactions in any of these areas.

The lungs of WT and KO C57BL/6 mice treated with anti-IL-5 failed to show eosinophils. The peribronchiolar and perivascular chronic inflammatory infiltrates in IL-4 KO mice were mild, while more severe inflammatory infiltrates were seen in WT mice (Fig. 6A). These animals also demonstrated severe thickening of the small vessel walls with mild chronic inflammatory cells and eosinophils (thin arrows, Fig. 6B). In addition, lymphocytes, neutrophils, eosinophils, and increased numbers of alveolar macrophages were dispersed throughout the lung parenchyma.

The lungs of WT BALB/c mice treated with anti-IL-5 antibody showed only minimal eosinophil numbers, while no eosinophils were detected in the lungs of IL-4 KO mice treated with anti-IL-5 antibody (Fig. 4D). However, both groups showed similar moderate amounts of peribronchiolar and perivascular chronic inflammatory infiltration.

C57BL/6 mice treated with anti-IFN- γ antibody revealed a few eosinophils in both WT and KO mice (Fig. 5E). However, in WT mice, peribronchiolar and perivascular inflammatory infiltration was slightly more prominent (Fig. 5E) than in the IL-4 KO mice. Small vessel walls showed marked thickening. Chronic inflammatory infiltrates including eosinophils and increased intra-alveolar macrophages could also be detected. These changes were similar to those seen in the WT mice treated with anti-IL-5 antibody (Fig. 6A–C).

The lungs of WT BALB/c mice treated with anti-IFN- γ antibody showed moderate to severe chronic peribronchiolar and perivascular inflammatory infiltration with numerous eosinophils (Fig. 5D). On the other hand, the IL-4 KO BALB/c mice, on treatment with anti-IFN- γ antibody, showed only a few eosinophils (Fig. 5C). The inflammatory response was also milder with mild to moderate peribronchiolar and perivascular chronic inflammatory infiltration.

Discussion

Exposure of mice to *Af* antigen results in immune and inflammatory responses of the lungs similar to those in human ABPA (1, 2, 6). The two strains of WT mice used in the present study demonstrated comparable pulmonary inflammation, total serum IgE levels, and *Af*-specific IgG antibody responses. The eosinophils were more numerous in the peripheral blood and lungs of *Af*-exposed BALB/c than in C57BL/6 mice. In both strains of WT mice, the response was predominantly of the Th2 type with enhanced total serum IgE, *Af*-specific IgG1, peripheral blood eosinophilia, and the lung inflammatory response (7, 12, 14, 15).

The IL-4 KO mice of both strains failed to demonstrate any detectable serum IgE, while *Af*-specific IgG1 levels were much lower than those detected in the respective WT animals. The eosinophil response was also reduced in these animals. However, the IgG2a antibody against *Af* was higher in all the IL-4 KO mice than in the WT animals. IgG2a is regulated by IFN- γ and is considered to be a Th1 response (12, 17, 18). Thus, the high total serum IgE, *Af*-specific IgG1, and low IgG2a responses indicate that the predominant response in WT animals exposed to *Af* antigen is of the Th2 type while in IL-4 KO animals, the response is predominantly of the Th1 type as a result of the lack of IL-4.

The suppression of IL-5 in both strains of WT animals resulted in the complete ablation of eosinophil levels. However, this response did not significantly affect the pulmonary inflammatory or serum antibody responses. However, anti-IFN- γ treatment in both groups of IL-4 KO animals showed some striking differences, particularly in eosinophil infiltration, and major differences in antibody responses. The eosinophils showed an increase in the lungs and peripheral blood of WT mice treated with anti-IFN- γ antibody. The histologic findings of the animals treated with anti-IFN- γ or anti-IL-5 antibody demonstrated no major differences. Thus, the enhanced eosinophil numbers due to anti-IFN- γ treatment or decreased eosinophils in response to anti-IL-5 treatment did not contribute to the pulmonary inflammation.

Of the several parameters studied, IgE, IgG2a, IgG2b, and eosinophils have no major effect on the overall pulmonary inflammation. IL-4 KO mice demonstrated moderate infiltration of eosinophils and *Af*-specific serum IgG1 levels (12). The intensity of the pulmonary pathologic response demonstrated only minor differences among the different groups of mice and the treatments they received. However, the overall response was predominantly of the Th1 type in both strains of IL-4 KO mice. On the other hand, anti-IFN- γ antibody treatment resulted in a slightly enhanced Th2 response, as evidenced by more severe inflammation and pulmonary infiltrates in the IL-4 KO mice. These results suggest that the Th1 response alone without IgE and eosinophils may result in pulmonary inflammation. However, the inflammation may be increased by an active Th2 response with accumulation of eosinophils and probable release of other inflammatory molecules. The significant role of IgE may be enhanced when mast-cell factors are released in response to stimulation resulting from interaction of IgE with mast cells and allergens. In the IL-4 KO mice, the lack of IL-4 resulted in a Th1 response, but the inflammation in the lungs was comparable to that of WT

animals. This suggests that the *Af*-induced response is probably the result of a combined effect of Th1 and Th2 cytokines. These results also confirm that the multiple pathways for Th2 responses reported in patients may be involved in the allergic response (19–21). It is apparent that the *Af* antigen is capable of eliciting a mixed response, representing both Th1 and Th2. The existence of a mixed effect is supported by our unpublished results, which indicate that certain purified antigens can effect a predominantly Th2 or Th1 response (unpublished results), although such a clear polarization is demonstrable only in

animals with prior immunomodulation, such as IL-4 or IFN- γ depletion, and probably does not exist in human ABPA.

Acknowledgments This investigation was partly supported by VA Medical Research and by the National Institutes of Health (AI 42349). DNAX Research Institute is supported by the Schering-Plough Corporation. The technical assistance of Laura Castillo, Nancy Elms, and Abe Resnick and the editorial assistance of Donna Schrubbe are gratefully acknowledged.

References

1. Kurup VP, Kumar A. Immunodiagnosis of aspergillosis. *Clin Microbiol Rev* 1991;**4**:439–456.
2. Fink JN, Kurup VP. Allergic bronchopulmonary aspergillosis. In: Barnes PJ, Grunstein MM, Leff AR, Woolcock AJ, editors. *Asthma*. Philadelphia: Lippincott-Raven, 1997:2077–2087.
3. Patterson R, Greenberger PA, Roberts ML. *Allergic bronchopulmonary aspergillosis*. Providence, RI: Oceanside Publications, 1995.
4. Kurup VP, Choi HY, Resnick A, Kalbfleisch, Fink JN. Immunopathological response of C57BL/6 and C3H/HeN mice to *Aspergillus fumigatus* antigens. *Int Arch Allergy Appl Immunol* 1990;**91**:145–154.
5. Murali PS, Dai G-Q, Kumar A, Fink JN, Kurup VP. *Aspergillus* antigen-induced eosinophil differentiation in a murine model. *Infect Immun* 1992;**60**:1952–1956.
6. Kurup VP, Mauze S, Choi HY, Seymour BWP, Coffman RL. A murine model of allergic bronchopulmonary aspergillosis with elevated eosinophils and IgE. *J Immunol* 1992;**148**:3783–3788.
7. Kurup VP, Seymour BWP, Choi HY, Coffman RL. Particulate *Aspergillus fumigatus* antigens elicit a Th2 response in BALB/c mice. *J Allergy Clin Immunol* 1994;**93**:1013–1020.
8. Kurup VP, Choi HY, Murali PS, Coffman RL. IgE and eosinophil regulation in a murine model of allergic aspergillosis. *J Leukoc Biol* 1994;**56**:593–598.
9. Mehlhop PD, van De Rijn M, Goldberg AB, et al. Allergen-induced bronchial hyperreactivity and eosinophilic inflammation occur in the absence of IgE in a mouse model of asthma. *Proc Natl Acad Sci U S A* 1997;**94**:1344–1349.
10. Grunig G, Corry DB, Leach MW, Seymour BWP, Kurup VP, Rennick DM. Interleukin-10 is a natural suppressor of cytokine production and inflammation in a murine model of allergic bronchopulmonary aspergillosis. *J Exp Med* 1997;**185**:1089–1099.
11. Kurup VP, Murali PS, Guo J, et al. Anti-interleukin (IL)-4 and IL-5 antibodies downregulate IgE and eosinophilia in mice exposed to *Aspergillus* antigens. *Allergy* 1997;**52**:1215–1221.
12. Kurup VP, Guo J, Murali PS, Choi HY, Fink JN. Immunopathologic responses to *Aspergillus* antigen in interleukin-4 knockout mice. *J Lab Clin Med* 1997;**130**:567–575.
13. Murali PS, Kurup VP, Guo J, Fink JN. Development of bone marrow eosinophilia in mice induced by *Aspergillus fumigatus* antigens. *Clin Immunol Immunopathol* 1997;**84**:216–220.
14. Chu HW, Wang JM, Bontel M, et al. Increased expression of intercellular adhesion molecule-1 (ICAM-1) in a murine model of pulmonary eosinophilia and high IgE level. *Clin Exp Immunol* 1995;**100**:319–324.
15. Chu HW, Wang JM, Bontel M, et al. Immunohistochemical detection of GM-CSF, IL-4 and IL-5 in a murine model of allergic bronchopulmonary aspergillosis. *Clin Exp Allergy* 1996;**26**:461–468.
16. Kuhn R, Rajewsky K, Muller W. Generation and analysis of interleukin-4 deficient mice. *Science* 1991;**254**:707–710.
17. Finkelman FD. Lymphokine control of *in vivo* immunoglobulin isotype selection. *Ann Rev Immunol* 1990;**8**:303–333.
18. Coffman RL, Carty T. A T-cell activity that enhances polyclonal IgE production and its inhibition by interferon- γ . *J Immunol* 1986;**136**:949–954.
19. Coffman RL, von der Weid T. Multiple pathways for the initiation of T-helper 2 (Th2) response. *J Exp Med* 1997;**185**:373–375.
20. Chauhan B, Knutsen AP, Hutcheson PS, Slavin RG, Bellone CJ. T-cell subsets, epitope mapping and HLA restriction in patients with allergic bronchopulmonary aspergillosis. *J Clin Invest* 1996;**97**:2324–2331.
21. Knutsen AP, Mueller KR, Levine AD, Chouhan B, Hutcheson PS, Slavin RG. Asp f I CD4+ TH2-like T-cell lines in allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol* 1994;**94**:215–221.