

Eosinophil recruitment into the respiratory epithelium following antigenic challenge in hyper-IgE mice is accompanied by interleukin 5-dependent bronchial hyperresponsiveness

(asthma/airway eosinophilia/mouse model)

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ABSTRACT A murine model for antigen-induced bronchial hyperreactivity (BHR) and airway eosinophilia, two hallmarks of asthma, was developed using ovalbumin-immunized mice, which produce large amounts of IgE (named BP2, “Bons Producteurs 2,” for High Line of Selection 2). A single intranasal ovalbumin challenge failed to modify the bronchial responses, despite the intense eosinophil recruitment into the bronchoalveolar lavage fluid and airways. When mice were challenged twice a day for 2 days or once a day for 10 days, BHR in response to i.v. 5-hydroxytryptamine or to inhaled methacholine was induced in BP2 mice but not in BALB/c mice. Histological examination showed that eosinophils reached the respiratory epithelium after multiple ovalbumin challenges in BP2 mice but remained in the bronchial submucosa in BALB/c mice. Total IgE titers in serum were augmented significantly with immunization in both strains, but much more so in BP2 mice. Interleukin 5 (IL-5) titers in serum and bronchoalveolar lavage fluid of BP2 mice were augmented by the antigenic provocation, and a specific anti-IL-5 neutralizing antibody suppressed altogether airway eosinophilia and BHR, indicating a participation of IL-5 in its development. Our results indicate that the recruitment of eosinophils to the airways alone does not induce BHR in mice and that the selective effect on BP2 mice is related to their increased IgE titers associated with antigen-driven eosinophil migration to the epithelium, following formation and secretion of IL-5.

Bronchial hyperreactivity (BHR), a major characteristic of asthma, is associated with eosinophilic airway inflammation (1–3). By releasing their secretory products, eosinophils are claimed to damage bronchial epithelium and thus to induce BHR (3). This correlation has been demonstrated for guinea pigs (4), but until recently few studies have focused on the development of an eosinophil-dependent BHR model in mice. This may be very important, since such a model would allow exploration of the immunological mechanisms involved in BHR, particularly by taking advantage of the availability of mice strains and of specific tools. We demonstrated that Swiss, CBA, and hyper-eosinophilic interleukin 5 (IL-5) transgenic mice do not show BHR upon antigenic challenge, despite the marked eosinophil infiltration in airways (5), suggesting the need for factors other than eosinophil infiltration *per se*. The purpose of our study was to develop a model of BHR using a mice selection that produces high antibody titers in response to complex antigens [referred to as BP2, “Bons Producteurs 2”; see Biozzi *et al.* (6)] and that indeed showed very high IgE titers. Following repeated antigenic challenges, these animals became hyper-eosinophilic and hyperresponsive to nonspecific

bronchoconstrictor substances. Our results suggest that BHR depends on an enhanced IL-5-driven production of eosinophils that locate in the respiratory mucosa, provided the microenvironment contains enough IgE antibodies.

MATERIALS AND METHODS

Immunization and Provocation Procedures. Male BP2 (kindly provided by Denise Mouton, Institut Curie–Centre National de la Recherche Scientifique, Paris and by “Centre d’Elevage R. Janvier”, BP5, 53940 Le Genest Saint-Isle, France) and BALB/c mice (Iffa-Credo and “Centre d’Elevage R. Janvier”), 8–10 weeks of age, were immunized s.c. twice at a 7-day interval with 0.4 ml of a solution of 250 μ g of ovalbumin (OA) per ml (Immunobiologicals, Lisle, IL) mixed with 4 mg of Al(OH)₃ per ml (Merck). One week after the second injection, mice were challenged intranasally under light ether anesthesia with 10 μ g of OA in 50 μ l of 0.9% NaCl (saline) as indicated in *Results*. Control mice were challenged with the vehicle.

Evaluation of Bronchoconstriction. In a first procedure, mice were prepared as described (5), using the computerized pulmonary analyzer (Mumed PR800 system, UK) adapted to mice. To evaluate the effect of antigenic challenge on airway responsiveness, 5-hydroxytryptamine (5-HT; Sigma) was injected into the cannulated jugular vein at 10, 20, 40, 80, 160, and 320 μ g/kg in a volume of 100 μ l during 10 sec at 5-min intervals. The results were expressed as PD30, PD60, and PD90 (the amount of 5-HT needed to augment the bronchial resistance by 30%, 60%, and 90%). In a second procedure, unrestrained conscious mice were placed in a whole body plethysmographic chamber (Buxco Electronics, Sharon, CT) to analyze the respiratory waveforms. After a few minutes for stabilization, an aerosol of methacholine (3×10^{-2} M in the aerosolator; Aldrich) was delivered during 20 and 60 sec, at a 10-min interval. The airway resistance was expressed as $Penh = [Te \text{ (expiratory time)}/40\% \text{ of } Tr \text{ (relaxation time)} - 1] \times Pef \text{ (peak expiratory flow)}/Pif \text{ (peak inspiratory flow)} \times 0.67$, according to the recommendations of the manufacturer. To calculate the $\Delta Penh$ (difference between the basal and maximal value), the average of five maximal values was used.

Quantification of Serum IgE Levels. The total IgE titers were measured by ELISA according to Ledermann *et al.* (7).

Histological Evaluation. Lungs were prepared and processed for conventional histological studies. For optic microscopy, the microsections were stained with hematoxylin/eosin and for cyanide-resistant peroxidase (8). For electron micros-

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Abbreviations: BALF, bronchoalveolar lavage fluid; BP2, “Bons Producteurs 2”; BHR, bronchial hyperreactivity; OA, ovalbumin; 5-HT, 5-hydroxytryptamine; PD30, PD60, and PD90, doses of 5-HT required to increase airway resistance by 30%, 60%, and 90%; IL, interleukin.
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copy, ultrathin sections were prepared with an LKB microtome, stained with uranyl acetate/lead citrate, and observed with a JEM-100CXII electron microscope.

For immunohistochemistry, 6- μ m sections were prepared from OTC-inflated lungs and stained for murine CD4⁺ and CD8⁺ molecules using rat anti-murine CD4 antibody (L3/T4; Tebu, France) and rat anti-murine CD8 antibody (Ly-2; Tebu, France) and revealed with the alkaline phosphatase anti-alkaline phosphatase technique (9).

Statistical Analysis. The significance of differences was determined with the unpaired Student's *t* test.

RESULTS

Effect of Immunization and Antigen Challenge on the Cell Composition of the BALF. Total cell numbers (macrophages essentially) in the BALF of saline-challenged mice amounted to $0.85 \pm 0.06 \times 10^5$ cells per ml for BP2 and $1.23 \pm 0.1 \times 10^5$ cells per ml for BALB/c, eosinophils being absent. After a single antigen challenge, these numbers increased time-dependently, peaking at 96 hr (BP2, 3.95 ± 0.46 and $1.86 \pm 0.24 \times 10^5$ cells per ml; BALB/c, 2.70 ± 0.33 and $1.03 \pm 0.24 \times 10^5$ cells per ml, for total number and eosinophil number, respectively). These single-challenged mice failed to display BHR (see below) and, accordingly, more intense and/or prolonged stimuli were tested. Mice were challenged either twice a day for 2 days (group indicated as "2 \times 2 days") or once a day for 10 days (group indicated as "1 \times 10 days"). When the BALF was evaluated 24 hr after the last antigen challenge, the cell counts in BALF were increased above those following a single challenge. In particular, the 1 \times 10 days group contained approximately 4.92 ± 0.7 (total cells) and 3.05 ± 0.54 (eosinophils) $\times 10^5$ cells per ml for BP2 and 6.02 ± 0.7 (total cells) and 2.99 ± 0.21 (eosinophils) $\times 10^5$ cells per ml for BALB/c, respectively. Lymphocytes were absent in controls but were present in both groups of multichallenged mice, neutrophil counts being unaffected.

Effect of Antigen Challenge on Bronchial Responsiveness to i.v. 5-HT in Anesthetized Mice. Single-challenged BP2 mice showed no significant augmentation of bronchial responses to 5-HT (Fig. 1A for 24 hr; other time points not shown). However, BHR was induced following repeated challenge with antigen (Fig. 1A). By contrast, BHR was not uncovered in BALB/c mice (Fig. 1B), even though the eosinophil numbers were comparable to those of BP2. BHR was also absent in response to methacholine in BALB/c mice, no matter what the provocation protocol (data not shown). No statistical correlation (by linear regression analysis) was found between eosinophil numbers in BALF and the intensity of bronchoconstriction in all OA-multichallenged BP2 mice, whether from the groups 2 \times 2 days and 1 \times 10 days.

Effect of Immunization on the IgE Serum Titers of BALB/c and BP2 Mice. Nonimmunized BP2 mice contained $3.35 \pm 2.57 \mu$ g of total IgE per ml and BALB/c contained $0.18 \pm 0.05 \mu$ g/ml. Immunization induced a significant augmentation of those titers in both strains, the total amount reaching $24.96 \pm 5.84 \mu$ g/ml and $0.68 \pm 0.11 \mu$ g/ml, in BP2 ($P < 0.05$) and BALB/c ($P < 0.05$) mice, respectively.

Effect of Antigen Challenge on Bronchial Reactivity to Inhaled Methacholine in Nonanesthetized Mice. Our inability to induce BHR in BALB/c mice might be attributed to failure of 5-HT to reach the appropriate sites in the airways, since it was injected i.v. A group of immunized and nonanesthetized mice challenged using the protocol 2 \times 2 days was thus evaluated. OA-challenged BP2 mice showed a significant augmentation of airway resistance in response to methacholine as compared to control animals, whereas, once again, no BHR was observed in BALB/c mice (Fig. 2).

Effect of Immunization and Antigen Challenge on the Lung Histology. No histological abnormalities were observed in

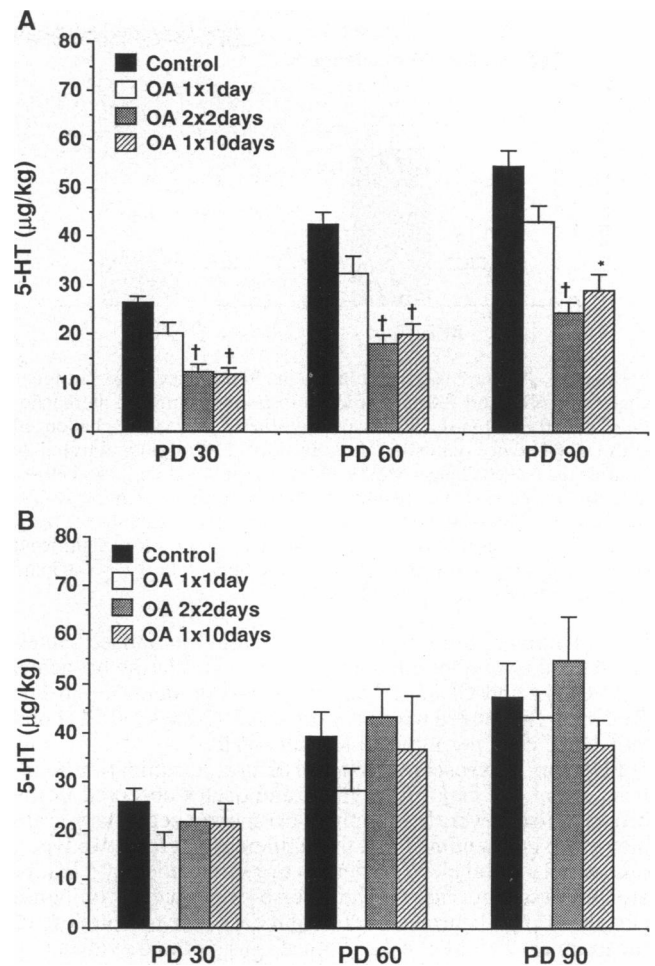


FIG. 1. Airway responsiveness in immunized and antigen-challenged BP2 (A) and BALB/c (B) mice following different protocols for antigen challenge. In the protocol 1 \times 1 day, 10 μ g of OA in 50 μ l of 0.9% NaCl was instilled at \approx 9 a.m. and BHR was evaluated after 24 hr (see text). In the protocol 2 \times 2 days, OA as above was instilled at 9 a.m. and at 5 p.m.—i.e., at an 8-hr time interval, for 2 days. On the 3rd day, BHR was evaluated. When the protocol 1 \times 10 days was used, OA as above was instilled at 9 a.m. for 10 days. On the 11th day, BHR was evaluated. Controls were saline-challenged mice of each group, which were pooled. Only the BP2 mice challenged 4 (■) or 10 (▨) times showed a significant augmentation (\dagger , $P < 0.001$, or $*$, $P < 0.005$, respectively) of the intensity of bronchoconstriction, compared to control mice. Each value represents the mean \pm SEM of 5–14 mice.

lungs from control mice of both strains (Fig. 3a). In antigen-challenged mice, granulocytes (mainly eosinophils and a few neutrophils), lymphocytes, plasma cells, and some macrophages accumulated in the peribronchial and perivascular tissues. A moderate submucosal eosinophil infiltration was observed in BP2 and BALB/c mice (30 ± 6 and 29 ± 4 cells per mm, respectively) challenged once and killed after 24 hr, with practically no eosinophils present in the epithelium. More eosinophils were found in the submucosa of 2 \times 2 days mice (120 ± 7 cells per mm for BP2 and 46 ± 10 cells per mm for BALB/c). Under those conditions, the epithelium of BALB/c mice was still eosinophil-free, but eosinophils clearly infiltrated that of BP2 mice (23 ± 7 cells per mm) (Fig. 3b). This epithelial and submucosal eosinophil infiltration in BP2 mice was better appreciated in sections stained for eosinophil peroxidase. Finally, plasma cells were more abundant in the submucosa of BP2 mice (Fig. 3d) as compared to BALB/c mice.

Both mice strains showed a multifocal perivascular and peribronchial eosinophilic alveolitis with eosinophils, macrophages, and few giant cells, even after a single antigenic chal-

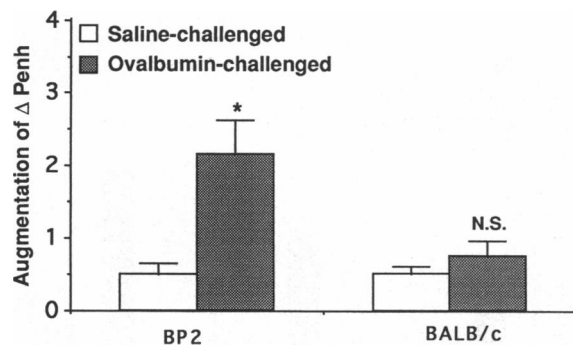


FIG. 2. Airway responsiveness in immunized and antigen-challenged BP2 and BALB/c mice in response to inhaled methacholine (3×10^{-2} M) in the absence of anesthesia. Mice were challenged with OA (■) twice daily for 2 days (protocol 2×2 days) and tested 24 hr after the last challenge. Methacholine was inhaled for 20 and 60 sec at a 10-min interval. The results for 20 sec are shown; similar results were obtained for 60 sec. The results are expressed in Δ Penh (see text). Each value represents the mean \pm SEM of 6–10 mice. Significant differences compared with control (□) are indicated: *, $P < 0.05$ and nonsignificance is expressed as N.S.

lunge. Under conditions where lungs from immunized saline-challenged mice contained no T lymphocytes in the bronchial wall, CD4⁺ and CD8⁺ T lymphocytes were identified in two BP2 mice challenged with the protocol 2×2 days (30 CD4 and 19 CD8 T cells per mm of bronchial wall).

Electron Microscopy. Ultrastructural examination confirmed that the majority of the granulocytes observed in the bronchial walls were eosinophils. Eosinophil secondary granules in the epithelium and in the submucosa were of two types, one with a central electron-dense core surrounded by a lighter area and a second showing the reverse—i.e., an electron-dense periphery and a central electron-light core. The morphology of the granules was identical in both strains, with no evidence of degranulation or for differences in number of granules. Thus, an average of 22.7 ± 1.7 and 19.44 ± 3 granules per eosinophil section of BP2 mice was scored in the submucosa ($n = 18$ cells) and in the epithelium ($n = 9$ cells), respectively. Similar values were obtained for eosinophils of BALB/c mice counted in the submucosa.

Interference of the Anti-IL-5 Antibody TRFK-5 with Eosinophilia and BHR. Approximately 150 pg of IL-5 per ml was found in the serum and 300 pg/ml in the BALF of BP2 mice 1–6 hr after the fourth antigenic provocation (protocol 2×2 days). This led us to evaluate the anti-IL-5 antibody TRFK-5. In these experiments, OA challenges augmented the total cell numbers in the BALF of IgG isotype-treated mice to $6.3 \pm 0.8 \times 10^5$ cells per ml ($P < 0.001$), a number comparable to that of saline-treated animals (Fig. 4A). This increment was accounted for by a marked increase in the proportion of eosinophils ($57.5\% \pm 2.5\%$ of total cell numbers). TRFK-5, injected i.v. at 0.5 mg per mouse 1 hr before each OA challenge, reduced significantly the total BALF cell counts, which were accounted for essentially by decreased eosinophil numbers (isotype, $3.54 \pm 0.38 \times 10^5$ cells per ml; TRFK-5, $0.58 \pm 0.25 \times 10^5$ cells per ml; $P < 0.05$) (Fig. 4A). Histological observations confirmed that the administration of TRFK-5 inhibited the epithelial (from 6.8 to 0.7 cells per mm) and submucosal (from 55.7 to 14.2 cells per mm) eosinophil infiltration in two mice.

As expected, OA challenges in saline or IgG isotype-treated mice resulted in BHR (Fig. 4B). TRFK-5 inhibited the antigen-induced BHR for 5-HT in anesthetized (Fig. 4B) and for methacholine in nonanesthetized antigen-challenged mice (Fig. 4C).

DISCUSSION

Eosinophils are recruited to the airways of asthmatic patients and are considered important effector cells in BHR, a hall-

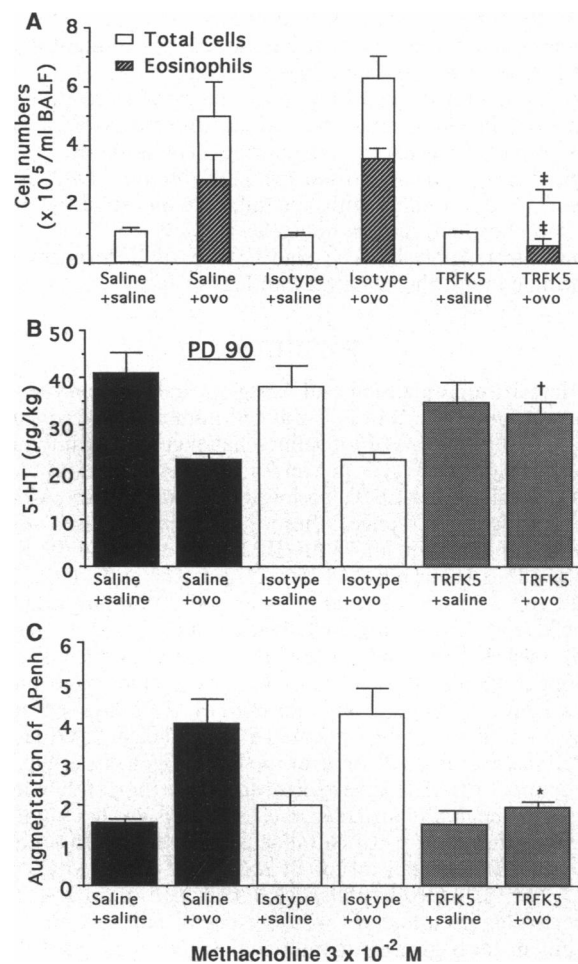


FIG. 4. Effect of the anti-IL-5 antibody TRFK-5 on eosinophil recruitment into the BALF (A) and on BHR (B and C) in immunized BP2 mice. The protocol 2×2 days was used. Purified IL-5 antibody from TRFK-5 cells was injected i.v. at 0.5 mg per mouse to immunized BP2 mice, 1 hr before each of the four OA (ovo) challenges. The same volume of control antibody (rat IgG) was injected into control mice. Bronchoalveolar lavage and BHR were evaluated 24 hr after the last antigen challenge. BHR with 5-HT was expressed as PD90 (see text) in anesthetized mice and BHR with methacholine was expressed as Δ Penh (see text) in nonanesthetized mice. Each value represents the mean \pm SEM of 4–12 mice. Significant differences compared with control isotype (challenged with OA) are indicated: *, $P < 0.05$; †, $P < 0.005$; ‡, $P < 0.001$.

mark of asthma (10). Although BHR is reported to correlate with the number of eosinophils in lungs staining positively with a monoclonal antibody that recognizes secreted eosinophil cationic protein (10–13), there is no formal evidence for a causal relationship between eosinophil recruitment *per se* and BHR in humans (14, 15), sheep (16), and guinea pigs (4). Few studies have addressed the correlation between eosinophil recruitment to the airways and BHR in mice, possibly because the evaluation of their pulmonary functions was considered difficult or unreliable. Nevertheless, murine models should be invaluable for dissecting the mechanisms of BHR, since murine basic immunology is well defined. This led us to search for and to identify mice that displayed intense anaphylactic responses, in addition to antigen-induced hyper-eosinophilia. We took advantage of the availability of high-responder mice (detailed in ref. 6) selected by bidirectional breeding for antibody responsiveness to sheep erythrocytes in terms of agglutinin titers, which have been shown to be homozygous for the character considered in F₁₄–F₁₇ generations (6, 17). These mice developed BHR, which was detected by two methods. In

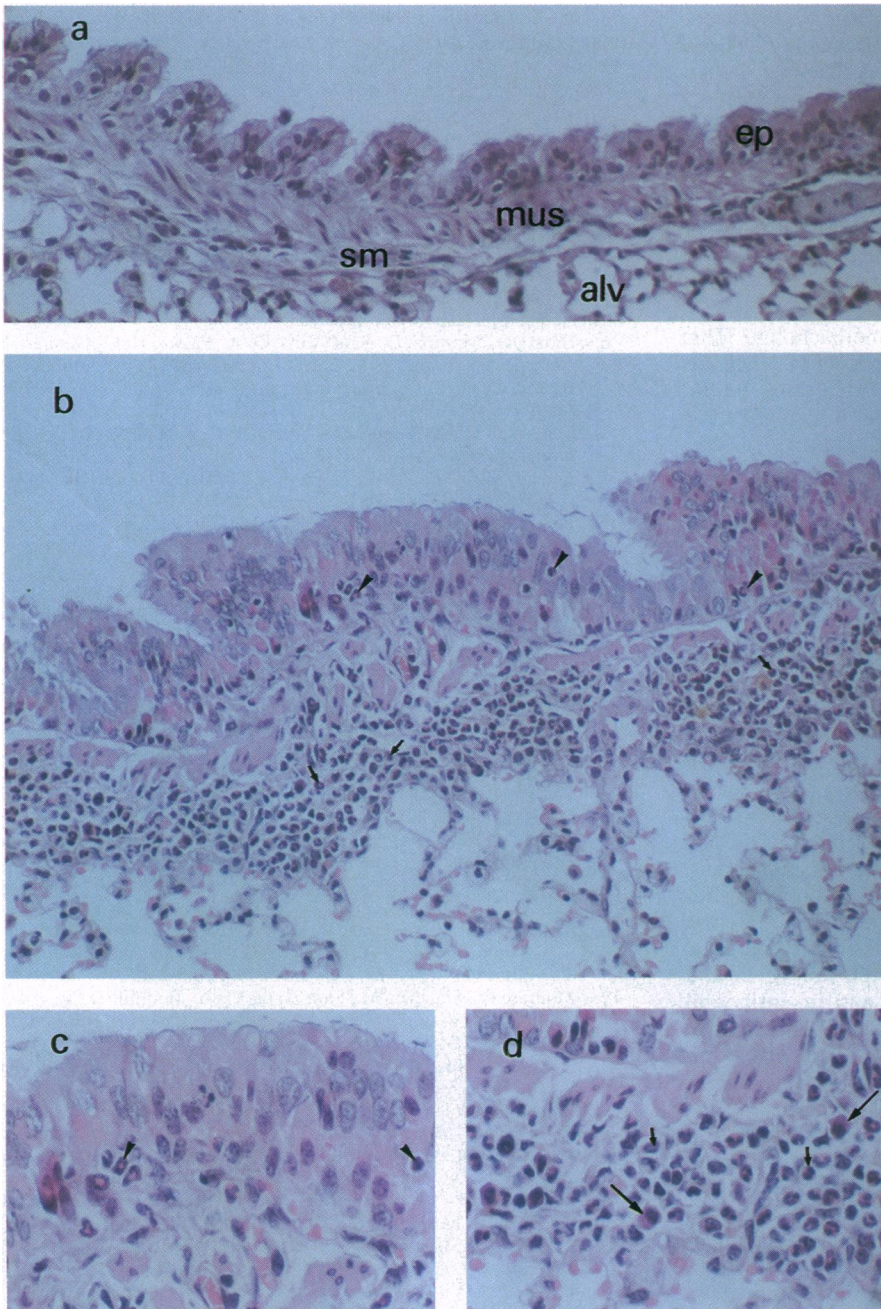


FIG. 3. (a) Lobar bronchus from BP2 mice immunized with OA and challenged with saline. Control BALB/c mice showed the same morphology as BP2 mice. Note the absence of inflammatory cell infiltration. ep, Epithelium; mus, bronchial muscle; sm, submucosa; alv, alveolus. ($\times 70$.) (b) Lobar bronchus from BP2 mice challenged with OA according to protocol 2 \times 2 days. A marked eosinophil infiltration of the submucosa (three short arrows) and of the mucosa (three arrowheads) is noted. ($\times 70$.) (c) Higher magnification of the section in b showing the mucosa containing numerous infiltrating eosinophils (two arrowheads). ($\times 280$.) (d) Higher magnification of the mucosa and submucosa showing the dominant infiltrating eosinophils (short arrows) but also a number of plasma cells (long arrows). ($\times 280$.) All sections were stained with hematoxylin/eosin.

a first one, 5-HT was administered i.v. to anesthetized mice (5, 18), and in the second, methacholine was administered by aerosol to awake animals (19). This is important since bronchoconstrictor responses may differ according to the route of administration of the agonists, which determines the first line cell species that is encountered. Furthermore, since eosinophils activated *in situ* might induce BHR by affecting the respiratory epithelium (3), it seemed logical to deliver the bronchoconstrictor agent directly into the airways. Both methods provided similar results.

BP2 mice differed from BALB/c mice in at least four different ways. (i) A first and essential difference is that only BP2 mice displayed BHR following multiple antigenic challenges. (ii) They showed very high levels of serum IgE, which doubled after immunization, whereas BALB/c mice had considerably lower amounts, which, as expected, also augmented upon immunization. (iii) BP2 mice underwent an intense anaphylactic bronchoconstriction when challenged with i.v. OA, whereas BALB/c were unresponsive (not shown). (iv)

Eosinophils were identified in the epithelium of BP2 mice after antigen challenges, being absent in BALB/c mice. In previous studies, we also failed to identify eosinophils in the epithelium of antigen-challenged mice from other strains, including IL-5 transgenic animals, even though the BALF and the submucosa were heavily enriched with eosinophils (5). The basic difference between BP2 and other mice in terms of eosinophils thus appears to relate to their topographic distribution and not to absolute counts. It was tempting to speculate that eosinophils stimulated at the vicinity of the targeted respiratory epithelium, in a hyper-IgE environment, are likely to become activated, particularly after repeated antigenic challenges. Nevertheless, the secondary granules of the eosinophils found in the epithelium and in the submucosa did not show morphological evidence of degranulation, according to published criteria (20, 21). It is thus unlikely that eosinophils participate in the induction of BHR in BP2 mice, via the secretion of cationic proteins such as "Major Basic Protein" as in guinea pigs (4), and it also remains to be shown whether an augmented

production of lipid mediators may occur. Eosinophils from immunized and aerosol-challenged guinea pigs are indeed primed to produce larger amounts of leukotriene C₄ than control animals (22). On the other hand, the respiratory epithelium might express adhesion proteins in the hyper-IgE environment in BP2 mice, leading to the recruitment and activation of eosinophils. Indeed, we demonstrated that an antibody against very late activation antigen 4 suppresses the antigen-induced airway eosinophil recruitment and *in situ* activation and the associated BHR in guinea pigs (23).

Our results contrast to those of Renz *et al.* (18), who showed that BALB/c mice immunized by inhaled OA for a long time interval display BHR in response to *i.v.* methacholine. This may result from different protocols, particularly since no eosinophils or other inflammatory cells were found in the airways of these mice at the time of BHR but might have occurred before. BHR persists in BP2 mice for at least 16 days, a time when eosinophils are absent (not shown).

In immunized BP2 mice, the IgE titers were very elevated and the number of plasma cells identified in the bronchial submucosa was increased, two possibly related events. Increased IgE titers may be involved in the induction of BHR, particularly since an anti-IgE antibody inhibits altogether airway eosinophilia and BHR in our model (data not shown). IgE production is dependent on IL-4 (24), a Th2 lymphocyte-derived cytokine. IL-4 is also involved in eosinophil recruitment to the airways upon antigen challenge (25), but it is clear that IL-5 is responsible for eosinophil recruitment to the airways in the mice strains studied and for BHR, when applicable. Indeed, IL-5 was present in the BALF and serum of the BP2 mice after antigen challenge and the anti-IL-5 antibody TRFK-5 suppressed altogether eosinophilia in airways and tissues and BHR. In allergen-challenged guinea pigs, anti-IL-5 antibody also suppressed airway eosinophilia (26) and BHR (27, 28). CD4⁺ T lymphocytes that migrate into the lungs after antigen challenge (29) are responsible for the local production of IL-4 and IL-5, which are important in B- and T-lymphocyte development and function and in eosinophil activation and differentiation (30). In CD4⁺ deficient or immune-depleted mice, BALF eosinophilia and airway inflammation in response to antigen provocation were absent (25, 31) and BHR was reduced (25). In confirmation, we identified CD4⁺ T cells in the bronchial wall after antigen challenge in BP2 mice, suggesting that these cells participate in the induction of airway eosinophilia, and accordingly of BHR, by releasing cytokines.

As a conclusion, repeated intranasal antigenic challenges induce eosinophil recruitment to the lungs and to the BALF in BALB/c and in BP2 mice, but BHR develops selectively in the latter. Since eosinophil recruitment to the lungs and BHR were inhibited by *in vivo* IL-5 neutralization, eosinophils are clearly necessary, but not sufficient, for BHR. Our finding that eosinophils locate in the respiratory epithelium only in hyper-IgE BP2 mice, which become hyperresponsive, suggests that BHR involves the interaction between eosinophils recruited and/or primed by IL-5 and a component of the epithelium in the presence of an altered microenvironment, including high IgE titers. Interference with one of the sequential events involved—*i.e.*, excessive eosinophil production or recruitment, the selective expression of adhesion proteins on the endothelial or epithelial surfaces, the production or the effects of IgE, or finally the production or effects of eosinophil-borne mediators—should be sufficient to suppress BHR.

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- Boushey, H. A., Holtzman, M. J., Sheller, J. R. & Nadel, J. A. (1980) *Am. Rev. Respir. Dis.* **121**, 389–413.
- Corrigan, C. J. & Kay, A. B. (1992) *Immunol. Today* **13**, 501–507.
- Gleich, G. J. (1990) *J. Allergy Clin. Immunol.* **85**, 422–436.
- Pretolani, M., Ruffié, C., Joseph, D., Campos, M. G., Church, M. K., Lefort, J. & Vargaftig, B. B. (1994) *Am. J. Respir. Crit. Care Med.* **149**, 1167–1174.
- Lefort, J., Bachelet, C. M., Leduc, D. & Vargaftig, B. B. (1995) *J. Allergy Clin. Immunol.*, in press.
- Biozzi, G., Mouton, D., Sant'Anna, O. A., Passos, H. C., Gennari, M., Reis, M. H., Ferreira, V. C. A., Heumann, A. M., Bouthillier, Y., Ibanez, O. M., Stiffel, C. & Siqueira, M. (1979) *Curr. Top. Microbiol. Immunol.* **85**, 31–98.
- Ledermann, F., Schlienger, C., Wagner, K. & Heusser, Ch. (1991) *J. Immunol. Methods* **141**, 263–275.
- Ten, R. M., Pease, L. R., McKean, D. J., Bell, M. P. & Gleich, G. J. (1989) *J. Exp. Med.* **169**, 1757–1769.
- Polak, J. M. & Noorden, S. V. (1986) in *Immunocytochemistry: Modern Methods and Applications* (Wright, Bristol, U.K.), 2nd Ed., pp. 47–48.
- Djukanovic, R., Roche, W. K., Wilson, J. W., Beasley, C. R. W., Twentyman, O. P., Howarth, P. H. & Holgate, S. T. (1990) *Am. Rev. Respir. Dis.* **142**, 434–457.
- Howarth, P. H., Bradding, P., Montefort, S., Peroni, D., Djukanovic, R., Carroll, M. P. & Holgate, S. T. (1994) *Am. J. Respir. Crit. Care Med.* **150**, S18–S22.
- Azzawi, M., Bradely, B., Jeffery, P. K., Frew, A. J., Wardlaw, A. J., Knowles, G., Assoufi, B., Collins, J. V., Durham, S. & Kay, A. B. (1990) *Am. Rev. Respir. Dis.* **142**, 1407–1413.
- Tai, P. C., Spry, C. J. F., Peterson, C., Venge, P. & Olsson, I. (1984) *Nature (London)* **309**, 182–184.
- Gleich, G. J., Frigas, E., Loegering, D. A., Wassom, D. L. & Steinmuller, D. (1979) *J. Immunol.* **123**, 2925–2927.
- Frigas, E., Loegering, D. A. & Gleich, B. S. (1980) *Lab. Invest.* **42**, 35–43.
- Abraham, W. M., Sielczak, M. W., Ahmed, A., Cortes, A., Lauredo, I. T., Kim, J., Pepinsky, B., Benjamin, C. D., Leone, D. R., Lobb, R. R. & Weller, P. F. (1994) *J. Clin. Invest.* **93**, 776–787.
- Mouton, D., Siqueira, M., Sant'Anna, O. A., Bouthillier, Y., Ibanez, O., Ferreira, V. C. A., Mevel, J. C., Reis, M. H., Piatti, R. M., Stiffel, C. & Biozzi, G. (1988) *Eur. J. Immunol.* **18**, 41–49.
- Renz, H., Smith, H. R., Henson, J. E., Ray, S., Irvin, C. G. & Gelfand, E. W. (1992) *J. Allergy Clin. Immunol.* **89**, 1127–1138.
- Chand, N., Nolan, K., Pillar, J., Lomask, M., Diamantis, W. & Sofia, R. D. (1992) *Allergy* **48**, 230–235.
- Beasley, R., Roche, W. R., Roberts, J. A. & Holgate, S. T. (1989) *Am. Rev. Respir. Dis.* **139**, 806–817.
- Dvorak, A. M. (1994) in *Eosinophils in Allergy and Inflammation*, eds. Gleich, G. J. & Kay, A. B. (Marcel, New York), pp. 159–209.
- Pretolani, M., Joseph, D., Lobb, R. R. & Vargaftig, B. B. (1994) *Am. J. Respir. Crit. Care Med.* **151**, A221.
- Pretolani, M., Ruffié, M., Lapa e Silva, J. R., Joseph, D., Lobb, R. R. & Vargaftig, B. B. (1994) *J. Exp. Med.* **180**, 795–805.
- Kopf, M., Le Gros, G., Bachmann, M., Lamers, M. C., Bluethmann, H. & Köhler, G. (1993) *Nature (London)* **362**, 245–248.
- Brusselle, G. G., Kips, J. C., Tavernier, J. H., Van Der Heyden, J. G., Cuvelier, C. A., Pauwels, R. A. & Bluethmann, H. (1994) *Clin. Exp. Allergy* **24**, 73–80.
- Chand, N., Nolan, K., Pillar, J., Lomask, M., Diamantis, W. & Sofia, R. D. (1992) *Allergy* **48**, 230–235.
- Nakajima, H., Iwamoto, I., Tomoe, S., Matsumura, R., Tomioka, H., Takatsu, K. & Yoshida, S. (1992) *Am. Rev. Respir. Dis.* **146**, 374–377.
- Van Oosterhout, A. J. M., Ladenius, A. R. C., Savelkoul, H. F. J., Ark, I. V., Delsman, K. C. & Nijkamp, F. P. (1993) *Am. Rev. Respir. Dis.* **147**, 548–552.
- Gerblich, A. A., Salik, H. & Schuyler, M. R. (1991) *Am. Rev. Respir. Dis.* **143**, 533–537.
- Garlich, C. G., Falcone, A., Kung, T. T., Stelts, D., Peline, K. J., Beavis, A. J., Smith, S. R., Egan, R. W. & Umland, S. P. (1995) *Clin. Immunol. Immunopathol.* **75**, 75–83.
- Gavett, S. H., Chen, X., Finkelman, F. & Wills-Karp, M. (1994) *Am. J. Respir. Crit. Care Med.* **10**, 587–593.