

## Suppression of immediate and late responses to antigen by a non-anaphylactogenic anti-IgE antibody in a murine model of asthma

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*Suppression of immediate and late responses to antigen by a non-anaphylactogenic anti-IgE antibody in a murine model of asthma. S. Haile, J. Lefort, S.Y. Eum, C. Dumarey, M. Huerre, C. Heusser, B.B. Vargaftig. ©ERS Journals Ltd 1999.*

**ABSTRACT:** Eosinophils are recruited to the airways during allergic reactions, but animal models have shown that their mere presence is not sufficient for the development of bronchopulmonary hyperreactivity. Other factors, such as immunoglobulin (Ig)E, seem to be required.

Using mice selected for the production of large amounts of IgE, the effects of antibody neutralization of IgE on antigen-induced lung recruitment of eosinophils and induction of bronchopulmonary hyperreactivity and of other indicators of inflammation were studied.

A monoclonal non-anaphylactogenic rat anti-mouse IgE (mAb1-5), given within 24 h of the challenge with antigen, reduced tissue eosinophilia, the recruitment of IgE-bearing cells identified as basophils, mucous cell metaplasia, anaphylactic bronchoconstriction and bronchopulmonary hyperreactivity. mAb1-5 inhibited interleukin (IL)-4 titres in the bronchoalveolar lavage fluid, but not those of IL-5.

Inhibition by mAb1-5 may result from competitive displacement of immunoglobulin E from its different receptors, thus preventing cell stimulation. Moreover, the inhibition of the massive recruitment of immunoglobulin E-bearing basophils into the lungs within hours after challenge and of interleukin-4 production by mAb1-5 may be important factors leading to the reduction of pulmonary eosinophilia and bronchopulmonary hyperreactivity. Thus, immunoglobulin (Ig)E and allergic IgE-bearing cells seem to play an essential role in the initial development of the late allergic airway responses.

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Immunoglobulin (Ig)E-mediated release of pharmacologically active substances from cells possessing the high affinity receptor for IgE (FcεRI) is considered the cause of the immediate type hypersensitivity reaction, designated the early asthmatic response (EAR), when expressed in the airways [1]. Asthmatic patients frequently exhibit high serum titres of IgE, which correlate with bronchopulmonary hyperreactivity (BHR) [2], a nonspecific augmentation of airway responses to unrelated stimuli, including cholinergic agents. IgE exerts its effect *via* FcεRI-bearing cells, such as basophils and mast cells, and also *via* low affinity receptor (FcεRII)-bearing cells, such as B-lymphocytes and monocytes.

The cross-linking of IgE bound to FcεRI on mast cell and basophil membranes mediates the release of pharmacologically active substances, such as eicosanoids, histamine and interleukins [3, 4], which may be implicated in the late asthmatic response (LAR) [4]. FcεRI, which was thought to be present on mast cells and basophils only, has recently been identified on antigen-presenting cells and is also implicated in the IgE-facilitated antigen presentation [5]. Even before the discovery of these functions for IgE receptors, neutralization of IgE, either through the administration of anti-IgE antibodies or the induction of

the production of autoantibodies to IgE [6] was envisaged to reduce allergic inflammation.

It has been demonstrated that ovalbumin (OVA)-immunized mice from one of the "Biozzi" selections (called BP2 mice) become hyperresponsive to the bronchoconstrictive effects of *i.v.* serotonin and aerosolized methacholine after 3–4 intranasal OVA challenges [7]. Under similar conditions of immunization and challenge, BALB/c, CBA/J or hypereosinophilic interleukin (IL)-5 transgenic mice do not develop BHR, despite the OVA-driven recruitment of eosinophils into the airways [8]. Thus, even though eosinophils are usually associated with allergic BHR, their presence alone seems not to be sufficient for its induction. Clearly, additional factors, such as IgE, are required for the development of eosinophil-related BHR. This hypothesis is supported by the recent demonstration that a monoclonal antibody (mAb) to IgE (mAb1-5) reduces allergen-induced pulmonary eosinophilia [9] and BHR [10]. In humans, the parenteral injection of a non-anaphylactogenic anti-IgE antibody was also reported to considerably reduce the titres of circulating free IgE, inhibit the late-phase responses and reduce sputum eosinophilia [11].

In the present study, a protocol based on a single intranasal antigenic provocation of BP2 mice with OVA

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allowed the investigation of the effects of IgE-neutralization on BHR, on IL-4 and IL-5 protein in serum and bronchoalveolar lavage fluid (BALF), and on OVA-induced mucous cell metaplasia. Furthermore, because the majority of the IgE molecules are bound to cells expressing FcεRI, and since cell-bound IgE has a longer half-life than circulating IgE [12], it was also investigated as to which cells express cell-bound IgE and whether the anti-IgE antibody neutralizes this expression.

## Materials and methods

### Animals

BP2 and BALB/c (Centre d'Élevage R. Janvier, France) mice aged 6–8 weeks were immunized subcutaneously twice at a one-week interval with 0.4 mL 0.9% NaCl containing 100 µg OVA (Immunobiologicals, Lisle, IL, USA) and 1.6 mg alum (Merck, Darmstadt, Germany). One week after the second immunization, *i.e.* at day 14, mice were anaesthetized lightly with ether and challenged intranasally with 10 µg OVA in 50 µL alum-free 0.9% NaCl solution or with 0.9% NaCl solution as a control.

### Administration of the anti-IgE antibody mAb1-5

BP2 mice were injected (*i.v.*) with 0.33 mg of rat anti-IgE antibody (mAb1-5) or of rat IgG (control) 5 min and 24 or 72 h before the *i.v.* injection of OVA. To study the effect of IgE-neutralization on BHR, mice received mAb1-5 or normal rat IgG as a control *i.v.* using three protocols: 1) 1 mg before each of the two immunizations (at days 1 and 7), 2) 1 mg 24 h before the intranasal challenge, and 3) four injections of 0.5 mg each; 1 h before and 6, 24, and 48 h after challenge (day 14, 15 and 16, respectively). BHR was evaluated and blood samples were collected from the posterior vena cava for determining the titres of serum IgE and cytokines at 1, 6, 24 and 72 h after OVA challenge. Each study group included at least six mice.

### Evaluation of OVA-specific IgE titres in the serum

OVA-specific IgE levels in the serum were measured using a standard enzyme-linked immunosorbent assay (ELISA) as described previously [13]. Briefly, 96-well microtitre plates were coated with OVA (20 mg·mL<sup>-1</sup> in bicarbonate buffer at pH 9.6). After overnight incubation at 4°C, plates were washed and blocked at room temperature (22°C) with phosphate-buffered saline (PBS) containing 10% foetal calf serum for 30 min. Diluted serum samples were incubated overnight at 4°C. After washing, a monoclonal rat anti-mouse IgE (EM95-3) diluted to 1% in PBS containing 0.1% Tween buffer was added and incubated for 1 h. This was followed by washing and incubation with peroxidase-conjugated mouse anti-rat IgG. The reaction was developed with *o*-phenylenediamine dihydrochloride substrate (Sigma, St Louis, MO, USA) in phosphate buffer and the plates read with a microplate reader at 490 nm.

### Evaluation of T helper 2 cytokines in BALF and serum

IL-4 was measured with a commercial ELISA (Valbio-tech, Paris, France). Briefly, 96-well plates were coated with 2 µg·mL<sup>-1</sup> of rat anti-mouse IL-4 (BVD4-1D11). To these were added dilutions of recombinant IL-4 (rIL-4) standard (7–1,000 pg·mL<sup>-1</sup>) or of the sample, followed by a biotinylated rat anti-IL-4 antibody (BVD6-24G2) at 0.5 mg·mL<sup>-1</sup>. The reaction was revealed with *o*-phenylenediamine dihydrochloride substrate (Sigma) in phosphate buffer and read at 490 nm. The lower limit of detection of this assay for IL-4 is ~5 pg·mL<sup>-1</sup> of the sample.

IL-5 titres were measured using the immunometric assay as described previously [14]. Briefly, 96-well plates were coated with 10 mg·mL<sup>-1</sup> of rat anti-mouse IL-5 (TRFK-4) to which an rIL-5 standard (7.8–1,000 pg·mL<sup>-1</sup>) or the sample was added, followed by an acetylcholinesterase-labelled rat anti-mouse IL-5 antibody (TRFK-5) at 10 Ellman units·mL<sup>-1</sup>. Absorbance was read at 405 nm. The lower limit of detection of this assay for IL-5 is ~5 pg·mL<sup>-1</sup> of the sample.

### Functional studies

BHR was evaluated on unrestrained conscious mice as described previously [15]. Mice were placed in a barometric plethysmographic chamber (Buxco Electronics, Sharon, CT, USA) and respiratory parameters were measured before and after an aerosol of methacholine (Sigma-Aldrich, Stenheim, Germany) at  $3 \times 10^{-2}$  M in the aerosolator delivered for 20 s. The bronchopulmonary resistance was expressed as enhanced pause (Penh), calculated as: (expiratory time (*tE*)/40% of relaxation time (*trel*) - 1) × peak expiratory flow (PEF)/peak inspiratory flow (PIF) × 0.67, according to the manufacturer's recommendations. Every 20 s an average value of Penh was recorded. For the graphic representation, each value was expressed for every minute. Accordingly, each point represents the average of three values (fig. 1).

To study the effects of IgE on anaphylactic bronchoconstriction, mice were anaesthetized with urethane (*i.p.* 15 mg·10 g body weight<sup>-1</sup>), the trachea cannulated and connected to a pulmonary function analyser (Mumed PR800 system; Mumed, Chesham House, London, UK) at a ventilation volume flow of 0.2 mL·10 g<sup>-1</sup> and a frequency of 100 breaths·min<sup>-1</sup>. After an injection of serotonin at 20 µg·kg<sup>-1</sup> and 40 µg·kg<sup>-1</sup> at a 5 min interval to record standard responses, mice were injected *i.v.* with 100 µL of a solution of 50 mg·kg<sup>-1</sup> OVA (*via* a cannulated jugular vein) and respiratory functions were evaluated. Results were calculated as the percentage increase of the bronchial resistance (*R*) which is the ratio of (*R*<sub>max</sub>-*R*<sub>basal</sub>)/*R*<sub>basal</sub> × 100, where *R*<sub>max</sub> is maximal resistance and *R*<sub>basal</sub> is basal resistance.

### Histological studies of the lung

After evaluating bronchoconstriction as described above, mice were deeply anaesthetized with urethane (*i.p.* 15 mg·10 g body weight<sup>-1</sup>), and the abdominal cavity was opened. Blood samples for serum were collected from the caudal vena cava. The trachea was cannulated and the lungs were inflated with 1 mL of 50% Optimum Cutting

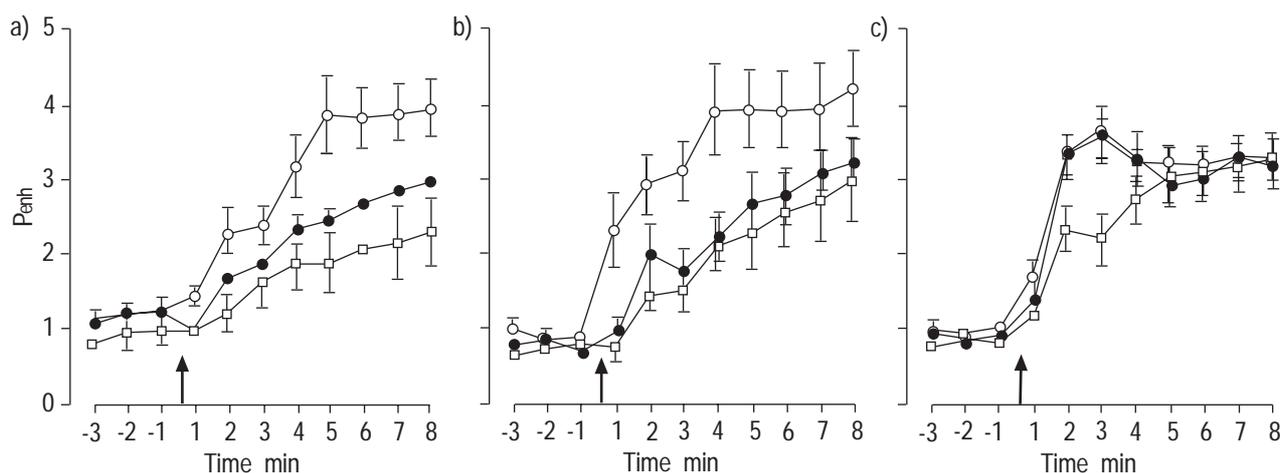


Fig. 1. — Time-dependent effects of a monoclonal non-anaphylactogenic rat anti-mouse immunoglobulin (IgE) antibody (mAb1-5) on bronchial hyperreactivity (BHR) (measured as enhanced pause (Penh)) in response to methacholine inhalation in BP2 mice. Mice received *i.v.* injections of 1 mg mAb1-5 or rat IgG as a control and were challenged with ovalbumin (OVA) 24 h later. BHR was measured 6 (a), 24 (b) and 72 (c) h after challenge. ○: rat IgG-injected and OVA-challenged animals; ●: mAb1-5-injected and OVA-challenged animals; □: rat IgG-injected and saline-challenged controls. Arrow represents the moment of methacholine inhalation. The differences between the values of Penh at 6 and 24 h in treated and control mice were significant. At 72 h the protective effect of mAb1-5 was partially over.

Temperature (OCT; Sakura Finetek, Torrance, CA, USA) in saline solution. The left lobe was separated, placed in a freezing tube and immediately frozen in liquid nitrogen for cryostat sectioning. The remaining right lobes were fixed in 10% formaldehyde for conventional histological preparations (preliminary studies had shown that the fixation in formaldehyde in the presence of OCT did not affect the histological quality of the sample). Lungs fixed in formaldehyde were embedded in paraffin blocks, 4  $\mu$ m sections prepared and stained with haematoxylin and eosin as well as with alcian blue–periodic acid–Schiff reaction to observe cellular mucus contents. A group of mice was used for the collection of BALF from the cannulated trachea and lungs were washed with 4 mL saline solution ( $8 \times 0.5$  mL each). After cell counting, a cytospin was prepared with standard apparatus. The frozen lung lobes were cut in a cryostat to obtain a longitudinal section of the major intrapulmonary bronchus, and 6- $\mu$ m thick sections were prepared, fixed in acetone for 10 min, dried and either used immediately or stored at  $-20^{\circ}\text{C}$  until further use. Cytospin preparations were fixed and stored identically.

#### Histochemical identification of cells

Eosinophils were stained on frozen sections for eosinophil peroxidase (EPO) as described previously [16]. Briefly, acetone-fixed sections were incubated with a solution containing diaminobenzidine, sodium cyanide and hydrogen peroxide (all from Sigma) at neutral pH, for 10 min, washed, counterstained and mounted. From preliminary studies, it was confirmed that this EPO staining in mice is very specific to eosinophils since it did not stain neutrophils or other cells as tested in blood, bone marrow and inflammatory infiltrates (data not shown).

Mast cells were stained using a histochemical reaction for nonspecific esterase as described previously [17]. Briefly, acetone-fixed sections were incubated with a solution containing the substrate, naphthol AS-D acetate (5 mg dissolved in 0.5 mL dimethylformamide), 25 mL distilled water, 25 mL Tris buffer at pH 7.1, and 30 mg Fast-blue

RR salt (all from Sigma), washed and mounted with aquamount (Gurr, BDH Laboratory, Poole, UK).

#### Immunohistochemical identification of cells

Immunohistochemical staining was performed on frozen sections and cytospin preparations using the indirect by labelled antibody staining method to identify IgE-bearing cells using the monoclonal rat anti-mouse IgE antibody (EM95-3). Briefly, sections were first incubated with the specific rat anti-mouse antibody or with normal rat IgG as a control for 1 h, followed by washing and incubation with biotinylated rabbit anti-rat antibody (Dako A/S, Glostrup, Denmark) for 45 min. After washing, samples were incubated with alkaline phosphatase-labelled streptavidin (Dako A/S) for 45 min. The reaction was revealed with a substrate solution containing Fast-red, naphthol AS-MX phosphate, and levamisole (all from Sigma) at pH 8.2. For double staining, samples were sequentially incubated with the two specific rat anti-mouse antibodies, and the remaining steps were applied in the same manner except that the reaction of the first antibody was revealed using Fast-red and the second was revealed with Fast-blue BB salt. Double immunohistochemical staining with mAb EM95-3 and anti-neutrophil mAb (NIMP-R14) or anti-B lymphocytes mAb (B220), and combined immunohistochemical and histochemical stain for IgE, for eosinophils and for mast cells were performed to identify the nature of the IgE-bearing cells.

Double or competitive staining of cells with the two anti-IgE antibodies mAb1-5 followed by mAb EM95-3, and mAb EM95-3 followed by mAb1-5, were performed on lung sections and cytospin preparations of BALF collected 24 h after OVA challenge.

#### Sources of antibodies

Anti-IgE mAb1-5 was generated by C. Heusser [18]. Anti-IgE mAb EM95-3 was provided by Y. Chvatchko

(Ares-Serono Pharmaceutical Research Institute, Geneva, Switzerland), anti-neutrophil mAb NIMP-R14 and anti-B lymphocyte mAb B220 by G. Milon (Institut Pasteur, Paris, France). Anti-IL-4 mAb BVD4-1D11 and mAb BV-D6-24G2 were purchased from Valbiotech, Paris, France. Anti-IL-5 mAb TRFK-4 and mAb TRFK-5 were purified from ascitic fluids (cloned hybridomas provided by P. Minoprio, Institut Pasteur, Paris, France).

#### Cell counts and statistics

Eosinophils were counted as the number of cells per millimetre length of the epithelium. Mast cells were scarce and localized on the first 2–3 mm of the longitudinally sectioned major intrapulmonary bronchiole; they were counted as the number of cells per whole section of the lung. Other cells which are distributed uniformly, were counted as cells per mm<sup>2</sup> of the lung section. Considering the stereological distribution of cells in the tissue, this counting method is to be referred to as semiquantitative. Results are presented as mean±SEM. Significance levels were calculated using the nonparametric Mann–Whitney U-test, and  $p < 0.05$  was taken as a significant difference between data [19].

## Results

#### Serum IgE titres

In order to investigate the relationship between biological effects and IgE-neutralization and/or elimination, the serum levels of specific IgE were assessed. Titres of IgE in the serum of immunized mice varied between 500 and 3,200 units. The administration of mAb1-5 either before immunization or before challenge with the antigen induced a significant reduction (65–100%) in OVA-specific IgE titres which remained at very low or undetectable levels for at least 18 days.

#### Effects of the neutralization of IgE on anaphylactic bronchoconstriction and BHR

Intravenous OVA-induced anaphylactic bronchoconstriction was prevented by mAb1-5 injected 5 min to 72 h before challenge (fig. 2) in BP2 mice. Since BALB/c mice did not undergo anaphylactic bronchoconstriction even though anaphylactic shock was observed (cyanosis and death), no results are reported for this standard strain.

A substantial BHR was observed in response to aerosolized methacholine 24 h after intranasal antigenic challenge in BP2 mice. MAb1-5 suppressed BHR when administered within the last 24 h before challenge (fig. 1), but not when administered before immunization (data not shown).

#### IgE staining on cells and effects of antibody neutralization of IgE

IgE-dependent activation of mast cells and basophils leads to the release of stored amines and newly produced cytokines, and is considered a potential trigger for EAR and subsequent LAR [20]. It was therefore investigated

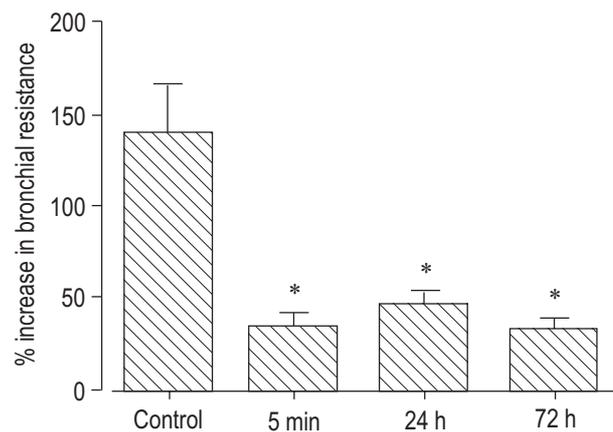


Fig. 2. – Effect of a monoclonal non-anaphylactogenic rat anti-mouse immunoglobulin E (mAb1-5) on ovalbumin (OVA)-induced anaphylactic bronchoconstriction in BP2 mice. MAb1-5 was administered at 5 min 24 h and 72 h before the induction of bronchial anaphylaxis with OVA. Results are expressed as percentage increase in bronchial resistance as compared to the basal responses to serotonin measured before the *i.v.* injection of OVA (50 mg·kg body weight<sup>-1</sup>). Results represent mean±SEM. \*:  $p < 0.05$ .

whether IgE-bearing cells are recruited into the lungs. Staining of lung sections from OVA-challenged mice with the anti-IgE mAb EM95-3 revealed B lymphocytes, mast cells and other intensively stained cells, which were uniformly distributed throughout the section and showed a polymorphonuclear (PMN) cell morphology. In cyto-spin preparations of the BALF, this PMN morphology was confirmed. These cells were negative for EPO, for the anti-neutrophil mAb NIMP-R14 (fig. 3A and B), for the B-lymphocyte marker B220, and for chloroacetate-esterase staining, which is expressed by mast cells [21]. These observations strongly suggest that the IgE-bearing PMN cells are basophils.

MAb1-5 administered before immunization or before challenge significantly inhibited the IgE staining of PMN cells in lung tissue (fig. 3D and fig. 4). In addition, mAb1-5 suppressed the IgE staining of lung mast cells (fig. 3F) without affecting their numbers (data not shown). MAb1-5 also inhibited the IgE-staining of B lymphocytes. In principle, the reduction of pulmonary basophils may have resulted either from the prevention of surface IgE binding or from the inhibition of basophil infiltration into the lungs. MAb1-5 does not bind to receptor-bound IgE [18]. In order to investigate whether mAb1-5 was able to clear IgE from the surface of pulmonary cells, immunostaining of lung sections after preincubation with mAb1-5 was performed. Preincubation of sections from OVA-challenged mice with mAb1-5 did suppress IgE staining with mAb EM95-3, another anti-IgE.

#### Interference of IgE-neutralization with the recruitment of eosinophils into the airways

When injected 24 h before challenge, mAb1-5 reduced the inflammatory infiltrates in the lungs and in particular inhibited airway eosinophilia at 6, 24, and 72 h post-challenge, whereas a control antibody was ineffective (fig. 5a). When mAb1-5 was administered 1 h before challenge and lungs were examined 72 h thereafter, a marked

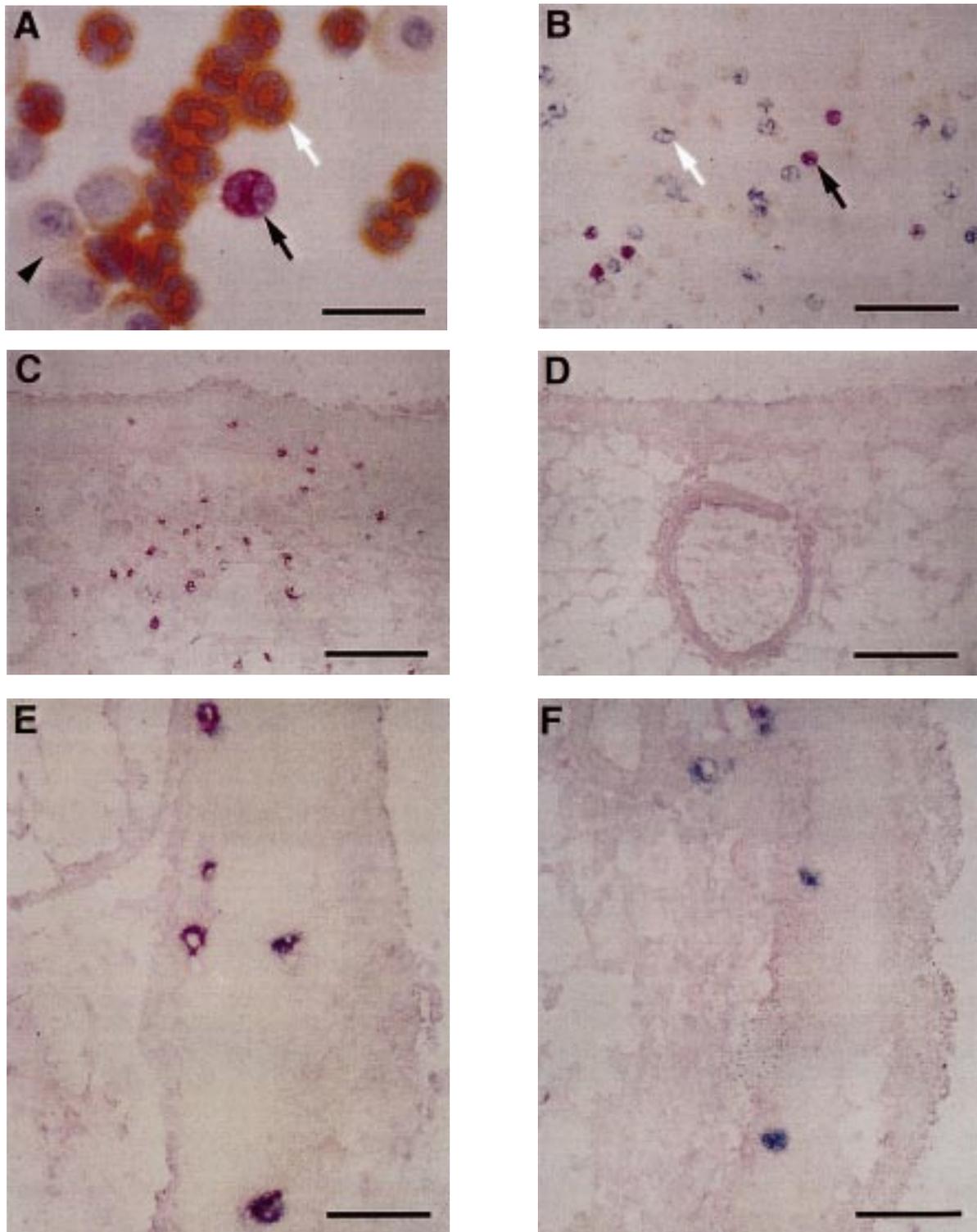


Fig. 3. – Immunohistochemical staining for cell-bound immunoglobulin (Ig)E using the rat anti-mouse anti-IgE monoclonal antibody EM95-3 on polymorphonuclear (PMN) leukocytes and mast cells in the lung and bronchoalveolar lavage fluid (BALF) of ovalbumin (OVA)-challenged or monoclonal non-anaphylactogenic rat anti-mouse IgE antibody (mAb1-5)-treated and OVA-challenged BP2 mice. A: cytopsin preparation of BALF stained histochemically for eosinophil peroxidase (EPO; brown colour) and immunohistochemically for IgE (red colour), showing IgE-positive EPO-negative PMN cells identified as basophils (black arrow). EPO-positive IgE-negative cells are eosinophils (white arrow). Some macrophages are also present (arrow head). B: double immunohistochemical stain showing IgE-positive PMN cells or basophils (black arrow) and mAb NIMP-R14-positive neutrophils (white arrow). C: IgE-positive PMN cells uniformly distributed in the lung of OVA-challenged BP2 mice. D: complete loss of IgE-staining in mice treated with 1 mg mAb1-5 and challenged with OVA thereafter. E: IgE-(red) and chloroacetate-esterase-(blue) positive mast cells in the bronchiole of OVA-challenged BP2 mice. F: complete loss of IgE-staining on mast cells (chloroacetate-esterase-positive) of the bronchiole of mice treated with 1 mg mAb1-5 and challenged with OVA thereafter. (Internal scale bars A=25  $\mu$ m; B=125  $\mu$ m; C and D=250  $\mu$ m; E and F=62.5  $\mu$ m.)

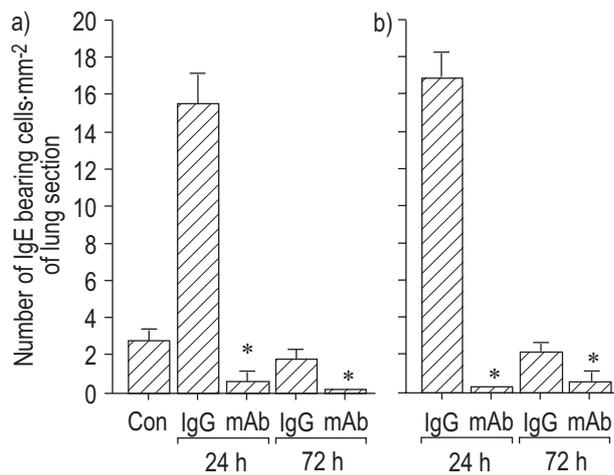


Fig. 4. – Effect of a monoclonal non-anaphylactogenic rat anti-mouse immunoglobulin (IgE) antibody (mAb) on the number of IgE-positive polymorphonuclear cells or basophils compared with a rat IgG control. Immunohistochemical staining for IgE was performed with a monoclonal rat anti-mouse IgE antibody (EM95-3) on frozen lung section, 24 or 72 h after ovalbumin (OVA) challenge. Antibodies injected 24 h before OVA challenge (a) and antibodies injected before each immunization (b). Con: rat IgG with saline challenge. Reading was performed at 24 h after challenge. Results represent mean $\pm$ SEM. \*:  $p < 0.05$ .

reduction of tissue eosinophilia was noted (fig. 5b), whereas mice that received mAb1-5 only before immunization, but not before challenge, displayed tissue eosinophilia as intensively as their controls (fig. 5c).

#### Antibody neutralization of IgE inhibits IL-4 but not IL-5 in the BALF

Both IL-4 and IL-5 are important mediators of hyper-eosinophilia [22]. IL-4 was detected in the BALF of OVA-challenged mice, with a peak production at 24 h post-challenge. IL-5 was detected in serum and in BALF with a peak production at 6 h and 24 h, respectively (data not shown). Treatment with mAb1-5 considerably reduced OVA-induced IL-4 levels in the BALF, but had no significant effects on IL-5 (fig. 6).

#### Antibody neutralization of IgE and epithelial mucus contents

Antigen provocation resulted in intense metaplasia of epithelial cells into mucus-secreting cells, as has been described previously [23]. The major epithelial lesion observed was a marked mucous metaplasia of the epithelium of the major bronchiole of mice studied 24 and 72 h after OVA challenge. The Clara cells, which make up up to 50% of the epithelial cells of the major bronchiole of mice, contained mucus granules when examined at 72 h post-challenge. These mucus-containing cells were reduced in numbers in mice treated with mAb1-5 24-h before challenge and examined 72 h later (fig. 7). Again, mice which received mAb1-5 before immunization showed no reduction of mucus-containing cells (data not shown).

### Discussion

These results raise the important problem of the role of IgE for the development of BHR and, more generally, of

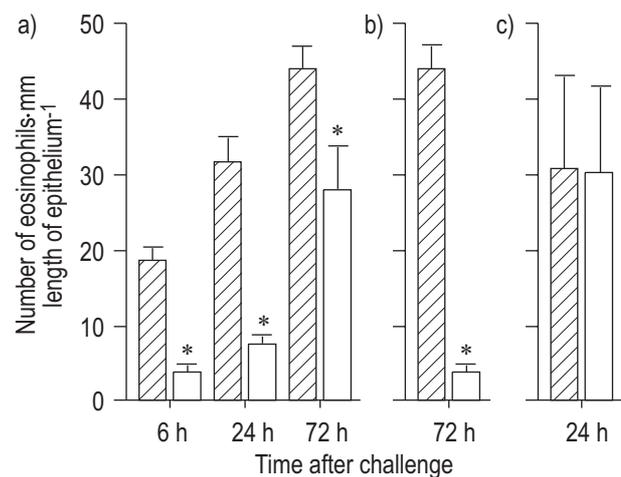


Fig. 5. – Effect of a monoclonal non-anaphylactogenic rat anti-mouse immunoglobulin (IgE) antibody (mAb1-5;  $\square$ ) and rat IgG as a control ( $\text{▨}$ ) on tissue eosinophilia. a) mAb1-5 was administered *i.v.* 24 h before ovalbumin (OVA) challenge and bronchial eosinophilia observed at different times after challenge. b) mAb1-5 was administered *i.v.* 1 h before challenge and bronchial eosinophilia observed at 72 h after challenge. c) mAb1-5 was administered *i.v.* 24 h before each immunization and bronchial eosinophilia observed at 24 h after challenge. Eosinophils were differentially stained for eosinophil peroxidase using diaminobenzidine diluted in phosphate buffer at neutral pH. Since eosinophils preferentially infiltrate peribronchial and perivascular areas, they were counted per millimetre length of the major intrapulmonary bronchiole. Results are presented as mean $\pm$ SEM. \*:  $p < 0.05$ .

the relation between early and late responses to allergen, *i.e.* between EAR and LAR. Anaphylactic bronchoconstriction only observed in BP2 mice and absent in BALB/c, even though anaphylactic death was induced, was inhibited when mAb1-5 was administered 5 min to 72 h earlier. Characteristic parameters of the LAR, such as lymphocytic and eosinophilic inflammation, recruitment of basophils, mucous cell metaplasia, and BHR were inhibited by mAb1-5 administered within the 24 h preceding the antigenic challenge. The fact that mAb1-5 inhibited the anaphylactic bronchoconstriction as well as LAR suggests that IgE is important for both airway responses. The link between IgE and functional disturbances may thus not only involve eosinophil recruitment, as shown in earlier studies [9], but also basophils and mast cells, which secrete the T-helper (Th)2 cytokines IL-4 and IL-5 [3, 24]. It is unlikely that eosinophils are the direct target for mAb1-5, since they barely showed IgE on their surface, in agreement with the fact that they lack surface IgE receptors [25].

The implication of basophils in asthma in humans was stressed by KOSHINO *et al.* [26] who showed that antigen induces a marked recruitment of IgE-positive basophils to the lungs. In the present study, basophils were recruited within the first 3 h after challenge, well before the massive infiltration of eosinophils which started after 6 h [27]. In mice, recruitment of basophils into the lungs has not been described before. GERSHWIN *et al.* [28], using immunofluorescence techniques noted the recruitment of IgE-containing cells into the lung of mice receiving aerosolized OVA and ozone. They concluded that these cells were lymphocytes. In the present study, the IgE-positive cells had a PMN morphology, and did not contain mast cell esterases or the B lymphocyte antigen B220, so were identified as basophils.

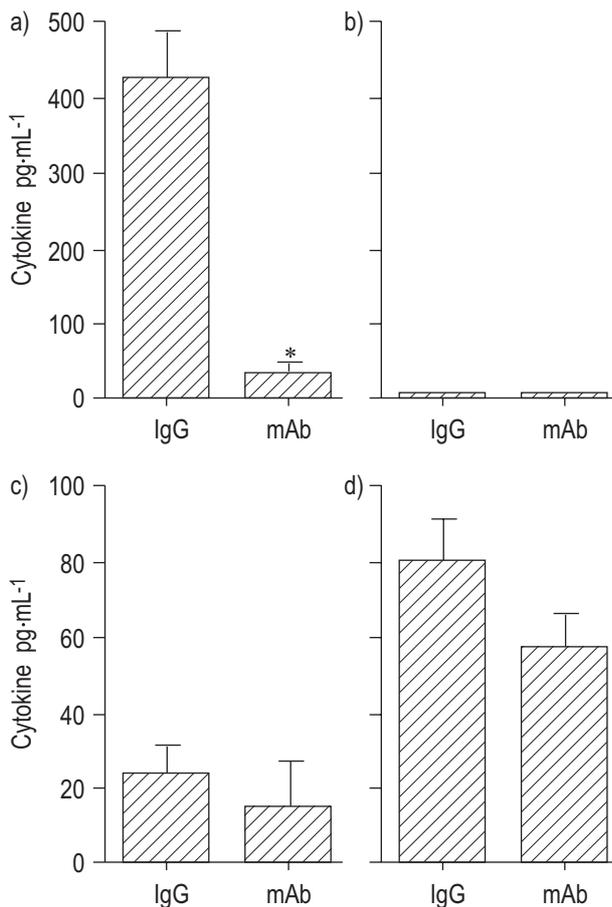


Fig. 6. – Effect of a monoclonal non-anaphylactogenic rat anti-mouse immunoglobulin (IgE antibody (mAb1-5) or of a rat IgG as a control on the ovalbumin (OVA)-induced levels for interleukin (IL)-4 in bronchoalveolar lavage fluid (BALF) (a) and serum (b) and for IL-5 in BALF (c) and serum (d) in BP2 mice. Mice received *i.v.* 1 mg of mAb1-5 24-h before challenge. IL-4 was not detected in the serum using this method. The lower threshold of detection of the two cytokines was ~5 pg·mL<sup>-1</sup>. Results are presented as mean±SEM. \*: p<0.05.

Human basophils and mast cells as well as murine mast cells release IL-4 in response to activation of the FcεRI receptor with IgE [3, 29, 30]. Moreover, FcεRI-expressing and IL-4 secreting cells from murine spleen and bone marrow are enriched in basophils [24]. This suggests that murine basophils and mast cells show similar functions, although recently the capacity of human mast cells to secrete IL-4 has been questioned [31].

Two mechanisms may contribute to IL-4 suppression. On the one hand, anti-IgE can prevent IgE-facilitated antigen uptake and processing by antigen-presenting cells, resulting in reduced Th2 cytokine production by lung T cells, as has been shown [9], a mechanism operating *via* FcεRII. On the other hand, it is likely that competition of mAb1-5 with the FcεRI receptor for IgE determinants inhibits the IgE-dependent stimulation of basophils and mast cells and thus suppresses the release of IL-4 with, as a consequence, a reduction in eosinophil recruitment.

Mucus hypersecretion is one of the manifestations of chronic allergic and non-allergic inflammation of the airways [32]. It has recently been shown that anti-IL-4 receptor antibodies inhibit mucus production in mice [23]. Accordingly, the reduction in the number of mucus-

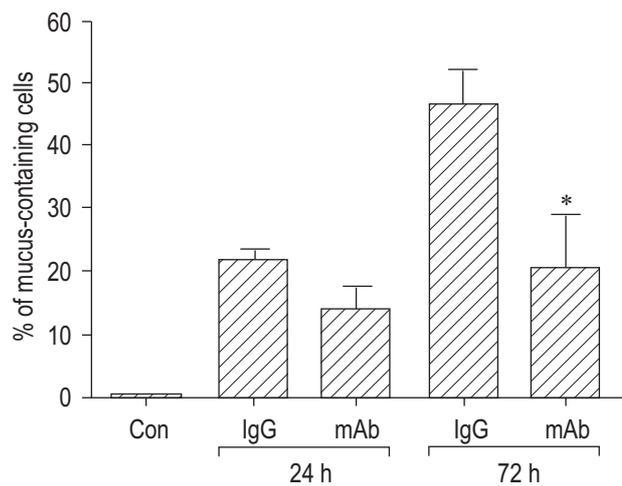


Fig. 7. – Effects of a monoclonal non-anaphylactogenic rat anti-mouse immunoglobulin (IgE antibody (mAb1-5) on antigen-induced epithelial cell mucus contents compared to rat IgG control in ovalbumin-challenged mice. Con: rat IgG with saline challenge. Histological evaluation was performed at 24 and 72 h post-challenge. Mucus was stained by the periodic acid–Schiff (PAS)/alcian blue (AB) reaction. The percentage of stained cells over total epithelial cells of the major intrapulmonary bronchiole was used to compare the different treatment. AB stains acidic mucus and sialic acid-containing glycoproteins, while PAS stains neutral mucus containing sulphated glycoproteins. Results are presented as mean±SEM. \*: p<0.05.

containing cells observed in these experiments may result from inhibition of IL-4 production by mAb1-5. By contrast, very recently COHN *et al.* [33], demonstrated that mucus production is not prevented in IL-4-deficient mice and suggested that Th2 lymphocytes stimulate mucus production probably through cytokines other than IL-4. Recent studies have indicated a role for IL-13 in addition to IL-4 in mucus metaplasia [34, 35].

The general mechanism of the inhibitory effects of anti-IgE antibodies has been discussed [9, 36]. As mentioned before, COYLE *et al.* [9] suggested that mAb1-5 decreases inflammatory cell infiltration by preventing IgE–CD23-mediated antigen presentation, as demonstrated in CD23-deficient mice, resulting in inhibition of Th2-type cytokine production by lung T cells. However, the complete absence of IgE-staining on all lung cells and the demonstration that mAb1-5 was able to deplete IgE from all these cells rather suggests that mast cell and pulmonary basophil functions may also have been affected by anti-IgE treatment. Competition and removal of IgE from its FcεRI receptor by a non-anaphylactogenic anti-IgE has already been shown with the anti-human IgE antibody, BSW17 [37]. A similar mechanism may have contributed to the absence of IgE-staining on basophils and mast cells in this study. Inhibition of basophil and mast cell function by mAb1-5 is in line with the observation that mAb1-5 suppressed bronchoconstriction and selectively reduced IL-4 levels in BALF and serum, in agreement with the observation that murine [38] and human [3] basophils are a major source of IL-4.

It was anticipated that neutralization of IgE with mAb1-5 injected before immunization would also inhibit OVA-induced changes, which, surprisingly, was not the case. This failure is in agreement with the findings of MEHLHOP *et al.* [39] that IgE-deficient mice develop antigen-dependent

eosinophilia as intensively as their controls. Moreover, DOMBROWICZ *et al.* [40] demonstrated that mice lacking the FcεRI receptor undergo systemic anaphylactic responses through IgG<sub>1</sub> and FcγRIII.

Finally, MACCLASHAN *et al.* [41] showed that the reduction of the immunoglobulin E levels in the serum down-regulates the expression of FcεRI on human basophils. A shift from immunoglobulin E to immunoglobulin G-dependent mechanisms or other still unknown mechanisms may have contributed to the late-phase responses in the mice receiving mAb1-5 before immunization. Nevertheless, mAb1-5 given at a time near to the antigenic challenge, significantly reduced immunoglobulin E, pulmonary eosinophilia, mucus content in the epithelial cells, and interleukin-4 in the bronchoalveolar lavage fluid, which indicates that immunoglobulin E is somehow involved in the early establishment of these changes.

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