

## Bronchial Reactivity in Relation to Immune Responses in Rats after Subcutaneous Sensitization without Adjuvant

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Rats of BNxWi/Fu strain were immunized by daily subcutaneous (s.c.) injections of antigen without the use of adjuvant for two 2-week periods with a 4-week interval. The bronchial responses to airway and intravenous (i.v.) antigen challenge were measured during and after the immunization period. These responses were compared with both the humoral and the cell-mediated immune response of the animals. Immunization induced bronchial reactivity of the animals to antigen after airway and i.v. challenge. This reactivity persisted for 7 weeks following the second immunization period. Specific IgE antibodies were detected in serum, bronchial fluid and in supernatants from cultured peripheral lymph node cells. The immunization also resulted in an IgG antibody response. Neither the amount of IgE, IgG2a, nor of any other isotype, correlated with the bronchial reactivity in the animals. Antigen stimulation of lymph node cells mixed with syngeneic spleen cells induced proliferation. This procedure also resulted in a maturation of mast cells in 3-week cultures. The immunization also resulted in some increase in the number of mast cells around vessels in the lung. There was no correlation between these parameters of cell-mediated immunity and either antibody responses or bronchial reactivity.

**Key words:** Asthma; bronchial reactivity; immune responses; lymphocyte reactivity; mast cells.

*Accepted for publication 18 October 1983*

### CLINICAL ASPECTS

Relevant experimental animal models reflecting the various compartments of the immune system are essential for an understanding of how various factors interact in the allergic inflammation. The allergic asthmatic is presumably sensitized to antigen in the absence of adjuvant, and an animal model of this disease should allow analysis of the bronchial response after inhalation of allergens in appropriately sensitized animals, e.g. by repeated small doses of antigen without using adjuvants. This would allow studies of various parameters of immunity, e.g. antibody production and cell-mediated immunity, and the effects of this on mast cell maturation. The present communication describes such a model and demonstrates that bronchial reactivity as well as IgE antibody responses can be achieved by prolonged intermittent antigen administration without using any adjuvant.

The serum levels of IgE antibodies in rats have been shown to correlate poorly to the evoking of allergic inflammatory reactions after challenge in the lungs (3, 6, 7, 9, 22, 24) and skin (7, 15). Other factors will contribute to the allergic reaction in the lungs, such as local IgE antibodies and antibodies of other isotypes, mast cells, mucous cells and cell-mediated im-

munity as well as unspecific hyperreactivity. In experimental models the development of such factors are affected by the use of adjuvants (13). Bronchial reactivity as a measure of allergy has often been studied in animals sensitized with antigen in combination with various adjuvants, e.g. aluminium hydroxide (6, 8, 9, 23). Such sensitization procedures cause an uncon-

trolled systemic inflammation (13, 14) with unknown effects on the bronchial reactivity. In addition, the sensitization regimen, including route of administration and choice of adjuvant, affects the type of humoral antibody response induced, i.e. antibody isotypes as well as antibody avidity (13). Recent work has shown that IgE antibody formation in rats and mice can be induced without adjuvant by daily administration of antigen (2, 11, 12, 17). In addition to antibody responses, cell-mediated immunity may be influenced by the immunization protocol (13, 24). Variations in the immunological responses may be expected to affect the results of bronchial challenge.

In this study we evaluated the bronchial response in rats immunized with daily subcutaneous administration of antigen. The results were related to the development of humoral and cell-mediated immune responses and to maturation and appearance of mast cells in the lungs.

## MATERIAL AND METHODS

### *Animals*

BNxWi/Fu rats of both sexes, weighing 200–350 g were used (Prof. O. Sjögren, University of Lund, Sweden). The immunizations were started at an age of 10–14 weeks.

### *Immunizations*

The rats were immunized according to a previously described protocol (2). Briefly, the rats were given 100 ng s.c. ovalbumin (OA) (grade V, SIGMA Chem. Co., St. Louis, Mo.) in 0.25 ml saline daily Monday through Friday for 2-week periods with an interval of 4 weeks. At various times during the test period, animals were tested for immune responses, subjected to bronchial challenge or sacrificed for histological examination of the lungs.

### *Respiratory measurements*

Respiratory measurements were performed on rats anaesthetized with Inactin® (125 mg/kg,

intraperitoneally, Promota, Hamburg, West Germany) as previously described in detail (3). Briefly, the animals were given pancuronium bromide (0.5 mg/kg i.v.) and ventilated with a Palmer animal respirator (80 strokes/min, tidal volume 9 ml/kg, later adjusted to give an arterial  $PCO_2$  of approximately 4.50 kPa). Transpulmonary pressure (TPP) was measured as the difference between tracheal and intrapleural pressures using a Statham differential transducer (PM 131 TC). Mean arterial blood pressure (MAP) was measured via a catheter introduced into the carotid artery and an IMP-transducer and, like TPP, was recorded on an UV recorder. In addition to the arterial catheter a jugular vein was also catheterized. Arterial blood samples were analysed on an IL automatic blood gas analyser.

The animals were challenged with antigen in aerosol from a solution (10 mg OA/ml in saline) using an Ultrasonic nebulizer model 108 (Engström Medical AB, Stockholm, Sweden). The nebulized antigen was administered for 10 min into the intake of the Palmer pump resulting in a total delivery of 5 ml antigen solution into the airways. After recovery within 1 h, the animals were challenged a second time, now i.v. with 5 mg OA in a volume of 0.5 ml saline. Control experiments included challenge with dog serum albumin (DSA) (Sigma) instead of OA in sensitized animals.

### *Sampling*

Blood samples for antibody analysis were taken under ether anaesthesia from the retroorbital plexus or, in animals subjected to bronchial challenge, from an arterial catheter before challenge (see below). Bronchial washings were done after maximum bleeding using 6 ml Hank's balanced salt solution containing 1% EDTA. The lungs were rinsed 3 times with 2 ml of the solution, which was inflated three times into the surgically exposed trachea (1). To study cell-mediated immunity axillary, brachial and mediastinal lymph nodes and the spleen were dissected from ether anaesthetized animals after maximum bleeding. The lungs were removed for histological examination.

### *Antibody determination*

The IgE antibody responses were recorded with the radioallergosorbent test, RAST, as previously described (20) using specific rabbit-anti-rat-IgE antibodies. The RAST results were calculated as bound radioactivity in % of total activity. For serum the dilutions used were 1/20 whereas the bronchial fluids and the culture supernatants were analysed undiluted. For serum and bronchial fluid the results were expressed as ratios between test and control samples. The levels of IgG, IgG2a, IgM and IgA antibodies were determined with the enzyme-linked immunosorbent assay, ELISA. The gammaglobulin fraction of specific rabbit anti-rat-IgG, IgG2a, IgM and IgA sera (Nordic Labs, Tilbury, the Netherlands) were utilized after conjugation with alkaline phosphatase (Sigma). The enzyme reaction time was 100 min and the optical density for different serum dilution was measured at 405 nm using a Titer-tek multiscan spectrophotometer (Flow Laboratories) as previously described (3). The serum dilution giving twice the optical density of a control serum was considered as the antibody titer.

### *Cell-mediated immunity*

Single cell suspensions were prepared from dissected axillary, brachial and mediastinal lymph nodes by perfusion of the nodes with medium. Cells from the different lymph nodes were pooled in Hank's balanced salt solution (Flow Laboratories). Lymph node cells were cultured, alone or with spleen cells ( $2 \times 10^6/\text{ml}$ ) in McCoy's medium 5A, pH = 7.4 supplemented with 10% fetal calf serum, 2 mM glutamine (Svenska Flow, Solna, Sweden),  $5 \times 10^{-6}$  M  $\beta$ -mercaptoethanol and gentamycin as previously described (3). For lymphoblast stimulation 200  $\mu\text{l}$  aliquots were incubated for 5 days in 5%  $\text{CO}_2$  and 95% moisture in round bottom well microplates (Falcon Plastics) with leukoagglutinin (Pharmacia) at optimal concentrations of 5 and 10  $\mu\text{g}/\text{ml}$  or with 30  $\mu\text{g}/\text{ml}$  of specific antigen. During the final day of incubation 0.01  $\mu\text{g}$   $^3\text{H}$ -thymidine was added for 6 h. The cultures

were harvested automatically in a Titertek cell harvester and the incorporated  $^3\text{H}$ -thymidine was measured.

To study mast cell maturation  $4 \times 10^6$  of lymph node cells and of mixed lymph node and spleen cells were cultured in 2 ml medium in Falcon tubes (3) in the presence of mitogen or antigen in the above concentrations. Half of the medium was replaced twice every week with fresh medium containing stimulating agent. Supernatant fluids were collected after 4 and 7 days for antibody determination. Cytospin preparations were also made at these times, as well as after 2, 3 and 4 weeks, using a Cytospin centrifuge (Shandon). The slides were stained with Alcian blue Eosin and analysed for content of mature mast cells. The results given are from the 3 week-cultures which gave optimum mast cell numbers.

### *Histological examination*

The lungs were processed as previously described (1). Briefly, they were immersed in Carnoy's fixative, embedded in acryl plastic (Sorvall, Du Pont Co., Newton, CT., USA). Sections, 1  $\mu\text{m}$  thick, were stained with acid toluidine blue. The numbers of mast cells on the specimen were examined blindly at  $\times 10$  objective magnification and scored as previously described (3) as negative, low numbers (+ = 1–5 mast cells per visual field), moderate numbers (++ = 6–10 mast cells) or high numbers (+++ = > 10 mast cells).

## RESULTS

### *Bronchial reactivity*

OA-sensitized animals, but not the non-immunized controls showed increased TPP following challenge with OA via the airways as well as i.v. (Fig. 1). Intravenous challenge induced stronger bronchial reactions than airway challenge. No bronchial response was observed in control experiments in which animals sensitized with OA were challenged with DSA (data not shown). The bronchial reactivity to OA appeared after a single treatment period of 2 weeks. Moderate

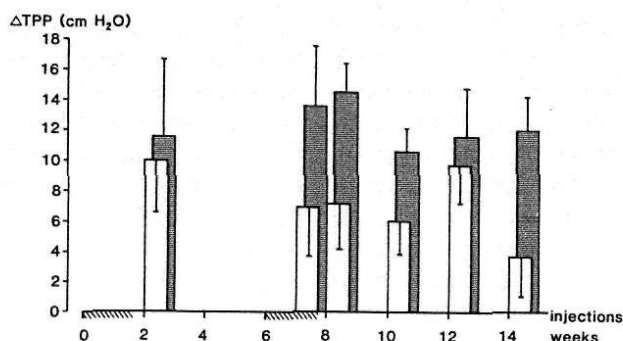


Fig. 1. Mean changes ( $\pm$  SD) in transpulmonary pressure (TPP) after aerosol ( $\square$ ) and i.v. ( $\blacksquare$ ) antigen challenge in rats sensitized for various time periods.  $n = 4-6$ .

fluctuations in the magnitude of the response were seen later during the study period. There was no obvious relation between the bronchial response to airway and i.v. provocation. After airway challenge there was slightly less bronchial reactivity in animals sensitized for two 2-week periods compared with rats sensitized only for one period. The reactions after i.v. challenge were similar in these animals and they were not influenced by immunization protocol. The reactivity subsided slightly with time, and 7 weeks after the second immunization period only a minor response was observed after airway challenge.

#### Blood pressure and blood gases

The baseline value for the mean arterial pressure (MAP) was  $136 \pm 28$  mmHg (mean  $\pm$  SD) for the whole group of animals. No significant differences were observed during the test period of 15 weeks. After airway challenge of immunized animals a moderate decrease in MAP was observed ranging from 0–50 mmHg. Following i.v. challenge a more pronounced and sustained decrease in MAP was observed. The decrease ranged from 20 to 100 mmHg. No such changes were seen in non-immunized animals after challenge.

The blood gas values were similar in non-challenged animals irrespective of immunization. The baseline values were for  $\text{PaO}_2$   $11.4 \pm 1.8$  kPa,  $\text{PaCO}_2$   $4.35 \pm 0.40$  kPa and  $\text{pH}$   $7.47 \pm 0.06$  kPa. Airway challenge of immunized ani-

mals but not of non-immunized animals resulted in a decrease in  $\text{PaO}_2$  of 3.0–4.5 kPa and, simultaneously, a slight although consistent decrease in  $\text{PaCO}_2$  of approximately 0.30 kPa. There was no change in arterial pH following airway challenge. No obvious correlation could be found between the changes in TPP and blood gases in individual animals.

#### Antibodies

Specific IgE antibodies were detected in serum (Fig. 2a), bronchial fluid (Fig. 2b) and in supernatants from cultured peripheral lymph node cells (Fig. 2c) of sensitized animals during and after the second immunization period. In the animals analysed for IgE in serum, bronchial fluid and culture supernatant, the kinetics of the response was similar in the bronchial fluid and the supernatants. The serum IgG antibody responses varied among the animals but seemed to peak during the second immunization period (Fig. 3). Neither the amount of specific IgE nor any other antibody isotype correlated with bronchial reactivity in the animals.

#### Cell-mediated immunity

There was increased spontaneous proliferation of the regional lymph node cells throughout the observation period although the individual responses varied (Fig. 4). Addition of antigen or mitogen resulted in very little further stimulation. In contrast, lymph node cells, mixed with

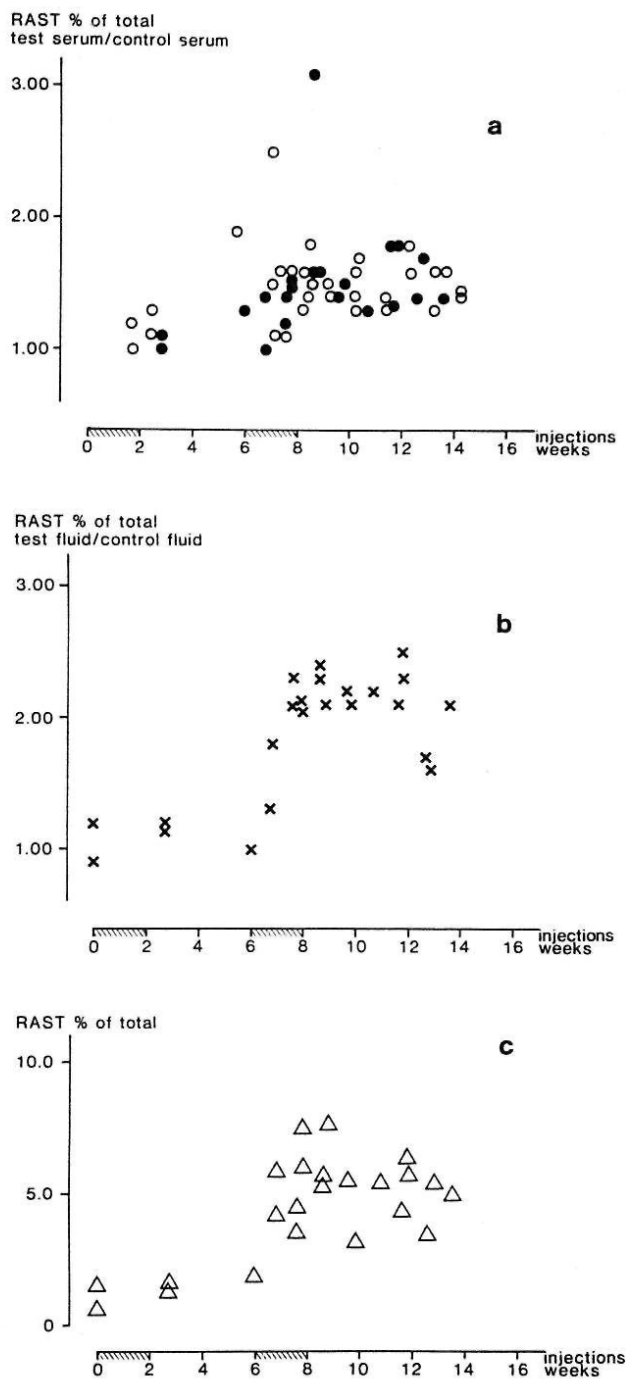


Fig. 2. IgE antibody responses determined with RAST in a) serum, b) bronchial fluid, c) culture supernatants of axillary, brachial and mediastinal lymph node cells. In a) the filled symbols indicate animals which also were analysed for IgE antibodies in bronchial fluid and in the supernatant fluids of cell cultures.

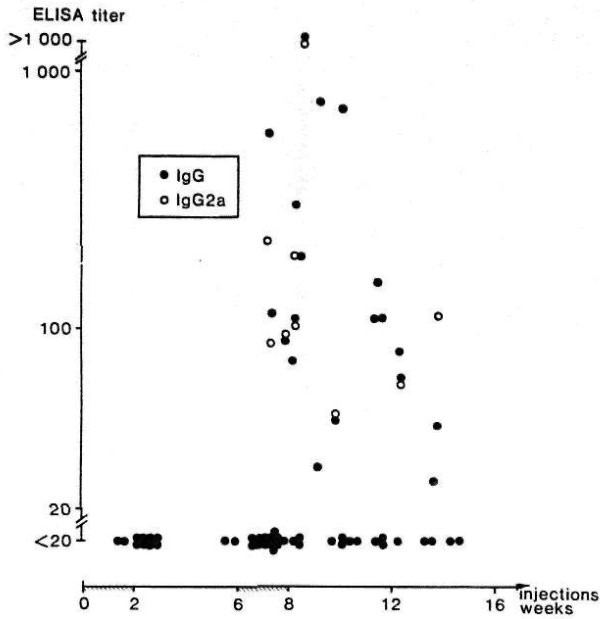


Fig. 3. IgG (●) and IgG2a (○) antibody levels in serum as determined with the ELISA.

syngeneic spleen cells showed only little spontaneous proliferation, whereas, in these mixed cultures, addition of antigen or mitogen induced a considerable blast transformation. This pattern was obvious already after the first immunization period.

During the second period of immunization considerable numbers of mast cells were seen in peripheral lymph node cells mixed with syngeneic spleen cells cultures with antigen or mitogen for up to 4 weeks (Fig. 5). Lymph node cells cultured alone did not show any

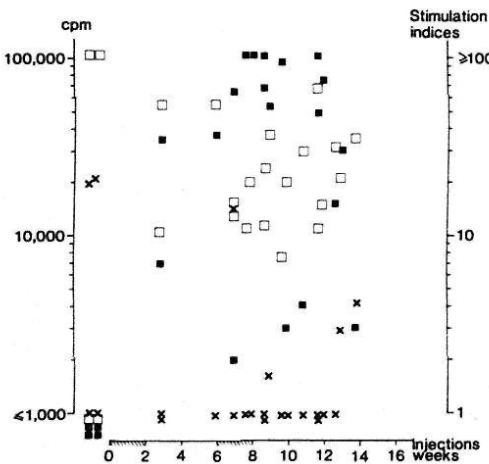


Fig. 4. Lymphoblastogenesis given as cpm of lymph node cells cultured alone (□) and mixed with spleen cells (×) without stimulation. In addition, the stimulation indices of lymph node cells mixed with spleen cells and stimulated with OA (■) are shown. The values are given in cpm.

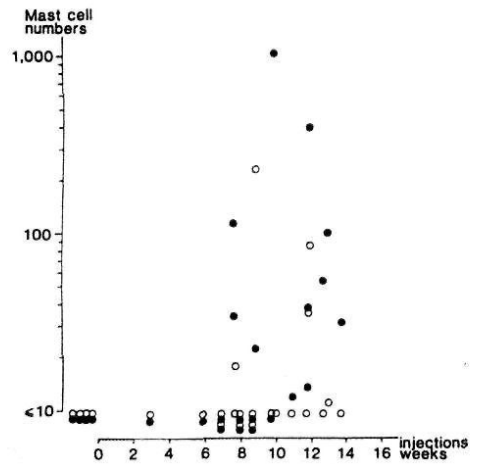


Fig. 5. Mast cell numbers in 3 week-cultures of lymph node cells mixed with spleen cells without stimulation (○) and stimulated with OA (●).

Table 1

Mast cells around blood vessels in the lung as demonstrated in histological sections. The results are summarized as a histological score

| Week No. | n | Histological score of individual animals |     |     |    |    |    |
|----------|---|------------------------------------------|-----|-----|----|----|----|
| 0        | 4 | +                                        | +   | +   | +  |    |    |
| 1        | 3 | -                                        | +   | +   |    |    |    |
| 3        | 6 | +                                        | ++  | ++  | ++ | ++ | ++ |
| 6        | 3 | ++                                       | ++  | +++ |    |    |    |
| 7        | 6 | +                                        | +   | +   | ++ | ++ | ++ |
| 8-9      | 6 | +                                        | +   | +   | +  | +  | ++ |
| 10       | 2 | +                                        | ++  |     |    |    |    |
| 11       | 3 | -                                        | +   | +   |    |    |    |
| 12       | 2 | ++                                       | +++ |     |    |    |    |

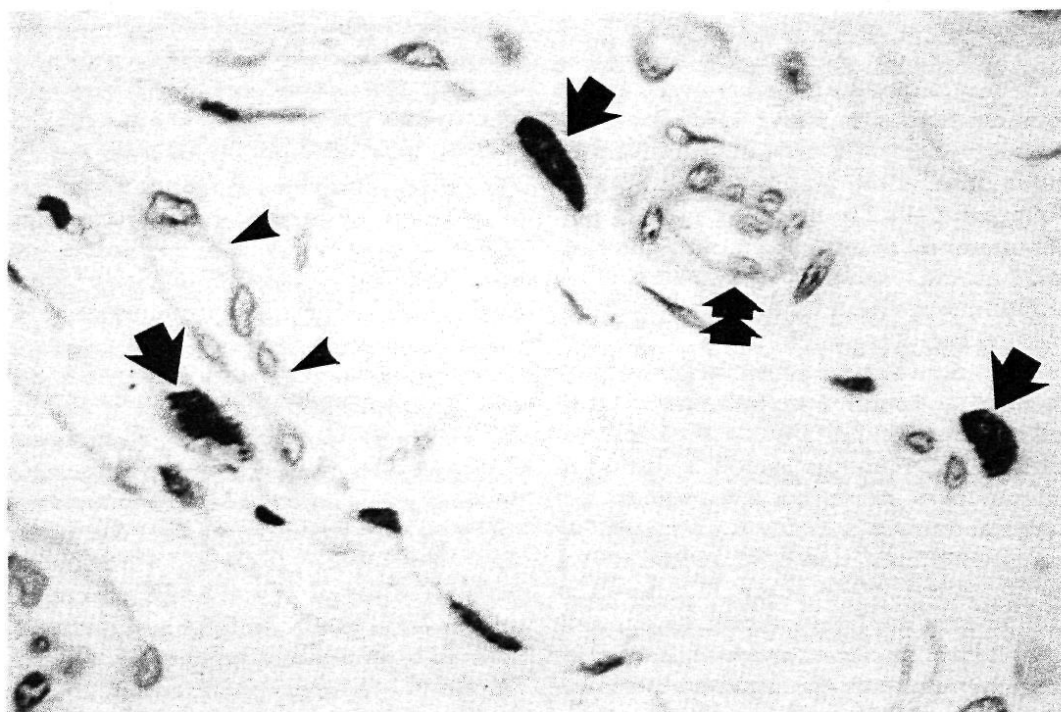


Fig. 6. Section of lung from a rat immunized s.c. daily for 2 weeks with 100 ng OA. Mast cells (arrow) are seen around small vessels morphologically resembling venules (arrowhead) and arterioles (double arrow). Magnification  $\times 500$ .

mast cell maturation. There was no relation between mast cell maturation and proliferative responses in the cultures. Neither was any relation found between the parameters of cell-mediated immunity, and either antibody responses or bronchial reactivity.

#### *Histological examination*

The first period of daily immunization induced increased numbers of mast cells in the lungs. The numbers were similar in the rats during the following 4 weeks but then declined during

the second immunization period. Two animals analysed 3 weeks after the second period again showed increased mast cell numbers (Table 1). The mast cells were preferentially localized to the immediate vicinity of vessels, morphologically recognized as venules (Fig. 6). In contrast, the mast cells in control animals were located in the adipose tissue surrounding large vessels and to a lesser extent around bronchioli.

## DISCUSSION

The present study demonstrates that a state of bronchial hypersensitivity to antigen can be induced by daily s.c. injection of low doses of antigen without any adjuvant. The state of hypersensitivity appeared during the second week of immunization and, although diminishing with time, it persisted for the entire experimental period of 15 weeks. Challenge of the airways resulted in a changed ventilation/perfusion ratio in the lung as reflected by the decreased  $\text{PaO}_2$ . The decreased  $\text{PaCO}_2$  is possibly explained by systemic effects of the challenge causing a decreased cardiac output. This possibility was supported by the observed decrease in arterial pressure.

As described by other authors (e.g. 3, 6, 7, 22, 23) there was no correlation between the magnitude of the bronchial responsiveness and the levels of IgE antibodies induced in individual animals. Thus, during the first treatment period, when strong bronchial reactivity was observed, the levels of specific IgE antibodies were low or undetectable in serum, bronchial fluid and supernatants from cultured lymph node cells. During the second immunization period, increased levels of IgE antibodies were recorded, particularly in the supernatants from cultured regional lymphoid cells and in the bronchial washings. The IgE antibody levels in serum, however, were generally low. This supports the findings by others that IgE antibodies are produced regionally (5, 18). On the other hand, the mast cells which were abundant around the vessels in the lung during the early phase of immunization declined in numbers with time. Possibly, minute amounts of IgE antibodies not detected in the assays, acting in con-

cert with mast cells induced by the immunization procedure, were sufficient to induce an allergic reaction after challenge, even early during the observation period. It should be pointed out that the localization of mast cells in the lungs after the s.c. immunization is quite different from that seen after aerosol immunization, which drives the mast cells towards the bronchioli and also to the interstitium (1, 3). We also measured the serum levels of IgG2a antibodies. This subclass has been claimed to possess reaginic activity in the rat (19). However, there was no correlation between either the levels of IgG2a or the levels of IgG antibodies and airway responsiveness. This supports the recent findings by Dahlbäck et al. (9).

Another possible mediator of antigen-specific allergic inflammation is the T-cell derived factor with "IgE-like" activity on basophils and mast cells, recently described in relation to cutaneous basophil hypersensitivity by Askenase and co-workers (4). In addition to specific parameters of immunity, unspecific factors including the degree of hyperreactivity of the airways and the releasability of mediator substances from effector cells (16) may also play a role in bronchial responses.

The magnitude of the responses to intravenous challenge could possibly have been influenced by reactions to the preceding airway challenge. This possibility is less likely, since there was no correlation between magnitude of reactions after airway provocation and subsequent intravenous challenge. Furthermore, during the second immunization period the reactions after airway challenge appeared to be followed by an increased responsiveness to subsequent i.v. challenge. The decreased reactivity to airway provocation during the second treatment period occurred despite increasing IgE antibody levels and increasing numbers of mast cell precursors in the regional lymph nodes. The responses to i.v. challenge were possibly influenced by the formation of immune complexes with anaphylactic properties as discussed in a previous paper (3). The influence of such immune complexes might explain the slightly increased reactivity to i.v. challenge during the later phases of the study. On the other hand,

increased levels of specific IgG antibodies could possibly also decrease the absorption of antigen (10) and, consequently, the reaction to airway challenge (21, 25, 26).

This study shows that antigen-specific bronchial reactivity can be achieved in rats after daily s.c. injections of small amounts of antigen. There was no significant correlation between the magnitude of the physiological responses and cell-mediated or humoral immunity, or appearance of mast cells and mast cell progenitors in the regional lymph nodes and the lungs. The animal model allows studies of parameters of immunity and physiological responses, related to the allergic inflammatory reaction of the lung.

#### ACKNOWLEDGEMENTS

The technical assistance of Jenny Borwell, Ingrid Hammarström and Magnus Lundberg is greatly appreciated.

#### REFERENCES

- Ahlstedt, S., Björkstén, B., Nygren, H. & Smedegård, G.: Induction of humoral immunity and pulmonary mast cells in mice and rats after immunization with aerosolized antigen. *Immunology* 48, 247-254, 1983.
- Ahlstedt, S. & Björkstén, B.: Specific antibody responses in rats and mice after daily immunization without adjuvant. *Int. Arch. Allergy Appl. Immunol.* 71, 293-299, 1983.
- Ahlstedt, S., Smedegård, G., Nygren, H. & Björkstén, B.: Immune response in rats immunized with aerosolized antigen. Antibody formation, lymphoblastic responses, and mast cell and mucous cell development in relation to bronchial reactivity. *Int. Arch. Allergy Appl. Immunol.* 72, 71-78, 1983.
- Askenase, P. W., Rosenstein, R. W. & Ptak, W.: T-cells produce an antigen binding factor with in vivo activity analogous to IgE antibody. *J. Exp. Med.* 157, 862-873, 1983.
- Befus, A. D., Johnston, N., Berman, L. & Bienenstock, J.: Relationship between tissue sensitization and IgE antibody production in rats infected with the nematode *Nippostrongylus brasiliensis*. *Int. Arch. Allergy Appl. Immunol.* 67, 213-218, 1982.
- Carswell, F. & Oliver, J.: The respiratory response in sensitized rats to aerosol challenge. *Immunol.* 34, 465-470, 1978.
- Church, M. K.: Correlation of anaphylactic bronchoconstriction with circulating reagin antibody levels and active cutaneous anaphylaxis in the rat. *Immunology* 29, 627-634, 1975.
- Dahlbäck, M.: Antigen-induced bronchial anaphylaxis in actively sensitized SD rats. *Allergy* 36, 583-595, 1981.
- Dahlbäck, M., Pauwels, R., Bazin, H. & Bergstrand, H.: Relation between bronchial reactivity to antigen in vivo and serum IgE and IgG2a antibody levels in rats. *Allergy* 37, 569-580, 1982.
- Geoghean, W. D., Dawson, C. A. & Calvanico, N. J.: The effect of IgG and IgG fragments on the absorption of inhaled antigens across the air-blood barrier of isolated perfused rabbit lungs. *Immunology* 44, 331-337, 1981.
- Hall, E., Ahlstedt, S. & Kristofferson, A.: Boosterable IgE antibody response in mice without the use of adjuvant. *Int. Arch. Allergy Appl. Immunol.* 67, 96-99, 1982.
- Holt, P., Rose, A. H., Batty, J. E. & Turner, K. J.: Induction of adjuvant-independent IgE responses in inbred mice: primary, secondary and persistent IgE responses to ovalbumin and ovomucoid. *Int. Arch. Allergy Appl. Immunol.* 65, 42-50, 1981.
- Immunological adjuvants. Report of a WHO Scientific group I. Tech. Rep. Ser. 595, Geneva 1976.
- Ishizaka, K.: Cellular events in the IgE antibody response. In Kunkel & Dixon (eds.): *Adv. Immunol.* vol. 23, p. 1. Academic Press, New York, 1976.
- Jarrett, E. E. & Steward, D. C.: The significance of circulating IgE. Correlation of the amount of circulating reaginic antibody with cutaneous sensitivity in the rat. *Immunology* 24, 37-45, 1973.
- Lichtenstein, L. M. & Conroy, M. C.: The "releasability" of mediators from human basophils and granulocytes. *Excerpta Medica* 414, 109-115, 1977.
- Manning, J. K. & Drewes-Prochniak, M.: IgE antibody responses induced by repeated administration of antigens without adjuvant. *Proc. Soc. Exp. Biol. Med.* 170, 367-372, 1982.
- Mayrhofer, G., Bazin, H. & Gownas, J. L.: Nature of cells binding anti-IgE in rats immunized with *Nippostrongylus brasiliensis*. IgE synthesis in regional nodes and concentration in mucosal mast cells. *Eur. J. Immunol.* 6, 537-545, 1976.
- Morse, H. C., Block, K. J. & Austen, K. F.: Biological properties of rat antibodies. II. Time course and appearance of antibodies involved in antigen induced release of slow reacting substance (SRS-A<sup>rat</sup>); association of this activity with rat IgG. *J. Immunol.* 101, 658-663, 1968.
- Nordvall, S. L., Grimmer, Ø., Karlsson, T. & Björkstén, B.: Characterization of the mouse and rat IgE antibody responses to timothy pollen by means of crossed radio-immunoelectrophoresis. *Allergy* 37, 259-264, 1982.
- Patterson, R., Tannenbaum, J. J., Pruzansky, J. J. & Nelson, V. L.: Canine antiragweed serum. Demonstration of "blocking" activity by the in vivo and in vitro techniques. *J. Allergy* 36, 138-146, 1966.
- Pauwels, R.: Relationship between bronchial reactivity and immediate hypersensitivity. *Eur. J. Respir. Dis.* 63, 63-86, 1982.
- Piechuta, H., Smith, M. E., Share, N. N. & Holme, G.: The respiratory response of sensitized rats to challenge with antigen aerosols. *Immunology* 38, 385-392, 1979.
- Revillard, J. P.: Delayed hypersensitivity. In Bach, J. F.

- (ed.): Immunology, 2nd ed., pp. 365–398. John Wiley and Sons, New York, Chichester, Brisbane, Toronto, Singapore, 1982.
25. Shampain, M. P., Behrens, B. L., Larson, G. L. & Hanson, P. M.: An animal model of late pulmonary responses to *Alternaria* challenge. *Am. Rev. Respir. Dis.* 126, 493–498, 1982.
26. Swineford, O. & Wang, L.: Specific desensitization. IV. Passive desensitization with heterologous antiserum protects against intravenous but not against inhaled antigens. V. Active desensitization with antigen protects against both inhaled and intravenous antigens. *Ann. Allergy* 22, 423–428, 1964.

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