

Relation between Bronchial Reactivity to Antigen *in Vivo* and Serum IgE and IgG_{2a} Antibody Levels in Rats

MAGNUS DAHLBÄCK,¹ ROMAIN PAUWELS,² HERVE BAZIN³ and HÅKAN BERGSTRAND¹

¹Research and Development Laboratories, AB Draco, Lund, Sweden, ²Dept. of Respiratory Diseases, State University, Ghent, and ³Experimental Immunology Unit, University of Louvain, Brussels, Belgium

Sprague Dawley rats were immunized with graded doses of ovalbumin (OA) together with alum. The capacity of the animals to produce a bronchial anaphylactic response to intravenous antigen challenge was related to serum OA-IgE and OA-IgG_{2a} antibody levels estimated by radioimmunoassay. A significant correlation between bronchial anaphylactic response capacity and OA-IgE antibody levels was found under a few, but not all, of several carefully chosen experimental conditions. No correlation was demonstrable between the *in vivo* reactivity of the animals and their serum levels of OA-specific IgG_{2a} antibodies.

Key words: bronchial anaphylaxis; IgE and IgG_{2a} antibody levels; ovalbumin sensitized; SD rats.

Accepted for publication 15 May 1982

CLINICAL ASPECTS

It is now established in humans that immediate type allergic reactions are primarily mediated by IgE antibodies. The present investigation examines whether the rat is a suitable experimental model for studies of the correlation between bronchial anaphylactic response and IgE serum antibody levels. The results show that the degree of bronchial anaphylactic response was correlated to serum titre of OA-IgE antibodies only under certain well-defined circumstances but not to OA-IgG_{2a} antibodies.

It is now firmly established in humans that immediate type allergic reactions are primarily mediated by a special class of antibodies known as IgE. In allergic disorders, the levels of IgE antibodies to the pertinent allergen(s) are usually elevated. Pharmacological reduction of high IgE antibody levels should therefore be a possible approach to the treatment of atopic disorders (15).

The rat should conceivably be a suitable experimental animal in the search for agents with IgE antibody reducing properties, because when appropriately sensitized, rats produce IgE antibodies that can be accurately determined by radioimmunoassay. Furthermore, the animals are sufficiently large to permit relevant studies on the physiological significance of a reduced IgE antibody response.

However, the literature contains a number of reports indicating that there is no simple correlation in this species between the titre of IgE antibodies and the degree of allergic tissue reactivity. Thus, antigen-induced release of histamine *in vitro* from mast cells, lung tissue, mesenteric tissue, or skin does not necessarily vary with serum titres of IgE antibodies estimated by passive cutaneous anaphylactic (PCA) tests or radioimmunoassay (23, 27, 30, 31, 32). Similarly, the correlation between antigen-induced bronchial anaphylactic reactivity and serum reagent titres is not unequivocal (5, 6, 24, 29). Several possible explanations for these discrepancies have been suggested but the item is not clarified.

We recently described in some detail the temporal pattern of development of the bron-

chial anaphylactic response capacity *in vivo* in Sprague Dawley (SD) rats (9). The animals used in those studies were immunized according to various regimens chosen to stimulate preferentially IgE antibody synthesis. In the present investigation the degree of response in this test was studied for any correlation with the serum titre of OA-IgE antibodies, OA-IgG_{2a} antibodies, the level of total IgE, or the ratio OA-IgE antibody/total IgE.

MATERIAL AND METHODS

Animals and immunization

Outbred male Sprague Dawley (SD) rats of SPF status were used, preliminary experiments having shown this strain to be suitable for respiratory measurements. The animals were obtained from Møllegaards Avlcentrum, Ejby, Skensved, Denmark, weight about 200 g. They were reared under standard animal housing conditions. Sensitization was performed by either of the following two procedures.

A. Intraperitoneal injections of the indicated dose of ovalbumin (OA; Sigma grade III, crystallized and lyophilized) in 0.5 ml saline containing 100 mg of dried and resuspended Al(OH)₃ (F 2200 Reheis Chemical Company, obtained through AB Astra, Södertälje). At least 1 h before injection the adjuvant was resuspended in the antigen solution.

B. In each of the four footpads intracutaneous injections of 0.05 ml were given of an emulsion of equal parts of OA-solution and FCA (Difco Lab, Detroit, Michigan).

Tests of anaphylactic reactivity in vivo

Respiratory measurements

At the indicated time after immunization, the animals were taken to test. They were anaesthetized with 50 mg/kg i.p. pentobarbital (Mebumal®, ACO, Sweden); 5 mg/kg succinylcholine chloride (Celocurin®, Vitrum, Sweden) was administered i.p. to prevent spontaneous

respiration. Respiratory measurements were made essentially as described by Stotland & Share (29).

The trachea was cannulated and the animal was ventilated with a Braun constant respiratory pump in a closed system. Tracheal pressure was recorded through a side arm of the cannula connected to a Satham P23AC pressure transducer. The respiratory rate was set at 80 strokes/min with a tracheal pressure of 10 cm H₂O (stroke volume range 2–3.75 ml). Throughout the experiment the mean arterial blood pressure was recorded by a Satham P23AC pressure transducer via a catheter in the right carotid artery. The blood pressure pulses were transmitted to a tachogram, where the heart rate was registered. All the recordings were made on a Grass polygraph model 7B. The animals were challenged with the intravenous injection of a specified dose of OA in 0.5 ml saline through a catheter in the right jugular vein. 300 µg OA was injected initially. The resulting increase in the tracheal pressure was recorded. As soon as this response had developed (within 1–2 min) 5000 µg OA was injected and the resulting response was taken as the maximum attainable. Separate experiments showed that the preceding injection of 300 µg OA under the present circumstances did not influence the response to the 5000 µg dose. We are aware of the fact that this does not apply to bronchial anaphylactic reactions in the rat under all experimental conditions.

OA-IgE antibody and total serum IgE levels were measured according to procedures described in detail elsewhere (20, 21). Blood was withdrawn from each animal by orbital bleeding or from the jugular vein at the indicated time after immunization; serum was stored at –20°C until examined.

Measurement of OA-IgG_{2a} antibody levels. The radioimmunoassay for the measurement of ovalbumin-specific IgG_{2a} levels was performed as for measuring specific IgE levels (1). The quantity of IgG_{2a}-antibodies was expressed as a percentage of the total amount of radioactivity added. All the samples obtained in each ex-

periment were analysed during the same immunoassay run.

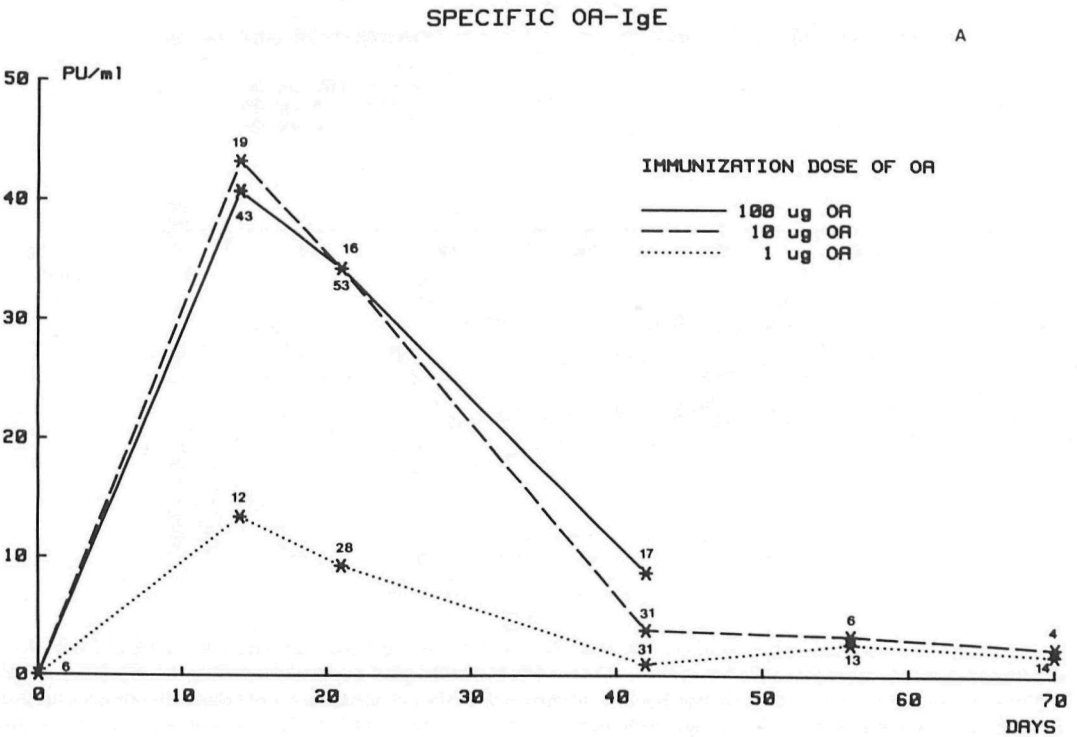
RESULTS

Serum levels of OA-IgE antibodies, total IgE, and the ratio of OA-IgE/total IgE as a function of OA dose and time after immunization

We previously reported in detail the pattern of temporal development of the response capacity in SD rats immunized i.p. with varying doses of OA and 100 mg Al(OH)₃ with respect to bronchial anaphylactic reactivity (9). Fig. 1 in the present report shows the temporal development, in such animals, of OA-IgE antibody levels (frame A), of total IgE levels (frame B), and of the calculated ratio OA-IgE antibody to total IgE (frame C) in rats immunized with 1, 10, or 100 µg OA together with 100 mg Al(OH)₃. The rats that provided the sera and whose numbers are included in these graphs, were those examined for bronchial anaphylactic

response capacity (9). It is clear from this figure that the pattern of development of the capacity to produce OA-IgE antibodies was similar in animals given 1 µg OA, 10 µg OA, or 100 µg OA, the magnitude of the response being significantly higher in the last two groups of animals than in the first. The maximum response of total IgE levels was observed on day 21 in all the groups; at that time, animals given 1 µg OA show the highest total IgE level.

The difference in total IgE levels at this time between the groups given 1 µg OA and 10 µg OA was significant ($P < 0.01$; Wilcoxon test). This led to the interesting observation that (Fig. 1C) the ratio OA-IgE/total IgE apparently followed a biphasic temporal pattern of development after injection of 1 µg and of 10 µg OA. Thus, this calculated ratio was almost two orders of magnitude higher on day 14 and day 56 than on day 42 for these two immunization regimens. However, this was not so in animals immunized with 100 µg OA.



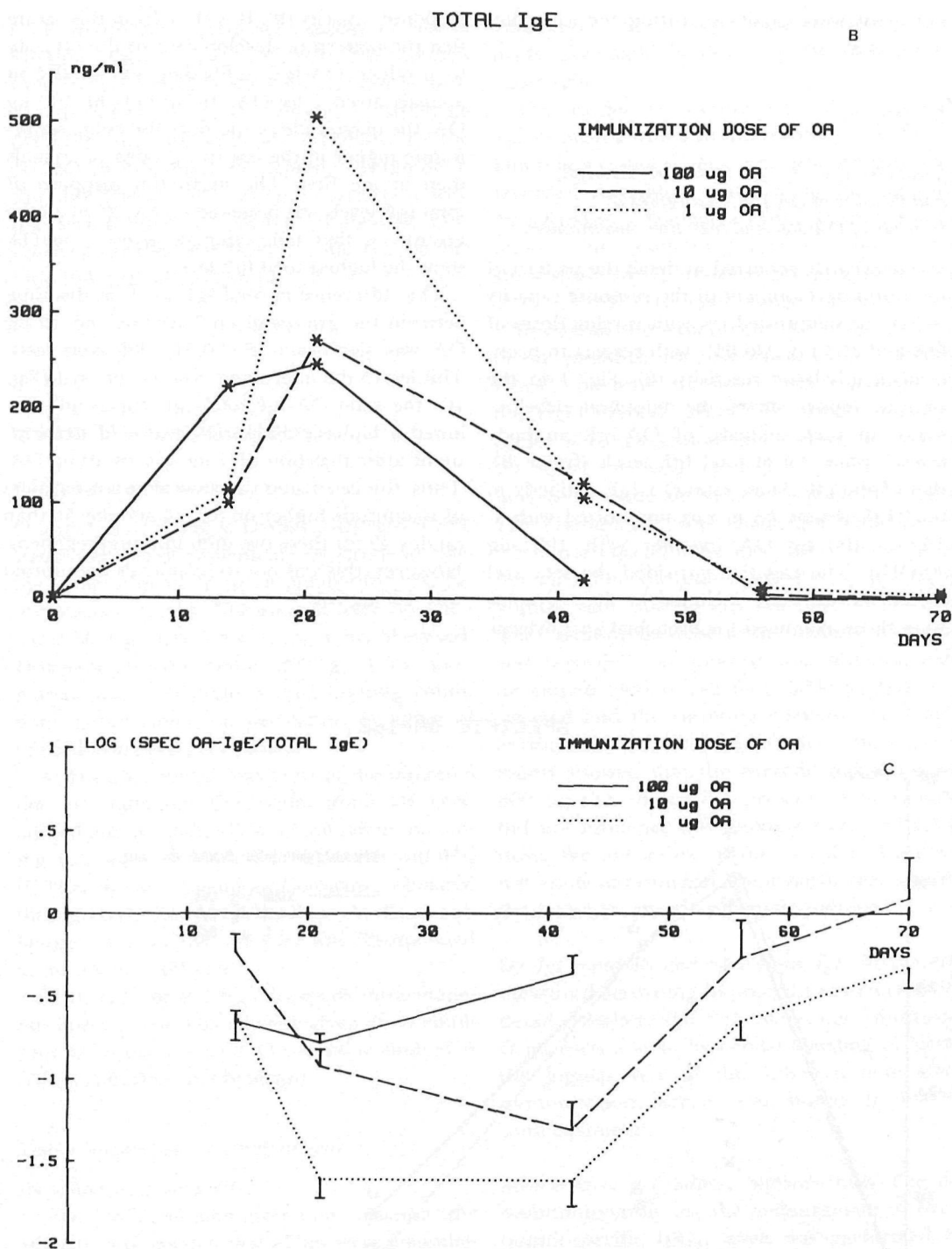


Fig. 1. Temporal development of levels of OA-IgE antibodies (A, previous page), total IgE (frame B), and log of ratio (R) of specific OA-IgE/total IgE (frame C) in SD rats immunized with 1, 10 or 100 μ g OA together with 100 mg dried and resuspended $Al(OH)_3$. In frames A and B, each point represents the median value of the indicated number of animals; in frame C, the mean values $\pm$ SEM are given (ratios R are normally distributed).

Relation of serum levels of OA-IgE antibodies, total IgE, and the ratio OA-IgE/total IgE to anaphylactic reactivity

In Fig. 2, the median of the serum levels of OA-IgE antibodies and the mean of the ratio OA-IgE/total IgE of selected groups of animals are compared with the mean response of the pertinent groups with respect to bronchial anaphylactic reactivity *in vivo*. Fig. 2 represents the data collected from animals given 10 μ g OA in 100 mg Al(OH)₃. Note that the OA-IgE antibody levels and the ratio OA-IgE/total IgE decreased with time, whereas the bronchial

anaphylactic reactivity following a low challenge dose initially decreased slightly but then persisted unchanged, and that recorded after a high challenge dose increased with time.

Fig. 3 shows the data obtained with animals tested 42 days after immunization with 1, 10, or 100 μ g OA. Note that now the OA-IgE antibody levels and the ratio OA-IgE/total IgE increased with the immunization doses of OA, whereas the bronchial anaphylactic reactivity *in vivo* following a low, but not a high, challenge dose of OA seemed to decrease with increasing immunization doses of OA.

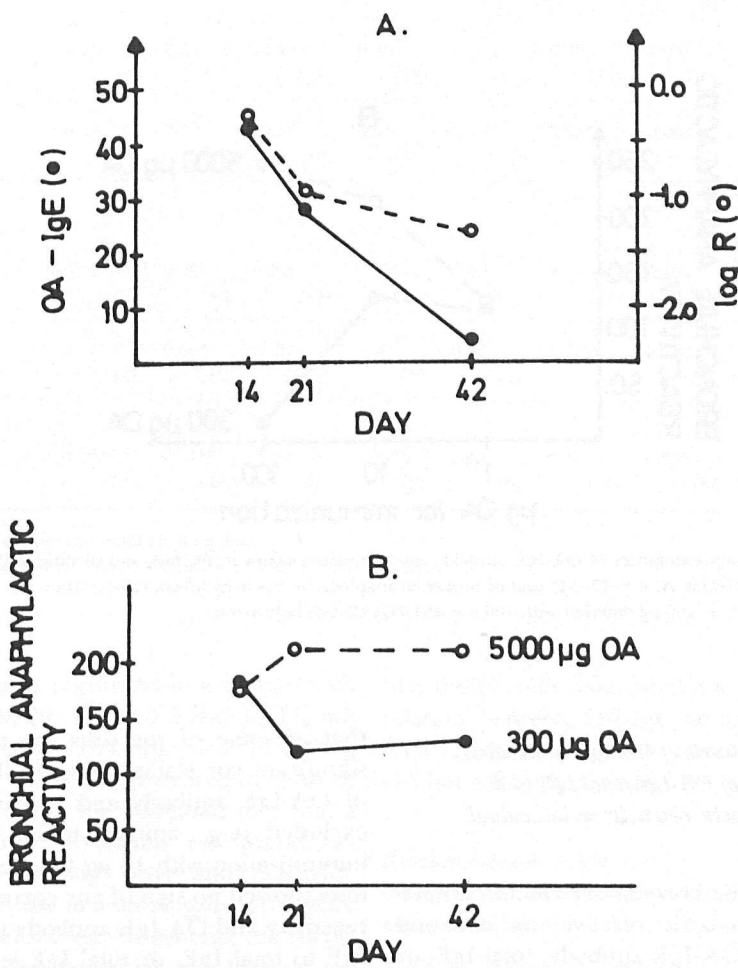


Fig. 2. A schematic representation of the temporal development of OA-IgE antibody levels (median values in PU/ml), and ratio R (OA-IgE/total IgE; mean values shown) (frame A; $n = 10-25$), and of bronchial anaphylactic reactivity (mean values, data from (9)) (frame B; $n = 44-50$) in SD rats immunized with 10 μ g OA together with 100 mg Al(OH)₃.

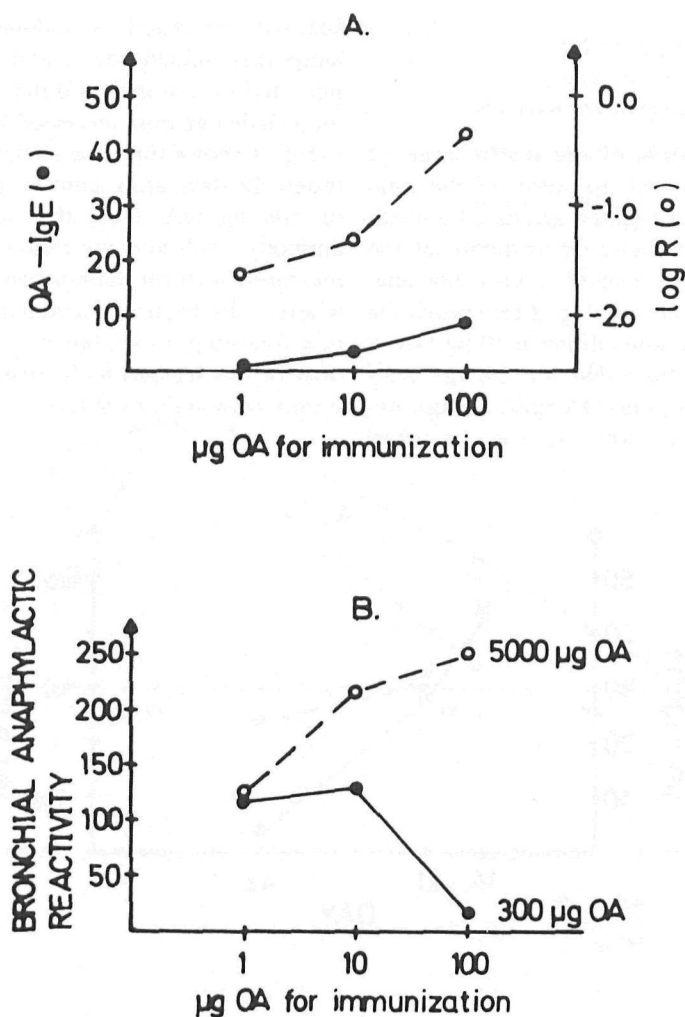


Fig. 3. A schematic representation of OA-IgE antibody levels (median values in PU/ml), and of ratio R (OA-IgE/total IgE; mean values shown) (frame A; $n = 17-31$), and of bronchial anaphylactic reactivity (mean values) (frame B; $n = 23-44$) in rats immunized with 1, 10, or 100 μg together with 100 mg $\text{Al}(\text{OH})_3$ 42 days before test.

Relation of serum levels of OA-IgE antibodies, total IgE, or ratio of OA-IgE/total IgE and bronchial anaphylactic reactivity in individual SD rats

Table 1 shows the correlations found between bronchial anaphylactic reactivity *in vivo* and serum levels of OA-IgE antibody, total IgE, or the ratio of OA-IgE antibody to total IgE in rats immunized with 1 μg , 10 μg , or 100 μg of OA 14, 21, or 42 days before the test. It is clear

that in some of the tests the possibility of a significant correlation between the serum level of OA-IgE antibody and reactivity cannot be excluded (e.g., animals tested 14 days after immunization with 10 μg OA), but most of the tests showed no sign of any correlation between reactivity and OA-IgE antibody titre, ratio OA-IgE to total IgE, or total IgE level. It is noteworthy that several animals immunized with 100 μg of OA and $\text{Al}(\text{OH})_3$ 42 days before the test showed significant levels of OA-IgE anti-

Table 1

Coefficients (r) of correlation between bronchial anaphylactic reactivity (in each animal recorded after a challenge dose of 300 μ g OA: $\Delta P\%$, or at a subsequent challenge with 5000 μ g OA: ΔP max %) and specific OA-IgE antibody level, total IgE, or ratio R defined as specific OA-IgE antibody/total IgE

	Immunization dose and time of test								
	1 μ g OA			10 μ g OA			100 μ OA		
	14 days	21 days	42 days	14 days	21 days	42 days	14 days	21 days	42 days
Spec. OA-IgE vs									
$\Delta P\%$	-0.44 (12)	-0.21 (10)	0.13 (13)	0.59** (18)	0.16 (11)	0.56 (11)	0.05 (30)	0.13 (31)	-0.17 (17)
ΔP max %	0.42 (12)	0.45 (10)	0.16 (13)	0.26 (18)	-0.42 (11)	0.64* (11)	0.03 (27)	0.44** (29)	0.82*** (17)
Total IgE vs									
$\Delta P\%$	-0.20 (12)	0.02 (10)	0.33 (13)	0.20 (18)	0.04 (11)	0.29 (11)	-0.05 (30)	0.17 (31)	0.05 (17)
ΔP max %	0.45 (12)	-0.32 (10)	0.13 (13)	0.04 (18)	-0.19 (11)	0.62* (11)	-0.40* (27)	-0.07 (29)	0.31 (17)
Log $\frac{\text{Spec. OA-IgE}}{\text{Tot. IgE}}$ vs									
$\Delta P\%$	-0.52 (12)	0.02 (10)	-0.08 (13)	0.48* (18)	0.09 (11)	-0.55 (11)	0.10 (30)	-0.07 (31)	-0.26 (17)
ΔP max %	0.31 (12)	0.66* (10)	0.32 (13)	0.10 (18)	-0.22 (11)	-0.77** (11)	0.26 (27)	0.41* (29)	0.37 (17)
Spec. OA-IgE vs Tot. IgE	0.76** (12)	0.51** (28)	0.26 (31)	0.59** (19)	0.43 (16)	-0.22 (31)	0.33* (43)	0.37* (53)	0.36 (17)

Figures in brackets denote the number of animals.

Probability levels *0.01 < P < 0.05; **0.001 < P < 0.01; *** P < 0.001.

bodies (the figures registered in a group of six animals were 29, 20, 17, 14, 7.5 and 3.7 PU/ml) yet did not react to a challenge dose of 300 μ g OA. However, when the provocation dose of OA in these animals was increased to 5 mg, a highly significant correlation was found between OA-IgE antibody titre and reactivity. This was not the case in animals given 10 μ g OA 14 or 21 days before test. In general, the correlation between the bronchial anaphylactic reactivity and antibody level was not increased by substituting the ratio of OA-IgE antibodies to total IgE for the OA-IgE. It is also noteworthy

that the present data showed a convincing correlation between OA-IgE antibody levels and total IgE level in animals immunized with 1 μ g OA but not in those given 100 μ g OA.

Relation between serum level of OA-IgG_{2a} antibodies and bronchial anaphylactic reactivity in vivo

Table 2 shows that the bronchial anaphylactic response capacity *in vivo* is not correlated to serum levels of specific IgG_{2a} antibody. Animals injected with various doses of OA in FCA

Table 2

Bronchial anaphylactic reactivity (BAR) (mean $\pm$ SEM) of % increase in intratracheal pressure following a challenge dose of 300 μ g OA (Δ P %) or a subsequent challenge with 5000 μ g OA (Δ P max %) and specific OA-IgG_{2a} serum antibody levels (median) in selected groups of animals immunized as indicated

Immunization regimen	Day(s) for test	BAR		OA-IgE PU/ml	IgG _{2a} (% of total radioactivity)	(n)
		Δ P %	Δ P max %			
10 μ g OA + FCA	14	49 $\pm$ 20	205 $\pm$ 10	6.5	39	(6)
100 μ g OA + FCA	15	20 $\pm$ 3	203 $\pm$ 8	7.5	59	(6)
10 mg OA + FCA	14	29 $\pm$ 5	200 $\pm$ 31	1.4	54	(6)
10 μ g OA + Al(OH) ₃	41–43	177 $\pm$ 23	203 $\pm$ 13	7	25	(11)

Figures in brackets denote the number of animals in each group.

showed a poor bronchial anaphylactic response capacity (at a low challenge dose of OA) but high OA-IgG_{2a} antibody levels; those given a low immunization dose of OA together with Al(OH)₃ reacted vigorously to the same challenge dose despite low OA-IgG_{2a} antibody levels.

DISCUSSION

Our investigation was concerned with the relation, in rats, between serum levels of specific IgE- and IgG_{2a} antibodies, on the one hand, and bronchial anaphylactic response capacity *in vivo*, on the other. A number of previously published studies have failed to demonstrate a simple correlation between serum IgE antibody titres, histamine release from a variety of tissues *in vitro* and/or antigen-induced anaphylactic reactivity *in vivo* (e.g. 5, 6, 24, 29). With this in mind, it is clearly noteworthy that a highly significant correlation between serum OA-IgE antibody levels and bronchial anaphylactic reactivity, precipitated by a high dose of antigen, was in fact recorded by us in animals immunized with 100 μ g OA 42 days before test, although the serum OA-IgE antibody titres at that point of time were already declining. A correlation, though less close, was also found between bronchial anaphylaxis (precipitated by a low antigen dose) and OA-IgE antibody levels in animals given 10 μ g OA 14 days before the test. However, under other very similar experimental

conditions no correlation was observed, despite high serum levels of OA-IgE antibody and considerable bronchial anaphylactic reactivity. How can this difference be explained?

Possible general explanations should first be considered to account for the lack of correlation between response capacity and OA-IgE antibody level under certain experimental conditions.

Firstly, Conroy et al. (7), who used basophilic leukocytes, observed that only a small number of cell-fixed IgE antibodies were necessary to prepare these cells for histamine release. Furthermore, cells from individuals having approximately the same number of IgE varied widely in their capacity to release histamine when challenged with anti-IgE. The authors concluded that an intrinsic property of human basophils, which they referred to as "releasability", is an important parameter in determining mediator release and that this releasability differs from individual to individual. However, available data are not yet sufficient to establish that such a parameter is important in the determination of the degree of rat mast cell histamine release *in vitro*.

Secondly, many factors may interfere with mediator release and that this releasability differs from individual to individual. However, and mediators of inflammation (3, 25). It is also well known that histamine release from rat peritoneal mast cells *in vitro*, induced by IgE-dependent secretagogues, is markedly en-

hanced by certain phospholipids, e.g., phosphatidyl serine (16) and that on incubation *in vitro* with human basophils certain viruses enhance ragweed antigen E or anti-IgE induced histamine release (11).

Thirdly, some normal individuals with specific IgE antibodies, and manifesting antigen-induced histamine release from basophilic leukocytes, do not exhibit any manifestation of disease on natural exposure to allergen (19). Furthermore, there is no correlation between severity of disease and serum level of specific IgE antibody (4, 26); especially in asthmatics, the mild immediate type of allergen-induced bronchospasm seems to depend more on non-specific bronchial hyperreactivity than on the level of circulating specific IgE antibody (26). In rats bronchial reactivity to administered exogenous serotonin varies from strain to strain (22).

Fourthly, in our investigation antigen was administered intravenously, preliminary experiments having indicated that, in our hands, even high doses (10 mg/ml) of OA administered as an aerosol failed to precipitate a response. Antigen given i.v. may have induced changes in lung mechanics through mediators released from extrapulmonary organs and have reached the lung through the circulation.

It is clear that all such mechanisms might conceivably contribute to a weak correlation between degree of release and antibody level. However, the existence of a highly significant positive correlation between OA-IgE serum antibody levels and response capacity under some, but not all, of the conditions studied suggests that nonspecific factors such as releasability, endogenous hormones, degree of bronchial reactivity and route of antigen administration are of little value in the search for an explanation of the lack of correlation under certain circumstances. This we consider a crucial point.

It would rather seem that the lack of a general correlation between serum antibody level and bronchial anaphylactic response capacity has an immunological basis. Firstly, is a correlation masked by the longer half-life of tissue-bound IgE compared with serum IgE? Sydbom & Karlsson (30) observed a significant, although

not convincing, correlation between peritoneal mast cell response capacity and OA-IgE antibody level on day 11 after immunization, but not later. However, in our experiments, serum levels of OA-IgE were still high 3 weeks after immunization with both 1 and 10 μ g OA, indicating that any such masking cannot be the major reason for the lack of correlation at that point in time. Furthermore, serum levels of OA-IgE antibody were lower in the animals immunized 42 days before the test with 100 μ g OA (those showing a highly significant correlation) than in most groups of animals examined 14 or 21 days after immunization.

Secondly, is the correlation dependent on the level of nonspecific IgE? In the human basophilic leukocyte system, Zeiss et al. (33) found no correlation between the level of allergen-specific IgE in plasma and the number of molecules of allergen-specific IgE per basophil, but rather a correlation between the ratio of allergen specific IgE to total serum IgE and the last-mentioned variable. Cell reactivity, i.e., the highest attainable degree of histamine release at any antigen concentration, was dependent on the number of allergen-specific IgE molecules per basophil, but cell sensitivity, i.e., the lowest antigen concentration inducing a defined degree of histamine release, was not. However, in the present system, we did not find a better correlation with bronchial anaphylactic response to a low or to a high antigen provocation dose when the ratio OA-IgE antibody to total IgE was substituted for the OA-IgE antibody level. Thus, from data published by Jarrett et al. (13), who showed that active anaphylactic reactions in rats are not influenced by *Nippostrongylus brasiliensis*-induced increases in non-specific IgE; and from the present data, it seems that the effect of total IgE is not sufficient to explain the above-mentioned lack of correlation.

Thirdly, Jarrett & Stewart (12) suggested that detectable levels of IgE antibodies might not appear in serum until IgE fixing sites in the pertinent tissues have been saturated, i.e., fixation could conceivably withdraw OA-IgE antibodies from the circulation. However, in our experiments, animals immunized with 10 or

100 μ g OA showed high serum levels of OA-IgE antibodies but there was still no convincing correlation between OA-IgE titre and bronchial anaphylactic reaction to a low challenge dose in any of these groups, except in those mentioned above.

Fourthly, *in vivo* elimination by blocking non-reaginic antibody of the challenge dose of antigen from the circulation could conceivably mask a correlation. Blocking antibody could have another profile of production or avidity maturation than reaginic antibody. However, in the present experiments this is not a likely explanation because even following a low challenge dose of antigen, there is a sufficient amount of it present to precipitate a vigorous physiological response (see (9)). Furthermore, increasing the challenge dose more than tenfold did not increase the degree of correlation (except for animals immunized with 100 μ g OA 42 days before test).

Fifthly, a protein antigen and an outbred rat strain were used in our experiments. It is not clear to what extent the outcome of the experiments is influenced by the effect of genetic polymorphism on clonotypic expression of IgE and IgG_{2a}, average affinity of antibodies present and degree of heterogeneity of antigenic determinants on OA.

We previously showed (9) that animals immunized with 10 μ g OA and 100 mg Al(OH)₃ tended to develop a bronchial anaphylactic response capacity, as revealed by a low challenge dose of OA according to a "biphasic" pattern; a high degree of reactivity on day 14 is followed by a lower degree of reactivity which is of similar magnitude on day 21 and 42 (Fig. 3). A qualitative difference in the antibodies mediating the two parts of the temporal response development curve is suggested by the finding that some anti-allergic agents are able to inhibit the bronchial anaphylactic reactivity late, but not early, after immunization (9). A "biphasic" pattern of reaginic antibody synthesis is apparently not a novel observation, since data published by Petillo & Smith (23) (Table 1) and by Jarrett et al. (14) may also be interpreted as showing the existence of a "biphasic" time course of the appearance of reaginic antibodies

in the serum of rats immunized with alum precipitated OA. In our hands, in the early part of the bronchial anaphylactic response capacity curve, the reactivity of rats immunized with 10 μ g OA showed some degree of correlation with the OA-IgE antibody levels, indicating that IgE-antibodies could, at least partly, mediate the response at this time. No correlation was demonstrable between reactivity and OA-IgE antibody levels in animals tested during the latter part of the temporal response curve, or in the group of animals immunized with only 1 μ g OA. But it is not known why. In this context, it should be pointed out that Church (6) reported that both the severity of an active anaphylactic bronchoconstriction and the level of circulating reaginic antibodies (PCA test) in rats sensitized with *Nippostrongylus brasiliensis* did not reach a maximum until 5 weeks after the injection. As pointed out by Church (6), these data deviate from those of Jarrett & Stewart (12) on the temporal development of the active cutaneous anaphylactic reaction; the last-mentioned data conform to the temporal profile of IgE antibody synthesis in the present report.

Thus, the possibilities of several homocytotropic antibodies contributing to the response cannot be dismissed. In this context the results of Yamamoto et al. (32) deserve consideration. These authors found that within individual animals the relative degrees of antigen-induced histamine release from actively sensitized lung tissue, mesenteric tissue, and skin were not correlated to each other or to the level of circulating antigen-specific reagin. Our experiments (2) showed that the development of lung tissue response capacity (estimated by antigen-induced release of histamine *in vitro*) can apparently be qualitatively and temporally differentiated from that of serosal mast cells.

The short-term sensitizing homocytotropic IgG_{2a} antibody (for review see 28) is a conceivable candidate for mediating at least part of the bronchial anaphylactic responses either 14–21 days or 6 weeks after immunization. However, the role played by this kind of antibody can be questioned. Firstly, we examined rats immunized with various amounts of OA in Freund's complete adjuvant. This regimen is known to

induce high levels of OA-IgG_{2a} antibodies, which was confirmed. However, such animals showed no more than a weak bronchial anaphylactic response capacity following a low challenge dose of OA.

Secondly, we found no bronchial anaphylactic responses to a challenge dose of 300 µg of OA 42 days after immunization when the immunization dose of OA was increased from 10 to 100 µg OA (Fig. 2). There is only little information available about the dependence of the level or the pattern of development and persistence of IgG_{2a} antibody responses in rats on the antigen dose used at immunization. However, like the synthesis of mouse IgG₁ antibody (18) one would hardly expect that reactivity due to IgG_{2a} antibody activity would decrease with increasing immunization doses. However, such a phenomenon is well known in IgE antibody synthesis.

It must be stressed that we have not yet succeeded in consistently transferring responses with serum passively. Thus, unfortunately, we cannot clearly state whether the antibodies mediating the anaphylactic responses are thermolabile. Characterization of the homocytotropic antibodies responsible for the bronchial anaphylactic reactivities at various points in time obviously requires further research.

Together with a number of other reports our observations in rats suggest that more than one type of reagenic antibody might be responsible for sensitization of lung tissue for anaphylactic reactivity. We have previously reported data indicating a dissociation in the development of lung and tracheal tissue response capacity in SD rats (2) with respect to histamine release from chopped tissue *in vitro*. Lehrer & Bazin (17) recently reported that rat IgE myeloma proteins are heterogeneous in PCA inhibitory capacity. Also in humans the existence of IgE-antibody subclasses has been considered on the basis of a differential capacity to passively sensitize lung tissue and basophils (8, 10). Our peculiar observation that the ratio OA-IgE/total IgE in animals given 1 µg or 10 µg OA follows a biphasic temporal pattern of development (Fig. 1C) could be interpreted as strengthening the possibility of subclasses of IgE with different kinetics of production.

In conclusion, our data indicate that lung tissue response capacity *in vivo* is correlated to OA-IgE antibody levels provided the experimental conditions are carefully controlled. However, no correlation between tissue reactivity *in vivo* and serum OA-IgE antibody level is demonstrable under a number of other experimental conditions. The reason for this is not clear, but one possible explanation is the existence of several reagenic antibody subclasses contributing to the response.

ACKNOWLEDGEMENTS

Ulla Larsson kindly typed the manuscript.

This work was supported in part by grants from FGWO Belgium No. 3.0008.80 and contract NIH AI 12840.

REFERENCES

1. Bazin, H. & Pauwels, R.: The rat as experimental model of human immediate type hypersensitivity. *Progr. Allergy* 1982. (In press).
2. Bergstrand, H., Frick, I. M., Lindqvist, V., Lundqvist, B., Nyström, I. & Schurmann, A.: Antigen-induced release of histamine from rat tissues *in vitro*: Dissociation in development of serosal mast cell, lung tissue and tracheal tissue response capacity. *Int. Arch. Allergy* 68, 342–351, 1982.
3. Bourne, H. R., Lichtenstein, L. M., Melmon, K. L., Henney, C. S., Weinstein, Y. & Shearer, G. M.: Modulation of inflammation and immunity by cyclic AMP. *Science* 184, 19–28, 1974.
4. Bruce, C. A., Norman, P. S., Rosenthal, R. R. & Lichtenstein, L. M.: The role of ragweed pollen in autumnal asthma. *J. Allergy Clin. Immunol.* 59, 449–459, 1977.
5. Carswell, F. & Oliver, J.: The respiratory response in sensitized rats to aerosol challenge. *Immunology* 34, 465–470, 1978.
6. Church, M. K.: Correlation of anaphylactic bronchoconstriction with circulating reagenic antibody level and active cutaneous anaphylaxis in the rat. *Immunology* 29, 527–534, 1975.
7. Conroy, M. C., Adkinson, N. F. & Lichtenstein, L. M.: Measurement of IgE on human basophils: relation to serum IgE and anti-IgE induced histamine release. *J. Immunol.* 118, 1317–1321, 1977.
8. Conroy, M. C., Adkinson, N. F. Jr. & Lichtenstein, L. M.: Passive sensitization of human basophils: Evidence for heterogeneity in the IgE molecule. *Int. Arch. Allergy Appl. Immunol.* 60, 106–109, 1979.
9. Dahlbäck, M.: Antigen-induced bronchial anaphylaxis in actively sensitized SD rats: Effects of immunization and

- provocation doses of antigen, and of pretreatment with DSCG, theophylline, terbutaline and a new antiallergic xanthine derivative, D 4026. *Allergy* 36, 583–595, 1981.
10. Godfrey, R. C., Gradidge, C. F., Hawksley, M. R. & Elliot, E. V.: Differences in binding affinity of human IgE for receptors in chopped human lung. *Immunology* 35, 581–587, 1978.
 11. Ida, S., Hooks, J. J., Siraganian, R. P. & Nothius, A. L.: Enhancement of IgE-mediated histamine release from human basophils by viruses: Role of interferon. *J. Exp. Med.* 145, 892–906, 1977.
 12. Jarrett, E. E. & Stewart, D. C.: The significance of circulating IgE. Correlation of amount of circulating reaginic antibody with cutaneous sensitivity in the rat. *Immunology* 24, 37–45, 1973.
 13. Jarrett, E. E. E., Mackenzie, S. & Bennich, H.: Parasite-induced nonspecific IgE does not protect against allergic reactions. *Nature* 283, 302–304, 1980.
 14. Jarrett, E. E. E., Hall, E., Karlsson, T. & Bennich, H.: Adjuvants in the induction and enhancement of rat IgE responses. *Clin. Exp. Immunol.* 39, 183–189, 1980.
 15. Katz, D. H.: Prospects for the clinical control of IgE synthesis. In Schwartz, R. S. (ed.): *Progress in clinical immunology*, Vol. 4, pp. 127–150, Grune & Stratton, New York, 1980.
 16. Kazimierzczak, W. & Diamant, B.: Mechanisms of histamine release in anaphylactic and anaphylactoid reactions. *Progr. Allergy* 24, 295–365, 1978.
 17. Lehrer, S. B. & Bazin, H.: Heterogeneity of IgE myeloma passive cutaneous anaphylaxis (PCA) inhibition. Abstr. 13.4.12 IV Int. Congr. Immunol. Paris, 1980.
 18. Levine, B. B. & Vaz, N. M.: Effect of combinations of inbred strain, antigen, and antigen dose on immune responsiveness and reagent production in the mouse. *Int. Arch. Allergy* 39, 156–171, 1970.
 19. Marsh, D. H., Lichtenstein, L. M. & Norman, P. S.: Induction of IgE-mediated immediate hypersensitivity to group I rye grass pollen allergen and allergoids in non-allergic man. *Immunology* 22, 1013–1028, 1972.
 20. Pauwels, R., Bazin, H., Platteau, B. & Van Der Straeten, M.: In vitro measurement of specific rat IgE antibodies. *Ann. Immunol. (Inst. Pasteur)* 128C, 675–685, 1977.
 21. Pauwels, R., Bazin, H., Platteau, B. & Van Der Straeten, M.: The measurement of total serum IgE levels in rats. *J. Immunol. Methods* 18, 133–140, 1977.
 22. Pauwels, R., Bazin, H. & Van Der Straeten, M.: Relation between non specific bronchial reactivity and the immediate type bronchoconstriction in the rat. Abstr. 13.1.20 IV Int. Congr. Immunol. Paris, 1980.
 23. Petillo, J. & Smith, S. R.: Reaginic response in rats to alum precipitated antigens. I. Time course of appearance of anti-egg albumin reagins and potentiation of the response with *Nippostrongylus brasiliensis*. *Int. Arch. Allergy* 44, 309–320, 1973.
 24. Piechuta, H., Smith, M. E., Share, N. N. & Holme, G.: The respiratory response of sensitized rats to challenge with antigen aerosol. *Immunology* 38, 385–392, 1979.
 25. Plaut, M. & Lichtenstein, L. M.: Pharmacologic control of mediator release. In Bach, M. K. (ed.): *Immediate hypersensitivity modern concepts and developments*, pp. 503–532. Marcel Dekker, Inc., New York and Basel, 1978.
 26. Popa, V.: Respiratory allergy to ragweed: Correlation of bronchial responses to allergen with bronchial responses to histamine and circulating immunoglobulin E. *J. Allergy Clin. Immunol.* 65, 389–397, 1980.
 27. Smith, S. R., Petillo, J. & Hwang, A.: Further studies with the potential reagin response to egg albumin. *Int. Arch. Allergy* 43, 145–187, 1972.
 28. Stechschulte, D. J.: Non-IgE homocytotropic antibody in animals. In Bach, M. K. (ed.): *Immediate hypersensitivity. Modern concepts and developments*, pp. 259–276. Marcel Dekker Inc., New York and Basel, 1978.
 29. Stotland, L. M. & Share, N. N.: Active bronchial anaphylaxis in the rat. *Can. J. Physiol. Pharmacol.* 52, 1114–1118, 1974.
 30. Sydbom, A. & Karlsson, T.: Relationship between serum IgE levels and anaphylactic histamine release from isolated rat mast cells. *Acta Physiol. Scand.* 107, 313–318, 1979.
 31. Wilson, R. J. M. & Block, K. J.: Homocytotropic antibody response in the rat infected with the nematode, *Nippostrongylus brasiliensis*. II: Characteristics of the immune response. *J. Immunol.* 100, 622–628, 1968.
 32. Yamamoto, S., Seo, K., Fujiwara, Y., Maeda, M. & Yamura, T.: Correlation between in vitro anaphylactic histamine release from tissues and reagent titer in rat. *Int. Arch. Allergy* 47, 329–338, 1974.
 33. Zeiss, C. R., Pruzansky, J. J., Levitz, D. & Wang, J.: The quantification of IgE antibody specific for ragweed antigen E (AgE) on the basophil surface in patients with ragweed pollenosis. *Immunology* 35, 237–246, 1978.

Address:

Magnus Dahlbäck
 Research and Development Laboratories
 AB Draco
 Box 1707
 S-221 01 Lund
 Sweden

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.