

Histamine Release from Human Leucocytes by IgG4 Subclass in the Sera of Allergic Children

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Our studies on histamine release from normal washed leucocytes sensitised with sera of children allergic to various inhaled and ingested allergens show that besides IgE, IgG4 present in these sera was capable of sensitising leucocytes of normal donors, and these leucocytes released histamine on challenge with anti-IgG4. By contrast, antisera of other subclasses of IgG released very small amounts of histamine from sensitised leucocytes. A discordant correlation between skin tests and RAST IgE was observed in some children. Significantly greater amounts of histamine were released from normal leucocytes sensitised with serum from these children when challenged with anti-IgG4 compared to anti-IgE. These studies indicate that IgG4 merits further study as a reaginic marker in atopic diseases of the children with possible prognostic significance.

Key words: allergen; histamine release; homocytotropic antibody; leucocytes.

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It has been clearly established now that IgE is a principal reaginic antibody in man. This property of IgE in mediating immediate type hypersensitivity has resulted in the gradual acceptance of an immunological definition of the atopic person as being, "one who readily makes IgE antibodies to allergens to which he or she is exposed" (6, 7). However, in a number of patients, IgE is not raised, and allergen specific IgE has not been found despite positive skin tests to allergens (13). Investigations during the 1960's suggested that there was, in addition to IgE, a homocytotropic antibody in man which belonged to the IgG class of immunoglobulin. Research to date on IgG type homocytotropic antibody has been mainly confined to adults and the results are controversial (10, 11, 12, 13). Atopic allergy is more prevalent in children compared to adults. It has been reported (1) that above normal concentration of IgG4 in serum may indicate a poor prognosis in childhood asthma. Despite such reports, there is a paucity of data on the

role of IgG4 in childhood allergy. We have carried out experiments to ascertain whether sera of children with atopic disease contained IgG4 immunoglobulin capable of sensitizing leucocytes of normal non-allergic donors and whether such leucocytes released histamine on challenge with anti-IgG4. Results from our preliminary investigations on histamine release, RAST IgE and skin tests, together with data from the non-atopic control population, suggest that IgG4 merits a further study in atopic children.

MATERIAL AND METHODS

Patients

Thirty children aged between 1 and 14 years (mean age 6.5 years) were included in the study. Some of these were inpatients and others were attending the asthma and gastroenterology clinics at the Queen Elizabeth Hospital for Children. The criteria for atopic disease were as

follows: 1) flexural distribution of the eczema at some time during the disease, 2) history of asthma or hay fever, 3) family history of asthma, atopic eczema, or hay fever, 4) positive prick tests to common allergens (i.e. pollens, animal fur, house dust mite), 5) positive RAST-IgE to the skin test positive allergens, and 6) serum IgE concentrations > 180 units/ml.

Swelling of the face, reddening of the ears and slight swelling of abdomen were seen in those children who manifested allergy to cow milk, fish, eggs and wheat. None had history of abdominal pains or diarrhoea following ingestion of foods.

Of the 30 children, 10 were classified as allergic to foods such as cow milk and egg white, eight gave history of allergy to cow milk and eggs, and two were allergic to cow milk only. Six of the patients allergic to cow milk and eggs also had severe wheezing episodes and were found to be skin test positive with allergens such as pollen, animal fur, house dust, and house dust mite. In the group presenting with eczema (6 patients of 30) one was allergic to foods, three had eczema with asthma and two had eczema only. Fourteen children (included in this group were two children with allergic rhinitis) gave history of allergy to pollens, house dust mite, cat and dog dander.

Controls

Because of the ethical problem involved in taking blood from healthy children for research purposes, sera from 10 hospitalised children, age range 2 to 14 years (mean 5.7 years) were used as controls. These children were referred to the children's hospital for a variety of disorders, none of which were of an apparently atopic nature. These children were having blood samples taken for other reasons. All sera were stored at -70°C .

Skin tests

Skin tests were performed as per instructions of Bencard leaflet for allergy diagnosis and scored either as 1, 2, 3, 4, 5, or +, ++, +++,

++++, depending on the size of the weal.

Antisera

Heavy chain specific antisera directed against human IgG subclasses were supplied by the Department of Pathology at the University of Birmingham (UK). Rabbit anti-IgE was obtained from Mercia Brocades Ltd., (West Byfleet, Surrey, UK).

Buffers

Following buffers were used: Tris-A-buffer pH 7.3 contained Tris (hydroxymethyl aminomethane 0.025 M; NaCl 0.120 M; kcl 0.005 M; and human serum albumin 0.3 mg/ml). Tris-ACM buffer which was used for the histamine release reaction contained all the constituents of Tris-A-buffer and calcium and magnesium ions at final concentrations of 6×10^{-4} M and 1×10^{-3} M, respectively. Sensitising buffer consisted of Tris A, heparin 10 $\mu\text{g/ml}$, EDTA at a concentration of 4×10^{-3} M and 0.5 ml of reaginic or control serum in a total volume of 5 ml.

Polymorphonuclear leucocytes

Normal polymorphonuclear leucocytes were obtained from non-allergic adults aged between 22 and 35. Blood collected in EDTA bottles was mixed with dextran in 0.9% saline (6 ml dextran 110:10 ml blood) in polycarbonate tubes and allowed to stand at room temperature for 60 to 90 min. When the erythrocytes had sedimented sufficiently, the leucocyte-rich supernatant fluid was carefully aspirated and transferred to polycarbonate tubes. These were centrifuged at 100 g for 15 min at 4°C and the plasma was discarded. The button of cells was gently suspended with a siliconized Pasteur pipette and washed three times in chilled Tris-A-buffer. Leucocytes were then passively sensitised with non-atopic and atopic serum by suspending the leucocytes in a prewarmed sensitising buffer at 37°C (4). Sensitised leucocytes were then washed twice and assayed for

their capacity to release histamine on challenge with antisera to IgG subclasses and anti-IgE. In a separate experiment, leucocytes from nine normal individuals were washed three times and challenged with antisera to human IgE and IgG4 and percentage of total cellular histamine release was recorded.

Histamine assay

The spectrophotofluorometric technique (9) with modifications (5) was used. The reaction mixture supernates were deproteinized with perchloric acid, extracted into alkalized butanol, and then re-extracted from heptane-butanol into 0.1 N HCl. The acid phase was removed and the histamine conjugated with o-phthalaldehyde to form a fluorophore. In view of the instability of histamine in alkaline solutions, the extraction of the test sample into butanol was modified so as to add the sodium hydroxide as the last reagent in this step. The tubes were then shaken immediately. In this manner, significant and unpredictable losses of histamine were avoided. Formation of the fluorophore was carried out for 40 min at 4°C. Fluorescence was measured with a Perkin Elmer spectrofluorimeter model LC 1000, the excitation wavelength was 360 nm and emission wavelength was 450 nm. A reference calibration

curve with histamine dihydrochloride was constructed for each experiment. The percentage of histamine released, i.e. the fraction of the histamine released from sensitised leucocytes on challenge with a given antiserum, was calculated with the aid of the expression:

$$\frac{E-CB}{CC-CB} \times 100$$

where E represents histamine release in (ng) in the experimental tube and CC (cell complete) which measures the total histamine content of the sensitized cells whereas CB (cell blank) measures the non-specific histamine release in the presence of reagents used. All the tubes, i.e. experimental, cell blank and cell complete, were set up in triplicate.

Allergen-specific IgE antibodies were measured by the radioallergosorbent test (RAST) using the commercial kit (Pharmacia UK Ltd.). Antisera were titrated out to establish optimal dilution needed to effect maximal release of histamine from sensitised leucocytes.

Radioimmunoassay

A Phadebas IgE test kit (Pharmacia UK) was used to determine whether IgG subclass antisera contained any cross-reacting anti-IgE antibodies.

Table 1

Anti-IgE and anti-IgG4 induced histamine release (%) from leucocytes of normal individuals before and after sensitisation with non-atopic sera

Number	Anti-IgE		Anti-IgG4	
	Unsensitised	Sensitised	Unsensitised	Sensitised
1	25	28	25	26
2	20	18	20	24
3	20	18	21	19
4	25	28	21	22
5	14	15	13	15
6	22	20	20	22
7	13	10	15	20
8	25	25	22	25
9	8	10	10	10
10	29	26	-	-
Mean	20.1	19.8	18.6	20.3

Table 2

Histamine release from leucocytes of nine normal individuals upon challenge with antihuman IgE and IgG4 antisera

Number	Percentage release	
	Anti-IgE	Anti-IgG4
1	36	25
2	4	36
3	33	N.R.
4	27	N.R.
5	45	51
6	12	26
7	N.R.	8
8	8	4
9	35	26
Mean	22.2 %	19.6 %

N.R.: No release of histamine means no release above the non-specific release of histamine when sensitised leucocytes are challenged with relevant antisera.

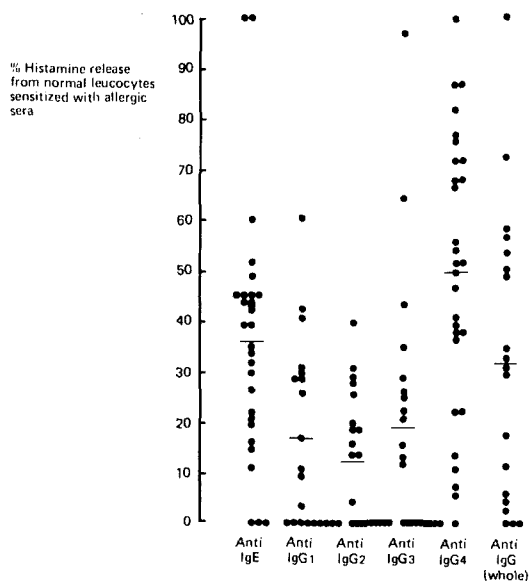


Fig. 1. Histamine release from leucocytes of normal donors sensitised with reaginic sera of allergic children and challenged with antibodies to IgE, IgG1, IgG2, IgG3, IgG4 and IgG (whole).

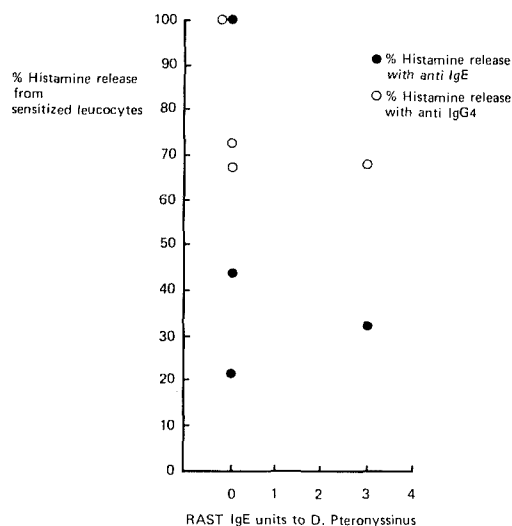


Fig. 2. RAST IgE versus histamine release from normal leucocytes sensitised with sera of children with strong skin reactions to *D. pteronyssinus*.

RESULTS

^{125} I-IgE did not bind detectable amounts of any L6IgG subclass antisera when tested in a radioimmunoassay. It would therefore seem unlikely that IgG subclass antisera were contaminated

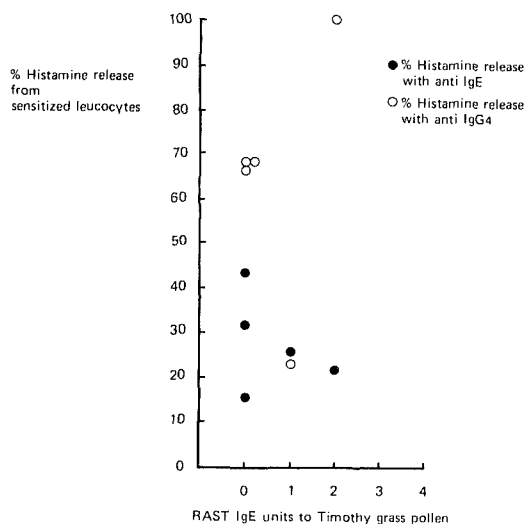


Fig. 3. RAST IgE versus histamine release from normal leucocytes sensitised with sera of children with strong skin reactivity to Timothy grass pollen.

with anti-IgE. Maximal amounts of histamine were released with 1:1000 dilution of anti-IgE serum, whereas anti-IgG4 serum released maximal amounts of histamine at a dilution of 1:100. Fig. 1 shows the histamine release from the leucocytes sensitised with allergic sera. Significant amounts of histamine were released from the leucocytes sensitised with allergic sera when challenged with antihuman IgE and antihuman IgG4, while lower responses were obtained with antisera to other subclasses, mean release with anti-IgG1 = 17 %, anti-IgG2 = 12.5 %, anti-IgG3 = 19 % and anti-IgG4 = 50 %. In many cases, a greater amount of histamine was released on challenge with antihuman IgG4 than with antihuman IgE. In others, antihuman IgE released an amount of histamine equal to or greater than antihuman IgG4. By contrast, sera from 10 non-atopic children failed to prepare leucocytes for a similar response (Table 1). Low levels of histamine release were obtained from washed normal donor leucocytes on challenge with anti-IgE and anti-IgG4, with a mean release of 22.2 % with anti-IgE and 19.6 % with anti-IgG4 (Table 2). A discordant correlation is seen between RAST and release of histamine (a point illustrated by Figs. 2-5). These were instances in which the

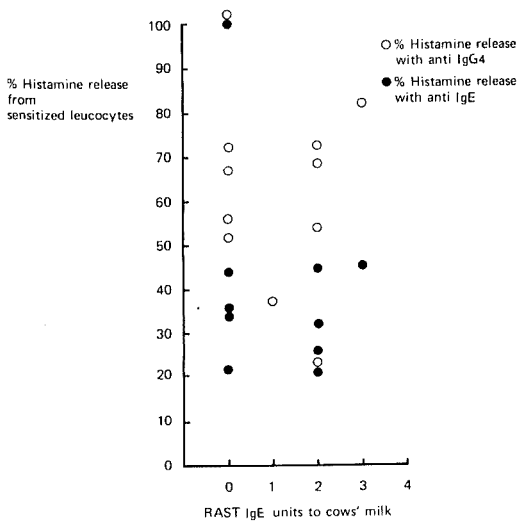


Fig. 4. RAST IgE versus histamine release from normal leucocytes sensitised with sera of children allergic to cow milk.

RAST with relevant allergens is negative or weakly positive, but in which there is a significant release of histamine from sensitised normal leucocytes on challenge with anti-IgG4 compared to anti-IgE. Patients allergic to *D. pteronyssinus* and Timothy pollen gave strong skin reactions. Children allergic to cow milk and egg white showed symptoms of food allergies as described under the Material and Methods section.

DISCUSSION

Although leucocytes for passive sensitisation were obtained from apparently normal adult donors who denied any history of allergy, there were instances (Table 2) when a considerably higher percentage of total cellular histamine was released from unsensitised leucocytes. It is possible that some of these normal donors were mildly atopic without clinical symptoms but who, nevertheless, tended to produce appropriate antibodies in moderate quantities which reacted with appropriate antisera to trigger release of considerably larger amounts of their cellular histamine. Variation in histamine release from leucocytes after passive sensitisation has been reported (3). Despite the mark-

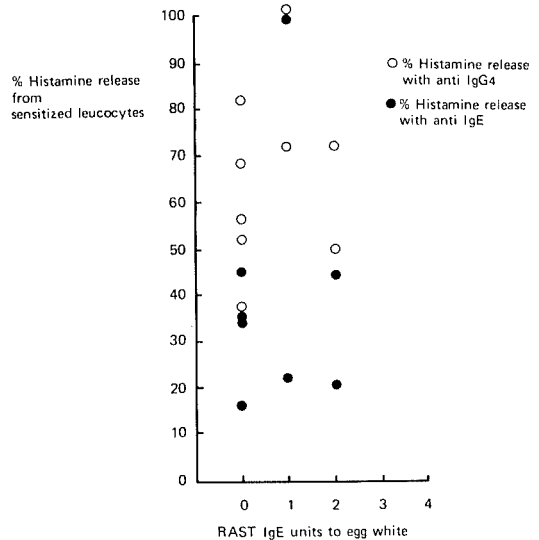


Fig. 5. RAST IgE versus histamine release from normal leucocytes sensitised with sera of children allergic to egg white.

edly individual characteristics of leucocytes, results of the present study suggest that normal leucocytes may be primed with allergic sera, and after washing may be induced to release histamine on challenge with anti-IgE and anti-IgG4. From these studies it may be inferred that atopic children who present clinically with pronounced eczema, hay fever and asthma have the ability to produce IgG4 antibody besides IgE antibody. High concentrations of IgE and IgG4 have been reported in a relatively high proportion of adults with atopic dermatitis (8). This may be explained by the fact that atopic children who produce both IgE and IgG4 and who present clinically with pronounced eczema, hay fever and asthma may retain in adult life the ability to produce these immunoglobulins in high concentrations.

Imperfect correlations between skin tests and RAST and the significant amounts of histamine released when sensitised leucocytes are challenged with anti-IgG4 compared to anti-IgE in the patients who had strong skin reactions to *D. pteronyssinus* and Timothy pollen (Figs. 2 and 3) may be explained by the fact that skin test reactivity in these patients may be due to the presence of IgG4 reagins. Discordant correlations between skin tests and *in vitro* RAST

therefore do not necessarily imply that skin tests are false positive or more sensitive than RAST. Whereas RAST is specific for IgE antibody, the skin test and release of histamine make no distinction between antibody classes. Negative or weakly positive RAST IgE in patients clearly allergic to milk and egg and in whom anti-IgG4 released significantly greater amounts of histamine from sensitised leucocytes compared to anti-IgE (Figs. 4 and 5) may also be explained by the fact that allergy to certain ingested allergens is mediated by IgG4 rather than IgE. There is some evidence in adults to suggest that IgG4 antibodies are indeed involved in egg and milk allergy (2) and that subjective improvement was obtained by avoidance of egg and milk in these patients. The clinical relevance of the exact role of IgG4 in childhood allergy may become clearer by the pursuance of studies on the release of histamine together with measurement of subclass specific antibody levels. These studies are presently in progress.

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