

A method for the simultaneous determination of serum and cell-bound IgE

K. R. ALLEN, S. OSMOND and W. C. ALSTON

Department of Chemical Pathology, Frimley Park Hospital, Camberley, Surrey

Summary

A method for determining the amount of total IgE bound to leucocytes has been devised by measuring that released on incubating various cell number dilutions in acetate buffer pH 4.0. A linear relationship between IgE released and cell number was obtained over a limited concentration range. The method seemed to work satisfactorily with 50 ml of whole blood for those subjects with very high serum levels of total IgE. However, with subjects having a slightly elevated or normal serum IgE level it may be necessary to use twice the amount of blood.

Introduction

The cross-linking of IgE molecules on the surface of the mast cell by antigen (anaphylaxis) or by an IgE antibody (reversed anaphylaxis) is believed to be the triggering process leading to the release of chemical mediators of the allergic response (Ishizaka, Tomioka & Ishizaka, 1970; Ishizaka *et al.*, 1972; Ishizaka, Soto & Ishizaka, 1973; Becker *et al.*, 1973; Lewis *et al.*, 1975). An understanding of the mechanism of this process may eventually lead to various theoretical means of its modification. For example, it has been suggested that allergic diseases may be prevented or treated by inhibiting the attachment of the specific reaginic antibody to the mast cell. This could be achieved by: (a) inducing high IgE responsiveness by artificial infection with parasites; (b) treatment of the patient with myeloma IgE; (c) treatment with short chain peptides (fragments of the IgE heavy chain) (Editorial, 1976).

The purpose of these treatments is to displace or inhibit the attachment of the specific IgE molecules to the mast cell surface. If such therapy is introduced it may become important both in the investigation and monitoring of treatment in patients to determine the amount of IgE bound to the mast cells. Since methods have not yet been developed to perform this determination in man, we sought to develop a relatively simple laboratory method of measuring the amount of IgE both in the serum and that bound to basophil leucocytes, which have been shown to be the only blood cells with specific receptors for this immunoglobulin class (Ishizaka *et al.*, 1970, 1972, 1973; Becker *et al.*, 1973; Ishizaka & Ishizaka, 1974; Lewis *et al.*, 1975).

Methods

Preparation of basophil leucocyte fraction

This was carried out as described by Harris & Ukaejiofo (1970).

A Ficoll-Hypaque gradient was prepared, composed of 10 vol. of 33.9% Hypaque (sodium diacetoamido, 2,4,6,-triiodobenzoate) added to 24 vol. of 9% Ficoll (Sigma).

Blood, 55 ml, was taken from each patient into a syringe containing 0.5 ml of preservative-free heparin (1000 u/ml) as an anticoagulant. Aliquots of the blood, 10 ml, were then layered onto 10 ml of Ficoll-Hypaque in plastic universal containers and centrifuged for 20 min at 400 g. The buffy layer, containing basophils in a predominantly lymphocyte fraction, was removed and diluted 1:2 with physiological saline. The cells were then spun down at 70 g for 5 min and the supernatant discarded. Saline-albumin 2 ml (0.03% human serum albumin), was added to the cell pellet. This was gently resuspended to remove free serum IgE from the cell medium and the suspension was transferred to an LP3 tube. The cells were again spun down at 70 g for 5 min and resuspended in 2 ml of saline-albumin and a total white blood count performed on the suspension using a Coulter D cell counter.

Release of cell bound IgE

The cell suspension was divided, as in Table 1, and the volumes made up to 2 ml with saline-albumin. Cell pellets were then obtained by centrifuging the tubes at 70 g for

Table 1. The relationship between total IgE (iu) released and cell number. The mononuclear cells were separated by the Ficoll gradient technique (Harris & Ukaejiofo, 1970)

Patient	Cell suspension (ml)	Total mononuclear leucocytes ($\times 10^6$)	Total IgE released (iu)	
A	0.8	20.96	6.6	7.7
	0.6	15.72	3.3	3.9
	0.4	10.48	2.2	2.2
B	1.0	13.50	4.1	4.4
	0.5	6.75	2.8	2.8
	0.3	4.05	1.9	1.7
C	1.0	21.00	5.2	5.0
	0.5	10.05	3.9	3.9
	0.3	6.30	3.0	3.0
D	1.0	12.27	9.9	8.8
	0.5	6.14	3.8	3.3
	0.2	2.45	1.9	1.1
E	1.0	20.30	15.1	13.8
	0.5	10.15	9.4	10.5
	0.25	5.08	3.9	3.3
	0.10	2.03	1.1	1.1
F	1.0	19.9	8.8	8.8
	0.5	9.95	5.5	5.0
	0.25	4.98	1.9	1.4
	0.10	1.99	0.8	0.8

5 min and decanting the supernatants. Acetate buffer, 500 μ l of 0.05 mol/l, pH 4.0, containing 0.085 mol/l NaCl, 0.005 mol/l KCl, 0.01 mol/l EDTA and 0.03% human serum albumin were added to each cell pellet after precooling the buffer to 0°C. The cells were kept in contact with the buffer at this temperature for 10 min. After this time, the tubes were spun down at 900 *g* for 2 min and the supernatants completely decanted into LP3 tubes containing 50 μ l of 0.06 mol/l Tris. The resulting solutions, being adjusted in this way to pH 7.4, contained the cell bound IgE and were kept frozen at -15°C until the radioimmunoassay was performed.

Radioimmunoassay

Both cell-bound and serum IgE were measured for each patient using the Phadebas IgE Kit (Pharmacia).

Haematological studies

Glass slide smears of the cell suspensions were made after first centrifuging 100 μ l of the suspensions and resuspending the cell pellet in homologous plasma. The smears were stained with May-Grunwald and Giemsa stains and differential counts performed to determine the proportion of basophils extracted.

Patients

Two groups of patients were investigated to evaluate the method, patients A, B and C having seasonal allergic rhinitis and patients D and E suffering from atopic eczema. A normal non-atopic subject was also included in the study, patient F.

Results

Basophil content of leucocyte suspensions

The basophil counts for the cell suspensions obtained from each patient are given in Table 2. Ishizaka & Ishizaka (1974) obtained basophil counts of 1.1–5.3% using a similar cell separation technique. The values which we obtained for the basophils as a percentage of the total leucocyte count correspond to a range of 2–5%.

Table 2. The level of serum IgE (iu/ml) and number of basophils in the mononuclear leucocyte suspensions obtained from each patient

Patient	Condition	Serum IgE (iu/ml)	Number of basophils in the mononuclear leucocyte suspension (cells/mm ³)
A	Allergic rhinitis	85	524
B	Allergic rhinitis	35	270
C	Allergic rhinitis	50	630
D	Atopic eczema	2600	245
E	Atopic eczema	2200	1020
F	Non-atopic	280	597

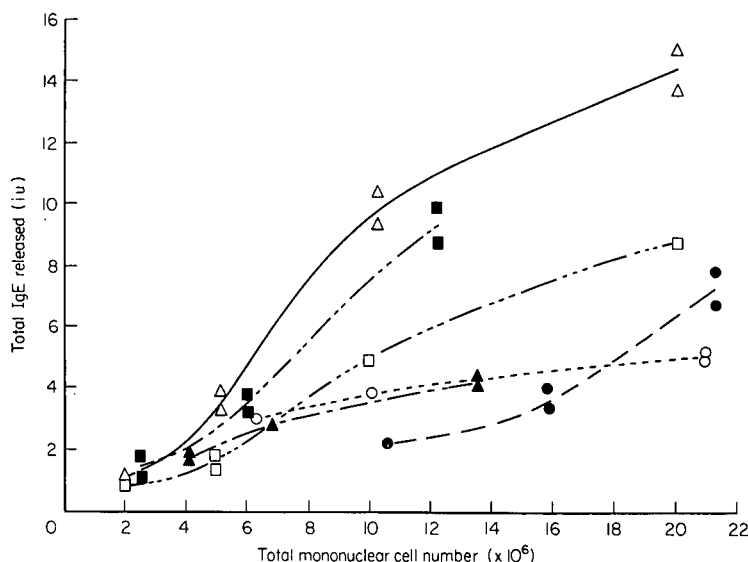


Fig. 1. Patients: A, ●; B, ▲; C, ○; D, ■; E, △; F, □.

Serum IgE

The serum values for IgE are expressed in Table 1 and were measured to make a comparison with the cell-bound levels. The normal range for serum IgE is given as 100–273 u/ml as determined by Nye *et al.* (1975).

Cell-bound IgE

The cell-bound IgE, measured in duplicate, is expressed in Table 2 as the total IgE released. Graphs were drawn of these values against the total cell numbers (Fig. 1).

Discussion

The method which we developed of measuring cell bound IgE took advantage of two separate techniques:

(1) The Ficoll–Hypaque method of removing the bulk of the granulocytes to form a basophil-enriched mononuclear cell fraction (Harris & Ukaejiofo, 1970).

(2) The discovery that when basophil leucocytes are incubated in an acetate buffer pH 4.0 at 0°C for 10 min the attached IgE molecules become dissociated from the cells (Ishizaka & Ishizaka, 1974).

It was considered that by releasing the IgE molecules into an aqueous environment, determination of their concentration would be more directly comparable to that used for serum IgE (Pharmacia Kit method).

There were two difficulties to overcome when developing the technique. A minimum number of cells is necessary in order to measure accurately the total IgE. Also, it was essential to check that a linear relationship between cell number and total IgE dissociated was being obtained. Although the general shape of the curves was sigmoid it is clear that this linear relationship was obtainable over a limited concentration range with each subject. It is reasonable to suppose that at the lower end of the scale the sensitivity of the IgE determination is limiting. However, at the upper end of the scale it is possible that increasing buffering power of the greater cell number also increases

the pH of the incubation medium above pH 4.0, or possibly the elution of the IgE molecules from the increasingly dense mass of cells becomes progressively less efficient. Better recoveries of the IgE molecules by elution of the cells with several changes of buffer were limited by the decreasing sensitivity due to dilution of the final assay. As might be expected, the curves of the subjects D and E with the highest levels of serum IgE, are the furthest to the left of the IgE via cell number plot, i.e. have the highest number of IgE molecules bound per cell and hence the highest number of receptor sites occupied. The curves of A, B and C, the hay fever subjects with the very much lower levels of serum IgE, are much more flat having many fewer molecules bound per cell.

Acknowledgment

The authors wish to acknowledge the help of Dr R. Felix, Consultant Dermatologist, Frimley Park Hospital, who kindly allowed us to study his patients.

References

- BECKER, K.E., ISHIZAKA, T., METZGER, H., ISHIZAKA, K. & GRIMLEY, P.M. (1973) Surface IgE on human basophils during histamine release. *Journal of Experimental Medicine*, **138**, 394.
- EDITORIAL (1976) IgE, parasites, and allergy. *Lancet*, **i**, 894.
- HARRIS, R.G. & UKAEJIOFO, E.O. (1970) Tissue typing using a routine one-step lymphocyte separation procedure. *British Journal of Haematology*, **18**, 229.
- ISHIZAKA, K., TOMIOKA, H. & ISHIZAKA, T. (1970) Presence of IgE and IgG molecules on human leucocytes. *Journal of Immunology*, **105**, 6, 1459.
- ISHIZAKA, T., DE BERNARDO, R., TOMIOKA, H., LICHENSTEIN, L.M. & ISHIZAKA, K. (1972). Identification of basophil granulocytes as a site of allergic histamine release. *Journal of Immunology*, **108**, 4, 1000.
- ISHIZAKA, T. & ISHIZAKA, K. (1974) Dissociation of IgE molecules from basophil receptors at acid pH. *Journal of Immunology*, **112**, 1078.
- ISHIZAKA, T., SOTO, C.S. & ISHIZAKA, K. (1973) Number of IgE molecules and their receptors on human basophil granulocytes. *Journal of Immunology*, **111**, 500.
- LEWIS, R., EOETZL, E.J., WASSERMAN, S.I., VALONE, F.H., RUBIN, R.R.H. & AUSTEN, K.F. (1975) The release of four mediators of immediate hypersensitivity from human leukaemic basophils. *Journal of Immunology*, **114**, 87.
- NYE, L., MERRETT, T.G., LANDON, J. & WHITE, R.J. (1975) A detailed investigation of circulating IgE levels in a normal population. *Clinical Allergy*, **5**, 13.

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.