

IgE LEVELS IN THE SERA OF ASTHMATIC CHILDREN

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SUMMARY

The serum IgE concentrations of children with varying grades of asthma were determined. The mean serum IgE level in each grade corresponded with the severity of asthma in that grade, there being a significantly increased mean serum IgE level in children in the most severely affected grades. Several individuals with pronounced asthma had anomalously low serum IgE levels and conversely a few subjects with no, or minimal clinical evidence of asthma had disproportionately high levels of IgE. An immunopathological interpretation of these findings is discussed.

INTRODUCTION

There has recently been considerable interest in IgE following its identification as a distinct class of immunoglobulin, and its involvement as the major antibody of reaginic type in immediate-type hypersensitivity (Type 1) (Symposium on IgE and Atopic Allergy, 1970). Serum levels of IgE are known to be elevated (>200 ng/ml) in allergic patients (Johansson, 1967), and also in patients infected with *Ascaris* (Johansson, Mellbin & Vahlquist, 1968) and other nematodes. (Hogarth-Scott, Johansson & Bennich, 1969).

A longitudinal epidemiological study of asthmatic and wheezing school children in Melbourne has shown a broad spectrum of clinical manifestations, ranging from those who have a few transient attacks, to those who have severe, persistent asthma (Williams & McNicol, 1969). Examination of a wide range of clinical, biochemical, biological and physiological data in these children strongly supports the hypothesis that these children suffer from a common disorder. These asthmatic children have been grouped into varying grades of severity in terms of frequency of episodes, persistence of history, and clinical and physiological manifestations of asthma (McNicol, Williams & Gillam, 1970; Gillam, McNicol & Williams, 1970).

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It was considered important to determine the IgE levels in the sera of children from each of these grades and from a control group of subjects, there being no previous report of IgE levels in a random sample of children with asthma, nor any attempt to relate IgE levels to different clinical patterns of asthma.

An improved, indirect, radio-immunodiffusion technique was used for the determination of IgE.

MATERIALS AND METHODS

Sera. Samples taken from 10-year-old children suffering from clinically demonstrable asthma of various grades of severity, and from non-asthmatic children, were provided by the Research Foundation of the Royal Children's Hospital, Melbourne. The gradings of asthmatic children were based on the following definitions. Grade 0 (control), children who had never wheezed; Grade 1, children who had wheezed not more than five times; Grade 2, children who had experienced more than five asthmatic attacks, but had not wheezed in the 12 months before examination at 10 years of age; Grade 3, children who had experienced more than twenty asthmatic episodes and had wheezed within 12 months before examination. More detailed clinical and physiological information relating to these gradings is given by McNicol *et al.* (1970).

IgE determination. The technique of radioactive single radial immunodiffusion as described by Rowe (1969) was used to determine serum IgE concentrations with the following minor modifications. (i) The gel was made according to the methods of Fahey & McKelvey (1965). (ii) The ring diameter on the X-ray film was measured by the use of a Nikon Shadowgraph Comparator. The accuracy of this instrument was ± 0.02 mm.

Sheep antihuman IgE, was kindly provided by Dr S. G. O. Johansson and Dr H. Bennich, Blödcentralen, Uppsala, Sweden. 131 Iodine was obtained from the Australian Atomic Energy Commission, Lucas Heights, Sydney.

Reference preparation for IGE. Serum of an *Ascaris* sensitive patient (J.A.W.) known to contain homocytotropic antibody reactive with *Ascaris* allergens in PCA tests in monkeys, was used. It had an IgE titre, as determined by Dr S. G. O. Johansson (Hogarth-Scott, Johansson & Bennich, 1969), of 1785 ng/ml. In addition, a WHO Laboratory Standard serum with an IgE titre of 10,148 ng/ml, was used.

RESULTS

The calibration curve used for the calculation of serum IgE concentrations was derived by measuring the ring diameters of two-fold dilutions of the reference IgE standard following a diffusion time lapse of 48 hr as shown in Fig. 1. A linear relationship was observed by plotting the logarithm of the ring diameter against the logarithm of IgE concentration.

This relationship was valid for ring diameter measurements between 3.70 mm and 9.40 mm, which corresponded to an IgE concentration range of 37–2000 ng/ml. Values greater than 2000 were not included in the determination of the mean.

The frequency distributions of calculated serum IgE concentrations of patients in the four grades are represented by the histograms in Fig. 2 (a, b, c and d).

The frequencies are expressed as percentages of the total number of patients in each grade. Because differing range sizes were selected, the percentage in each range is shown by the area under the histogram.

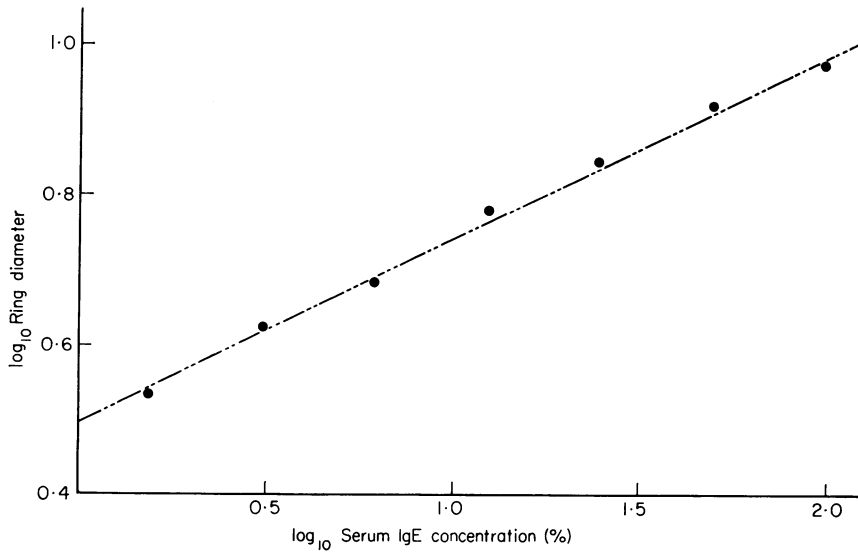


FIG. 1. The calibration curve used for the calculation of serum IgE concentrations using the J.A.W. standard serum. A linear relationship was observed by plotting the logarithm of the ring diameter against the logarithm of serum IgE concentration. Undiluted standard serum was assigned a concentration value of 100%.

Fig. 2 shows that with increasing grade number there was a consistent increase in the percentage of patients with serum IgE concentrations in the higher ranges.

There was no significant difference (*t*-tests) between arithmetic means of consecutive gradings. However, analysis of variance showed an overall significant upward trend in means ($P < 0.01$) with increasing grade number.

It can also be seen from Fig. 2 that some children in Grade 3 have low serum IgE concentrations and that a few children in Grade 0 and Grade 1 have high serum IgE concentrations when compared with the mean values for a given grade. Thus an increase in the frequency and severity of asthma is usually, but not invariably, associated with increase in the serum IgE concentration.

DISCUSSION

The linear relationship between the logarithm of the IgE concentration and the ring diameter at a particular diffusion time is typical of results obtained in the conventional single radial diffusion system (Mancini, Carbonara & Heremans, 1965; Clausen, 1969). Although Rowe (1969) demonstrated a linear relationship between concentrations of antigen and the square of the ring diameter, this applies only after diffusion of antigen from the well has ceased, i.e. after the ring diameter has reached its equilibrium value. We found that 48 hr was not sufficient for all concentrations of IgE to achieve their equilibrium ring diameters, hence a linear relationship between IgE concentration and the square of ring diameter was not observed.

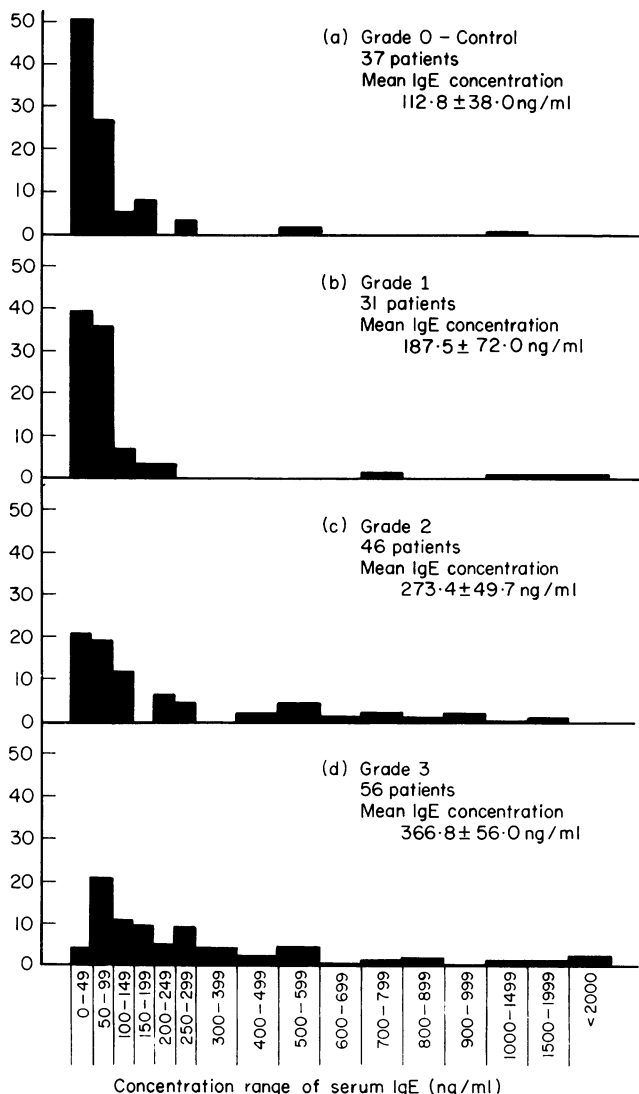


FIG. 2. A comparison between four grades of asthmatic patients (including a control group ○) showing the percentages of patients within particular serum IgE concentration ranges.

The linear relationship between the logarithm of ring diameter and logarithm of concentration empirically gave the best fit to our data. It must be stressed that, with every set of IgE determinations, a calibration curve should be derived which will apply only to that particular group of estimations.

Currently, there are two methods available for the *in vitro* determination of IgE: the radioactive radial immunodiffusion technique employed in this paper, and the radio immunosorbent test (RIST) (Wide, Bennich & Johansson, 1967). Although there is no doubt that both methods are capable of detecting very small quantities of purified protein, the exact

relationship between the two techniques remains to be clarified, especially since each method depends, to some extent, on a different physical property. Nevertheless, Johansson has used the second technique to demonstrate a six-fold increase in serum IgE level of patients suffering from allergic asthma (Johansson, 1968). Our findings, using the first technique, confirm these results and provide further clinical information which shows a correlation between the level of IgE and the frequency and severity of asthma. Rowe & Wood (1970) have recently published independent but similar studies on normal adults and children and children with allergic asthma. Their findings agree closely with the results reported here.

The recent identification of IgE and its involvement in the allergic state has represented an advance in the clarification of the immunopathology of immediate-type hypersensitivity (Type 1). The present results suggest that assay of IgE may have a useful clinical application in the assessment of asthma. The increased serum IgE levels found in association with increasing frequency and severity of asthma probably indicate an increasing production of this immunoglobulin as an essential part of the asthmatic state in children.

However, some controls and mild asthmatic subjects showed very significantly elevated serum levels of IgE, while some severe asthmatics had normal or very low levels of IgE. These anomalies may be accounted for on the following basis: First, subjects with a raised level of IgE who had no asthma, or mild asthma years previously, may have had other allergic manifestations, i.e. urticaria or nematodiasis. The control, and other study groups, were selected solely on the criteria of the presence or absence of asthma, the frequency of episodes, and the persistence of the history at the time of examination. Second, when considering those subjects with severe asthma and a normal level of IgE, it should be understood that serum levels of IgE may not necessarily reflect the antibody status at the cell surface. Current immunopathological interpretation suggests that IgE effects its clinical manifestations in the cell-associated state, rather than as free antibody in the plasma (Simons, Hosking & Hogarth-Scott, 1970). This is in contradistinction to IgG, and its postulated role of blocking antibody following desensitization procedures. There are no *a priori* grounds for assuming that the serum level may parallel the allergic status of the sensitized cell *in vivo*.

Finally, IgE levels represent a general increase in this class of immunoglobulin rather than an increase in specifically-reactive antibody. Further analysis of the elevated IgE level into specifically-reactive components may help identify specific allergens which are responsible for precipitating or aggravating attacks of asthma.

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