

Serum IgE levels in healthy children quantified by a sandwich technique (PRIST*)

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Summary

Serum IgE was estimated in 226 healthy children aged 0–14 years without obvious atopic disease in their first degree relatives. A paper disc radioimmunoassay technique (PRIST) was used. A number of criteria for selection of cases were established. This led to the exclusion of forty-two potentially atopic children. The remaining group of 184 children is considered representative of normal children and may therefore be used for reference purposes. The IgE levels were found to be lower than in previous investigations, and this is attributed to the refined technique and the method of selection.

Introduction

During the middle of the 1960s it was shown independently by Ishizaka, Ishizaka & Hornbrook and by Johansson & Bennich (Ishizaka *et al.*, 1966; Johansson & Bennich, 1967) that reaginic antibodies belong to an until then unknown immunoglobulin class, later called IgE (Bennich *et al.*, 1968). Since then an intense interest has been devoted to reaginic antibodies in healthy, as well as in diseased, individuals. Elevated serum concentrations of IgE in asthma were reported by Johansson (1967). The hope was expressed that IgE determinations would make it possible to discriminate between atopic and non-atopic individuals (Berg & Johansson, 1969a).

The IgE level in children has been shown to increase with age, but wide variations are found between apparently healthy children of the same age group (Johansson, 1968; Berg & Johansson, 1969b). Because of the very low concentrations radioimmunological methods are usually used for the IgE determinations (Wide & Porath, 1966; Wide, 1971; Johansson, Bennich & Berg, 1972). Recently interest has been focused on conditions characterized by extremely low IgE levels, such as atopic allergy in small infants, and the possibility of predicting the development of such conditions at an early stage (Polmar, Robinson & Minnefor, 1972; Foucard, 1974; Hamburger *et al.*, 1974). Methodological improvements enable a more accurate measurement of the low IgE

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levels usually present in the newborn and infants. The present study was motivated by the urgent need to establish the normal IgE values of childhood.

Materials and methods

Selection of serum samples

Children 0–14 years of age were accepted for the study if they fulfilled several conditions. They were selected from consenting families at the Department of Obstetrics (newborns and infants up to 12 months of age), at Child Welfare Centres (1–4 years) and schools (7–14 years) in the district of Linköping, Sweden. About 130 infants of 0–1 year of age were thus selected for examination at random from the 824 non-atopic families, who volunteered out of 1827 families questioned. Similarly about 110 children of 2–14 years of age were accepted for examination. Age and sex distribution is shown in Table 1.

Table 1. Age and sex distribution of children included in the study

Age	Tolerated age variation ±	Number in the initial material		Number in the 'gross' normal material		Number in the 'net' normal material	
		Boys/girls	Total	Boys/girls	Total	Boys/girls	Total
0 days	(cord serum)	15/11	26	15/10	25	14/10	24
6 weeks	6 days	7/14	21	7/13	20	5/12	17
3 months	8 days	14/6	20	11/6	17	9/6	15
6 months	2 weeks	10/10	20	9/10	19	6/9	15
9 months	3 weeks	14/6	20	13/6	19	10/6	16
1 year	4 weeks	14/4	18	11/4	15	9/3	12
2 years	6 weeks	13/7	20	12/7	19	11/7	18
3 years	2 months	6/5	11	5/4	9	3/3	6
4 years	3 months	6/3	9	6/3	9	6/1	7
7 years	6 months	10/9	19	10/9	19	10/8	18
10 years	6 months	11/9	20	9/8	17	9/8	17
14 years	1 year	15/7	22	14/6	20	14/5	19
Total		135/91	226	122/86	208	106/78	184

A total of ten medical criteria for participation were set up. Of these numbers 1–7 below mainly concerned the case history and numbers 8–9 the actual status. The tenth criterion was based on the results of immunochemical screening of a group selected at random among those primarily accepted.

- (1) No atopic disease in the child or its first degree relatives
- (2) No symptoms of food allergy in the child
- (3) No symptoms or signs of contact allergy
- (4) No unexpected reactions noticed at vaccinations
- (5) No abnormal reactions to insect-bites
- (6) No tendency to frequent infections
- (7) No infection during the last 14 days
- (8) No early symptoms of infectious disease at the time of examination

- (9) Normal growth, physical development and nutritional status
- (10) No specific reaginic antibodies detected in serum at RAST-screening

A total number of 226 serum samples from apparently healthy children (those fulfilling the criteria 1-9) were thus collected (Fig. 1). Five children happened to be sampled twice during infancy (e.g. cord blood and at 3 months). Age and sex distribution are shown in Table 1.

A RAST-screening was performed in order to eliminate those with specific reagins in the serum. Six children were excluded because of positive RAST-analyses.

When the results of the IgE-analyses were known, case histories at the time of the initial examination were rechecked and further examined in thirty-three children with high IgE levels. It was then found that another twelve children had to be excluded

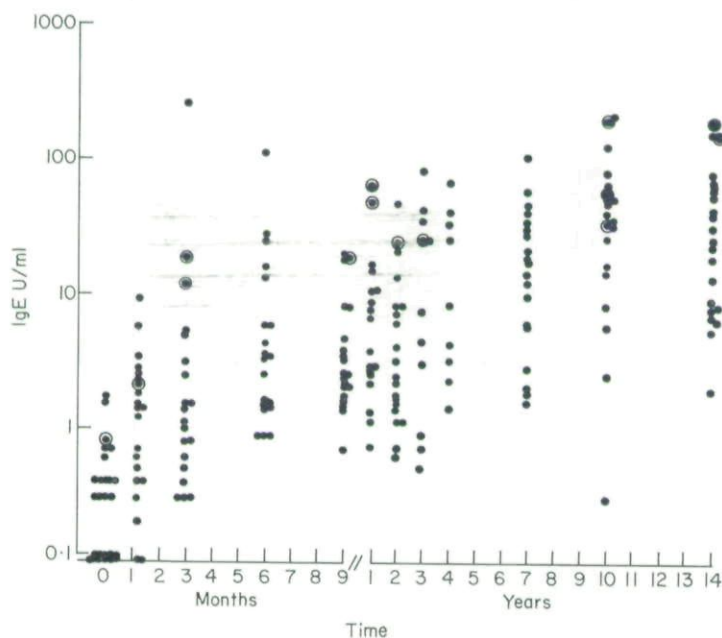


Fig. 1. Distribution of serum IgE concentrations in participants initially selected. ⊙; afterwards not found to fulfil criteria for normal with respect to allergy.

because they did not fulfil the criteria set up for participation: in eleven of the children, one of the parents had suffered from atopic manifestations during infancy or early childhood and one child had a history of infantile eczema. The remaining 208 children constitute the 'gross' normal material for this study.

About 18 months after the initial blood sampling, fifty-three of the children were reinvestigated. These were selected from the 'gross' normal material on the basis of an apparently raised IgE level for their age. The reinvestigation included, in addition to case history and physical examination, a repeated determination of IgE and RAST as well as skin prick tests as described in detail in a study of the predictive value of IgE (Kjellman, to be published). Of the fifty-three children re-examined, twenty-four had developed atopic diseases (Table 2) and were thus excluded. The remaining 184 children are presented as 'net' normal material.

Table 2. Atopic diseases which developed in the 'gross' normal material before re-examination 18 months after the initial examination

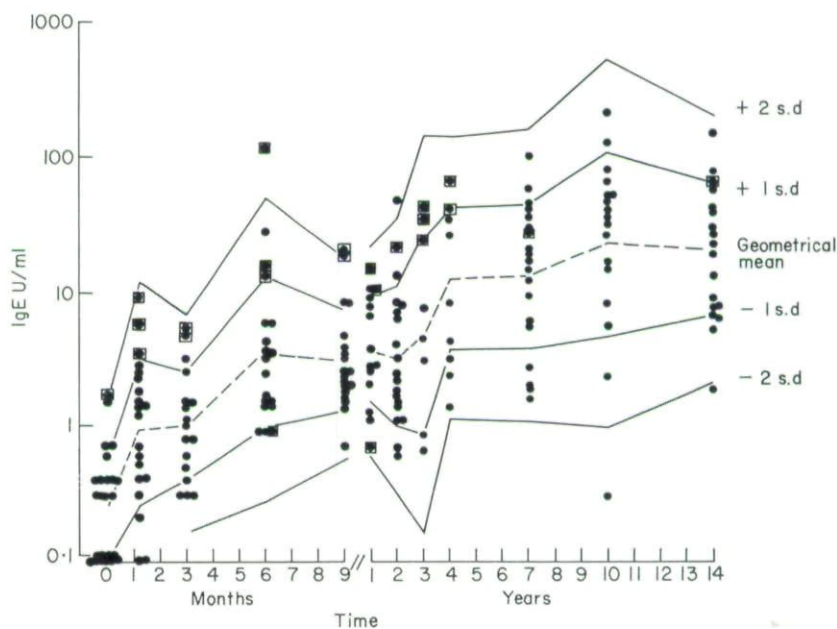
Age group	Bronchial asthma	Allergic rhinitis	Atopic dermatitis	Gastrointestinal allergy	Urticaria	Total
0-1 year	2	0	9	3	2	16
2-14 years	1	4	2	0	1	8
Total	3	4	11	3	3	24

Determination of IgE

The determination of IgE concentration in the serum was made by direct sandwich radioimmunoassay (PRIST) (Ceska & Lundkvist, 1972) as described in detail (Johansson, Berglund & Kjellman, 1976). The standard was calibrated against the British Research Standard for human serum IgE, 68/341 (Rowe *et al.*, 1970). IgE was expressed in U/ml serum (1 U/ml = 1 kU/l).

Determination of specific IgE-antibodies

Antibodies were determined by means of RAST (Phadebas RAST®, Pharmacia Diagnostics AB, Sweden). One hundred randomly selected initial samples from children 6 weeks to 4 years of age were subjected to RAST-analyses with respect to allergy to cow's milk, egg white and fish. Similarly 100 samples from children 1-14 years of age were tested for IgE-antibodies against birch and timothy pollen.

**Fig. 2.** Distribution of serum IgE concentrations of the 'gross' normal material. □; cases with signs of allergic manifestations developed during the 18 months' observation period.

Statistics

In order to compensate for the positively skewed distribution of the IgE values the statistical calculations were made after e-log conversion of the IgE results. Geometric mean, ± 1 and 2 s.d. were calculated for each age group.

Results

The distribution of IgE levels of the 226 children initially selected is shown in Fig. 1. The variation within the respective age group was considerable, especially from 3 years of age and upwards. The eighteen children who did not fulfil the criteria for inclusion at the early re-examination are indicated in the figure.

The 'gross' normal material shown in Fig. 2 consists of the remaining 208 children. In the figure the twenty-four children who had developed atopic symptoms during the 18 months' observation period are indicated (Table 2). The variation in the IgE values was less in most age groups than in the initial material.

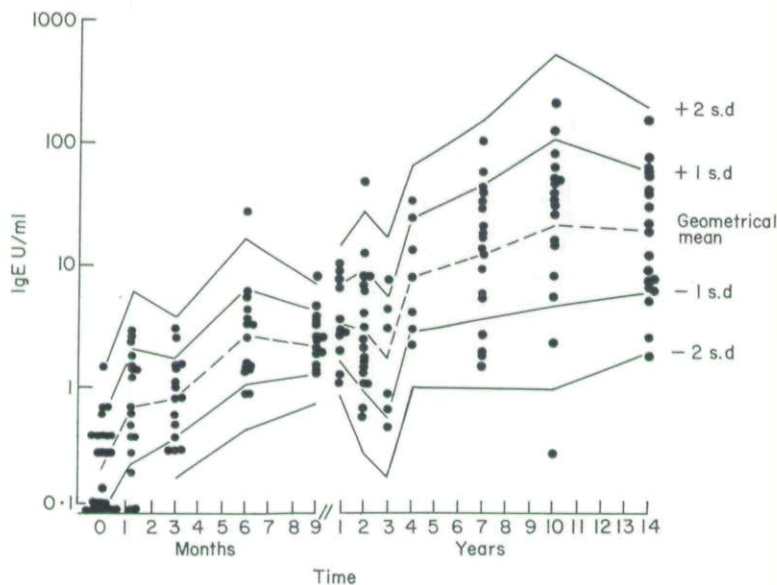


Fig. 3. Distribution of serum IgE concentrations of the 'net' normal material.

The 'net' normal material comprising 184 children is illustrated in Fig. 3 and Table 3. There was a smaller variation in the IgE values within most age groups and a relatively slow increase in the IgE values with age. Considerable variation was still evident at the age of 10. At this age geometrical mean and $+1$ and $+2$ s.d. were highest ($+2$ s.d. = 570 U/ml). Up to the age of 3 years $+2$ s.d. was below 30 U/ml. In most age groups the lowest IgE level obtained was below 1 U/ml; at the most it was 2.4 U/ml. Four children whose initial IgE levels were above $+2$ s.d. for their age are presented in Table 4.

Discussion

In 1972 it was suggested that the published normal IgE values for the newborn and infants, as determined by radioimmunological inhibition techniques, were over-

estimated due to serum proteins interfering in the determinations (Johansson, Bennich & Berg, 1972). The PRIST technique used in this study permits more accurate determination of IgE, especially in the low part of the range (Johansson, Berglund & Kjellman, 1976). The improved method for IgE determinations probably explains most of the discrepancies between the 'normal' range previously reported from our

Table 3. 'Net' normal values with regard to serum IgE (U/ml) in children

Age	<i>n</i>	Range U/ml	Geometric mean U/ml	Geometric mean - s.d.	Geometric mean + s.d.	Geometric mean - 2 s.d.	Geometric mean + 2 s.d.
0 days	24	<0.1-1.5	0.22	0.09	0.53	0.04	1.28
6 weeks	17	<0.1-2.8	0.69	0.23	2.05	0.08	6.12
3 months	15	0.3-3.1	0.82	0.39	1.76	0.18	3.76
6 months	15	0.9-28.0	2.68	1.09	6.60	0.44	16.26
9 months	16	0.7-8.1	2.36	1.34	4.16	0.76	7.31
1 year	12	1.1-10.2	3.49	1.67	7.29	0.80	15.22
2 years	18	1.1-49.0	3.03	0.97	9.46	0.31	29.48
3 years	6	0.5-7.7	1.80	0.59	5.51	0.19	16.86
4 years	7	2.4-34.8	8.58	3.03	24.31	1.07	68.86
7 years	18	1.6-60.0	12.89	3.64	45.60	1.03	161.32
10 years	17	0.3-215	23.66	4.82	116.20	0.98	570.61
14 years	19	1.9-159	20.07	6.43	62.58	2.06	195.18

Table 4. Children whose initial IgE was above +2 s.d. but who were without obvious atopic diseases at re-examination

Age group	Sex	Recent case history	Findings at re-examination		
			IgE	RAST	Prick test
Cord serum	Boy	Otitis media 4 times Pseudo-croup 3 times	More than +2 s.d.	Negative	Not done
6 months	Girl	Keratosi pilaris —as had her mother			
9 months	Boy	Refuses to eat egg	More than +1 s.d.	Negative	Negative
2 years	Girl	More often and more protracted respiratory infections than her siblings; dryer skin	More than +2 s.d.	Negative	Negative

group (Johansson, 1968; Berg & Johansson, 1969a, b) and the present values. IgE levels similar to ours were recorded in healthy children by Polmar, Robinson & Minnefor (1972) and Polmar, Waldmann & Terry (1973) using a double antibody technique. Using the Phadebas® IgE Test (Pharmacia Diagnostics AB, Uppsala, Sweden), Gerrard *et al.* (1974) found rather higher geometrical mean levels of IgE in *unselected* children from 2 years and upwards.

A detailed investigation of circulating IgE levels in a normal population of *adults* recently appeared (Nye *et al.*, 1975). Blood samples from 102 individuals 18-83 years of age were investigated by a double antibody technique. The mean IgE level was 36.3 U/ml—much lower than that found in most earlier studies on adults. A more

careful selection of participants and a sensitive technique probably account for the results. Using PRIST, however, Johansson has recently reported, preliminarily, a mean of 15 U/ml (Johansson, 1975). Nye *et al.* compared results obtained with the double antibody technique with those of the Phadebas IgE Test; there was an excellent correlation for samples with IgE levels above 100 U/ml but a poor one with lower levels. The findings are comparable with results from our group (Johansson, Berglund & Kjellman, 1976) using Phadebas IgE Test and PRIST.

The use of IgE as a parameter for atopic allergy, either for diagnostic (Berg & Johansson, 1969a, b) or predictive purposes (Foucard, 1974; Hamburger *et al.*, 1974; Kjellman, to be published) implies prior knowledge of the relevant IgE values in healthy children.

The selection of the participating children in this study aimed at obtaining a sufficient number of healthy, non-atopic individuals. We were especially interested in children 0-1 year of age ($n=125$ in the initial material). The risk of developing atopic symptoms is very high during the first year of life, in fact at least as high as during the following 6 years. Halpern *et al.* found in 1973 that 8% of 1753 children included in a prospective study developed atopic symptoms before 1 year of age and that in total 12.4% had done so before 7 years of age. Any patient material not selected on the basis of a follow-up (as done in the present study) is likely to include pathological IgE levels because of hidden potential atopics with reaginic antibodies in the serum before the appearance of the symptoms (Foucard, 1973; Kjellman, to be published).

In the present study, fifteen children (13%) from the age group 0-1 year developed atopic symptoms before the re-examination. Nine of the 101 children from the age group 2-14 years also developed atopic diseases. Thus there were twenty-four atopic children 0-14 years (11.5%) in this selected 'gross' normal material. Fifteen of them were boys. The 'net' normal material (Fig. 3, Table 3) includes 184 highly selected children 0-14 years of age without obvious atopic symptoms at the time of blood sampling and as far as is known even during the following 18 months.

Heredity is another important factor to consider when normal material is selected. About two-thirds of children with atopic diseases have first degree relatives with atopic symptoms at present or in the past (Orgel, 1975). Koivikko (1974) studied children with asthma and reported atopic first degree relatives in 68% of the children and atopy in one relative in 84%. Cases with a family history of atopic disease have to be excluded from any material representative of normal children. In this study eleven children were excluded on a hereditary basis at a second interview.

In spite of our exclusion of all detected atopic children some atopics may still be found in the 'net' normal material. The four children who had initial IgE levels above +2 s.d. but did not show obvious atopic symptoms before the re-examination nevertheless displayed symptoms and signs that would possibly be attributed to reagin mediated mechanisms (Table 4). Keratosis pilaris may well be an atopic disorder (Kahn, 1975) and about a quarter of food dislikes are due to clinical food allergy (Rowe & Rowe, 1972). It is also possible that some individuals are 'good reagin producers' but lack an 'organ factor' like the increased bronchial sensitivity of asthmatics. They could have high IgE levels without obvious symptoms. Most of the atopics, however, are excluded from the 'net' normal material.

We recommend that IgE results obtained with the direct sandwich technique (PRIST) in patients with possible atopic diseases, should be compared with the results

of the 'net' normal material presented in Fig. 3 and Table 3. A child then found to have a high IgE level is very likely to have or soon to develop a clinically significant atopic allergic disorder (Kjellman, to be published).

Acknowledgments

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