

IgE hidden in immune complexes with anti-IgE autoantibodies in children with asthma

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Serum levels of IgE, anti-IgE autoantibodies (Abs), and IgE/IgG anti-IgE immune complexes (ICs) were measured in 110 children with asthma and 90 healthy control children. Significantly enhanced levels of IgE/anti-IgE IC were detected in children with asthma. However, only a weak correlation was found between anti-IgE auto-Abs serum levels and the degree of lung function abnormalities in children with asthma. However, children with asthma with low serum IgE levels had elevated IC serum levels of IgE/anti-IgE auto-Abs, suggesting that IgE might be hidden within these ICs and is therefore not measurable in vitro. The significant elevation of IgE/anti-IgE IC serum levels raises the question whether IgE within ICs is neutralized or might still be involved in immunologic mechanisms responsible for clinical symptoms of bronchial asthma. (J ALLERGY CLIN IMMUNOL 1991;88:793-801.)

Key words: Anti-IgE, asthma, autoantibody, child, IgE, IgE-determination, immune complex

There is yet a tendency to classify individuals with increased IgE levels as individuals with atopic (extrinsic) asthma and individuals with normal IgE levels as having intrinsic asthma.¹ Although some authors demonstrated a correlation between the frequency of asthma symptoms and IgE serum levels,¹⁻³ whether clinical symptoms in BA, especially in childhood asthma, are related to immunologic laboratory determinations, such as total and specific IgE serum levels, is still controversial. Different cutoff levels of serum IgE were reported to be predictive for the development of BA,^{2,4} and the presence and amount of serum IgE Abs to inhalant allergens was considered to be an important risk factor for acute asthma attacks,⁵ establishing the child's allergic predisposition⁶ and leading to "in vitro diagnosis" of allergy^{1,7} or prognosis for children suffering from BA.⁸ However, the validity of this relationship is questioned definitively.⁴ Finally, determination of IgE serum levels was used to distinguish children with asthma from children with other wheezing respiratory diseases,⁷ although that asthma

Abbreviations used

BA:	Bronchial asthma
Ab:	Antibody
IC:	Immune complex
TGV:	Thoracic gas volume
R _{aw} :	Airway resistance
TBS:	Tris-buffered saline

in infants is inherited independently of allergy and that it is primarily triggered by viral infections is now recognized.⁹ However, since epidemiologic studies demonstrated that IgE Abs to common environmental allergens occurred in a considerable number of subjects without allergic symptoms or that such nonallergic subjects demonstrated some degree of skin test reactivity to common focal allergens,³ perhaps normal IgE levels neither rule out asthma nor make the development of future allergies unlikely.⁸

Based on the observation that severe asthmatic symptoms or even anaphylactogenic shocks occurred in patients with only low IgE serum levels, the hypothesis that in such cases IgE may be hidden within ICs with anti-IgE auto-Abs became of interest.¹⁰⁻¹⁴ Anti-IgE auto-Abs can be determined by various assay systems.^{10, 11, 15-22} They appear to belong mainly to various immunoglobulin classes^{11, 14, 16, 23} and occur mostly in the form of complexes with IgE. Anti-IgE

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TABLE I. Mean values of TGV and R_{aw} in different lung-function groups of children with asthma

Functional disorder	N	TGV	P_{aw}
		Mean $\pm$ SD (%)	Mean $\pm$ SD (%)
None*	50	92.6 $\pm$ 14.3	105.5 $\pm$ 18.4
Hyperinflated†	7	152.1 $\pm$ 24.8	95.8 $\pm$ 31.0
Mixed type‡	5	140.3 $\pm$ 8.25	155.6 $\pm$ 12.9
Obstructive§	48	94.3 $\pm$ 16.1	166.6 $\pm$ 26.4
All	110	99.3 $\pm$ 23.0	133.8 $\pm$ 38.3

Values in percent and corrected for age. Normal range, 80% to 130%.

*TGV and R_{aw} <130%.

†TGV >130% and R_{aw} <130%.

‡TGV and R_{aw} >130%.

§TGV <130% and R_{aw} >130%.

auto-Abs have been associated with the Kawasaki syndrome or Prausnitz-Küster reactions²⁵⁻²⁷ and parasitic infections, hyper-IgE syndrome, and different atopic disorders.²⁸⁻³³ Studies on anti-IgE auto-Abs in BA of mainly adult patients revealed a correlation between elevated IgE and anti-IgE auto-Abs,^{10, 11, 14-17, 34, 35} but anti-IgE levels have been demonstrated to fail to discriminate between atopic and nonatopic subjects with asthma.²⁵ Enhanced levels have been found in patients who underwent unsuccessful hyposensitization therapy,³⁶ but low levels of anti-IgE in all human sera can be found.^{12, 13, 37-40}

Therefore, based on the assumption that anti-IgE auto-Abs may interfere with the measurement of IgE^{14, 41, 42} and that IgE determinations may be underestimated because IgE was "hidden" within ICs of IgE and anti-IgE auto-Abs,^{11, 14} the present study was undertaken to investigate whether anti-IgE auto-Abs also might occur in children with asthma and may have been a reason for the controversy on "absolute" levels of total or specific IgE or its pathophysiologic role.

MATERIAL AND METHODS

Patients and sera

One hundred ten children with asthma (46 female and 64 male children), aged from 5 to 16 years (mean age, 9.5 ± 2.7 years), observed at the outpatient clinic, Department of Pediatrics, University of Bern, were included in this study. Asthma was diagnosed when a history of recurrent wheeze and cough and laboratory documentation of airway obstruction, which reversed with sympathomimetics, were present. Patients receiving therapy with oral xanthines and systemic steroids were not included in the study. Lung-function tests by whole-body plethysmography were performed on the same day as serum samples were collected and stored at -20°C . In addition, 90 children

(44 female and 46 male children) aged from 4 to 16 years (mean age, 10.5 ± 3.2 years), admitted to the hospital for other reasons than pulmonary disorders, served as the control group.

Lung-function tests

After a medical interview and a physical examination, functional abnormalities were evaluated by whole-body plethysmography. TGV at resting level was taken as the measure of pulmonary hyperinflation, and R_{aw} , maximal expiratory flow at 50% vital capacity, respectively, as a measure of bronchial obstruction. The measurements were performed with the patient seated in an air-conditioned room with a constant volume, variable pressure, whole-body plethysmograph (E. Jaeger GmbH, Würzburg, Germany). According to these functional data, the patients were divided into four functional groups: (1) lung-function data within the range of normal (TGV and R_{aw} , <130% predicted), (2) pulmonary hyperinflation (TGV, >130% predicted), (3) mixed type (TGV and R_{aw} , >130% predicted), and (4) bronchial obstruction (R_{aw} , >130% predicted).

Determination of immunoglobulins

Total IgG, IgA, and IgM were measured by a commercial rate nephelometry test (Beckmann Instruments, Inc., Galway, Ireland). For determination of IgE, we used a sandwich RIA based on two different monoclonal anti-IgE Abs.⁴³⁻⁴⁶

Additionally, we determined the serum IgE in the children with asthma by the commercial Phadebas PRIST (Pharmacia Diagnostics AB, Uppsala, Sweden).

Dot-immunobinding assay for detection of free anti-IgE Abs and IgE/anti-IgE complexes

Natural anti-IgE Abs capable of directly recognizing purified solid-phase IgE were termed "free" anti-IgE Abs. To assess these "free" anti-IgE Abs in serum samples, we dotted 1 μl volumes of purified human myeloma IgE (PS, 50 $\mu\text{g}/\text{ml}$) onto nitrocellulose (0.45 mm, HA 304 FO; Millipore, Molsheim, France). IgE/anti-IgE ICs were measured by dotting the murine monoclonal antihuman IgE Ab, Le 27 (DeII specific⁴⁶) onto nitrocellulose (500 $\mu\text{g}/\text{ml}$). Nitrocellulose strips were incubated in TBS-casein to prevent unspecific protein adsorption, followed by incubation with diluted serum (dilution 1:4 in TBS-casein) at room temperature on a shaker (4010, Köttermann, Schönengrund, Switzerland) overnight. After three washings with TBS, bound Abs were detected with a peroxidase-conjugated monoclonal anti-IgG Ab (BS17, Biotest AG, Frankfurt, Germany, diluted in TBS-casein). The strips were washed three times with TBS and developed with a solution of 16.6% of 3 mg/ml 4-chloro-1-naphthol (Merck, Darmstadt, Germany) in methanol, and 0.042% hydrogen peroxide (stock solution, 30% in water) in TBS for 30 minutes. After a wash with distilled water, strips were dried, and the color intensity of the dots was measured with a hand-held reflection densitometer (Gretag Ltd., Regensburg, Switzerland) at 600 nm to obtain relative densitometric units. This relative color density was compared to a standard of directly dotted human IgG (Nordic Laboratories, Tilburg, The Netherlands) revealed by the same anti-IgG Ab. A regression

TABLE II. Serum immunoglobulin levels in children with asthma

Age† (yr)	N	IgG*		IgA*		IgM*	
		Mean ± SD (gm/L)	Norm† (gm/L)	Mean ± SD (gm/L)	Norm (gm/L)	Mean ± SD (gm/L)	Norm (gm/L)
5 + 6	12	9.58 ± 2.5	7.5-11.9	1.17 ± 0.6	0.65-1.53	1.36 ± 0.7	0.73-1.44
7 + 8	28	9.69 ± 3.3	8.1-12.5	1.10 ± 0.6	0.79-1.72	1.23 ± 0.6	0.77-1.49
9 + 10	28	9.98 ± 1.5	8.5-12.9	1.36 ± 0.5	0.9-1.87	1.01 ± 0.5	0.8-1.55
11 + 12	20	9.89 ± 1.9	8.9-13.1	1.38 ± 0.6	0.99-2.05	1.26 ± 0.4	0.8-1.56
>12	22	10.55 ± 3	8.9-13.1	1.67 ± 0.8	0.99-2.05	1.19 ± 0.5	0.8-1.56
5-16	110	10.05 ± 2.4	8.5-12.9	1.35 ± 0.6	0.9-1.87	1.2 ± 0.5	0.8-1.55

*Immunoglobulin levels determined by rate nephelometry (METHOD AND MATERIALS).

†Age distribution and normal values according to a normal in-house standard as described.³⁴

TABLE III. Serum levels of IgE, anti-IgE, and IgE/anti-IgE ICs in children with asthma and control children

	Children		Probability (Student's <i>t</i>)
	Control (N = 90)	With asthma (N = 10)	
	Mean ± SD	Mean ± SD	
IgE* (U/ml)	73.55 ± 172.9	206.13 ± 228.9	<i>p</i> < 0.001
Anti-IgE autoAbs† (ng/ml)	1.51 ± 3.2	3.79 ± 10.1	<i>p</i> = 0.02
IgE/anti-IgE ICs† (ng/ml)	28.31 ± 26.2	77.94 ± 57.4	<i>p</i> < 0.001

*IgE determined by a sandwich RIA based on two different monoclonal anti-IgE Abs as described.

†IgG anti-IgE auto-Abs and IgE/anti-IgE ICs were determined by a dot-immunobinding procedure. IgG anti-IgE auto-Abs are defined as Abs capable of recognizing solid-phase myeloma IgE (PS). IgE/anti-IgE ICs are defined as ICs caught by the monoclonal anti-IgE Ab, Le 27. Both are then revealed by a monoclonal anti-IgG Ab.

curve was calculated and used to estimate IgG concentrations.

Determination of specific IgE by commercial Phadezym RAST

The sera of the children with asthma were tested by RAST (Pharmacia Diagnostic AG) for the following allergens: house dust mites, grass pollen, tree pollen, and animal epithelia.

Statistics

Hypothesis tests with means and pooled variances between the same variables of two groups were used to perform a Student's *t* test for calculating a *t* value and its associated probability (computer program, Microstat-II Release 2.0, Ecosoft, Inc.).

RESULTS

Lung function and immunologic characterization of patients

The values of R_{aw} and TGV expressed in percent of predicted values within the four functional groups are presented in Table I. Fifty children with asthma

(45.5%) presented with normal lung functions. Only 48 (43.6%) of the 110 children with asthma had increased R_{aw} and only 12 children (10.9%) had pulmonary hyperinflation. Symptoms were well controlled with an inhalative treatment with a combination of either β_2 -mimetics and cromoglycate or β -mimetics and topical steroids. For control purposes, we compared IgG, IgA, and IgM levels in children with asthma with the normal reference values of a normal child population.³⁴ In Table II, the levels of total IgG, IgA, and IgM of the children with asthma are presented within the normal range.

Anti-IgE auto-Abs in children with asthma and control children

The values of IgE, anti-IgE auto-Abs, and IgE/anti-IgE IC levels are presented in Table III. All three levels were significantly higher in children with asthma compared to levels in control children (*p* < 0.001), although a smaller significance (*p* = 0.02) was found for "free" anti-IgE auto-Abs. ("Free" is used here for Abs capable of binding to solid-phase IgE but may

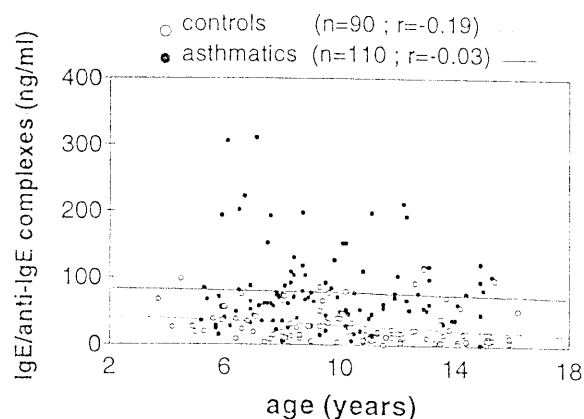


FIG. 1. Serum levels of IgE/anti-IgE ICs in relation to the age of children; data of 90 control children ($\circ$); data of 110 children with asthma ($\bullet$). Each point is the mean of triplicate determinations in a dot-immunobinding assay as described in MATERIAL AND METHODS. Linear regression lines with the indicated correlation coefficient (r) are also illustrated.

still include ICs.) No detectable "free" anti-IgE Ab levels were observed in 43 children with asthma out of 110, and in 40 control children out of 90. This finding may be due to the low sensitivity of the assay (0.05 ng/ml) used for detecting the "free" anti-IgE Abs. ICs consisting of IgE and anti-IgE auto-Abs were found in all individuals studied, although these were in variable concentrations. Thus, it might be assumed that a certain proportion of anti-IgE auto-Abs has no free valency to recognize solid-phase IgE.

Next, we analyzed serum IgE Ab and anti-IgE auto-Ab levels in relation to the age of the blood donors. In agreement with the literature, we did not find an overall correlation (children with asthma, $r = 0.01$; control children, $r = 0.06$), but an increasing tendency for IgE in 7- to 10-year-old children (data not presented) and a small decreasing tendency for IgE/anti-IgE IC serum levels in control children and children with asthma (Fig. 1). Additionally, we observed an inverse trend between free anti-IgE and age of children with asthma ($r = -0.21$; $p < 0.05$).

Comparing serum IgE to anti-IgE auto-Ab levels, we did not find a correlation ($r = 0.04$ in children with asthma; $r = 0.1$ in control children) and also no correlation with IgE/anti-IgE ICs (control children, $r = 0.1$; with asthma, $r = 0.16$). However, there was a significant correlation ($p < 0.001$) between anti-IgE auto-Abs and corresponding IgE/anti-IgE IC levels both in control children ($r = 0.48$; $N = 90$) and children with asthma ($r = 0.42$; $N = 110$).

Frequency distribution of anti-IgE in children with asthma and healthy control children

To analyze further the significantly higher mean values of anti-IgE auto-Abs in the children with asthma, we examined their frequency distribution. A considerable difference in the frequency distribution of IgE serum levels between control children and children with asthma is illustrated in Fig. 2. In the control group, we observed a broad distribution between 0 to 100 U/ml of IgE, whereas in the group of children with asthma, we found a bimodal distribution with an additional population peaking at approximately 400 U/ml. For IgE/anti-IgE ICs, we found a broad distribution in control children only, but in the children with asthma, we found a monomodal distribution of a relative narrow range, which clearly differed from the distribution of the control children. When the distribution of "free" anti-IgE auto-Abs was examined, we did not observe differences between control children and children with asthma. IC levels were well distributed in a monomodal fashion, peaking at 1.2 ng/ml in the control children and at 2.4 ng/ml in the children with asthma (frequency distribution not presented). Thus, 75% (83/110) of the children with asthma belonged to the population of patients with elevated ICs of IgE/anti-IgE (>40 ng/ml). The control group had only 21% (19/90) within the range of the children with asthma. In this context, it is important to mention that atopy was not an exclusion criteria for the control group. This nonexclusion implies that children with atopy remained within the control group and might contribute to the population with elevated immune complexes.

Anti-IgE serum levels in children with "low IgE" and in "high IgE"

Based on the observed frequency distribution of IgE serum levels in the children with asthma, we grouped them into 58 children with asthma with "low levels of IgE" (IgE <120 U/ml) and 52 children with asthma with "high levels of IgE" (IgE >120 U/ml) to investigate whether IgE serum levels may be linked to the levels of anti-IgE auto-Abs. As illustrated in Fig. 3, the groups with asthma and "low IgE" and "high IgE" were almost equally distributed. Interestingly in the group with asthma and "low IgE," the IgE/anti-IgE IC mean value differed significantly (control children, 26.7 ± 25.1 ng/ml; with asthma 65.3 ± 40.3 ng/ml; $p < 0.001$) from the control group despite the fact that their IgE serum levels did not differ (control children, 22.9 ± 24.7 U/ml; with asthma, 31.1 ± 28 U/ml). Ten control children with IgE >120 U/ml

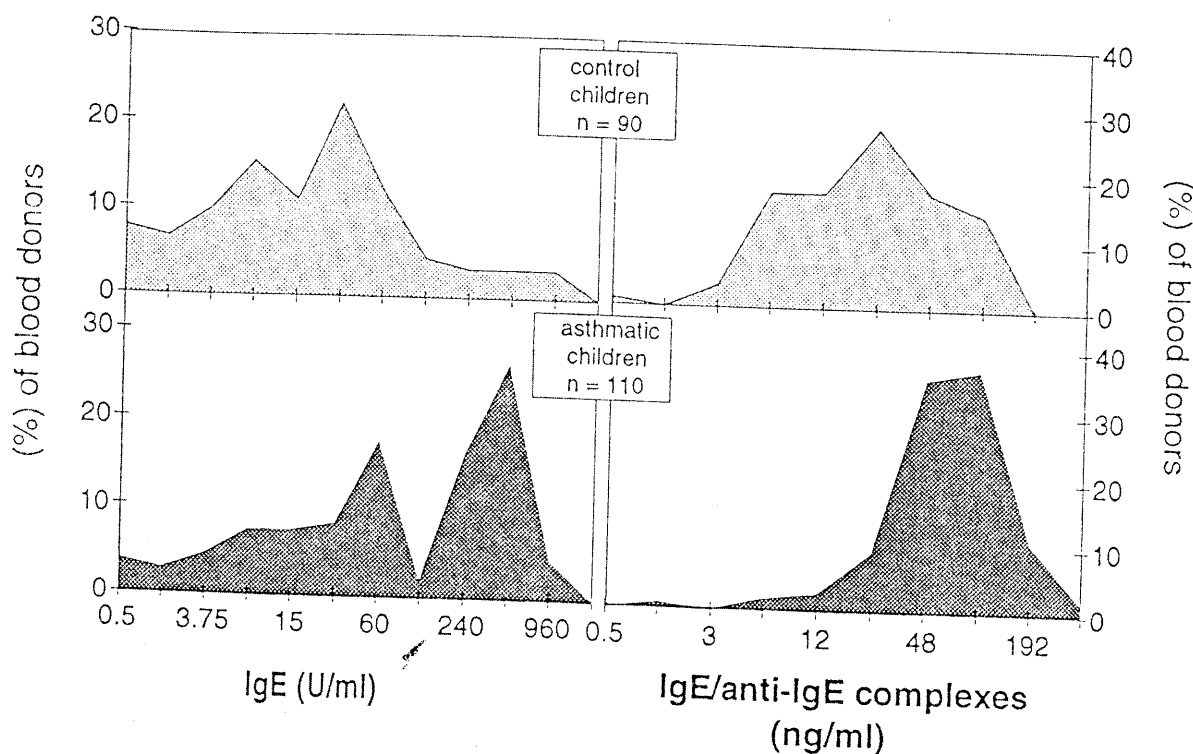


FIG. 2. Frequency distribution of IgE and IgE/anti-IgE ICs in sera of control children and children with asthma. IgE was determined by a sandwich RIA and IgE/anti-IgE ICs by a dot-immuno-binding assay as described in MATERIAL AND METHODS.

revealed similarly enhanced serum levels of IgE/anti-IgE ICs as the "high IgE" children with asthma. Again serum levels of "free" anti-IgE auto-Abs in "low IgE" children with asthma did not differ from levels of the "high IgE" children with asthma (data not presented).

We next studied the relation of measurable IgE to the levels of IgE within ICs. The children with asthma were divided into three groups according to their serum IgE distribution, as illustrated in Fig. 2. These three groups differed significantly in their serum IgE levels but not in their levels of ICs, as presented in Table III, but this arbitrary division clearly demonstrates that children with asthma with low levels of serum IgE have approximately five times more IgE within their ICs than measurable total IgE. In contrast, IgE is in excess of ICs in children with asthma with high IgE serum levels (Table IV).

Anti-IgE serum levels in children with asthma with experimental evidence of atopy

In this study, all serum IgE levels had been determined by a sandwich RIA based on two different monoclonal anti-IgE Abs. This assay correlates very well with the commercial Pharmacia PRIST.⁴⁴⁻⁴⁶ Since

IgE serum levels in the children with asthma were additionally determined by the commercial Phadebas PRIST (Pharmacia), we again evaluated the correlation for both assays. Interestingly, we again found a high significance ($p < 0.001$), but only a regression coefficient of 0.78. Based on our previous finding that anti-IgE Abs interfere with IgE determinations,¹⁴ it might be speculated that the increased anti-IgE auto-Ab levels of the group of children with asthma may have prevented precise IgE determination in this group.

To address this question we used the same selection criteria as presented in Table IV. Indeed, the commercial PRIST measured, in the children's group with low serum IgE, five times more IgE than our in-house RIA, based on monoclonal Abs (Table V). In contrast, only 70% more IgE is detected by the PRIST in sera derived from children with asthma with high serum IgE levels.

Additionally, all sera had also been analyzed by a Phadezym RAST with a panel of different allergens; 25 children with asthma (22.7%) were positive for minimally one of the allergens described above (RAST class ≥ 2); 21 children with asthma (19.1%)

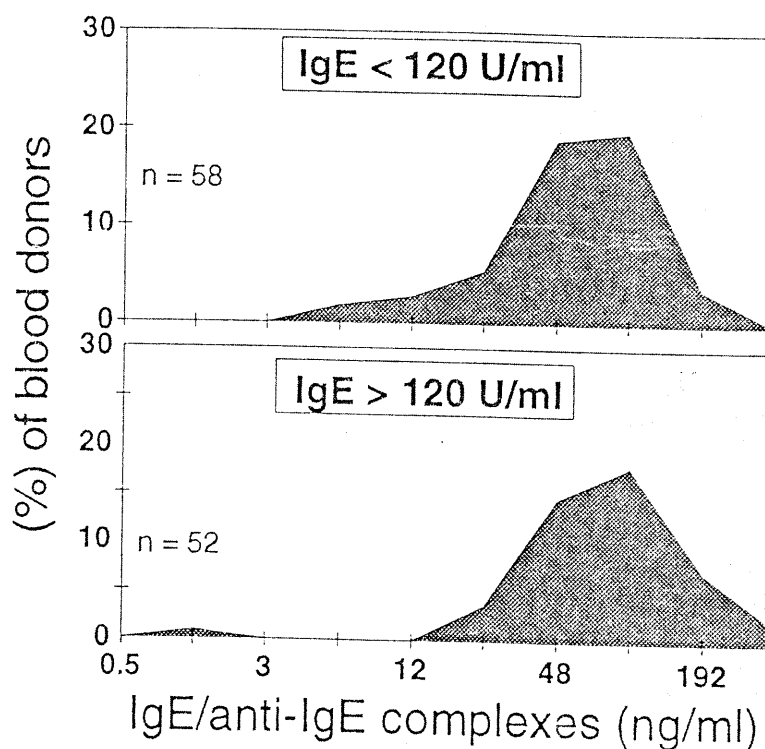


FIG. 3. Frequency distribution of IgE/anti-IgE ICs in children with asthma with low levels of IgE (<120 U/ml) and high levels of IgE (>120 U/ml). Based on IgE serum levels (cutoff, 120 U/ml, defined in Fig. 2), children with asthma were analyzed for their serum IgE/anti-IgE ICs.

had both the specific IgE (RAST ≥ 2) and total IgE (determined by Phadebas PRIST) above the upper normal values for their age (Pharmacia); 24 children (21.0%) had elevated IgE only, and 40 children (36.4%) had neither specific IgE nor IgE levels above normal values.

RAST class ≥ 2 or PRIST IgE above upper normal age values are commonly associated with an atopic base for BA in childhood; therefore we divided the group with asthma according to these criteria, yielding 70 "atopic" and 40 "nonatopic" children with asthma bronchiale. As expected, this classification, based on established commercial laboratory assays, revealed that most of the "high IgE" children with asthma defined by the monoclonal sandwich RIA were among the atopic group. However, atopic and nonatopic children had similarly increased IC levels (nonatopic, 75 ± 54.9 ; atopic, 82.1 ± 61.1).

Relation of pulmonary symptoms to IgE or auto-Ab levels

In addition, we compared TGV and R_{aw} to the corresponding serum levels of IgE, anti-IgE, and IgE/anti-IgE ICs in the children with asthma. There was no strong correlation between IgE or anti-IgE

auto-Ab levels and the two plethysmographic values, with the exception that an inverse trend between R_{aw} and IgE/anti-IgE ICs was found ($r = 0.22$; $p = 0.03$).

DISCUSSION

Despite the difficulties of defining asthma, clinicians usually have no difficulty in understanding what they mean by the term.⁴⁷ For most of these clinicians, diagnosis of asthma is based on recurrent wheezing or attacks of shortness of breath with wheezing.² Frequently, in the pathogenesis and development of this syndrome, the allergic reaction is believed to play a major role,^{2, 5} but much controversy exists over the exact nature of the association between atopy and childhood asthma, whether it is causative⁹ and over the predictive value of IgE determinations.

To clarify further the relationship between immunologic parameters and their clinical importance, we investigated the relationship between anti-IgE auto-Abs and the syndrome of childhood asthma. In agreement with several other studies,^{16, 38, 40} we did not find a strong correlation between the type and severity of lung functional disorders and auto-Ab levels. The major finding of our study is that elevated serum IgE

TABLE IV. Quantitative comparison of serum IgE with anti-IgE/IgE-ICs

Selection criteria*		IgE†		Anti-IgE/IgE ICs	Ratio (IgE/IC)
Serum IgE (U/ml)	N	Mean (ng/ml)	Mean (ng/ml)	Mean (ng/ml)	
0-15	23	12.2		61.1	0.2
15-120	35	116		68.2	1.7
120 max	52	967.4		92	10.5

Max, Maximum.

*Selection criteria are based on the distribution illustrated in Fig. 2.

†IgE determined by a sandwich RIA based on two monoclonal anti-IgE Abs, as described in MATERIAL AND METHODS.

levels were found within ICs in practically all children with asthma.

In our dot assay we used a monoclonal anti-IgE Ab (Le 27) as catching Ab, which will recognize only a certain proportion of circulating IgE/anti-IgE ICs because serum contains anti-IgE auto-Abs of a similar epitope specificity as Ab Le 27. For example, if another monoclonal anti-IgE Ab is used, such as BSW17, in most instances lower levels of ICs are found.^{18, 23, 48} In these sera of children with asthma with elevated anti-IgE auto-Ab levels, we could demonstrate that IgE determination by an assay based exclusively on monoclonal Abs yields indeed lower IgE levels than an assay based on polyclonal Abs (PRIST). This finding demonstrates that certain epitopes on IgE are not accessible for some anti-IgE Abs used in immunoassays. We assume that anti-IgE auto-Abs are responsible for covering of such epitopes. Therefore, it is obvious that an immunoassay based on two different monoclonal Abs is far easier to be inhibited because IgE will no longer be detected if only one of the two relevant epitopes is hidden. However, our data indicate that also polyclonal assay Abs may underestimate serum IgE levels. In the arbitrarily created group of children with low serum IgE (Tables IV and V), we measured 5 U/ml of IgE by monoclonal and 26.9 U/ml by polyclonal Abs. This finding means that by monoclonal Ab determination, only 20% of the IgE was actually detected, but polyclonal Abs detected 90% of the IgE (12.2 ng/ml [IgE] + 61.1 ng/ml [IgE in complexes] divided by 2.41 = 30.4 U/ml). This calculation is based on the assumption that the monoclonal Ab, Le 27, catches "all" ICs, which, of course, will not be the case because some auto-Abs also will block the "Le 27 epitope." Thus, if ICs are in excess of IgE measurable with polyclonal Abs, polyclonal Abs might also underestimate the true levels of IgE.

However, it is important to note that all statistical

TABLE V. IgE determination with polyclonal or monoclonal anti-IgE Abs

Selection criteria*	RIA-IgE†	PRIST-IgE‡	
	Mean (U/ml)	Mean (U/ml)	Δ%
IgE (U/ml)			
0-15	5	26.9	438
15-120	48.2	93.1	93
120 max	401.4	684.6	70

Max, Maximum.

*Selection criteria are based on the distribution illustrated in Fig. 2.

†Sandwich RIA based on two different monoclonal anti-IgE Abs as described.

‡Commercial Phadebas PRIST based on polyclonal anti-IgE Ab.

analysis in this study, based on IgE determination by our RIA, yielded the same statistical differences of IgE values obtained by the PRIST were used instead.

Our most interesting finding was the relative homogenous elevation of IgE/anti-IgE ICs in children with asthma. Approximately 50% of the group with asthma did not differ from the control group in respect to their IgE serum levels (with asthma and "low IgE") but demonstrated a striking difference in their IgE/anti-IgE IC serum levels ($p < 0.001$). In this respect, it is interesting to note that a similar distribution of serum IgE in children with asthma was observed by Shibasaki et al.² These investigators demonstrated a similar cutoff point of 120 U/ml of IgE, but all their children with asthma had serum IgE levels above this cutoff point. In contrast, we can clearly discriminate two populations of children with asthma with either low or high serum IgE levels, based on the commercial PRIST as well as the in-house sandwich assay.

It is now important to study whether IgE within ICs in children with asthma represent IgE that is still biologically functional or whether it is neutralized. We have evidence that such ICs also occur on human basophils and are either anaphylactogenic or blocking auto-Abs.⁴⁹⁻⁵¹ However, we have so far not developed assays to discriminate easily in serum between these two types of functional anti-IgE auto-Abs. The functional nature of auto-Abs in children with asthma might be envisaged to be more important than the total amount of auto-Abs.

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