Immunodeficiency and other clinical immunology

IgE against HIV proteins in clinically healthy children with HIV disease

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Elevated serum IgE was detected in 26% (7 of 30) of children with HIV infection. The majority of children with elevated IgE were of one ethnic group (Puerto Rican) (4 of 7), compared with only 9% (2 of 23) in the normal to low IgE group (p = 0.02). Most of the children with elevated IgE had decreased circulating CD4$^+$ T cells (5 of 7 or 71%); but none had opportunistic infections, and none failed to thrive. Although similar numbers of children with normal to low IgE had decreased circulating CD4$^+$ T cells (19 of 23 or 83%), this group had opportunistic infections (6 of 23 or 26%) and failure to thrive (7 of 30 or 30%). There was no difference in incidence of allergic symptoms between groups. IgE antibody against HIV protein was detected by Western blot technique in the sera of three children with elevated serum IgE. Thus we have identified a group of children with HIV infection and elevated serum IgE of predominantly one ethnic group, who are without opportunistic infections or failure to thrive, some of whom produce HIV-specific IgE. This suggests that IgE may play a protective (perhaps late compensatory) role in HIV disease in genetically predisposed individuals. (J Allergy Clin Immunol 1996;98:979-84.)

Key words: *HIV infection, IgE levels, opportunistic infections, CD4$^+$ T cells, Western blot*

It is recognized that serum IgE levels are elevated in adults with HIV infection,[1] but the exact role of IgE in HIV disease is unknown. The presence of IgE has been correlated with disease progression and has been reported to signal the onset of opportunistic infections (OIs) in patients with decreased CD4$^+$ blood T cells.[2] Nonspecific increases in antibody responses of all isotypes, including IgE, (i.e., so-called polyclonal activation) is a possible explanation for the presence of IgE in serum of persons with HIV infection; however, Wright et al.[1] have described preferential increases in serum IgE over immunoglobulins of other isotype classes in some adults with HIV infection.

Abbreviations used
CDC: Centers for Disease Control
OIs: Opportunistic infections

Specific allergens and antigens have been investigated as causes of the IgE elevation in HIV disease.

Increased incidences of allergic rhinitis, atopic dermatitis, and drug eruptions have been reported in adults with HIV infection.[1,3] Elevations in total serum IgE, however, have been detected equally in both allergic and nonallergic patients with HIV.[1,2] One study reports detection of IgE directed against aeroallergens in serum of selected adults with HIV infection and sinusitis;[4] however, other studies of adults with HIV infection have not detected IgE directed against either aeroallergens or food allergens.[3] Carini et al.[5] found that peripheral blood mononuclear cells from individuals infected with HIV released IgE-binding factor and proposed that

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this release, combined with other IgE regulatory factors released in excess, may be contributing to adverse drug reactions in patients with HIV disease.

Serum IgE directed against fungal antigens has been detected in some adults infected with HIV,[6] and Khalife et al.[7] have reported the presence of IgE anti-HIV proteins in serum of adults with HIV infection and hemophilia. The antibodies (HIV-IgE) were not found in patients with sexually transmitted disease or disease transmitted through intravenous drug use.[7]

A study of children with HIV infection reported that mean serum IgE level is elevated above that of noninfected age-matched control subjects, especially in advanced disease, but demonstrated no specific correlation with OI, allergic symptoms, or positive skin test responses to common aeroallergens.[8]

The interpretation of IgE in serum of persons infected with HIV is complicated by the fact that "normal values" for serum IgE are established in relation to allergic disease, and IgE in HIV disease is not always elevated in relation to allergic symptoms.

We studied serum IgE responses in children infected with HIV (intrapartum or transplacental infection) and found that 26% of the children had elevated serum IgE levels (>100 IU/ml for children 7 years or older and according to normal values established by Homburger et al.[9] for children younger than 7 years). The majority of these children had decreased numbers of circulating $CD4^+$ blood T cells; but none had OIs, and none failed to thrive. IgE directed against HIV proteins was detected in serum of 43% of the children with serum IgE elevation. Most of the children with elevated serum IgE levels and all of those with elevated IgE levels who had HIV-specific IgE were Puerto Rican, suggesting a genetic predisposition to the response.

METHODS

Patients

Peripheral blood was obtained from children with HIV infection (n = 30) who attended the Pediatric Immunology Clinic, Children's Medical Center of Brooklyn (State University of New York Health Science Center, Brooklyn) over a 2-month period. HIV infection was established by: (1) ELISA and positive pattern on Western blots (after 18 months of age) or (2) OI in HIV-positive infants (or other criteria of Centers for Disease Control [CDC]) before the age of 18 months. This study was approved by the institutional review board, and all blood samples were obtained with parental or guardian consent.

Immunofluorescence studies

$CD4^+$ T cells were stained with monoclonal mouse anti-human CD4 (Becton-Dickinson, Mountainview, Calif.) and enumerated by FACScan flow cytometry (Becton-Dickinson) as previously described.[10]

Serum immunoglobulins

Total immunoglobulins. Levels of total serum IgM, IgG, and IgA were determined by nephelometry (Beckman Array System, Fullerton, Calif.), and levels of total serum IgE were determined by radioimmunoassay (Kallestead, Chaska, Minn.). Serum IgE values were considered elevated for children 7 years or older if they were greater than 100 IU/ml (as established with age-matched control subjects in our immunoserology laboratory), and values were considered elevated for children younger than 7 years if they were above those established by Homburger et al (younger than 1 year, >89 IU/ml; 1 to 2 years, >132 IU/ml; 2 to 3 years, >100 IU/ml; 3 to 4 years, >144 IU/ml; 4 to 5 years, >149 IU/ml; and 5 to 7 years, >552 IU/ml.[9]

Western blot assay. Assays were carried out before and after IgG removal from serum. To remove IgG, serum was filtered through Protein-G-Sepharose columns (MINI.rapi.sep.M; Integrated Diagnostics, Baltimore, Md.). HIV Western blot strips (Cambridge Biotech Corp., Worcester, Mass.) were incubated with sera (± IgG) diluted 1:100 in blocking buffer containing Tris-buffered saline and powdered milk (5%) for 20 hours. A final volume of 2 ml was used for each sample for each incubation, all incubations were done on a shaker at room temperature, and all incubations were followed by three washes with 0.3% Tween in Tris-buffered saline. The second incubation was in mouse anti-human IgE (Dako, Carpenteria, Calif.), diluted (1:100) in phosphate-buffered saline for 1 hour. The final incubation was in sheep anti-mouse peroxidase-labeled antibody (Amersham International Ltd., Little Chalfont, U.K.), diluted (1:2000) in phosphate-buffered saline, for 1 hour. Strips were developed in 2 ml of a 3% H_2O_2 solution containing 3'3' diaminobenzidine (Sigma Chemical Co, St. Louis, Mo.) for 15 minutes, and the reaction was stopped with distilled water. The strips were read, dried, and mounted. Control sera were obtained from an HIV-seronegative atopic adult (serum IgE >500 IU/ml) and a child with HIV infection without specific IgE against HIV but total serum IgE greater than 2000 IU/ml.

Serum p24 antigen determination

Serum HIV-1 p24 was detected by using the HIV-1 p24 Core Profile ELISA System (Dupont NEN, Wilmington, Del.) on immune complex–disrupted samples (Dupont NEN ADD kit).

RESULTS

Children with HIV infection and elevated serum IgE

Peripheral blood was obtained from 30 children infected with HIV, who were seen for routine visits over a 2-month period at the Pediatric Immunology Clinic, State University of New York Health

TABLE I. Profiles of HIV-infected children with elevated or normal to low serum IgE

Patient No.	Ethnic origin	Age	Sex	Disease class			p24 (ng/ml)	CD4+ T cells/ mm³		Immunoglobulins				
				P2D1	P2D2, 3	FTT		Total	Percent	IgM	IgG	IgA	IgE	HIV-IgE
										(expressed in mg/dl)				(IU/ml)
1	P	12 yr	M	−	−	−	0	345	27	142	1440	394	312	P24
2	P	8 yr	F	−	−	−	0	408	12	107	1620	878	253	P24, gp160
3	P	11 yr	F	−	+	−	0	754	25	312	2480	541	324	P24, gp160
4	P	8 yr	M	−	−	−	110	52	2	123	1460	394	2475	Neg
5	A	10 yr	F	−	−	−	0	905	40	558	5960	7	1000	Neg
6	A	9 yr	F	−	−	−	ND	483	18	361	2714	564	130	Neg
7	A	10 yr	F	−	−	−	20	7	2	171	1750	461	121	Neg
8	A	3 yr	F	+		+	0	528	10	352	4580	366	6	p14, p17
9	A	8 yr	F	+		+	500	1	1	40	1100	275	6	Neg
10	A	4 yr	F	−	−	−	140	8	1	42	972	16	6	Neg
11	A	11 yr	F	−	+	−	0	7	1	156	1320	710	<5	Neg
12	A	7 yr	F	+		+	>500	5	1	262	2320	412	78	Neg
13	A	3 yr	M	−	−	−	ND	19	2	112	2440	931	89	Neg
14	A	13 yr	M	−	−	−	445	714	31	183	2640	293	<5	Neg
15	A	10 yr	F	−	+	−	ND	343	9	286	3540	828	10	Neg
16	A	13 yr	M	−	−	−	500	47	3	159	3040	649	10	Neg
17	A	9 yr	F	−	−	−	0	111	13	152	2128	297	42	ND
18	A	3 yr	F	−	+	+	ND	665	15	265	4360	164	5	ND
19	A	13 yr	M	−	+	−	ND	185	11	290	4590	372	<5	ND
20	A	4 yr	F	−	+	−	ND	857	31	145	4310	155	58	ND
21	P	11 yr	M	−	+	−	0	401	27	69	1350	388	87	ND
22	A	6 yr	F	−	−	−	125	303	13	361	2922	81	<5	ND
23	A	4 yr	F	−	−	−	ND	539	26	122	2850	172	82	ND
24	A	4 yr	F	−	−	−	ND	1001	35	143	2490	613	37	ND
25	A	2 yr	M	−	−	−	240	864	27	163	1840	197	57	ND
26	A	6 mo	M	+		+	ND	499	26	262	1740	41	29	ND
27	A	6 mo	M	+		+	ND	1018	17	299	1480	17	24	ND
28	P	7 mo	M	−	+	+	0	293	17	83	544	89	<5	Neg
29	A	18 mo	M	+		−	ND	327	13	29	1010	11	7	ND
30	A	2 yr	F	−	+	−	0	1225	28	374	3300	241	<5	ND

Clinical profiles of children with HIV infection and elevated serum IgE and normal to low serum IgE. Disease Classification: P2D1, 2, and 3 refer to CDC classification for HIV disease in children under the age of 13 years: *P2D1,* OIs; *P2D2,* unexplained recurrent serious bacterial infections; *P2D3,* other infectious diseases. Allergic symptoms include rhinitis, asthma, dermatitis, or urticarial drug reaction. Failure to thrive is defined as below the 5th percentile for height and weight for age.

FTT, Failure to thrive; *P,* Puerto Rican; *A,* African American; *ND,* not done.

Science Center in Brooklyn. Six of the 30 children were Puerto Rican, and 24 of the 30 children were African American (Table I). The IgE level was elevated (>100 IU/ml; ages, 8 to 12 years) in sera of 26% (7 of 30) of the children (121 to 2475 IU/ml) (Table I, patients 1 to 7). The majority of the children with elevated serum IgE were Puerto Rican (4 of 7). The children with elevated serum IgE ranged in age from 8 to 12 years, and the children with normal to low serum IgE ranged in age from 6 months to 13 years.

Peripheral blood of 70% of the children with elevated serum IgE had decreased $CD4^+$ T-cell counts (5 of 7). $CD4^+$ T-cell counts were considered decreased by the following criteria: less than 500 cells/mm^3 for children 6 years and older; less than 750 cells/mm^3 for children aged 2 to 6 years; less than 1000 cells/mm^3 for children aged 1 to 2 years; less than 1750 cells/mm^3 for children younger than 1 year.[11] Despite the decrease in $CD4^+$ T cells in the majority of children with elevated serum IgE, none had any documented episodes of OIs; that is, they did not fall into CDC class P2D1 (0 of 7 children). Only one of the seven children fell into CDC class P2D2 (recurrent bacterial infections) (Table I, patient 3), and none fell

TABLE II. Characteristics of children with HIV infection (n = 30)

	Elevated serum IgE (n = 7)		Normal to low IgE (n = 23)	
	No.	Percent	No.	Percent
OIs (P2D1)*	0/7	<1	6/23	25
Other serious infections (P2D2, P2D3)*	1/7	14	8/23	35
Failure to thrive	0/7	<1	7/23	30
Allergic symptoms	2/7	28	7/23	30
African American	3/7	43	21/23	91
Puerto Rican†	4/7	57	2/23	9

Summary of differences between children with HIV infection and elevated serum IgE and with normal to low serum IgE.
*P2D1, 2, and 3 are CDC classifications for HIV disease in children under the age of 13 years: *P2D1,* OIs; *P2D2,* unexplained recurrent serious bacterial infections; *P2D3,* other infectious diseases. Failure to thrive is defined as less than the 5th percentile for weight and height. Allergic is defined as exhibiting symptoms of rhinitis, asthma, dermatitis, or urticarial drug reaction.
*CDC classification.
†p = 0.02 (comparing incidence of Puerto Rican ethnicity in the IgE elevated group to that in the IgE normal to low group).

into class P2D3 (other infections). None of the children with elevated serum IgE met criteria for failure to thrive (0 of 7).

Blood of 83% of the children (19 of 23) with low to normal serum IgE levels contained decreased numbers of $CD4^+$ T cells. In contrast to the children with elevated serum IgE, however, 26% of those in the normal to low serum IgE group had documented episodes of OI, falling into CDC class P2D1 (6 of 23); 35% had other serious infections, falling into CDC classes P2D2 or P2D3 (8 of 23 children); and 30% (7 of 23) met criteria for failure to thrive.

The two groups demonstrated no differences in the incidence of allergic symptomatology (rhinitis, dermatitis, asthma, or urticarial reaction to medication) (elevated IgE, 2 of 7; and normal to low IgE, 7 of 23), and no difference in the incidence of lymphocytic interstitial pneumonitis or parotitis was seen (data not shown).

The ethnic breakdown of the elevated IgE group did not reflect that of the population served by the clinic. The majority of patients in the elevated IgE group were Puerto Rican (4 of 7), in comparison those in the normal to low IgE group, less than 10% of whom were Puerto Rican (2 of 23; p = 0.02) (data summarized in Table II).

The majority of the children with elevated IgE (4 of 7) (Fig. 1: patients 1, 2, 4, and 7) had IgM and IgG levels near or below the upper limit of normal for age-matched control subjects (ranges for age-matched control subjects, 7 to 12 years, established by seroimmunology laboratory: IgG, 608 to 1572; IgA, 45 to 236; IgM, 52 to 242).

Antigen-specific IgE directed against HIV peptides

Sera from children with HIV infection and elevated serum IgE (n = 7) (Table I, patients 1 to 7) and from children with HIV infection and normal to low IgE (n = 10) (Table I, patients 8 to 16 and 28) were tested for the presence of antigen-specific IgE directed against HIV proteins (IgE anti-HIV) in Western blot assays (Fig. 1). IgE anti-HIV was found in three of seven children with elevated serum IgE. Two of the children had IgE anti-p24 and IgE anti-gp160 (Table I, patients 2 and 3) (data for patient 2 shown in Fig. 1, No. 1), and one had only IgE anti-p24 (Fig. 1, No. 3). None of these three children had p24 antigenemia, although some of the children with elevated serum IgE and no HIV-specific IgE did have detectable p24 antigenemia (Table I, patients 4 and 7). The three children with elevated IgE and HIV-specific IgE were all Puerto Rican.

IgE anti-p24 and IgE anti-p17 were detected in serum of one African American child who did not have elevated serum IgE (6 IU/ml) (Table I, patient 8; Fig. 1, No. 2). Unlike the other children with IgE anti-HIV peptides in serum, this child had episodes of OI and demonstrated failure to thrive. There was no p24 antigenemia detectable in the serum of this child. None of the other children with normal to low serum IgE who were tested for the presence of IgE anti-HIV had positive Western blot results, either before or after IgG was removed from serum.

DISCUSSION

We have identified a group of children with HIV infection between the ages of 8 and 12 years (long-term survivors) with elevated serum IgE, most of whom have decreased numbers of $CD4^+$ T cells in peripheral blood, yet have had no OIs and do not fail to thrive. HIV-specific IgE was detected in the sera of approximately half of the children, all of whom were of the same ethnic origin, which suggests possible genetic predisposition to the IgE anti-HIV response.

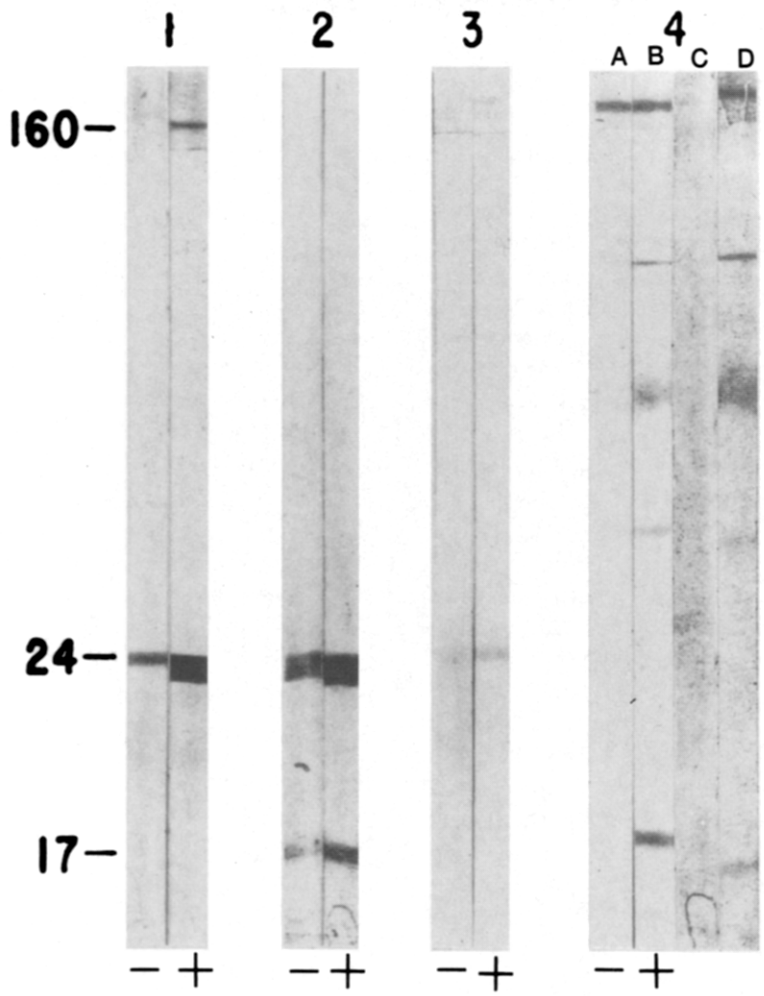

FIG. 1. HIV-specific IgE in serum of children with HIV infection, detected in Western blot assay before (+) and after (−) IgG was removed from sera. Patient 1, lanes (+) and (−) show data for one of two children (see Table I) with IgE anti-p24 and gp120; patient 2, a child with IgE anti-p24 and p17; patient 3, a child with IgE anti-p24; patient 4: control subject, a child with HIV infection and high total serum IgG and IgE but no IgE anti-HIV. *Strips A and B* demonstrate effectiveness of IgG removal. *Strip A* is after IgG removal; *strip B* is before. *Strips C and D* demonstrate specificity of anti-human IgE monoclonal antibody in this assay. *Strip C* was incubated with anti-IgE, and *strip D* was incubated with anti-IgG.

The existence of these children with elevated IgE and no OI or growth failure many years after infection with HIV raises several issues. Elevated serum IgE in adults with HIV infection has been noted to be a marker for poor prognosis by both. Wright et al.[1] and Israel-Biet et al.[2] Vigano et al.[8] also reported elevated mean IgE levels in groups of children with advanced HIV disease. Furthermore, T_{H2}-type cytokine patterns, which are linked to IgE responses,[12] have been associated with disease progression in HIV.[13] The apparent discrepancy between the aforementioned findings and our findings is most likely attributable to our inclusion of a group of children who produce IgE against HIV, a response previously reported only in patients with hemophilia.[7] The trends implied by the data from the children with elevated serum IgE actually led to the discovery of this group of children who demonstrate a specific IgE response against HIV. It may be that this response occurs preferentially in children, in certain ethnic groups, or in both.

We are certain that we are observing a specific IgE response, rather than a polyclonal activation, in the patients with IgE against HIV; but we have also observed near-normal levels of IgG and IgM in more than half of the children with elevated IgE, including some who have no IgE against HIV. In these children the evidence is also clearly against simple poly-

clonal activation. It appears that in agreement with the findings of Wright et al.,[1] some of our patients have a preferential increase in IgE above other serum immunoglobulin classes, even if we have not yet identified all of the specific antigens (opportunistic pathogens?).

Although previous studies have reported increased levels of serum IgE in patients with advanced HIV disease, these studies have not focused on patients with advanced HIV disease and decreased blood $CD4^+$ T cells or compared individuals with elevated serum IgE with those who have normal to low serum IgE. If elevation of IgE, and particularly production of IgE against HIV, is a late compensatory response, which only certain individuals are capable of producing, it may be a marker of HIV disease progression and still be beneficial.

It is tempting to speculate that the elevated IgE, particularly the HIV-specific IgE, contributes to the prolonged health of the children with HIV infection and elevated serum IgE identified in this study. However, it could be another factor, which operates in the presence of IgE, that is actually responsible for the healthier state. HIV-specific IgE was detected in serum of about 50% of the children with elevated serum IgE and in one child without elevated serum IgE; none of the children had detectable p24 antigenemia. However, it is not known whether their serum IgE levels are stable, increasing, or decreasing. The single child without total IgE elevation who had detectable IgE directed against p24 and p17 was failing to thrive and had OIs, possibly coinciding with a decrease in IgE anti-HIV. Prospective studies need to be undertaken to test this hypothesis.

The roles of IgE responses in HIV disease have not been fully studied, and such responses could include classic IgE-mediated immediate hypersensitivity,[4] antibody-dependent cell-mediated cytotoxicity (responses,[14] and other less well-characterized IgE responses to viral infections, as described, for example, in respiratory syncytial viral infections.[15] Further characterization of the IgE response against HIV is important because it may add to our armamentarium against HIV, as well as to our understanding of IgE responses against viral illnesses in general.

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