

IgE, CD8⁺CD60⁺ T Cells and IFN- α in Human Immunity to Parvovirus B19 in Selective IgA Deficiency

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ABSTRACT: Although IgE is implicated in viral immunity, its role in parvovirus B19 immunity and its relationship to other immunological states has not been studied. Total serum immunoglobulin levels, IgG and IgE anti-parvovirus B19, blood lymphocyte numbers, and epsilon and cytokine specific mRNA were determined in pediatric patients with normal serum IgA levels (IgA⁺) and selective IgA deficiency (IgA[−]) on days 0 (initial diagnosis) and 14, and 3 years after recovery (nephelometry, Western blot test, flow cytometry, reverse transcriptase–polymerase chain reaction). We found that both patients had serum IgM, IgG, IgE, and IgA levels within normal ranges on day 0 to 3 years, excluding IgG₁ and IgA in the IgA[−] patient, which were elevated and negative, respectively, and IgE in the IgA⁺ patient, which was elevated (>100 IU/ml). The serum IgA⁺ and IgA[−] patients made IgE (and IgG) anti-parvovirus B19 at all time points. Excluding CD8⁺CD60⁺ T cells, determinations of T, B, and NK lymphocyte subsets always were within normal ranges. In both patients, CD8⁺CD60⁺ T-cell numbers were within normal ranges on day 0, but dramatically in-

creased on day 14 (more than fivefold). At 3 years, they had returned to normal in the IgA⁺ patient, but remained high in the IgA[−] patient. On day 0 to 3 years, peripheral blood mononuclear cells of both patients expressed epsilon- and interferon (IFN)- α -specific mRNA. On day 0, the IgA⁺ patient expressed interleukin (IL)-4 and IL-10, but not IL-2, IFN- γ , or IL-6 mRNA; the IgA[−] patient expressed IL-6 and IL-10 mRNA, but not IL-4, IL-2, or IFN- γ mRNA. At 3 years, the IgA⁺ patient expressed mRNA for all cytokines, but the IgA[−] patient did not express mRNA for any of these cytokines. Our results suggest that IgE is important in parvovirus B19 immunity, and that IFN- α and CD8⁺CD60⁺ T cells may regulate IgE memory responses and isotype switching. *Human Immunology* 66, 1029–1038 (2005). © American Society for Histocompatibility and Immunogenetics, 2005. Published by Elsevier Inc.

KEYWORDS: parvovirus B19; IgE anti-parvovirus B19 Abs; selective IgA deficiency; CD8⁺CD60⁺ T cells; IFN- α

ABBREVIATIONS

IgE immunoglobulin E
PBMC peripheral blood mononuclear cells

PCR polymerase chain reaction
TMB 3,3',5,5'-tetramethylbenzidine

INTRODUCTION

Parvovirus B19 is a single-stranded DNA virus that causes erythema infectiosum, also known as the “fifth

disease” of childhood [1]. Usually mild flulike symptoms are observed. After 1–2 weeks, an exanthem appears on the cheeks and spreads over the entire body [1]. In normal adults, parvovirus B19 infection may progress asymptotically. However, complications including arthralgia and rheumatoid arthritis may occur [1].

It is becoming increasingly clear that IgE may play an important role in immunity to viruses. IgE anti-human immunodeficiency virus type 1 (HIV-1) [2–4], parvovirus B19 [5], parainfluenza [6], and respiratory syncytial virus [7, 8] have been described. Studies in our labora-

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tory were the first to describe a functional role for IgE anti-HIV-1 in that IgE anti HIV-1 antibodies strongly suppress virus replication *in vitro* [3], and possibly may contribute to nonprogressor status. In these studies, an IgE mediating cytotoxicity mechanism may be responsible for suppression of HIV-1 [3, 4].

It is well known that genomic DNA undergoes conventional VDJ recombination in the switch from expression of IgE to IgA [9]. Durkin *et al.* [10] demonstrated that surface IgE (sIgE)+ B lymphocytes appear to be lacking in adult germ-free rats in that their Peyer's patches contain numbers of cells with sIgE, 50% of which simultaneously express sIgA [10]. Studies of Auci *et al.* [11] demonstrated that peak antigen-specific IgE responses can be downregulated *in vivo*, whereas IgA responses can be upregulated by muramyl dipeptide or murabutide. In contrast, earlier studies of Weinstein and Cebra [9] demonstrated that infection of reovirus type I/Lang virus to stimulate acute *de novo* germinal center reactions in lymph node, sIgA+ B cells, which were the major non-sIgM/sIgD population found to arise in germinal centers of Peyer's patches, were not found in lymph node, whereas IgG₁ was detected [9]. Bos *et al.* [12] demonstrated that in IgA-producing hybridomas derived from peritoneal B cells that monoclonal IgA is encoded by both germ line and somatically mutated *VH* genes and is reactive with commensal bacteria [12]. In human primary immunodeficiency disease, such as ataxia telangiectasia, decreased levels of serum IgA, IgE, and IgG₂ and IgG₄ subclasses have been reported [13–15].

In the present study, we investigated the role of IgE in anti-parvovirus immunity in serum obtained from parvovirus B19-infected children. We studied T-cell subsets and cytokines expressed by peripheral blood mononuclear cells (PBMC) obtained from serum IgA-positive and IgA-deficient parvovirus B19-infected children on day 0 and 3 years after presentation of symptoms. Our results suggest that IgE anti-parvovirus B19 antibodies may play an important role in persistent immunity to parvovirus B19, and that interferon (IFN)- α and CD8⁺CD60⁺ T cells may regulate IgE responses and isotype switching.

MATERIALS AND METHODS

Patient Specimens

Peripheral blood (5 ml total) was obtained from a selective IgA-deficient (IgA <6.0 mg/dL) parvovirus B19-infected 5-year-old Caucasian boy with normal serum IgE levels (29 IU/ml), and from a parvovirus B19-infected 8-year-old Caucasian boy with normal serum IgA levels (37–58 mg/dl) and increased serum IgE levels (150–256 IU/mL) at day 0 (initial presentation) and 3 years after presentation of symptoms. The IgA-deficient

patient had negative skin prick to tree and grass pollens and ragweed allergens; the IgA-positive patient had positive skin prick to the above allergens. Parvovirus B19 infection was established by enzyme immunoassay (EIA) and positive pattern on Western blot tests. Each patient presented with a "slap cheek" rash over his face and a diffuse erythematous rash over the neck, ears, hands, elbows, and knees. No other coinfections were described by the patient history. No medications or immunotherapy were administered to either patient. Both children recovered from the infection of parvovirus B19 approximately 2–3 weeks after initial presentation.

The study was approved by the institutional review board of the SUNY Downstate Medical Center, Brooklyn, New York (study number 01-105), and the procedures followed were in accordance with institutional guidelines involving human subjects.

Blood

For studies of serum immunoglobulins, blood was collected into red top Monoject tubes (Sherwood Medical, St. Louis, MO, USA) and sent to Quest Diagnostics (Teterboro, NJ, USA) for Ig determinations.

For studies of surface markers, blood was collected into EDTA Monoject tubes and stored at room temperature for up to 2 hours when complete blood counts were performed (Sysmex, McGraw Park, IL, USA); flow microfluorometry studies (Coulter Epics XL/MCL) (Beckman Coulter, Miami, FL, USA) were performed within 3 hours.

Determination of Cell Surface Markers

Antibodies. Mouse anti-human monoclonal antibodies (mAbs) directly conjugated to fluorescein isothiocyanate (FITC) included CD45, CD45RA, CD23, Simultest CD3/CD4, Simultest CD3/CD8, Simultest CD3/CD19, CD3/CD16+CD56, and CD60. Mouse anti-human mAbs directly conjugated to phycoerythrin (PE) included CD8 and CD45RO. Labeling studies were carried out with CD8 antibody in conjunction with CD60 (double labeling). All mAbs and appropriately matched isotype controls were purchased from Becton Dickinson Biosciences (San Jose, CA, USA), except for CD60, which was purchased from Ancell (Bayport, MN, USA). All antibodies were titrated to obtain maximum staining efficiency according to the manufacturer's recommendation.

Immunofluorescence assay. Peripheral blood (100 μ l) was incubated with conjugated antibodies for 10 minutes at room temperature. Erythrocytes were lysed with whole blood lysing solution (ImmunoPrep; Beckman Coulter). Flow cytometric analysis was performed on a Coulter Epics XL/MCL flow cytometer that used System II software and CytoComp (Coulter). Forward and side scatter were used to identify the lymphocyte population and CD45 was used to establish an optimal lymphocyte gate.

A minimum of 15,000 events was collected. The gain on the photomultiplier tube detecting fluorescence intensity was adjusted so that 99% of cells with background fluorescence staining were scored between 10^0 and 10^1 on a four-decade log scale. Specific fluorescence was reported as the percentage of cells with relative fluorescence intensity scored above background. The total numbers of lymphocytes were calculated from the white blood cell count. Data are expressed as total lymphocytes per cubic millimeter or percentage of total cells.

Detection of Total Serum Immunoglobulins

Total serum immunoglobulins (IgM, IgG, and IgA) were determined by Quest Diagnostics by means of nephelometry, which was performed, according to standard procedures (Quest Diagnostics reference range for healthy child serum: IgM: 47–311 mg/dL; total IgG: 688–1533 mg/dL; IgG₁: 225–1100 mg/dL, IgG₂: 42–375 mg/dL, IgG₃: 9.1–106.9 mg/dl, IgG₄: 0.3–138 mg/dL; IgA: 41–368 mg/dL). Total serum IgE levels were detected by the UniCAP Total IgE Fluoroenzymeimmunoassay (Pharmacia & Upjohn Diagnostics, Kalamazoo, MI, USA), which was performed according to standard procedures at the Clinical Immunology Laboratory at SUNY Downstate (Brooklyn, NY, USA). Data are expressed as IU/mL (SUNY Downstate reference range for healthy child serum: IgE: 20–100 IU/mL).

Detection of Parvovirus B19 Antibodies in Serum

IgM and IgG. Serum IgM and IgG antibodies to parvovirus B19 were determined by EIA performed by Quest Diagnostics according to standard procedure. Data are reported as ratio report.

IgE. The presence of IgE anti-parvovirus B19 antibodies was determined as previously described [5]. Briefly, parvovirus B19 Western blot strips (Mikrogen, Martinsried, Germany) were incubated with sera diluted 1:100 in washing/diluting buffer (Tris-buffered saline, NaCl, Tween-20, 0.01% methylisothiazolone [Mikrogen], 0.1% oxypyrion, protein [Mikrogen]) on a shaker for 20 hours at room temperature. The strips were washed four times in washing/diluting buffer. Goat polyclonal anti-human IgE (ICN Biomedicals, Aurora, OH, USA), diluted 1:50 or 1:100 in wash buffer, was added to each well and incubated on a shaker for 1 hour at room temperature. The strips were washed four times in wash buffer. Rabbit anti-goat peroxidase-labeled antibody (ICN), diluted 1:1000 in washing/diluting buffer, was added to each well and incubated on a shaker for 1 hour at room temperature. The strips were washed again four times in washing/diluting buffer, and developed in 2 ml of a TMB (3,3',5,5'-tetramethylbenzidine) substrate solution. The reaction was stopped by replacing TMB solution with distilled water, and then strips were read,

dried, and mounted. In addition, IgG anti-parvovirus B19 Western blot (Mikrogen) was determined according to manufacturer's recommendation.

Detection of Cytokine-Specific and Epsilon-Specific mRNA

Total cellular RNA (2 µg/mL) was extracted from PBMC by polymerase chain reaction (PCR) as previously described [5] with TriZol reagent (Gibco/BRL), according to manufacturer's recommendation. Pellets were dissolved in TE buffer (10 mM Tris HCl (Sigma Chemical Company, St. Louis, MO, USA), pH 7.5, 1 mM EDTA, Sigma), and stored at -70°C in a Bio-Freezer (Forma Scientific, Marietta, OH, USA). Expression of interleukin (IL)-2, IL-4, IL-6, IL-10, and IFN- γ , IFN- α , and epsilon mRNA was determined by using the Advantage One-Step reverse transcriptase (RT)-PCR kit (Clontech, Palo Alto, CA, USA), according to manufacturer's recommendation. PCR was conducted by using primer pairs specific for IL-2, IL-4, IL-6, IL-10, IFN- γ , IFN- α , and epsilon (expected band sizes: 305, 344, 628, 328, 427, 303, and 149 bp, respectively) (Integrated DNA Technologies, Coralville, IA, USA). A beta-actin primer set (Integrated DNA Technologies) was used as an internal positive control. Negative controls, consisting of water (no DNA), but addition of primers, were included in every experiment. The PCR amplicons were separated by electrophoresis in a 1.8% agarose (Seakem LE) gel (FMC, Rockland, ME, USA), and visualized with ethidium bromide (Sigma). Correlation with cytokine protein levels were not determined.

RESULTS

Total Serum Immunoglobulins

IgM, IgG, IgA. Serum obtained from the IgA-deficient patient on day 0 and 3 years after presentation of symptoms contained normal levels of total IgM (97 and 101 mg/dl, respectively), IgG₂ (146 and 176 mg/dl, respectively), IgG₃ (68 and 60 mg/dl, respectively), and IgG₄ (25 and 22 mg/dl, respectively); however, total levels of serum IgA were low (<6.0 and <15.0 mg/dl, respectively), whereas total levels of serum IgG₁ were high (1190 and 1190 mg/dl, respectively). Serum obtained from IgA-positive patient on day 0 and 3 years after presentation of symptoms contained normal levels of total IgM (45 and 58 mg/ml, respectively) and IgA (37 and 78 mg/dl, respectively). On day 0, levels of IgG₁ (621 mg/dl), IgG₂ (87 mg/dl), IgG₃ (52 mg/dl), and IgG₄ (13 mg/dl) were within normal range (see Materials and Methods). At 3 years after presentation of symptoms, total levels of IgG were also within the normal range (977 mg/dl) (Quest reference range for healthy child serum: IgG: 694–1618 mg/dl) (Table 1).

TABLE 1 Immunoglobulin levels in serum of serum IgA deficient and IgA+ parvovirus B19 patients

Subject	Time	Immunoglobulin							IgE (IU/ml)
		IgM (mg/dl)	IgG ^a (mg/dl)	IgG ₁ (mg/dl)	IgG ₂ (mg/dl)	IgG ₃ (mg/dl)	IgG ₄ (mg/dl)	IgA (mg/dl)	
IgA–	Day 0	97	nt	1190 ^b	146	68	25	<6.0 ^c	29
	3 years	101	nt	1190 ^b	176	60	22	<15 ^c	18
IgA+	Day 0	45	nt	621	87	52	13	37	256
	3 years	58	977	nt	nt	nt	nt	78	173

^a Total IgG levels (mg/dl).^b Abnormally high laboratory value.^c Abnormally low laboratory value.

IgE. Normal levels of serum IgE were detected in serum obtained from the IgA-deficient patient on days 0 and 3 years after presentation of symptoms (29 and 18 IU/mL, respectively). In contrast, serum IgE levels from the IgA-positive patient were increased on days 0 and 3 years after presentation of symptoms (256 and 173 IU/mL, respectively) (Table 1).

Parvovirus B19–Specific Antibodies

IgM and IgG. On day 0, IgM and IgG anti-parvovirus B19 in serum of the IgA-deficient patient were 4.3 and 6.4 units, respectively (EIA). However, 3 years after presentation of symptoms, parvovirus B19 IgM Abs decreased to 0.4, whereas IgG remained unchanged at 6.6 units. On day 0, IgM and IgG anti-parvovirus B19 Abs from sera of the IgA-positive patient were 1.0 and 3.7 units, respectively. However, by day 14, IgM and IgG anti-parvovirus B19 Abs decreased to 0.5 and 3.0 units, respectively (reference range, IgM, IgG: 0–0.89). Data are reported as ratio report (Quest Diagnostics). Values above the reference range (>0.89) were judged positive.

IgG and IgE. IgG anti-parvovirus B19 antibodies were detected in serum from the IgA-deficient patient on day 0 as determined by EIA and Western blot test (Figure 1, lane 3). Further, serum also contained antigen-specific IgE directed against parvovirus B19 components NS-1 (nonstructural protein-1), VP-N (viral capsid protein-N), VP-C (viral capsid protein-C), and VP-1S (viral capsid protein 1S) on day 0 (Figure 1, lane 5). At 3 years after presentation of symptoms, serum contained IgG anti-VP-C; IgG and IgE anti-parvovirus B19 antibodies were directed against NS-1, VP-N, and VP-1S (Figure 1, lanes 4 and 6, respectively). In contrast, serum of the parvovirus B19–negative donor did not contain either IgG or IgE anti-NS-1, VP-N, VP-C, or VP-1S antigens (Figure 1, lane 2). Similarly, serum from the IgA-positive patient contained antigen-specific IgG and IgE directed against parvovirus B19 components VP-N, NS-1, VP-1S, and VP-C (Figure 2, lanes 3 and 4, respectively).

Distributions of Blood Lymphocyte Subpopulations

Excluding CD8⁺CD60⁺ T cells, T, B and NK lymphocyte subset distributions were normal at all times. In both IgA-positive and IgA-deficient patients, CD8⁺CD60⁺ T cell numbers did not increase on day 0 (0.8 and 0.5 /mm³, respectively), but increased on day 14 (>5 fold) (13 /mm³). At 3 years, they returned to normal in the IgA-positive patient (0.00 /mm³), but remained high (>10-fold) in the IgA-deficient patient (5.0 /mm³) (Table 2).

Cytokine and Epsilon mRNA Expression by PBMC

On days 0–3 years, PBMC of both patients expressed epsilon- and IFN- α -specific mRNA. On day 0, the IgA-deficient patient expressed IL-6 and IL-10 mRNA, but not IL-4, IL-2, or IFN- γ mRNA; the IgA-positive patient expressed IL-4 and IL-10, but not IL-2 or IFN- γ mRNA. At 3 years, the IgA-deficient patient did not express mRNA for any cytokines, but the IgA-positive patient expressed mRNA for all cytokines (Table 3).

DISCUSSION

In the present studies, we have demonstrated (1) the presence of IgE anti-parvovirus B19 antibodies in serum obtained from both IgA-deficient and IgA-positive, parvovirus-infected children, and its persistence 3 years after presentation of symptoms, (2) Th2 cytokine responses predominate early in IgE anti-parvovirus infection, (3) IFN- α and epsilon mRNA is expressed both early and late in parvovirus infection, and (4) CD8⁺CD60⁺ surface expression on T cells is increased on peripheral blood lymphocytes on day 14 after presentation of symptoms (IgA positive) and 3 years after presentation of symptoms (IgA negative).

Previous studies in our laboratory were the first to demonstrate that IgE anti-parvovirus B19 is produced by a parvovirus B19–infected child [5]. The presence of virus-specific IgE has been demonstrated in several viral infections. Our laboratory has previously identified IgE

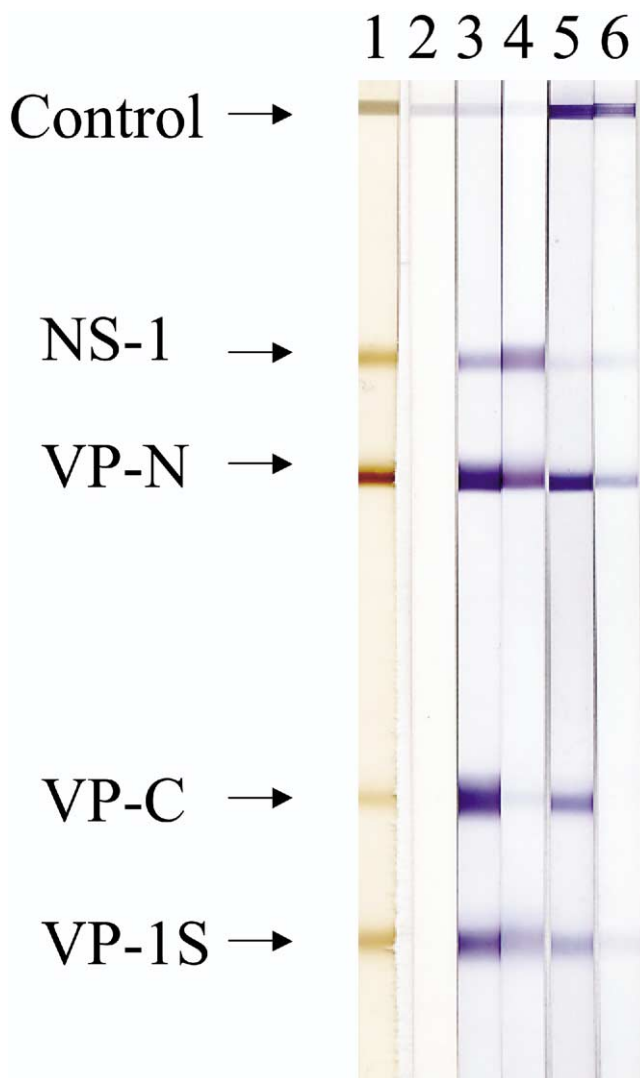


FIGURE 1 Western blot analysis of anti-parvovirus B19 antibodies in IgA deficiency. Serum obtained from the IgA-deficient patient was incubated with Western blot strips containing parvovirus B19 antigens NS-1, VP-N, VP-C, and VP-1S (see Materials and Methods). Lane 1—control strip showing representative bands. Lane 2—Serum from a parvovirus B19-negative donor. Lane 3—patient serum incubated with anti-human IgG (1:1000) at day 0 after presentation of symptoms. Lane 4—patient serum incubated with anti-human IgG (1:1000) at 3 years after presentation of symptoms. Lane 5—patient serum incubated with anti-human IgE (1:100) at day 0 after presentation of symptoms. Lane 6—patient serum incubated with anti-human IgE (1:100) at 3 years after presentation of symptoms. Control band represents anti-human immunoglobulin. Relative serum concentration of IgE compared with IgG is 1:1–100 million.

anti-HIV-1 antibodies in a cohort of HIV-1-infected nonprogressor children who had no detectable virus load, despite severely reduced [2, 3] or normal levels [4] of blood CD4⁺ T cells. In those studies, IgE anti-HIV antibodies appeared to be protective against HIV-1 dis-

ease by inhibiting virus production or mediating antibody-dependent cellular cytotoxicity against virus-infected cells [2–4]. Other investigators have identified IgE anti-respiratory syncytial virus (RSV) in nasopharyngeal secretions of infants infected with RSV [8]. Other studies have found elevated serum IgE levels and/or IgE antiviral antigens, including human T-cell lymphotropic virus type 1 [16], parainfluenza virus [17], Puumala virus [18], herpes simplex virus (HSV)-1, HSV-2, Epstein-Barr virus, and virus-associated encephalopathies [19]. The clinical implications of these IgE antiviral antibodies are unknown.

The relationship of IgE in IgA deficiency remains unclear. Studies of Buckley and Fiscus [20] demonstrated elevated serum IgE levels in IgA deficiency; however, many of these patients were atopic [20]. Studies of DeLaat *et al.* [21] reported concomitant IgG4-IgE deficiency in select IgA-deficient patients who also had circulating anti-IgA antibodies [21]. The presence of additional underlying immunodeficiency [22, 23], atopic [24–27], surgical [28], or drug-induced [29] states in apparent (selective) IgA deficiency suggest an altered state of immunoglobulin regulation in general [30, 31], making it difficult to establish a clear connection between IgA and IgE. IgA deficiency correlated with decreased serum IgE and increased IgG and IgM in a murine model of disease [32]. It is possible that IgE antiviral production may be affected by heavy chain class switching defects associated with IgA deficiency. Studies of Qiu and Stavnezer [33] have demonstrated that overexpression of B-cell-specific activator protein (BSAP)/Pax-5 can inhibit isotype switching to IgA and enhance switching to IgE *in vitro*. It is also likely that the relationship between IgA and IgE heavy chain production in IgA deficiency can vary and is influenced by many factors, including HLA [34]. The presence of elevated IgG₁ in the IgA-deficient patient on both day 0 and 3 years after presentation of symptoms may be related to the production of IgE anti-parvovirus B19 antibodies because these isotypes have been described to be regulated by similar promoters [35, 36] and can be independent of cytokines such as IL-4 [33].

In the present study, we demonstrated the existence of IgG and IgE anti-parvovirus B19 in serum of an IgA-deficient child. One of the most remarkable findings presented here is the long-term persistence of these antibodies, as well as decreased levels of total serum IgA and IgE. This result suggests that in addition to the persistence of IgE antiparvovirus antibodies in the IgA-deficient patient, there may be an IgE-specific memory component in parvovirus B19 infection that may play an important role in the control of secondary infection. Thus, this report may suggest a possible mechanism to explain the prolonged duration of protective immunity

against parvovirus reinfection in the human population. Previous studies in our laboratory were the first to demonstrate the presence of IgE anti-parvovirus B19 antibodies 7 months after infection in a nonimmunodeficient patient [5]. The fact that this study demonstrates the

presence and persistence of IgE anti-parvovirus B19 antibodies as late as 3 years after presentation of symptoms and in both healthy and immunodeficient patients elucidates a unique characteristic of IgE biology. Similar studies in our laboratory [3] in HIV-1 disease have revealed a persistence of HIV-1-specific IgE after 210 days, and retained its ability to suppress HIV-1 production *in vitro* [3]. Further, we have demonstrated that specific IgE anti-HIV-1 antibodies may protect against HIV-1 disease progression by promoting cytotoxic responses or by suppressing virus production [2–4]. Thus, the association of IgE with viral infection and immunity could be consistent with an important immune function of IgE.

Serum obtained from parvovirus-infected patients contained IgG and IgE antibodies to NS-1, VP-N, and VP-1S at day 0 and 3 years after presentation of symptoms. In the IgA-deficient patient, in contrast to IgG, there was no detectable IgE anti-VP-C antibodies present at 3 years after presentation of symptoms. VP-C represents the C-terminal end of VP-1/2 viral capsid protein of parvovirus. It could be that production of IgE antiviral antibodies is distinct from IgG production and is subject to independent antigen processing mechanisms. It is possible that IgE is influenced or displays a tendency to preferentially recognize conformational epitopes over time. This may explain why IgE anti-VP-C is not detectable at 3 years after presentation of symptoms because the VP-C antigen in our test system provides a linear epitope for antibody detection. Similar conformational requirement for ideal IgE antibody processing have been described in which cysteinylated glycosylation inhibiting factor (GIF), but not the wild-type GIF, possessed immunosuppressive effects on IgE antibody response *in vitro* [37]. The presence of IgG anti-VP-C antibodies in the IgA-deficient patient and the lack thereof in the IgA-positive patient may be due to differential antigen processing in immunodeficiency states. Similarly, differential response to antigens was demonstrated in dendritic cells in patients with common variable immunodeficiency [38]. In addition, anti-parvovi-

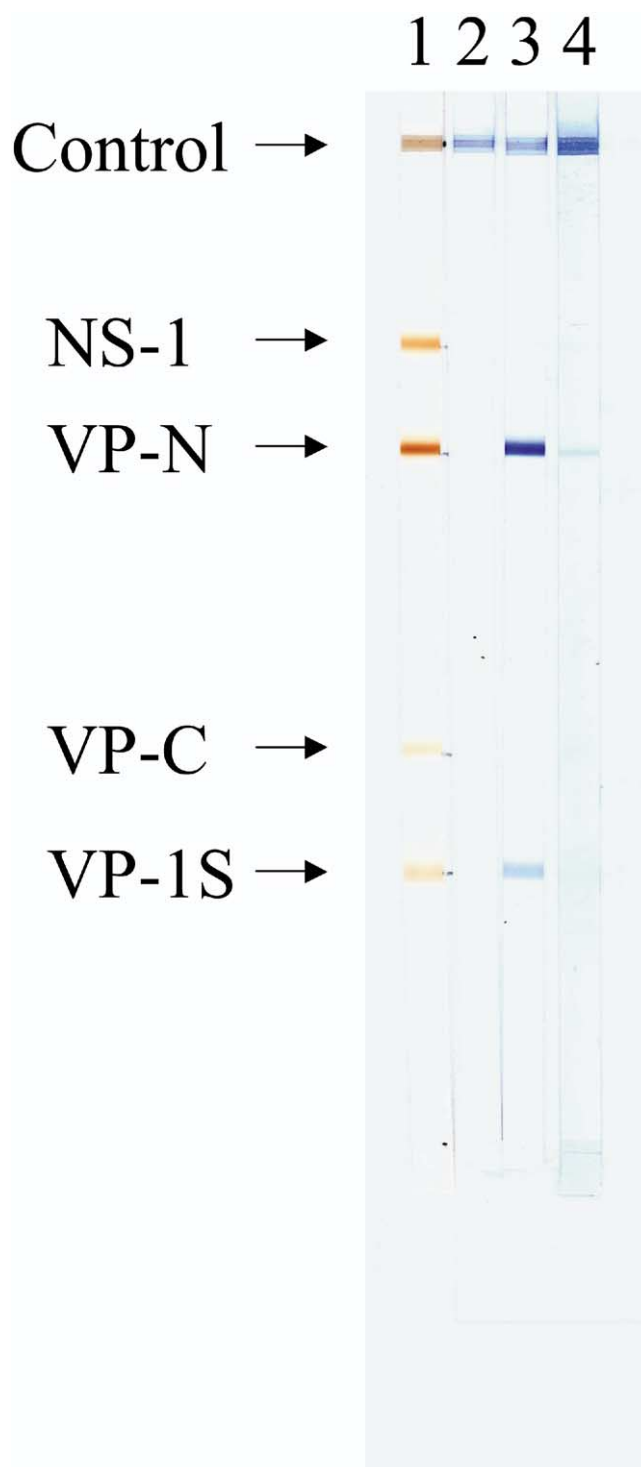


FIGURE 2 Western blot analysis of anti-parvovirus B19 antibodies. Serum obtained from the IgA-positive patient was incubated with Western blot strips containing parvovirus B19 antigens NS-1, VP-N, VP-C, and VP-1S (see Materials and Methods). Lane 1—control strip showing representative bands. Lane 2—serum from a parvovirus B19-negative donor. Lane 3—patient serum incubated with anti-human IgG (1:1000). Lane 4—patient serum incubated with anti-human IgE (1:100). Similar results were obtained at 3 years after presentation of symptoms. Control band represents anti-human immunoglobulin. Relative serum concentration of IgE compared with IgG is 1:1–100 million.

TABLE 2 Distributions of lymphocyte subpopulations in peripheral blood of serum IgA deficient and IgA+ parvovirus B19 patients^a

Subject	Time	CD3 ⁺ CD4 ⁺		CD3 ⁺ CD8 ⁺		CD8 ⁺ CD60 ⁺		CD16 ⁺ 56 ⁺		CD19 ⁺		CD23 ⁺	
		mm ³	%	mm ³	%	mm ³	%	mm ³	%	mm ³	%	mm ³	%
IgA [−]	Day 0	1286	46	653	23	0.5	13	115	4.0	477	17	115	4.0
	3 years	713	49	375	26	5.0	72	64	4.0	183	13	59	4.0
IgA ⁺	Day 0	703	50	419	30	0.8	0.06	64	5	154	11	40	3
	Day 14	859	47	548	30	13	0.7	92	5	180	10	77	4
	3 years	1011	48	650	31	0	0.00	81	4	298	14	68	3

^a The distributions of lymphocyte subpopulations in peripheral blood of serum IgA deficient and IgA+ parvovirus B19 patients were determined on days 0 and 14, and 3 years after symptom presentation by flow microfluorimetry (Coulter Epics XL/MCL). Data are expressed as mean total cells/mm³ or mean percentage (%) of positive cells.

rus B19 antibody responses may differ in children in that the unique region of VP1 appears to be a target for cell-mediated immune responses [39]. Furthermore, the presence of IgG reactivity against B19 VP1 is necessary to confirm past infection because antibody reactivity against “linear” epitopes of VP2 antigen is lost shortly after infections [39]. These studies suggest that immune responses against parvovirus infection can modulate over time and may differ in immunodeficiency states.

This is the first report demonstrating an increase in CD8⁺CD60⁺ T cell expression by peripheral blood lymphocytes in parvovirus B19 infection. A number of functions have been ascribed to CD60⁺ T cells. It has been described as a molecule/receptor [40–42] involved in activation of T cells in health and disease [43–46]. It has been suggested that CD60 on activated T or B cells may facilitate contact with other helper T cells, influencing either T-T [41] or T-B [42] cell interactions. Previous studies in our laboratory [47, 48] have demonstrated that CD8⁺CD60⁺ T cells are significantly increased in peripheral blood of serum IgE+ ragweed sensitized humans, compared with serum IgE negative nonatopic humans [47, 48]. Studies by Bogner *et al.* [49] reported a significant increase in numbers of CD8⁺CD60⁺ T cells in HIV-1 infected hemophiliac patients, but no correla-

tion with stage of HIV-1 infection, CD4⁺ T cell counts or viral load [49]. In the present studies, the dramatic increase in CD8⁺CD60⁺ T cells over 3 years in IgA deficiency implies that these cells may play an important role in regulation of parvovirus specific memory IgE responses *in vivo* and might provide epsilon helper activity.

Alternatively, Th1 and Th2 cytokines, may contribute to the immunopathogenesis of parvovirus B19 infection, and can be key mediators of viral elimination. In our study, cytokine specific mRNA expression in parvovirus B19 infection was compared on both day 0 and 3 years after presentation of symptoms. Analysis of the cytokine pattern, in the IgA-deficient patient revealed a Th2-like cytokine profile (IL-6+, IL-10+) on day 0, and an absence of these cytokines 3 years later. Similarly, for the IgA positive patient, Th-2 cytokines (IL-4+, IL-10+) prevailed on day 0. Although we demonstrate the presence of a Th-2 type cytokine response, other studies have reported Th-1 type (IFN-γ) [50] and Th-2 type responses (IL-6) [50] in parvovirus B19 infection. It is possible that cytokine responses to parvovirus B19 differ between adults and children. Lymphocytes isolated from convalescent adults produced a Th1 response (IL-2, IFN-γ), whereas lymphocytes isolated from recently infected

TABLE 3 Summary of cytokine production by peripheral blood mononuclear cells (PBMC) obtained from serum IgA deficient and IgA+ parvovirus B19 patients^a

Subject	Time	Cytokine					IFN-α	Epsilon
		Th-1		Th-2				
		IL-2	IFN-γ	IL-4	IL-6	IL-10		
IgA−	Day 0	−	−	−	+	+	+	+
	3 years	−	−	−	−	−	+	+
IgA+	Day 0	−	−	+	−	+	+	+
	3 years	+	+	+	+	+	+	+

^a Unfractionated PBMC from serum IgA deficient and IgA+ parvovirus B19 patients were evaluated for the presence (+) or absence (−) of Th1-type (IL-2, IFN-γ) cytokines and Th2-type (IL-4, IL-6, IL-10) cytokines, IFN-α, and epsilon. Expression of cytokine-specific mRNA production was determined by Advantage One-Step RT-PCR (Clontech), as described in Materials and Methods.

children had a deficient IFN- γ response to VP1 or VP2 epitopes [39]. Studies by others [50] demonstrated that acute parvovirus infection is characterized by an increase in monocytes, T cells, and natural killer cells [50]. The authors concluded that proinflammatory monokines and lymphokines play an important role in parvovirus disease manifestation. [50]. Our data suggest that it is also possible that during early viral infection, parvovirus B19 may suppress certain Th1-type cell responses, including IL-2 and IFN- γ production, and parvovirus B19 gene products might be involved in suppressing host immune responses and T cell activation.

However, in contrast to the IgA-deficient patient, Th-2 type cytokines (IL-4+, IL-10+) were present in the IgA-positive patient at days 0 and 3 years after presentation of symptoms. Interestingly, IL-4 was absent in the IgA-deficient patient at days 0 and 3 years after presentation of symptoms and that this patient demonstrated normal IgE production as evidenced by gene (positive epsilon mRNA) and protein (23 ± 5 IU/ml) expression. This suggests that, in IgA deficiency, IgE production can be independent of the presence of IL-4. It could be that this patient's IgE production is initiated and maintained by the presence of IFN- α because this cytokine is present at days 0 and 3 years after presentation of symptoms whereas IL-6 and IL-10 are only present at day 0. IFN- α was also present at both time points in the IgA positive patient. In this patient the presence of additional Th-2 type cytokines, IL-4, IL-6 and IL-10 at 3 years after presentation of symptoms, may serve to augment the IFN- α initiated IgE response. It is also possible that differences in cytokine mRNA responses may be caused by other infections that are common in children. No evidence of other coinfections were observed in these patients.

Furthermore, IFN- α appears to serve as a critical cytokine providing help for specific IgE responses because (1) in our previous studies, the CD8⁺CD60⁺ T helper cells for ragweed specific IgE responses by PBMC from serum IgE+ ragweed sensitized humans produced IFN- α , but not IL-4 or IL-13, *ex vivo* [47, 51]; (2) it provided isotype specific help for ~50% of the hapten specific IgE memory responses induced *in vitro* by Herrick *et al.* [52]; the remaining help was provided by IL-4; and (3) it increased specific IgE responses in isotype specific fashion when injected into mice at the peak of a hapten-specific IgE response; IL-4, depending on its concentration either helped or suppressed these responses, and IFN- γ suppressed them [53]. In addition, IFN- α increased IgE production by a human IgE secreting cell line (U266), whereas IL-4, IL-6 or IFN- γ suppressed IgE secretion by these cells [54].

Furthermore, the IFN- α initiated IgE response may be related to the expression of CD23. CD23 (Fc ϵ RII) is

the low affinity receptor for IgE and can be found expressed on cell surface or in shed/soluble form (sCD23) [55]. It is possible that IgE production in the IgA-deficient patient is initiated by IFN- α and maintained by sCD23 because cell surface CD23 expression decreases from day 0 to 3 years after presentation of symptoms, which could be due to an increase in the proportion of sCD23. Data from our laboratory [56] have demonstrated that IFN- α -mediated increases in sCD23 correlates with increased IgE production in a mouse atopy model system [56].

The presence of IFN- α on both days in both patients indicates that IFN- α may operate as a final effector to activate antiviral mechanisms within cells infected by parvovirus B19. It is well known that IFNs are potent immunomodulatory substances. They produce antiproliferative effects, induce antiviral resistance, and regulate immune responses [57]. Levels of IFN- α in healthy individuals are usually low, but in inflammatory conditions, IFN- α is secreted in the early stages of inflammation from antigen presenting cells [57]. In the present studies, it is possible that IFN- α may play a critical role as an IgE-specific immunomodulatory agent associated with pathogenicity or protection from parvovirus infection.

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