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Detection of IgE anti-parvovirus B19 and increased CD23+ B cells in parvovirus B19 infection: relation to Th₂ cytokines

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Abstract

The immune profile of a parvovirus B19-infected patient (male, 8 years old) was studied on day 0 (initial presentation) and on days 14 and 210 post symptom presentation (psp). Before infection, the patient was skin test positive to various allergens, including ragweed and tree and grass pollens, and had a serum IgE level of 150 IU/mL. On day 0, the patient was diagnosed as parvovirus B19 infected, as judged by the presence of IgG anti-parvovirus Abs in serum (EIA) and presentation of “slap cheek” rash. The patient’s serum IgE level increased from 150 IU/mL before infection to 256 IU/mL on day 0, was 233 IU/mL on day 14, and returned to preinfection levels on day 210. In contrast, there was little change in the levels of serum IgM, IgG, or IgA (nephelometry). IgE anti-parvovirus B19 protein (VP-N) was detected in serum (Western blot) on days 0, 14, and 210, despite the decrease in total IgE on day 210. Although there was no increase in total numbers of blood CD23+ B cells on day 0, by day 14 the numbers of these cells increased dramatically (93%), remaining high on day 210. In contrast, there were virtually no changes in total numbers of CD4+ and CD8+ T cells or CD16/56+ NK precursor cells on days 0–210. On day 0, when IgG and IgE anti-parvovirus were detected in serum, patient’s peripheral blood mononuclear cells (PBMC) expressed mRNA for the Th2 cytokines IL-4 and IL-10, but not for the Th1 cytokines IFN- γ or IL-2. However, by day 14 psp, PBMC expressed mRNA for the Th1 cytokines IFN- γ and IL-2, as well as for IL-4 and IL-10. This is the first demonstration of the existence of IgE anti-parvovirus B19 Ab. The presence of IgE anti-parvovirus B19 Ab in serum on day 0 and its persistence in serum 7 months psp suggests that IgE anti-parvovirus may be useful in prognosis of parvovirus B19 infection. Our results reinforce the idea that IgE, in general, may play a major role in anti-viral immunity, perhaps in conjunction with CD23+ cells. The results further suggest that clearance of this infection is accompanied by a switch to Th1 cytokines.

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Introduction

Previous studies in our laboratory were the first to identify the presence and function of IgE anti-HIV in serum of a subset of HIV-1 seropositive, nonprogressor pediatric patients who remained relatively healthy, despite decreased

numbers of peripheral blood CD4+ T cells [1,2]. Further, it has been demonstrated that specific IgE anti-HIV-1 antibodies may protect against HIV-1 disease progression by promoting cytotoxic responses or by suppressing virus production [1–3].

Studies by others [4–6] have identified immunoglobulin (Ig)E anti-parainfluenza virus [6] and IgE anti-respiratory syncytial virus (RSV) [4,5] in sera from pediatric patients [6]. However, studies of De Alarcon et al. [7] were unable to detect IgE anti-RSV-F(a) and -G(a) antibodies in nasal washes and sera from infants [7].

In the present study, we determined the presence of IgE

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anti-parvovirus B19 Ab in serum obtained from a parvovirus B19-infected pediatric patient and its relationship to Th1 or Th2 cytokines. The exact role of IgE in parvovirus infection is not known; however, the presence of IgE anti-parvovirus B19 Ab in serum on day 0 and its persistence 7 months post symptom presentation (psp) suggest that IgE anti-parvovirus may be useful in prognosis of parvovirus B19 infection and that IgE may play a major role in antiviral immunity.

Materials and methods

Patient specimens

Peripheral blood (5 ml total) was obtained from a pediatric parvovirus (B19) patient (male, 8 years old, Caucasian), with an elevated serum IgE level (>100 IU/ml). The patient had positive skin prick to various allergens, including tree and grass pollens and ragweed. Parvovirus B19 infection was established by: (1) EIA and (2) positive pattern on Western blots. The patient presented with a “slap cheek” rash over his face and with a diffuse erythematous rash over his neck, ears, hands, elbows, and knees and complained of achy joints. Informed consent was obtained from the child’s parents for the use of their son’s blood samples for an experimental study. The study was approved by the institutional review board of the SUNY Downstate Medical Center, Brooklyn, NY, and the procedures followed were in accordance with institutional guidelines involving human subjects.

Blood

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

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

Determination of cell surface markers

Antibodies

The following mouse anti-human monoclonal antibodies (mAbs) directly conjugated to fluorescein isothiocyanate (FITC): CD45, CD45RA, CD23, Simultest CD3/CD4, Simultest CD3/CD8, Simultest CD3/CD19, and CD3/CD16+CD56. The following mouse anti-human mAb directly conjugated to phycoerythrin (PE): CD45RO. All mAbs and appropriately matched isotype controls were purchased from Becton Dickinson BioSciences (San Jose, CA)

and titrated to obtain maximum staining efficiency according to manufacturer’s recommendations.

Immunofluorescence assay

Peripheral blood (100 μ L) was incubated with conjugated antibodies for 10 min 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 using System II software (Coulter) 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 were 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 4-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 (WBC) count. Data are expressed as total lymphocytes per cubic millimeter.

Total serum immunoglobulins

Total serum immunoglobulins (IgM, IgG, and IgA) were determined by Quest Diagnostics Incorporated (Teterboro, NJ), using nephelometry, which was performed according to standard procedure. The results are expressed in milligrams per decaliter (reference range for healthy child serum: IgM, 60–263 mg/dL; IgG, 694–1618 mg/dL; IgA, 69–378 mg/dL). Total serum IgE levels were detected by the UniCAP Total IgE Fluoroenzymeimmunoassay (Pharmacia & Upjohn Diagnostics, Kalamazoo, MI) which was performed according to standard procedure. Data are expressed as International Units per milliliter (reference range for healthy child serum: IgE, 20–100 IU/mL).

Parvovirus B19 serum antibody detection

a. IgM and IgG

Serum IgM and IgG antibodies to parvovirus B19 were determined by enzyme immunoassay (EIA) performed by Quest Diagnostics (Teterboro, NJ), according to standard procedure. Data are reported as ratio report.

b. IgE

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% MIT, 0.1% oxypyrion, protein) on a shaker for 20 h at room temperature. The strips were washed four times in washing/diluting buffer. Goat polyclonal anti-human IgE (ICN Biomedicals, Aurora, OH), diluted 1:50 or 1:100 in wash buffer, was added to each well and incubated on a shaker for 1 h 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 h at room temperature. The strips were washed again four times in washing/diluting buffer and developed in 2 mL of a 3, 3', 5, 5'-tetramethylbenzidine (TMB) 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.

Cytokine-specific mRNA

RNA extraction and polymerase chain reaction (PCR)

Total cellular RNA (2 µg/mL) was extracted from PBMC as previously described [8], using Trizol Reagent (GIBCO/BRL), according to manufacturer's recommendations. Pellets were dissolved in TE buffer (10 mM Tris-HCl (Sigma, St. Louis, MO), pH 7.5, 1 mM EDTA, Sigma), and stored at -70°C in a Bio-Freezer (Forma Scientific, Marietta, OH). Expression of interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10), and interferon-gamma (IFN-γ) mRNA was determined using the Advantage One-Step RT-PCR Kit (Clontech, Palo Alto, CA), according to manufacturer's recommendations. PCR was conducted using primer pairs specific for IL-2, IL-4, IL-6, IL-10, and IFN-γ (Expected band sizes: 305, 344, 628, 328, and 427 basepairs (bp), respectively). A beta-actin primer set 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) and visualized with ethidium bromide (Sigma).

Results

1. Clinical diagnosis of parvovirus B19

Symptoms

Clinical symptoms of parvovirus B19 patient included "slap cheek" rash over facial area, diffuse erythematous rash over body regions (neck, ears, hands, elbows, and knees), and arthralgias.

2. Serum immunoglobulins

a. Total IgM, IgG, and IgA

Serum obtained from parvovirus B19-infected patient contained similar levels of total IgM (45 and 49 mg/dL, respectively), IgG₁ (621 and 507 mg/dL, respectively), IgG₂ (87 and 109 mg/dL, respectively), IgG₃ (52 and 54 mg/dL, respectively), IgG₄ (13 and 13 mg/dL, respectively), and IgA (37 and 58 mg/dL, respectively) on days 0 and 14; these

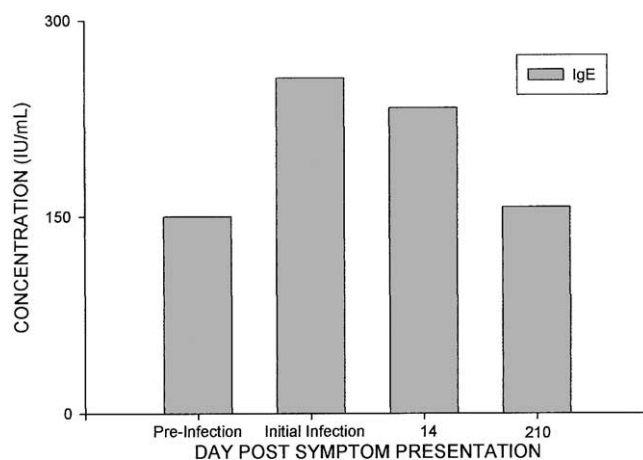


Fig. 1. Immunoglobulin levels in serum of parvovirus B19 patient. Total serum IgE levels were determined (total IgE fluoroenzymeimmunoassay) preinfection, on day 0 (initial infection), and on days 14 and 210 psp in serum of parvovirus B19 patient. Data are expressed as IU/mL.

levels were within normal range (see Materials and Methods).

b. Total IgE

Serum obtained from parvovirus B19-infected patient contained total serum IgE levels which increased from 150 IU/mL before infection to 256 IU/mL on day 0, were 233 IU/mL on day 14 psp, and returned to preinfection levels on day 210 psp, but were still elevated above the normal range (>100 IU/mL) (1,2) (Total IgE fluoroenzymeimmunoassay) (Fig. 1).

c. Anti-parvovirus B19 Abs: IgM and IgG

Serum obtained from parvovirus B19-infected patient was assayed for the presence of serum anti-parvovirus B19 Abs (IgM and IgG) in order to confirm diagnosis of parvovirus B19 infection in patient (EIA). On day 0 parvovirus B19 Abs (IgM and IgG) were 1.0 and 3.7 units, respectively. However, by day 14, parvovirus B19 Abs (IgM, IgG) decreased to 0.5 and 3.0 units, respectively. The reference range (for IgM and IgG) was 0.000–0.89. Data are reported as ratio report.

d. IgE anti-parvovirus B19

Serum obtained from the parvovirus B19-infected patient contained IgG anti-parvovirus B19 antibodies on days 0–210 psp as determined by EIA and also by Western blot (Fig. 2, lane 3). Further, patient serum also contained antigen-specific IgE directed against parvovirus B19 component VP-N (Fig. 2, lane 4). Western blot analysis also revealed faint bands representing IgE anti-NS-1, VP-1S, and VP-C (data not shown). Interestingly, on day 210 psp, patient serum contained both IgG and IgE anti-parvovirus B19 antibodies directed against VP-N and VP-1S (Fig. 2, lanes 5 and 6, respectively) and a faint band representing IgG anti-NS-1 (Fig. 2, lane 5). In contrast, serum of parvo-

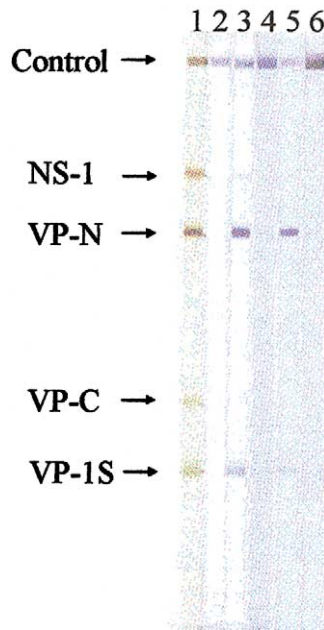


Fig. 2. Western blot analysis of anti-parvovirus B19 antibodies. Serum was incubated with Western blot strips containing parvovirus B19 antigens NS-1, VP-N, VP-C, and VP-1S (see Materials and Methods). Lane 1, control strip showing representative bands. Lane 2, serum from a parvovirus B19 negative donor. Lane 3, patient serum incubated with anti-human IgG (1:1000). Lane 4, patient serum incubated with anti-human IgE (1:100). Lane 5, patient serum incubated with anti-human IgG (1:1000) on day 210 psp. Lane 6, patient serum incubated with anti-human IgE (1:100) on day 210 psp. Results did not differ between days 0 and 14 psp (Lanes 3, 4). Control band represents anti-human immunoglobulin. The relative serum concentration of IgE compared with IgG is 1:1–100 million.

virus B19-negative donor did not contain either IgG or IgE anti-NS-1, VP-N, VP-C, or VP-1S antigens (Fig. 2, lane 2).

3. Distributions of blood lymphocyte subpopulations

On days 0, 14, and 210 psp distributions of lymphocyte subpopulations in peripheral blood of the same parvovirus B19 patient were determined. On days 0–210 psp, there was virtually no change in total numbers of T cells (CD3+CD4+, CD3+CD8+, CD45 RA+, and CD45RO+) and NK precursor cells (CD3/16+56+) (Table 1). In contrast, total numbers of both CD23-CD19+ and

Table 2

Summary of cytokine production by peripheral blood mononuclear cells (PBMC) from parvovirus B19 patient^a

Day	Cytokines				
	Th-1		Th-2		
	IL-2	IFN- γ	IL-4	IL-6	IL-10
0	–	–	+	–	+
14	+	+	+	–	+

^a Unfractionated PBMC from a representative parvovirus B19 patient was evaluated for the presence (+) or absence (–) of Th1 type (IL-2, IFN- γ) cytokines and Th2 type (IL-4, IL-6, IL-10) cytokines on day 0 and day 14 postinfection. Expression of cytokine-specific mRNA production was determined by Advantage One-Step RT-PCR (Clontech), as described under Materials and Methods.

CD23+CD19+ B cells increased on day 14 psp (17 and 93%, respectively). On day 210 psp, total numbers of CD23-CD19+ B cells further increased (86%), and CD23+CD19+ B cells remained unchanged from those of day 14 psp (Table 1).

4. Cytokine expression by PBMC

On day 0, the patient's PBMC expressed mRNA for the Th₂ cytokines IL-4 and IL-10, but not for the Th₁ cytokines IFN- γ or IL-2. However, by day 14, PBMC expressed mRNA for the Th₁ cytokines IFN- γ and IL-2, as well as for IL-4 and IL-10. PBMC did not express mRNA for IL-6 at either time point (Table 2).

Discussion

Several findings stand out from the results reported here. The present study is the first to demonstrate that (1) IgE anti-parvovirus B19 is produced by a parvovirus B19-infected child. (2) Th2 cytokine responses predominate early in IgE anti-parvovirus B19 infection, whereas late in parvovirus B19 infection Th1 and Th2 cytokine responses both are prevalent. (3) CD23 surface expression on B cells is increased on peripheral blood lymphocytes from a parvovirus B19-infected child.

This is the first report demonstrating an increase in CD23

Table 1

Distributions of lymphocyte subpopulations in peripheral blood of parvovirus B19 patient^a

Day	CD3+CD4+		CD3+CD8+		CD16+56+		CD45RA+		CD45RO+		CD19+		CD23+	
	mm ³	%	mm ³	%	mm ³	%	mm ³	%	mm ³	%	mm ³	%	mm ³	%
0	703	50	419	30	64	5	1014	72	724	52	154	11	40	3
14	859	47	548	30	92	5	1113	61	910	50	180	10	77	4
210	1080	45	768	32	72	3	1584	66	1008	42	336	14	72	3

^a The distributions of lymphocyte subpopulations in peripheral blood of a representative parvovirus B19 patient were determined by flow microfluorimetry (Coulter Epics XL/MCL) on days 0, 14, and 210 post symptom presentation (p.s.p.). Data are expressed as mean total cells/mm³ or mean percentage (%) of positive cells.

expression by peripheral blood lymphocytes from a parvovirus B19-infected child. A number of functions have been ascribed to CD23, including specific regulation of IgE production, IgE-mediated cytotoxicity and release of mediators, IgE-dependent antigen focusing, and promotion of B-cell growth [9]. In addition, CD23⁺ expression by peripheral blood leukocytes has been evaluated in diseases such as rheumatoid arthritis [10] and HIV-1 [11]. However, the latter studies found decreased numbers of CD23⁺ B cells [11,12] and increased numbers of CD23⁺ T cells [13,14].

Studies of Dugas et al. [15] have demonstrated that CD23 can be proteolytically cleaved from surfaces of CD23-expressing cells into biologically active soluble fragments, some of which retain the ability to bind IgE [15]. However, it has also been shown that CD23 (membrane bound and soluble forms) increases production of IgE by B cells by interacting with CD21 on B cell surfaces [16,17]. It could be that increased CD23 on B cells in parvovirus B19 infection may predispose these cells to play a role in aberrant inflammatory and allergic responses, which may be important in clearance of this virus. However, a definitive role for membrane-bound CD23 on B cells in parvovirus B19 infection has yet to be determined. Our results suggest that CD23⁺ B cells, under appropriate conditions, might provide an early protective advantage to the host. However, the increase in CD23 may also serve to regulate selective IgE responses [18], as well as provide an initial antiviral response, as demonstrated in parasitic infection [19,20].

Further, it appears that parvovirus B19 virus may potentiate IgE responses through interaction with B lymphocytes, with subsequent induction of class switching and synthesis of secreted IgE protein. Studies of Imani et al. [21] have shown that infection with MMR vaccine can induce IgE class switching in a human B cell line and freshly prepared PBL [21]. In addition, IgE may serve directly as an antigen-presenting cell (APC) in parvovirus B19 infection. This is in agreement with Mudde et al. [22], who have implicated IgE bound to its Fc receptor as an APC in atopy [22]. Since there are no other studies that have established a link between certain leukocytes and IgE-secreting B cells in the pathophysiology of parvovirus B19 infection, further studies in this area are required.

The presence of IgE has been demonstrated in several viral infections. Investigators [4,5] have identified IgE anti-RSV in nasopharyngeal secretions of infants infected with RSV [4,5], while others have found elevated serum IgE levels and/or IgE anti-non-HIV-1 viral antigens, including HTLV-1 [23], parainfluenza virus [24], Puumala virus [25], HSV-1, HSV-2, Epstein–Barr virus, and virus-associated encephalopathies [26]. The clinical implications of these IgE antiviral antibodies are not known, and no antiviral functions have been identified. In contrast, other viruses, such as the lactic dehydrogenase virus (LDV), suppress antigen-induced IgE production by decreasing IL-4 production [27]. Recent studies [28] have detected IgG and IgA

antibodies, but not IgE antibodies against Cowpea severe mosaic virus in mice [28], while others [29] have shown that *Mycobacterium tuberculosis* infection or viral infection may reduce IgE levels or suppress atopy in adult patients with tuberculosis [29]. In our study, the detection of IgE specific for parvovirus B19 may reflect the potential importance of this antibody response in the pathogenesis of parvovirus infection. Further, one can speculate that parvovirus B19 may behave similarly to viruses that elicit IgE antibodies.

In evaluation of suspected parvovirus B19 cases, a complete blood cell count and blood chemistry profile are useful additions to thorough history taking and physical examination [30]. However, diagnosis of acute and past infection of parvovirus B19 is based on detection of IgM and IgG antibodies [31]. Recombinant versions of parvovirus B19 viral capsid proteins VP1 and VP2 are used for immunodiagnostic assays, including either IgM or IgG EIA, immunofluorescence assays (IFA), or Western blot assays, for detection of antiviral antibodies [32]. Immunoglobulins specific for parvovirus B19 may have prognostic implications. Studies of Cassinotti et al. [33] demonstrated the prevalence of anti-B19 IgG antibody in patients with various forms of arthritis [33]. Others have studied the serological profiles and/or prevalence of human parvovirus B19 in patients with fibromyalgia [34], AIDS [35], and other infections [22]. In the present study, we demonstrated the existence of IgG and IgE anti-parvovirus B19 in serum of an IgE⁺ pediatric patient. In addition, we observed the presence of IgE anti-parvovirus B19 antibodies in serum from two other parvovirus B19-infected children who were also serum IgE⁺ (data not shown). One of the most remarkable findings presented here is the long-term persistence of these antibodies, as well as a significant decrease in levels of total serum IgE. This result suggests that there may be an IgE-specific memory component in parvovirus B19 infection. Similar studies [2], in HIV-1 disease, have shown a persistence of HIV-1-specific IgE after 210 days and retained its ability to suppress HIV-1 production in vitro [2]. Further, it has been demonstrated that specific IgE anti-HIV-1 antibodies may protect against HIV-1 disease progression by promoting cytotoxic responses or by suppressing virus production [1–3].

In addition to increased CD23 expression in peripheral blood and the presence of IgG and IgE anti-parvovirus B19 in serum of the infected patient, other factors, such as Th1 and Th2 cytokines, may contribute to the immunopathogenesis of parvovirus B19 infection. It is well known that these immunological factors play an important role in virus pathogenesis [36]. In our study, cytokine-specific mRNA expression was compared on both days 0 and 14 psp. Analysis of the cytokine pattern revealed a deviation of the cytokine profile Th-2-like (IL-2-, IFN- γ -) on day 0 to Th1-like (IL-2+, IFN- γ +) on day 14 psp. It is possible that during early viral infection, parvovirus B19 may suppress certain Th1-type cell responses, including IL-2 production, and parvovirus B19 gene products might be involved in suppressing

host immune responses and T cell activation. The presence of IFN- γ on day 14 psp may indicate that IFN- γ operates as a final effector to activate antiviral mechanism(s) within cells infected by parvovirus B19. The presence of these cytokines by day 14 psp may indicate that certain cells are not activated during the early immune response and that the process of Th1/Th2 selection may not begin until 10–14 days after exposure to the antigen (virus). Similarly, it has been shown [37,38] that patients chronically infected with hepatitis C virus (HCV) exhibit immune dysfunction with a Th2-dominant cytokine profile, while Th1 cytokines are prominent in those with self-limited HCV infection. Both Th1 and cytotoxic T lymphocyte (CTL) responses have been reported to play a crucial role in recovery from HCV infection [39,40]. Furthermore, in HIV-1 disease, the loss of the Th1 response has been correlated with disease progression and loss of protective cellular immunity [41]. Our observations suggest that IL-2 and IFN- γ expression on day 14 psp may represent the addition of an independent Th1 type cellular immunity required for efficient viral clearance.

The presence of antigen-specific IgE Abs in serum may represent an important marker of viral responses. The observation that IgE anti-parvovirus B19 is present when infection is first detected, and is still detectable 7 months later, suggests that IgE may play a major role in antiviral immunity, perhaps in conjunction with CD23+ cells.

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