

IgE Anti-*Borrelia burgdorferi* Components (p18, p31, p34, p41, p45, p60) and Increased Blood CD8⁺CD60⁺ T Cells in Children with Lyme Disease

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Abstract

Immunoglobulin (Ig) E may provide immunity against *Borrelia burgdorferi* infection (Lyme disease) in children which lasts throughout adulthood. We investigated the presence and persistence of IgE anti-*B. burgdorferi* antibodies (Abs) in paediatric patients infected with Lyme disease over time. Serum immunoglobulin levels, presence of IgG and IgE anti-*B. burgdorferi* components, and distributions of blood T, B and natural killer lymphocyte subsets were studied in *B. burgdorferi*-infected and -uninfected children (nephelometry, UniCAP Total IgE Fluoroenzymeimmunoassay, Western blot, flow cytometry). Total serum IgM, IgG, IgE and IgA levels, and distributions of blood lymphocytes (CD4⁺, CD8⁺, CD19⁺) of both groups, excluding CD8⁺CD60⁺ T cells, were within normal ranges. However, infected, but not uninfected children made IgG anti-*B. burgdorferi* proteins p18, p31, p34, p41, p45, but not IgG anti-p60, and IgE anti-*B. burgdorferi* proteins p31, p34, p41, p45, p60, but not IgE anti-p18. These proteins were also detected in an infected child 1 year post-infection. Interestingly, CD8⁺CD60⁺ T-cell numbers were significantly increased (fourfold) in infected, compared with uninfected, patients ($P = 0.001$). These results demonstrate that specific IgE anti-*B. burgdorferi* Abs are generated and persist in children with Lyme disease and that CD8⁺CD60⁺ T cells may play an important role in these responses.

Introduction

Immunoglobulin (Ig) E is involved in non-atopic diseases and may play an important role in immunity to viruses, as well as to specific spirochetes. Previous studies in our laboratory were the first to demonstrate the existence of IgE anti-parvovirus B19 in serum of a parvovirus B19-infected child, and its persistence in serum 7 months post-infection [1], as well as in IgA deficiency [2]. Earlier 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, non-progressor paediatric patients [3, 4], and that specific IgE anti-HIV-1 antibodies (Abs) may protect against HIV-1 disease progression by suppressing virus production [3–5].

The incidence of Lyme disease is increasing in the United States [6]. Lyme disease is caused by a tick-borne spirochete, *Borrelia burgdorferi* [7, 8]. This disease is charac-

terized by non-specific flu-like symptoms, and some patients develop skin lesions, erythema migrans, during disease course [7, 8]. While several reports exist on the presence of IgE anti-spirochete Abs in serum obtained from infected patients, there have been no reports of the presence or the antigenic specificity of IgE anti-*B. burgdorferi* Abs in infected paediatric patients or the persistence of such Abs over time. Although earlier studies of Benach *et al.* [9] have shown the presence of specific IgE Abs to *B. burgdorferi* in patients with Lyme disease [9], neither epitopic specificity nor the long-term presence of these Abs, were reported. In addition, IgE anti-spirochete Abs (*Treponema pallidum*) and spirochete tick vectors (*Ixodes ricinus* and *Argas reflexus*) have also been reported [10–12]. However, the role of IgE anti-spirochete Abs and the persistence of IgE anti-spirochete Abs remain unclear.

Modulation of lymphocyte responses have been described in Lyme disease. Studies by Zajkowska and

Hermanowska-Szapkowicz [13] have demonstrated that CD4/CD8 ratios are decreased after antibiotic therapy in patients with Lyme disease. However, in those studies there were no differences in infected versus control groups. Others studies [14] have shown that the majority of T cells generated after dendritic cells mediated *B. burgdorferi* antigen challenge were CD8⁺ T cells which were able to kill *B. burgdorferi* containing target cells. However, the role of CD8⁺ T cells in Lyme disease remains unclear and has been shown to correlate with disease depending on their cytokine secretion [15], and antigenic specificity profiles [16, 17] and have been postulated as tools for vaccine development in Lyme disease [16] and in other viruses [18]. CD8⁺/CD60⁺ T-cell subsets have been described in T-lymphocyte activation and have been found in blood and body fluids [19–22]. Studies in our laboratory have demonstrated that CD8⁺/CD60⁺ T cells may contain IgE isotype-specific memory cells [23–25], and are involved in IgE antiviral responses [2]. However, the role of CD8⁺/CD60⁺ T cells in Lyme disease remains unknown.

In the present study, we found (1) the presence of IgE anti-p31, -p34, -p41, -p45, -p60 components of *B. burgdorferi* from *B. burgdorferi*-infected paediatric patients, and persistence of these Abs 12 months post-symptom presentation (PSP) and (2) increased numbers of CD8⁺CD60⁺ T cells in infected patients. The presence of IgE anti-*B. burgdorferi* Abs in serum on day 0 (initial presentation) and its persistence 12 months PSP suggests that serodiagnosis of IgE anti-*B. burgdorferi* using Western blot analysis may provide additional information to detect peptides with defined IgE-binding specificities. The presence and persistence of an IgE-specific anti-spirochete response also suggests that IgE, along with the presence of CD8⁺CD60⁺ T cells, may play a role in anti-spirochete immunity which may persist over time.

Materials and methods

Patient specimens. Peripheral blood (5 ml total) was obtained from paediatric patients with Lyme disease (m/f, 2–14 years old; $n = 6$), with serum IgE levels ranging from 5 to 250 IU/ml. Patients had no known allergies, with the exception of one child who was allergic to pecans (radioallergosorbent test). Patients were infected either in Westchester, NY or Long Island, NY. Patients observed tick bites on their skin. Lyme disease infection was established by: (1) Ab capture enzyme immunoassay (EIA; with immunoblot) or/and (2) positive pattern on Western blots. IgG Western blots which have five (or more) of the 10 significant bands are considered positive for specific Ab to *B. burgdorferi* [26]. IgM Western blots which have two (or more) of the three significant bands are considered positive for specific Ab to *B. burgdorferi* [26].

Patients were initially evaluated during the acute phase of illness, after onset of symptoms. Associated symptoms observed in most patients (approximately 50%), included erythema migrans, joint inflammation or flu-like symptoms. Patients were treated with a 3-week course of either amoxicillin or doxycycline antibiotics. Treatment was consistent with published recommendations [6]. Non-*B. burgdorferi*-infected, normal healthy children ($n = 8$; m/f patients, age matched), with normal total IgE levels (<100 IU/ml) were used as controls. Informed consent was obtained from the children's parents for the use of their blood/serum samples for an experimental study.

Blood. For studies of serum immunoglobulins, blood was collected into red top monoject tubes (Sherwood Medical, St Louis, MO, USA) and sent to the Clinical Immunology/Immunoserology Laboratory at SUNY Downstate (Brooklyn, NY) for immunoglobulin determinations.

For studies of surface markers, blood was collected into ethylenediaminetetraacetic acid (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, USA); flow microfluorimetry studies (Coulter Epics XL/MCL; Beckman Coulter, Miami, FL, USA) were performed within 3 h.

Determination of cell surface markers. Antibodies Mouse anti-human monoclonal antibodies (MoAbs) directly conjugated to fluorescein isothiocyanate (FITC): CD45, CD25, CD23, Simultest CD3/CD4, Simultest CD3/CD8, Simultest CD3/CD19, CD3/CD16⁺CD56, CD60. Mouse anti-human MoAbs directly conjugated to phycoerythrin (PE): CD8. Labelling studies were carried out using CD8 Ab in conjunction with CD60 (double labelling). All MoAbs 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 Abs were titrated to obtain maximum staining efficiency (1:10–1:100 dilution) according to manufacturer's recommendation as previously described [1, 2].

Immunofluorescence assay Peripheral blood (100 µl) was incubated with conjugated Abs 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 number of lymphocytes were calculated from the white blood cell (WBC) count. Data are expressed as total lymphocytes/mm³.

Total serum immunoglobulins Total serum immunoglobulins (IgM, IgG and IgA) were measured by nephelometric quantitation, using the Beckman (Fullerton, CA, USA) array protein system and a Beckman array rate nephelometer according to the manufacturer's recommendations and clinical guidelines for quality control at the Clinical Immunology/Immunoserology Laboratory at SUNY Downstate (Brooklyn, NY). The results are expressed in mg/dl (reference range for healthy child serum: IgM, 47–311 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 procedure. Data are expressed as IU/ml (reference range for healthy child serum: IgE, 20–100 IU/ml.)

***B. burgdorferi* serum antibody detection.** IgM, IgG and IgA Serum IgM, IgG and IgA Abs to *B. burgdorferi* were determined by EIA performed by either IMUGEN reference diagnostic division (Norwood, MA, USA) or Quest Diagnostics (Teterboro, NJ, USA) according to standard procedure. Data are reported as ratio report.

IgE *Borrelia burgdorferi* Western blot strips (Immunetics, Cambridge, MA, USA) were incubated with sera diluted to 1:100 in diluting buffer, consisting of washing buffer (0.05% Polysorbate 20, 0.01 M phosphate-buffered saline) and non-fat dry milk (5%), in 1 ml final volume, on a shaker for 20 h at room temperature. The strips were washed three times in washing buffer. Goat polyclonal anti-human IgE (ICN Biomedicals, Aurora, OH, USA), diluted to 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 three times in wash buffer. Rabbit anti-goat peroxidase-labelled Ab (ICN), diluted to 1:1000 in washing buffer, was added to each well and incubated on a shaker for 1 h at room temperature. The strips were washed again three times in washing buffer, and developed in 1 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-*B. burgdorferi* Western blot (Immunetics) was determined according to manufacturer's recommendation.

Statistical analysis. Lymphocyte distributions and serum immunoglobulin levels of *B. burgdorferi* infected, and normal subjects were compared on each variable. Significance between variables was determined using Student's *t*-test. A *P*-value of <0.05 was considered

statistically significant for all comparisons. The degree of association between these measures was assessed using Pearson's correlations. Statistical analyses were performed at the Scientific Computing Center (SUNY Downstate Med Center, Brooklyn, NY), using SPSS for Windows, version 10.0 software (SPSS Inc., Chicago, IL, USA).

Results

Serum immunoglobulins

Total IgM, IgG and IgA

Serum obtained from *B. burgdorferi*-infected patients (*n* = 6) contained similar levels of total immunoglobulins compared with normal paediatric subjects (*n* = 8): IgM (82 ± 11, 73 ± 10 mg/dl, respectively), IgG (788 ± 128, 729 ± 55 mg/dl, respectively) and IgA (85 ± 21, 78 ± 15 mg/dl, respectively; Table 1); these levels were within normal reference range (see Materials and Methods).

Total IgE

Serum obtained from *B. burgdorferi*-infected patients (*n* = 6) contained similar levels of total serum IgE compared with normal paediatric subjects (*n* = 8; 49 ± 40, 46 ± 23 IU/ml, respectively; Table 1), these levels were within normal reference range (<100 IU/ml; total IgE fluoroenzymeimmunoassay).

Anti-*B. burgdorferi* Abs: IgM, IgG and IgA

Serum obtained from *B. burgdorferi*-infected patients was assayed for the presence of serum anti-*B. burgdorferi* Abs (IgM, IgG, IgA) in order to confirm diagnosis of *B. burgdorferi* infection in patients (EIA). All subjects' anti-*B. burgdorferi* Abs (IgM, IgG, IgA) were within normal reference range/below detection (<1), except two patients (A and B, respectively) whose anti-*B. burgdorferi* IgG Ab level was 7.5 and 3.54 units, respectively. Patient A's anti-*B. burgdorferi* IgG Ab level 1 year post-infection was 2.44 units. Normal reference range of specific total Abs:

Table 1 Immunoglobulin levels in serum of *Borrelia burgdorferi* patients^a.

Subject	IgM (mg/dl)	IgG (mg/dl)	IgA (mg/dl)	IgE (IU/ml)
Lyme infected	82 ± 11	788 ± 128	85 ± 21	49 ± 40
Normal	73 ± 10	729 ± 55	78 ± 15	46 ± 23

^aImmunoglobulin levels in Lyme-infected patients and healthy controls (nephelometry, Total IgE Fluoroenzymeimmunoassay) as determined by Quest Diagnostics. Data are expressed as either mg/dl (IgM, IgG, IgA) or IU/ml (IgE; ±SE).

<1 unit. Data are reported as ratio report (Quest Diagnostics).

IgE anti-*B. burgdorferi*

Serum obtained from *B. burgdorferi*-infected patients contained IgG anti-*B. burgdorferi* Abs as determined by Western blot (Fig. 1, lanes 3, 5, 7). Further, patients' serum also contained antigen-specific IgE directed against *B. burgdorferi* components p31, p34, p41 and p60 (Fig. 1, lanes 4, 6, 8). Western blot analysis also revealed faint bands representing IgE anti-p45. Interestingly, 1 year post-infection, patient A's serum contained IgG and IgE anti-*B. burgdorferi* Abs directed against p31, p34, p41 and p60 (Fig. 2, lanes 5 and 6, respectively). In contrast, serum obtained from a *B. burgdorferi*-negative subject (control) did not contain either IgG or IgE anti-*B. burgdorferi* antigens (Figs. 1 and 2, lane 2).

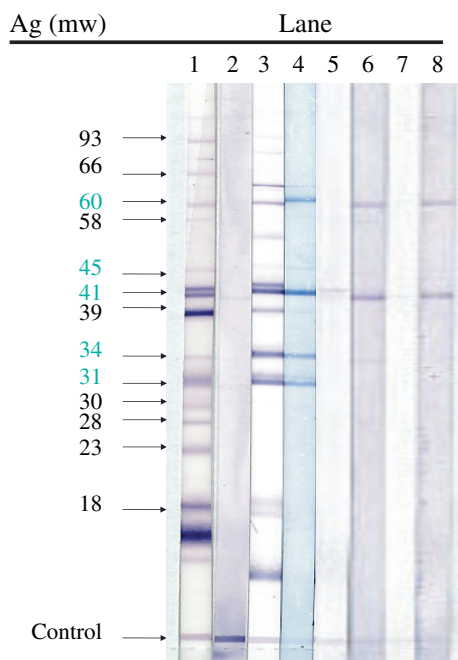


Figure 1 Western blot analysis of representative *Borrelia burgdorferi*-infected patients. Serum, obtained from spirochete-infected patients, was incubated with Western blot strips containing *B. burgdorferi* antigens. Lane 1 – positive control serum incubated with anti-human immunoglobulin (Ig) G depicting IgG anti-*B. burgdorferi* antigens (manufacturer control); lane 2 – negative control (*B. burgdorferi*-negative) serum incubated with anti-human IgE, similar results were obtained with anti-human IgG (data not shown); lane 3 – *B. burgdorferi*-infected patient A serum incubated with anti-human IgG; lane 4 – *B. burgdorferi*-infected patient A serum incubated with anti-human IgE; lane 5 – patient B serum incubated with anti-human IgG; lane 6 – patient B serum incubated with anti-human IgE; lane 7 – patient C serum incubated with anti-human IgG; lane 8 – patient C serum incubated with anti-human IgE. Data represent three of six *B. burgdorferi*-infected patients with similar results. Coloured numbers indicate IgE-specific *B. burgdorferi* antigens.

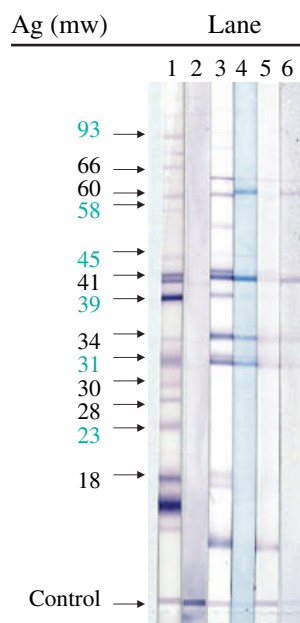


Figure 2 Western blot analysis of *Borrelia burgdorferi*-infected patient A at initial presentation and 1 year post-symptom presentation (PSP). Lane 1 – positive control serum incubated with anti-human IgG depicting IgG anti-*B. burgdorferi* antigens (manufacturer control); lane 2 – negative control serum incubated with anti-human immunoglobulin (Ig) E, similar results were obtained with anti-human IgG (data not shown); lane 3 – *B. burgdorferi*-infected patient A serum incubated with anti-human IgG at presentation (as in Fig. 1); lane 4 – *B. burgdorferi*-infected patient A serum incubated with anti-human IgE at presentation (as in Fig. 1); lane 5 – *B. burgdorferi*-infected patient serum A incubated with anti-human IgG at 1 year PSP; lane 6 – *B. burgdorferi*-infected patient serum A incubated with anti-human IgE at 1 year PSP. Coloured numbers indicate IgE-specific *B. burgdorferi* antigens.

Distribution of blood lymphocyte subpopulations

Lyme disease-infected ($n = 3$) and normal subjects ($n = 8$) had similar numbers of blood $CD3^+CD4^+$ ($48 \pm 4.0\%$, $45 \pm 2.0\%$, respectively) and $CD3^+CD8^+$ T cells ($18 \pm 2.0\%$, $25 \pm 2.0\%$, respectively), and $CD19^+$ ($15 \pm 1.0\%$, $16 \pm 2.0\%$, respectively) and $CD23^+$ B cells ($7 \pm 0.9\%$, $5 \pm 1.0\%$, respectively). However, $CD8^+CD60^+$ T-cell numbers were significantly increased in blood of Lyme disease-infected patients ($13 \pm 0.5\%$) compared with normal subjects ($3 \pm 0.9\%$; $P = 0.001$), as well as $CD25^+$ cells ($4 \pm 1.0\%$, $2 \pm 0.22\%$, respectively; $P = 0.010$). $CD16/56^+$ natural killer precursor cells ($12 \pm 3\%$, $7 \pm 1.0\%$, respectively; $P = 0.059$) were not significantly increased (Table 2).

Discussion

In the present study, we have shown that (1) IgE anti-p31, -p34, -p41, -p45, -p60 components of *B. burgdorferi* are present and persist in children with Lyme disease and

Subject	CD3 ⁺ CD4 ⁺	CD3 ⁺ CD8 ⁺	CD8 ⁺ CD60 ⁺	CD25 ⁺	CD16/56 ⁺	CD19 ⁺	CD23 ⁺
Lyme infected	48 ± 4	18 ± 2	13 ± 1 ^a	4 ± 0.1 ^b	12 ± 3	15 ± 1	8 ± 1
Normal	45 ± 2	25 ± 2	3 ± 1 ^a	2 ± 0.2 ^b	7 ± 1	16 ± 2	5 ± 1

^aLyme-infected patient value > normal patient value ($P = 0.001$).

^bLyme-infected patient value > normal patient value ($P = 0.01$).

^cThe distributions of lymphocyte subpopulations in peripheral blood of serum obtained from Lyme-infected patients and healthy controls were determined by flow microfluorimetry. Data are expressed as mean percentage (%) of positive cells (±SE).

Table 2 Distributions of lymphocyte subpopulations in peripheral blood of *Borrelia burgdorferi* patients^c.

(2) CD8⁺CD60⁺ T cells are increased in infected patients, compared with controls.

Immunoglobulin E has been shown to play a role in viral, spirochete and bacterial infections. Previous studies in our laboratory were the first to demonstrate that IgE is important in parvovirus B19 immunity [1, 2], and HIV-1 progression [3, 4]. The presence of IgE has been demonstrated in several viral infections including RSV [8, 9], HTLV-1 [11], Parainfluenza virus [12], Puumala virus [27], HSV-1, HSV-2 and Epstein-Barr virus [28]. The clinical implications of these IgE antiviral Abs are, as of yet, unknown. It has been shown that syphilis patients produce IgE Abs to *T. pallidum* [9, 10]; detection of anti-*Chlamydia pneumoniae* IgE has been reported in children with reactive airway disease [29]. IgE Abs to bacterial infections, such as staphylococcal disease have been reported in patients with allergies [30], and atopic dermatitis [31].

In the present study, all of the Lyme disease-infected children produced IgG anti-*B. burgdorferi* proteins p18, p31, p34, p41, p45, but not IgG anti-p60, and IgE anti-*B. burgdorferi* proteins p31, p34, p41, p45, p60, but not IgE anti-p18. A consistent feature among IgE anti-*B. burgdorferi* specificities in all patients analysed includes IgE anti-p41 (flagellin) and anti-p60 (heat shock protein) Abs. It is thought that IgM anti-p41 Abs are generated during early infection with subsequent generation of IgG anti-p41 Abs [32]. Conversely, anti-p60 Abs are generated later, and are generally of the IgG isotype [33]. In this context, it is interesting to observe the presence of IgE anti-p41 Ab in one patient, at initial presentation and 1 year later. It could be that IgE anti-spirochete Abs are generated during early infection and utilize a pathway independent of other immunoglobulin isotypes [34]. Similarly, selective antigen-specific IgE Ab production has been described [35] and may be T cell-independent [36]. It could be that IgE anti-p41 Abs may serve as useful markers of early *B. burgdorferi* infection either alone or in the context of other immunoglobulins such as IgM [37]. Furthermore, although the relationship of IgE/IgE anti-*B. burgdorferi* Abs to Lyme disease symptoms, i.e. erythema migrans, joint pain, remains unknown, one could postulate a potential prognostic value for these Abs similar to other immunoglobulins. Studies of Bacon *et al.*

[38] demonstrated that combinations of IgG responses in parallel with IgM responses to select antigens maintained higher specificity and were significantly more sensitive than individual immunoglobulins in detecting Abs to *B. burgdorferi* in patients with acute erythema migrans. Addition of IgE anti-*B. burgdorferi* Abs either alone or in combination with other anti-*B. burgdorferi* Abs may prove valuable in prognosticating disease severity.

Total serum IgE levels were within the normal range and did not correlate with clinical severity. Our results are in agreement with earlier studies of Benach *et al.* [9] who found that most of their Lyme disease-infected patients produced specific IgE Abs to *B. burgdorferi* and that development of IgE Abs appeared to be directed against a polypeptide with a molecular weight of 41,000 da [9]. In their study, total serum IgE levels were also within the normal range in all stages of the disease [9], and did not correlate with disease severity [9]. However, our present studies are the first to demonstrate that these proteins were also detected 1 year post-infection in one of the patients who had returned for a follow-up visit. It could be that in addition to the long-term persistence of IgE anti-*B. burgdorferi* Abs in infected patients, there may be an IgE-specific memory component in Lyme disease infection that may play an important role in the control of re-infection. Thus, the association of IgE with spirochete infection and immunity could be consistent with an important immune function of IgE.

This is also the first report demonstrating an increase in CD8⁺CD60⁺ T-cell expression by peripheral blood lymphocytes obtained from Lyme disease-infected children. CD60⁺ T cells have been described as a molecule/receptor [19, 20, 39] involved in T-cell activation in both health and disease states [21, 22, 40, 41], including atopy [23–25], cancer [41] and autoimmunity [22, 40]. Other studies in our laboratory found an increase in CD8⁺CD60⁺ T cells in Parvovirus B19 infection [2], and in atopy [23–25]. It has been suggested that CD60 on activated T or B cells may facilitate contact with other helper T cells, influencing either T–T [19] or T–B [20] cell interactions. Previous studies in our laboratory have shown that CD8⁺CD60⁺ T cells are significantly increased in peripheral blood of serum IgE+ ragweed-sensitized humans, compared with serum IgE-negative

non-atopic humans [23–25]. Studies by others have shown that CD8⁺CD60⁺ T cells are increased in other disease states such as HIV-1-infected haemophilic patients [42]. In the present study, the fourfold increase in CD8⁺CD60⁺ T cells Lyme disease-infected children may imply that these cells could play a role in regulation of *B. burgdorferi*-specific memory IgE responses *in vivo* and might provide initial anti-spirochete responses. Additional studies are warranted to better understand the utility of CD8⁺CD60⁺ T cells in Lyme disease.

The interpretation of serological results for patients who had previous Lyme disease infection is not well defined [43]. Studies of Kalish *et al.* [43] found that IgM and IgG Ab responses to *B. burgdorferi* may persist for 10–20 years, but these responses are not indicative of active infection [43]. In their studies [43] IgM and IgG responses were determined by a two-test approach (enzyme-linked immunosorbent assay and Western blot). In the present study, evaluation of suspected Lyme disease infection was determined based on EIA, and detection of *B. burgdorferi* IgE and IgG Abs (Western blot; see Materials and methods), a complete blood cell count and blood chemistry profile, in addition to thorough history taking and physical examination. We further evaluated suspected Lyme disease infection using an IgE-specific Western blot directed against *B. burgdorferi* protein components and these Ab responses persisted 1 year post-infection. Immunoglobulins specific for Lyme disease may have prognostic implications. Such an approach would find application for the design of IgE-specific serum-targeted Ab detection of spirochetes in children, especially when positive results using other assays are in question.

Specific author contributions

Dr Martin Bluth: PI and Western blot assays; Ms Jennie Robin and Mr Meir Ruditsky: Sample collection, data organization, Western blot assays, computer scanning; Drs Kevin Norowitz and Edna Pytlak: Provided Lyme-infected and control serum. Data analysis; Dr Nowakowski: Immunoglobulins determination; Dr Durkin: Lab Head. Flow cytometry analysis; Ms Seto Chice: Flow cytometry; Dr Tamar Smith-Norowitz: PI, Lab Head. Data and statistical analysis.

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