Case Study:
IgE Anti-Varicella Zoster Virus and Other Immune Responses Before, During, and After Shingles

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Abstract. Blood lymphocyte distributions, serum immunoglobulin and cytokine levels, and serum IgE and IgG anti-varicella zoster virus (VZV) levels were measured in an atopic girl (age 15 yr) who developed shingles 10 yr after infection with chicken pox. Before, during, and 5 months after the shingles episode, the child’s distributions of blood lymphocytes (excluding CD23+) and serum immunoglobulin levels (excluding IgE) were within the normal ranges. Her blood level of CD23+ lymphocytes decreased >50% during the shingles episode and remained low thereafter. Her serum level of IgE was elevated before and during shingles (154 and 168 IU/ml, respectively), but was reduced after recovery from shingles (<100 IU/ml). Before, during, and after shingles, her serum contained IgE and IgG anti-VZV antibodies. Before, during, and after shingles, low levels of IFN-γ were detected in serum, but neither IL-1β nor IL-4 were detected. Before shingles, low levels of IL-10 were detected in serum; during shingles, the serum level of IL-10 was increased 30-fold; it subsequently diminished at 5 mo after shingles. The role of IgE in immunity against varicella zoster virus (VZV) has not previously been studied. Our observations in this patient suggest that immunomodulation of IgE and accessory proteins may play a role in VZV pathogenesis.

Keywords: varicella zoster virus, shingles, IL-10, IgE, CD23+, anti-VZV antibodies

Introduction

IgE may play an important role in immunity to viruses. Previous studies in our laboratory were the first to demonstrate IgE anti-Parvovirus B19 in serum of a parvovirus B19 infected child, and its persistence in serum 7 mo post-infection [1], as well as in IgA deficiency [2]. Recent studies in our laboratory identified IgE anti-spirochete antibody (B. burgdorferi) and its persistence one yr later in serum of children infected with Lyme disease [3]. Earlier studies in our laboratory were the first to establish the presence and function of IgE anti-HIV in serum of a subset of HIV-1 seropositive, nonprogressor pediatric patients [4,5], and to show that these specific antibodies may protect against HIV-1 disease progression by suppressing virus production [4-6].

Varicella zoster virus (VZV) is a member of the Herpesviridae family, a group of large DNA viruses that replicate in the cytoplasm of virus-infected cells [7]. VZV becomes latent in sensory neurons after initial infection (chicken pox); reactivation of the infection may occur, causing herpes zoster (shingles) [7]. Serious complications of shingles include post-herpetic neuralgia, zoster multiplex, myelitis, herpes ophthalmicus, and zoster sine herpete [7].

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Studies in humans and animals have identified the presence of IgE anti-virus antibodies in several viral infections including respiratory syncytial virus (RSV) [8,9], parainfluenza [10], HTLV-1 [11], Puumala virus [12], HSV-1, HSV-2, Epstein-Barr virus [13], and blue tongue virus in cattle [14]. While several reports attest to the presence of serum IgG antibody to VZV and its correlation with immunity to varicella [15,16], there have been no reports of the presence or antigenic specificity of IgE anti-VZV in patients with a history of chicken pox, shingles, or vaccination against VZV. Some relevant herpesviridae include CMV [17], HHV-6 [18], and HHV-8 [19], which can cause chronic infections and immunosuppressive effects [13,19], but there have been no reported studies of the roles of IgE antibodies to these viruses.

Both humoral and cellular immune responses have been described in VZV. Harper and Grose [20] demonstrated IgM and IgG responses to VZV p32/p36 polypeptide complex after chicken pox and zoster, and to a lesser extent in recipients of VZV vaccine. They also detected an IgM response to VZV p32 during intrauterine VZV infection and subclinical VZV infection [20]. Infection with VZV elicits persistent cell-mediated immunity directed against the immediate early (IE62) protein and glycoprotein I (gpl) in healthy subjects [21]. However, modulation of specific lymphocyte responses, including Fcepsilon receptor II (CD23+) cells, has not been described in VZV infection or shingles. In contrast, Bluth et al [1] found that CD23 surface expression on B cells was increased in peripheral blood lymphocytes from a parvovirus B19-infected child [1]. Miller et al [22] reported that peripheral blood monocytes of AIDS patients express increased levels of CD23, compared to monocytes of healthy HIV-1 seronegative controls [22].

The exact role of IgE in VZV infection (chicken pox or shingles) is not known. However, our observation of IgE anti-VZV antibodies in serum samples from a child at 10 yr after chicken pox infection and 5 mo after recovery from shingles, suggests that IgE anti-VZV levels may be useful in prognosis of VZV infection and that IgE may play a major role in anti-viral immunity.

Materials and Methods

Clinical information. The subject of this study was an atopic pediatric patient (female, age 15 yr) at the SUNY Downstate Allergy Clinic, who presented with shingles and who had a history of chicken pox infection >10 yr previously. Blood specimens (5 ml) had been collected during past visits to the allergy clinic, prior to the onset of shingles but after the chicken pox infection; blood specimens (5 ml) were also collected during the acute phase of shingles and at 5 mo after recovery from shingles. The patient had known allergies to mixed trees and grasses, ragweed, weeds, and dust mites, based on skin prick tests. At the time of this study, the patient was not receiving allergy therapy or any other medications. Approval of this study was obtained from the SUNY Downstate Institutional Review Board, and the procedures followed were in accordance with institutional guidelines involving human subjects. Informed consent was obtained from the child’s parents and the patient signed a consent form for the use of her blood samples for research.

Shingles infection was established by the patient’s pediatrician through observation of a rash on the left side of the back, adjacent to the lower vertebra (T10 dermatome). The rash appeared as a small cluster of pink papules, which were painful. The past history of chicken pox infection was confirmed by positive titers for IgG varicella zoster virus (ELISA), assayed in our laboratory and also by Quest Diagnostics, Inc. (Teterboro, NJ). No other infections were noted in the patient’s history. No treatment was given for the shingles; the painful rash resolved after one month with no intervention.

For assays of serum immunoglobulins (Ig), blood was collected into red top monoyject tubes and sent to Quest Diagnostics, Inc. For flow cytometric studies, blood was collected into EDTA monoyject tubes and kept at room temperature for up to 2 hr.

Flow cytometry. The following antibodies were used in this study: mouse anti-human monoclonal antibodies directly conjugated to fluorescein isothiocyanate (FITC) (IgG1 anti-CD23; IgM anti-CD60); phycoerythrin (PE) (IgG2a anti-CD45RO); Simultest (FITC/PE-conjugated) reagents (CD3/CD4, CD3/CD8, CD3/CD19, CD3/CD16+CD56), and appropriately matched isotype control monoclonal antibodies (FITC-conjugated IgG1, PE-conjugated IgG2a, Simultest control gamma1/gamma2a, FITC-conjugated IgM). All antibodies were purchased from BD Biosciences, San Jose, CA, except IgM anti-CD60, which was from Ancell, Bayport, MN; the antibodies were used according to the manufacturers’ recommendations. Single- and double-labeling studies were carried out within 6 hr after blood was obtained. Conjugated antibodies (10 µl or 80 µl of titrated anti-CD60) directed against 1-2 markers, were singly or simultaneously added to blood (100 µl) in a 12 x 75 mm (5 ml) tube and incubated for 10 min at room temperature, after which erythrocytes were lysed with whole blood lysing reagent (Immunoprep, Beckman-Coulter, Hialeah, FL), and the cells were counted. Lymphocyte distributions were determined with a Coulter Epics XL/MCL flow cytometer using System II software.
The reaction was stopped by adding 1N H2SO4 (100 µl). The and developed in TMB substrate solution (100 µl) for 10 min.

For IgG, the samples were incubated with sera diluted 1:21 in sample diluent (TBS) for 30 min. Wells were then washed 3 times with wash buffer, and developed in TMB substrate solution (100 µl) for 10 min. The reaction was stopped by adding 1N H2SO4 (100 µl). The plates were read at 450 nm using an automated microplate densitometer (Model Elx800; Bio-Tek Instruments, Winooski, VT). For determination of IgG anti-VZV, the data were reported as an antibody index determined by dividing the optical density (OD) of each sample by a cut-off value. (Cut-off value = calibrator OD x calibration factor.) For determination of IgE anti-VZV, the data were reported as OD values after subtraction from the chromogen blank OD value (background).

VZV serum antibody detection: immunoblot/dot blot. The presence of IgE anti-varicella zoster virus antibodies was determined by immunoblot (dot blot). Briefly, Varicella Zoster Virus antigen (Fitzgerald Industries International, Inc., Concord, MA) (5 µl) (diluted 1:100 in diluent) was pipetted onto nitrocellulose membrane strips (BioRad Laboratories, Hercules, CA) and dried. The nitrocellulose membranes were then soaked in TBS-Tween 20 (0.05% Tween20 [Sigma] in Tris buffered saline [20 mM Tris-HCl, 150 mM NaCl, pH 7.5, Sigma] with 5% milk powder [Immunetics, Inc., Boston, MA]). Nitrocellulose membranes were then incubated with serum samples (100 µl) (diluted in 2 ml TBS-Tween 20) for 1 hr at room temperature, after which goat polyclonal anti-human IgE (ICN, diluted 1:1000 in TBS-Tween 20 with 1% powdered milk (1 ml final volume), was added to the membranes, and incubated for 1 hr on a shaker at room temperature. The membranes were washed 3 times with TBS-Tween 20. The membranes were subsequently incubated with rabbit anti-goat peroxidase labeled antibody (ICN, diluted 1:2000 in TBS-Tween 20 with 1% powdered milk for 1 hr on a shaker. The membranes were washed 3 times with TBS-Tween 20 and developed in TMB substrate solution (2 ml). After the membranes were removed from TMB solution, they were dried and scanned.

Cytokine determinations. Serum cytokines (IL-4, IL-10, IL-1β, IFN-γ) were determined by sandwich ELISA (Biosource, Camarillo, CA) according to the manufacturer’s protocol. Briefly, samples and standards were added to wells precoated with monoclonal murine antibodies, specific to human cytokines (IL-4, IL-10, IL-1β, or IFN-γ) and incubated for 2 hr, after which a biotin-conjugated polyclonal cytokine-specific conjugated streptavidin-HRP antibody (100 µl) (Biosource) was added, and incubated for 1 hr. Plates were washed 3 times, after which TMB substrate was added (100 µl) and incubated for 15 min. The reaction was terminated with 1N H2SO4 stop solution (100 µl). Plates were read using an automated microplate densitometer (Model Elx800; Bio-Tek Instruments). Absorbance was read at 450 nm within 30 min and sample concentrations were determined based on a standard curve. Data were reported as pg/ml or IU/ml. Normal serum reference values (mean ± SD, range, CV%) for cytokines (Biosource) were: IL-4: 1.6 ± 2.1 pg/ml, 0-13.1, 3.0%; IL-10: 2.5 ± 3.2 pg/ml, 0-112, 2.8%; IL-1β: 5.0 ± 8.0 pg/ml, 0-15, 4.5%; IFN-γ: 0.2 ± 0.3 IU/ml, 0-1.2, 3.7%.

Results

Serum immunoglobulins: IgM, IgG, IgA, and IgE. Serum obtained from the patient before, during, and 5 mo after the shingles infection contained similar levels of total IgM, total IgG, IgG1, IgG2,
IgG3, IgG4, and IgA (Table I). These levels were all within the normal ranges. Serum from the shingles patient contained total serum IgE levels that were high before and during the infection (168 IU/ml, 154 IU/ml, respectively). These levels decreased after the infection (92 IU/ml) (Table I).

Anti-VZV antibodies: IgG and IgM. Serum obtained from the shingles patient was assayed for VZV-specific antibodies (IgG and IgM) in order to confirm the diagnosis of past chicken pox infection and shingles infection. By ELISA tests in our laboratory, we found that before and during the shingles infection, VZV IgG Ab was 1.7 and 1.2, respectively. Five mo after the infection, VZV IgG Ab increased to 3.1 (Fig. 1). These results are reported as antibody index IgG; they are in agreement with the following results obtained by Quest Diagnostics: During infection, VZV antibodies (IgM and IgG) were 0.14 and 1.24 Units (index value), respectively. However, 5 months after infection, VZV antibodies (IgM and IgG) were <0.9 and 3.34 Units (index value), respectively.

Anti-VZV antibodies: IgE. Serum obtained from the shingles patient was assayed for serum IgE anti-VZV antibodies using ELISA and immunoblot/dot blot tests. Before and during shingles infection, VZV IgE Ab was 0.26 and 0.28, respectively. Five mo after the infection, VZV IgE Ab increased to 0.44. (Fig. 1). These data are reported as OD values. Immunoblot analysis also confirmed positive dot blot for IgE anti-VZV antibodies before and after shingles infection. In contrast, serum from a VZV negative child (chicken pox negative, shingles negative, unvaccinated to VZV) did not contain IgE anti-VZV antibodies or IgG anti-VZV antibodies. No reaction was observed in a control test without serum.

Blood lymphocyte subpopulations. Before, during, and after the shingles infection, the distributions of lymphocyte subpopulations (CD3+CD4+, CD3+CD8+, CD8+CD60+, CD16+CD56+, CD19+) in peripheral blood of the patient were all within the normal ranges. However, the numbers of CD23+ lymphocytes decreased >50% during

### Table 1. Immunoglobulin levels in serum of an atopic serum IgE+ child before, during, and after shingles infection.*

<table>
<thead>
<tr>
<th>Year</th>
<th>IgM (mg/dl)</th>
<th>total IgG (mg/dl)</th>
<th>IgG1 (mg/dl)</th>
<th>IgG2 (mg/dl)</th>
<th>IgG3 (mg/dl)</th>
<th>IgG4 (mg/dl)</th>
<th>total IgA (mg/dl)</th>
<th>total IgE (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 5 yr before</td>
<td>2001</td>
<td>66</td>
<td>713</td>
<td>469</td>
<td>195</td>
<td>85</td>
<td>2.4</td>
<td>96</td>
</tr>
<tr>
<td>Patient during shingles</td>
<td>2006</td>
<td>76</td>
<td>not tested</td>
<td>430</td>
<td>372</td>
<td>61</td>
<td>8.0</td>
<td>105</td>
</tr>
<tr>
<td>Patient 5 mo after shingles</td>
<td>2007</td>
<td>68</td>
<td>915</td>
<td>450</td>
<td>331</td>
<td>53</td>
<td>11</td>
<td>85</td>
</tr>
<tr>
<td>Reference ranges in children</td>
<td></td>
<td>47-311</td>
<td>688-1533</td>
<td>225-1100</td>
<td>42-375</td>
<td>9.1-106.9</td>
<td>0.3-138</td>
<td>41-368</td>
</tr>
</tbody>
</table>

* IgM, IgG, and IgA were analyzed by Quest Diagnostics, Inc., using nephelometric methods; IgE was analyzed by BioQuant, Inc., using an ELISA method. The reference ranges were provided by the respective analytical laboratories.

### Table 2. Lymphocyte subpopulations in peripheral blood of an atopic serum IgE+ child before, during, and after shingles, as determined by flow cytometry. Data are expressed as the mean total cells/mm3 and as the mean percent (%) of positive cells.

<table>
<thead>
<tr>
<th>Year</th>
<th>CD3+CD4+ cells/mm3 %</th>
<th>CD3+CD8+ cells/mm3 %</th>
<th>CD8+CD60+ cells/mm3 %</th>
<th>CD16+CD56+ cells/mm3 %</th>
<th>CD19+ cells/mm3 %</th>
<th>CD23+ cells/mm3 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001^a</td>
<td>1120 44</td>
<td>767 30</td>
<td>523 21</td>
<td>74 3.0</td>
<td>373 15</td>
<td>62 2.5</td>
</tr>
<tr>
<td>2006^b</td>
<td>859 43</td>
<td>708 35</td>
<td>485 24</td>
<td>47 2.0</td>
<td>264 13</td>
<td>28 1.0</td>
</tr>
<tr>
<td>2007^c</td>
<td>893 37</td>
<td>857 36</td>
<td>597 25</td>
<td>70 3.0</td>
<td>303 13</td>
<td>22 1.0</td>
</tr>
</tbody>
</table>

^a 5 yr before shingles infection; ^b during shingles infection; ^c 5 mo after shingles infection.
Serum IgE anti-varicella zoster antibody in shingles

Fig. 1. Serum total IgG and IgE levels (upper panels) in comparison to specific IgG and IgE anti-VZV antibody levels (lower panels) in a pediatric patient before, during, and after shingles infection.

Fig. 2. Serum levels (pg/ml) of cytokines (IFN-γ, IL-1β, IL-4, IL-10) determined by ELISA in a pediatric patient before, during, and after shingles infection. Dashed line indicates the assay detection threshold based on calibration curves.

Discussion

In this study we observed that: (a) IgE anti-VZV antibodies were produced by an atopic child with a prior history of chicken pox (>10 yr) and shingles (5 mo) infection; (b) peripheral blood lymphocyte CD23 expression was decreased during shingles, and remained low at 5 mo after recovery from shingles (Table 2).

Cytokines in serum. Before, during, and at 5 mo after shingles, low levels of IFN-γ were detected in serum (7.1, 8.7, and 9.8 pg/ml, respectively). In contrast, neither IL-1β nor IL-4 were detected (<2.0, <2.0 pg/ml, respectively). Before the shingles, a low level of IL-10 were detected in serum (2.0 pg/ml). However, during the shingles, the serum level of IL-10 increased 30-fold (60 pg/ml); by 5 months after shingles, the level of IL-10 decreased to 6.8 pg/ml (Fig. 2).
and remained low after the infection; and (c) a Th2 cytokine response, specifically serum IL-10 levels, became increased during the shingles infection.

This is the first study to demonstrate that CD23 expression by peripheral blood lymphocytes is decreased in a shingles-infected patient. CD23 has been implicated in regulation of IgE production, as well as IgE-mediated cytotoxicity [23]. As mentioned in the Introduction to this paper, Bluth et al [1] reported that CD23 surface expression on B cells is increased in peripheral blood lymphocytes of Parvovirus B19-infected patients and Miller et al [22] showed that CD23 expression by peripheral blood monocytes is increased in AIDS patients. Increased expression of CD23 by monocytes may be important in the immunopathogenesis of HIV-1 infection [22]. CD23-expressing monocytes have also been implicated in allergic diseases [24]. In a shingles patient in the present study, decreased expression of CD23 lymphocytes was associated with high serum IgE level, which suggests that IgE may suppress CD23 responses during and after shingles infection. Although it is established that cell surface and soluble (s) CD23 regulate IgE production [25], the relationship of these proteins in VZV infection is not known. It could be that CD23/sCD23 initially potentiate VZV-specific IgE antibodies and subsequently decline post-infection in an autoregulatory fashion. Our finding of decreased total serum IgE levels after shingles infection is in agreement with Bluth et al [1], who observed decreased serum IgE levels 210 days post-parvovirus infection. However our findings are in contrast to reports of increased levels of total serum IgE as HIV-1 disease progresses [22,26,27].

The presence of IgE anti-viral antibodies has been reported in several viral infections including Parvovirus B19 [1], HIV-1 [5], RSV [8,9], para-influenza virus [28], and Puumala virus [12]. The clinical implications of IgE anti-viral antibodies are unknown, and no antiviral functions have been found [1]. In the present study, detection of IgE specific for VZV suggests that this antibody response is potentially important in the pathogenesis of chicken pox or shingles infection. VZV may possibly behave in a similar fashion to viruses that elicit IgE antibodies.

Diagnosis of chicken pox or shingles cases involves a thorough patient history, physical examination, and blood chemistry profile [29]. Use of the VZV polymerase chain reaction, direct detection of virus in cell cultures, and testing for specific antibodies to VZV are recommended in special circumstances [29]. However, confirmation of past infection of chicken pox is mostly supported by serologic findings [30]. In our patient, IgG and IgE anti-VZV antibodies were detected using an IgG and IgE anti-VZV antibody ELISA. An immunoblot assay, generated in our laboratory, was used to confirm our ELISA results. If the ELISA result is negative, an immunoblot is unnecessary. However, use of immunoblots may improve the specificity of serologic testing for certain diseases.

Remarkable findings in our patient included the long-term persistence of IgE-specific antibodies for >10 yr after chicken pox infection and 5 mo after shingles infection, and the decreased level of total serum IgE at 5 mo after shingles. These results point to an IgE-specific component in chicken pox or shingles infection. Pellegrino et al [5] have shown that HIV-1 specific IgE persists after 210 days and is able to suppress HIV-1 production in vitro.

Herpes zoster is a viral infection that is caused by reactivation of varicella zoster virus [31]. Studies of Cho et al [31] and Buchbinder et al (32) suggest that impaired cellular immunity may be responsible for reactivation of the virus due to aging, stress, or genetic factors. However, cytokines and soluble factors may be involved in the pathogenesis of herpes zoster. Ongkosuwito et al [33] detected various immunoregulatory cytokines (IL-6, IL-10, IFN-γ) in ocular fluid samples from patients with uveitis. In the present study, serum IL-10 levels were high in our patient during shingles infection. IL-10 is an immunomodulatory cytokine that suppresses cell-mediated immunity by inhibiting antigen presenting cells [31]. Polymorphisms of the IL-10 promoter gene may be associated with susceptibility to infectious disease [31]. Furthermore, IL-10 potentiates humoral immune responses and can directly affect IgE regulation [34]. In contrast, IL-4 was undetectable in serum before, during, and after shingles infection. It is possible that in atopic individuals, there exists a baseline IgE reactivity or modality to many different antigens.
Upon infection with varicella virus, there might be a suppression of the baseline response, similar to a tolerance mechanism. This could explain the increase in IgE anti-VZV antibodies after infection, due to a direct response to VZV infection, but a major decrease in total serum IgE level as some form of suppression later on (5 mo post-infection). This is in agreement with studies of Nilsson et al [35] who reported that acquisition of Epstein-Barr virus (EBV) during the first 2 yr of life was associated with reduced risk of IgE sensitization; this effect was enhanced by cytomegalovirus co-infection. Viral infections have been implicated in influencing IgE-mediated sensitization, but their exact roles remain controversial [35-37].

While herpes zoster in children has never been common, more and more children are presenting the secondary form of the chicken pox infection, which appears as herpes zoster, a disease usually diagnosed in adults [38]. In addition, nowadays, chicken pox, which was traditionally considered a childhood disease, has an increased incidence in the adult population [38].

Our results suggest that: (a) IgE may play a role in anti-VZV immunity and their memory responses, and (b) these responses may be mediated by CD23 and/or IL-10. Taken together, IgE/antigen specific IgE and related responses may provide greater insight into VZV pathogenesis and viral infections in general. Further studies are necessary to elucidate the role of immunoglobulins in VZV infection and to determine whether IgE has any functional or immunomodulatory roles in this disease.

References