Disseminated Varicella Infection Caused by Varicella Vaccine Strain in a Child With Low Invariant Natural Killer T Cells and Diminished CD1d Expression

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Background. Live attenuated varicella vaccine is considered a safe vaccine with serious adverse effects reported only in immunocompromised children. We describe a severe life-threatening infection with varicella vaccine virus causing rash and pneumonitis in a 6-year-old boy with no apparent immunodeficiency.

Methods and Results. Polymerase chain reaction (PCR) analysis of vesicle swab samples demonstrated varicella zoster virus (VZV). Sequencing of the PCR product demonstrated 100% homology with human herpesvirus 3 strain VZV-Oka ORF62 gene. Routine immunologic investigations failed to demonstrate any abnormality. Total leukocyte, lymphocyte, and neutrophil counts and lymphocyte subsets were normal. Immunoglobulins, C3, C4, and CH50 were intact. Specific IgG to protein and polysaccharide antigens and to Epstein–Barr virus and cytomegalovirus were present. Normal lymphocyte proliferation to phytohemagglutinin and VZV antigens was detected. Neutrophil function and natural killer (NK) cell activity were normal. The analysis of invariant NK T (iNKT) cell numbers and function revealed diminished iNKT cells, reported once previously and unique to our patient, deficient expression of the cognate receptor, CD1d.

Conclusions. This report provides a further link between deficiency of the iNKT/CD1d pathway and increased susceptibility to varicella vaccine virus, suggesting an important role of this innate pathway in host defense against yet another member of the herpesvirus family.

Varicella zoster virus (VZV) is a member of the herpesvirus family that commonly causes varicella and herpes zoster infections in humans. Live attenuated varicella vaccine has been licensed for routine use in children in the United States since 1995. Since the introduction of the vaccine, severe complications of varicella immunization, such as extensive varicelliform rash and pneumonitis, have been rare and were described only in 7 children, 6 with an underlying immunodeficiency and 1 with Down syndrome [1–4]. Control of acute VZV infection requires intact innate and adaptive immune responses [5]. The invariant natural killer (NK) T (iNKT) cells are a unique subpopulation at the interface of these 2 arms of immunity, sharing some characteristics of adaptive, conventional T cells, but functionally belonging to innate immunity. They express invariant Vα24/β11 T-cell receptors [6] and recognize lipid antigens presented by the nonclassic major histocompatibility class I molecule, CD1d [7, 8]. Multiple naturally occurring lipid antigens able to stimulate iNKT-cell responses have been identified, including various glycolipids from microbial pathogens [9]. In addition, α-galactosyl ceramide (α-GalCer), a synthetic glycolipid derived from marine sponges, stimulates potent iNKT responses both in vitro and in

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PATIENTS AND METHODS

Case Report

Parental consent was obtained for all investigations and for publication of the case. A 6-year-old Caucasian boy with cerebral palsy presented with a 4-day history of low-grade fever, irritability, lethargy, and vesicular rash, 23 days after receiving varicella vaccination (Varivax; CSL). He had spastic quadriplegia with visual impairment, epilepsy, and severe developmental delay presumed to be due to birth asphyxia. He had chronic mild hypothermia (usual temperature, 33°C–35°C), possibly due to hypothalamic dysfunction, and was being treated with sodium valproate and topiramate for seizure control. He was the third child from a nonconsanguineous marriage and uneventful pregnancy. Both parents and 2 older siblings were healthy. There was no family history of early deaths from recurrent infections. His previous infective illnesses included recurrent otitis media, mild bronchitis with upper respiratory infections, and rotavirus gastroenteritis. He was allergic to penicillin. He had received all scheduled immunizations (including 2 doses of live measles vaccine at 1 and 4 years of age) without adverse effect. He had no recent exposure to natural wild-type varicella.

On admission he had a temperature of 36.5°C (elevated above his usual baseline) and generalized varicelliform eruption on his trunk and extremities, with characteristic vesicles in various stages of development (Figure 1). He had respiratory distress with a respiratory rate of 50 breaths per minute, intercostal recession, fine basal crackles, and persistent oxygen requirement. Another chest radiograph 5 days after admission showed progressive changes consistent with varicella pneumonitis (Figure 2). The patient’s skin lesions cleared slowly, but new lesions were still appearing 1 week after admission. Bullous cellulites developed on his left foot, from which no bacterial organisms were cultured. Intravenous clindamycin (10 mg/kg/8 h) was commenced. Acyclovir was replaced with valacyclovir after 8 days.

Seven days after admission, the boy had persistent extensive crackles in both lung fields and a continuing oxygen requirement, but his signs resolved gradually during the next 3 days, and he was well enough for discharge after 12 days of admission. There was no indication of acute central nervous system involvement during this illness, and seizure control remained stable. His thrombocytopenia was transient, reaching a nadir of 44 000 on admission and returning to normal within 1 month. Liver enzyme levels fell progressively, returning to normal within 3 months. Eight months later, the patient had another episode of pneumonia due to respiratory syncytial virus (RSV).
Analysis of VZV
To distinguish wild-type varicella from Oka vaccine strain, the polymerase chain reaction (PCR) analysis of a swab sample from a vesicle base was performed by the Queensland Health Scientific Services Virology Laboratory and further analyzed using the method of Loparev et al [21]. The amplified product was purified using QIAquick Gel Extraction Kit according to the manufacturer’s instructions (Qiagen). Products were sequenced in both directions using Applied Biosystems ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit. With use of the National Center for Biotechnology Information Nucleotide-Nucleotide Basic Local Alignment Search Tool, a 205–base pair sequence was compared with sequences held in GenBank.

Analysis of Cellular and Humoral Immunity
The patient’s immune competence was tested several weeks after complete resolution of acute illness, at the Department of Immunology, Pathology Queensland Central Laboratory, Brisbane, Australia. The testing was done with parental consent and according to the ethical and confidentiality requirements defined by National Pathology Accreditation Advisory Council and National Association of Testing Authorities, Australia. All healthy volunteers used as controls were deidentified before the testing. Immunoglobulin levels and specific antipathogen and anti-vaccine responses were analyzed by standard assays and were compared with laboratory-validated reference values. C3 and C4 levels were measured by nephelometry and total hemolytic activity of the classic complement pathway (CH50) by an in-house hemolytic assay. Lymphocyte subsets were determined by flow cytometry using BD FACSCalibur Multiset Clinical Software. Lymphocyte proliferation to phytohemagglutinin (PHA) and VZV antigens was measured by tritiated thymidine uptake. VZV antigen (ORYP 05) and cytomegalovirus (CMV)/VZV negative control antigen (OREE 05) were obtained from Dade Behring Marburg. Lymphocyte proliferation was expressed as index of stimulation (ratio between cultures stimulated with mitogen or VZV antigen and unstimulated cultures). Two known responders to PHA and VZV antigen were tested in parallel. The neutrophil oxidative burst was measured by reduction of dihydrorhodamine-123 in a flow cytometric assay. NK cell cytolytic activity was assessed by Central Sydney Laboratory Service in a standard K562 target cell lysis assay measuring chromium 51 release [22–24].

Flow Cytometry
The following monoclonal antibodies were purchased from BD Biosciences-Pharmingen: mouse anti-human CD3 allophycocyanin (SK7), 6B11 phycoerythrin (PE), CD1d PE (CD1d42), CD45 peridinin chlorophyll protein (2D1), and corresponding isotype controls: mouse immunoglobulin (Ig) G1 fluorescein isothiocyanate (FITC; X40), mouse IgG1 PE (X40), and mouse IgG1 allophycocyanin (X40). Mouse anti-human Vα24 FITC (C15) was purchased from Immunotech. The PE-labeled α-GalCer–loaded or unloaded (control) murine CD1d tetramers were generated by D. G. Pellicci, as described elsewhere [25]. The frequency of NK T cells was defined in the lymphoid gate and was expressed as total staining minus background for the corresponding fluorescence-minus-1 control. Approximately 250 000 CD45+/CD3+ events were acquired.

Functional Assay
A total of 0.2 × 10^6 CD3+ PB mononuclear cells (PBMCs)/well in a 96-well plate was stimulated with either α-GalCer (10 ng/mL; generous gift from Dale Godfrey, University of Melbourne, Australia) or PHA (5 and 10 μg/mL; Difco Laboratories). The interferon (IFN) γ levels in culture supernatants were measured by BD Human IFN-γ Flex Set (BD Biosciences) after 5 days for PHA and after 24 and 72 hours for α-GalCer stimulation.

PCR Analysis
Real-time PCR was performed using TaqMan Gene Expression Assays for human CD1D (assay ID, Hs00174321_m1) and carried out on a Rotor-Gene3000 (Corbett Research). The data were analyzed with Rotor-Gene V-5.0 software (Corbett Research). The CD1D complementary DNA (cDNA) copy numbers were normalized for variations in the efficiency of RNA extraction and cDNA transcription against the human actin-β (ACTB) housekeeping gene by TaqMan Gene Expression assays (assay ID, Hs99999903_m1).

RESULTS
PCR analysis of a swab from a vesicle base demonstrated the presence of VZV. Further analysis confirmed the presence of the vaccine strain of VZV. The sequencing of the purified PCR
product was found to be 100% homologous with human herpesvirus 3 strain VZV-Oka ORF62 gene (GenBank accession number, AY016449). When this sequence was compared with the sequence of the VZV Dumas strain (GenBank accession number X04370), a substitution of a C for a T at nucleotide position 106262 confirmed the VZV to be Oka vaccine strain (Figure 3).

Assessment of the patient’s immune function was performed several weeks after the resolution of acute illness, when the patient was well. The representative results are summarized in Table 1. Total leukocyte, lymphocyte, and neutrophil counts, lymphocyte subsets, and immunoglobulin levels were normal. Specific IgG antibodies to tetanus toxoid and Haemophilus influenzae type B were detected. Lymphocyte proliferation to PHA and VZV antigens was intact. The stimulation indexes for patient were 5.06 for PHA and 5.15 for VZV antigen, whereas controls demonstrated indexes of stimulation of 5.37 and 5.52 for PHA and 5.03 and 4.34 for VZV antigen. C3, C4, and CH50 were normal. Normal phagocyte oxidative burst and NK cell cytolytic activity were detected. Protective IgG responses to Epstein–Barr virus (EBV) and CMV were present. Specific anti-VZV IgG was detected in serum samples of the patient >1 year after illness.

Based on a similar case report published in 2003 by Levy et al [4], iNKT cells were enumerated in PB of the patient several months after the initial presentation, in parallel with 6 healthy controls (Figure 4). Similar results were obtained by 2 staining protocols (Figure 4A and 4B). Although a distinct population of iNKT cells was demonstrated in all controls (0.13%–0.28 %), the iNKT cells were significantly reduced in the patient (0.01% on each of 3 separate blood collections ~3 months apart; Figure 4C). To confirm flow cytometric data, a functional in vitro assay was performed. Control cultures of CD3+ PBMCs stimulated with the iNKT-specific agonist α-GalCer (Figure 4D) demonstrated significant IFN-γ secretion at 24 and 72 hours after stimulation (3.65- and 4.91-fold increase, respectively, from unstimulated levels), suggesting the presence of an intact iNKT response. By contrast, the response to stimulation with α-GalCer was significantly lower in cultures derived from the patient. Compared with the levels in unstimulated cultures, the IFN-γ levels were minimally elevated at 24 hours after stimulation with α-GalCer, and there was no further increase at 72 hours in the patient’s cultures (1.54- and 1.37-fold increase, respectively). This was due to the lower proportion of iNKT cells within the total CD3+ population in patient’s cultures, consistent with flow cytometric data. To assess IFN-γ production by conventional T cells in this patient, PB lymphoid cells were stimulated with polyclonal T-cell mitogen PHA. The IFN-γ levels in the patient’s cultures stimulated with 5 µg/mL PHA were 6.16-fold (Figure 4E) and 5.40-fold (data not shown) lower than those in control cultures, suggesting a possible defect in IFN-γ production by conventional T cells in this patient. However, the IFN-γ production by patient’s conventional T cells reached levels comparable to the control when 10 µg/mL PHA was used (1.19-fold [Figure 4E] and 1.15-fold [data not shown]).

To assess the expression of the cognate receptor for activation of CD1d-restricted iNKT cells, we measured surface CD1d expression by flow cytometry on APCs in the PB of the patient (>12 months after his recovery from acute illness) and 3 healthy controls. Although the human leukocyte antigen-DR–positive population in our control subjects expressed distinct levels of CD1d, the expression was significantly reduced in the patient (Figure 5A). Subsequent PCR analysis demonstrated presence of the CD1d messenger RNA (mRNA) in the patient’s PBMCs (Figure 5B).

**DISCUSSION**

Since the development of a live attenuated varicella vaccine in Japan in 1974, and its approval for use in the United States in 1995, the incidence of varicella in the United States has declined by as much as 90% [26]. Mortality due to varicella has decreased by ~66% [27]. A recent review reports that overall vaccine effectiveness in the United States is 44%–100% for the prevention of all varicella, 86%–100% for combined moderate and severe varicella, and 100% for severe varicella [28]. Adverse effects are usually minor and include fever, injection site reactions, and rash [29]. After licensure, serious adverse events have been reported at a rate of 1.4/100 000 doses distributed [29]. Detection of the vaccine strain of varicella in severe disseminated disease has occurred in only 7 previously reported cases, all in children with underlying immunodeficiency. The cases included HIV infection, high-dose corticosteroid therapy, severe combined immunodeficiency, DiGeorge syndrome, chemotherapy, and proposed numerical and functional deficiency of iNKT
cells [1–4, 30]. Rash and pneumonia have been attributed to vaccine virus in other reports without confirmation [31]. We considered the possibility that the pneumonia in this case was caused by an organism other than VZV. Bronchoalveolar lavage was not performed, but the progression and resolution of the pneumonia in the absence of an antibacterial other than flucloxacillin would make this unlikely. Furthermore, HIV-1 and HIV-2 infections were excluded.

Literature review and discussion with a clinical geneticist did not reveal any known predisposition to severe varicella in patients with cerebral palsy and seizures or chromosomal abnormalities. The potential immunosuppressive effects of the anticonvulsant medications [32–34] on routine immunologic parameters of the patient were not evident. However, their impact on selective T-cell functions (eg, cytokine production) cannot be excluded.

This patient was defined as immunocompetent by means of routine immunopathologic testing, and the only immunologic aberrancies detected were a profound reduction of iNKT-cell numbers, as described in a previous case report [4] and deficient expression of their cognate receptor CD1d, which has never been reported in this clinical scenario before this study. Presence of the CD1d mRNA in patient’s PBMCs made major deletions of the \( CD1D \) gene unlikely. However, this did not exclude the possibility of point mutations or small deletions or insertions that alter the open reading frame and thereby disrupt normal translation of CD1d mRNA. Furthermore, functional expression could also be compromised by mutations in major

<table>
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<th>Parameter</th>
<th>Patient’s Values</th>
<th>Reference Values</th>
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<tr>
<td>Leukocytes, ( 10^9/L )</td>
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<td>Lymphocytes, ( 10^9/L )</td>
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<td>1.50–7.00</td>
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<td>( CD3^+ ), % (( 10^9/L ))</td>
<td>58 (1.83)</td>
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<td>( CD3^+CD4^+ ), % (( 10^9/L ))</td>
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<td>27–57 (0.56–2.70)</td>
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<td>( CD3^+CD8^+ ), % (( 10^9/L ))</td>
<td>21 (0.66)</td>
<td>14–34 (0.33–1.40)</td>
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<td>( CD19^+ ), % (( 10^9/L ))</td>
<td>26 (0.82)</td>
<td>9–29 (0.20–1.30)</td>
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<td>Specific antibodies (IgG)</td>
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<td>Tetanus toxoid, IU/mL</td>
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<tr>
<td>( Haemophilus influenzae ) type B, ( \mu g/mL )</td>
<td>4.58</td>
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<td>Count, ( 10^9/L )</td>
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<tr>
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<tr>
<td>VZV</td>
<td>Present</td>
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Abbreviations: \( ^{51}Cr \), chromium 51; CMV, cytomegalovirus; DHR, dihydrorhodamine-123; EBV, Epstein–Barr virus; Ig, immunoglobulin; NK, natural killer; PHA, phytohemagglutinin; VZV, varicella zoster virus.

* Reference values are age-related, laboratory-validated normal values.
threonine-based signal residue in the cytoplasmic tail of the CD1d [35] or N-linked glycosylation sites [36], which control transport of this glycolipid to the cell surface and stabilize its surface expression.

Herpesviruses were reported to induce down-regulation of CD1d expression during lytic replication in vitro and hypothesized to use this escape mechanism during acute infections in vivo [37]. However, because the evaluation of the CD1d expression was performed more than 12 months after recovery from acute illness when our patient was well, we considered this to be an unlikely cause of low CD1d expression in this case.

The significance of the diminished frequencies of iNKT cells in our patient in comparison with healthy adult controls is uncertain. Although we were unable to compare both numbers and function of iNKT cells in our patient with those in healthy pediatric control subjects, these were studied in the report by Levy et al [4]. Both “healthy” and “diseased” pediatric control subjects (children with chronic viral infections and suspected but unidentified immunodeficiency) in their study demonstrated higher iNKT-cell numbers and function compared with healthy adults, and with Oka-VZV–infected patients.

However, it is of note that the low frequencies of iNKT cells in our patient were within the reference range for iNKT cells (0.008%–1.176%), recently established in 103 adult and pediatric healthy control subjects by Marsh et al [38]. We suggest that although the observed frequency of iNKT cells (0.01%) in PB may be considered “normal” for the majority of pediatric and adult subjects, this may be insufficient under suboptimal conditions.
conditions for their peripheral activation. The deficient expression of CD1d on APCs in this patient suggests impairment of the CD1d-restricted glycolipid antigen presentation pathway, which would consequently lead to deficient in vivo activation and expansion of iNKT cells. The low iNKT-cell frequency and suboptimal activation and expansion due to low expression of CD1d molecules may lead to relative insufficiency of this innate pathway in the early course of some viral infections.

The mechanisms triggered and used by iNKT cells in antiviral immunity are still controversial. Nonetheless, these “innate-like” cells are proposed to be an important early source of IFN-γ [39], a cytokine necessary for augmentation of adaptive CD8+ antiviral responses. This pathway may be critical in young children, in whom IFN-γ production by naive CD4+ T cells, required for enhancement of CD8+ responses, is impaired compared with adults [40].

An important role for iNKT cells in antiviral immunity, especially against herpesviruses, has been demonstrated in mouse strains deficient in iNKT cells or the CD1d-restricted antigen presentation pathway, which is required for their thymic selection and peripheral activation [15, 16]. Children with X-linked or autosomal recessive mutations of genes encoding signaling proteins in developing iNKT cells suffer from extreme susceptibility to EBV [17–19]. Most commonly, boys with X-linked mutations of SH2D1A encoding signaling lymphocyte activation molecule–associated protein (SAP), lack iNKT cells and develop X-linked lymphoproliferative (XLP) syndrome manifested by fulminant primary EBV infections with frequent fatal outcomes [41]. Because EBV-specific IgG antibodies were detected in our patient, suggesting previous contact with EBV and protective immunity without severe complications, we considered a diagnosis of XLP syndrome in this boy unlikely. This was confirmed by an intact intracellular expression of SAP (data not shown).

There is substantial evidence that iNKT cells are also involved in immune clearance of RSV, hepatitis B and C virus, HIV, and coxsackievirus [20], perhaps explaining the episode of RSV pneumonia after VZV infection in our patient. His previous uneventful EBV infection and measles vaccination suggested that innate immune mechanisms occurring in response to VZV may differ from those in response to EBV and other viruses.

Reduced frequencies of iNKT cells have been reported in patients with Omenn syndrome [42], Wiskott–Aldrich syndrome [43], common variable immunodeficiency [44], and autoimmune polyendocrine syndrome type 1 [45, 46]. Patients with these syndromes should not be immunized with potentially fatal live vaccines; therefore, there are no data on their vulnerability to VZV vaccine. Likewise, there are no reports on susceptibility to VZV vaccine strain among patients with XLP syndrome.

We also considered the possibility that both cases of disseminated VZV infection described by Levy et al [4] and ourselves have an additional but currently unidentified abnormality affecting other components of innate or adaptive immunity, which, under the appropriate constellation of clinical factors and in conjunction with iNKT/CD1d deficiency, predisposed these children to severe complications from the VZV vaccine.

In our patient, the reduced IFN-γ response to submaximal dose of PHA suggested an additional defect in IFN-γ production by conventional T cells. This raises the possibility of a subtle functional defect in conventional T cells of this patient, which could become clinically significant under suboptimal stimulation in vitro or an increased demand in vivo. In association with iNKT/CD1d insufficiency, this could predispose this child to more severe viral infections.

To our knowledge, this is the second case demonstrating that severe, life-threatening infection may occur after vaccination with the Oka strain of VZV in a subset of children who appear to be immunocompetent by routine immunologic investigations but have a selective reduction of iNKT cells. In our patient, this was combined with the deficient expression of CD1d and possible subclinical functional impairment of conventional T cells. This deficiency is not recognized as a clinical entity and is likely to be extremely rare, so routine use of this vaccine should be
continued [47]. Currently, analysis of frequencies of iNKT cells, CD1d expression, and cytokine production by T cells is confined to research laboratories and is not part of a routine testing of immune competence. We propose that examination of these parameters could be useful when investigating unexplained childhood cases of increased susceptibility to VZV and possibly other viruses. This may help to further define the still poorly understood role of iNKT cells in antiviral defense in humans.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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