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Absence of Circulating Natural Killer and Primed CD8$^+$ Cells in Life-Threatening Varicella

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Five pediatric patients with no history of immunodeficiency had a life-threatening course of varicella. Strikingly, natural killer (NK) cells were absent from the circulation in all children, and, despite active viral infection, up to 98% of the CD8$^+$ cells were naive. Primary immunodeficiencies were excluded—NK cells and primed CD8$^+$ cells reappeared in the circulation, granzymes were detectable in plasma early during infection, and no abnormalities could be detected in interleukin-15 receptor function. Our data indicate that varicella-zoster virus (VZV) has a unique capability to seclude primed CD8$^+$ cells and NK cells from the circulating lymphocyte pool. This may be the consequence of an overwhelming immune response to VZV that is influenced by factors such as infectious dose, age, and the presence of maternal antibodies during infancy. Because both homozygous twin sisters in the study had a severe course of varicella, particular genetic factors may contribute to severe varicella.

Primary infection with varicella-zoster virus (VZV) results in varicella (chickenpox), a highly contagious disease characterized by fever, malaise, and a generalized vesicular rash (exanthem). After primary infection, the virus remains latent in dorsal root ganglia. In countries of temperate climates where VZV vaccination is not implemented in routine childhood vaccinations, such as The Netherlands, >95% of children become infected before they are 5 years old. Varicella is usually a benign and self-limiting illness, although it may lead to discomfort. Nonetheless, complications are seen in otherwise healthy children, such as bacterial superinfection of the cutaneous lesions, VZV pneumonia, cerebellar ataxia, and viral encephalitis [1]. In immunocompromised individuals, the risk of these complications is highly increased [1].

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Before the appearance of the vesicular exanthem, abundant VZV replication is suppressed by the innate immune system. However, the innate immune system is incapable of containing the virus completely. The adaptive immune response is subsequently activated by the clonal expansion of VZV-specific T and B cells that interfere with viral replication and spread of the virus. VZV-specific CD4$^+$ cells secrete cytokines, such as interleukin (IL)–2 and interferons (IFNs), and may exhibit major histocompatibility class II–restricted cytotoxicity [2–4]. So far, VZV-specific CD8$^+$ cells could not be detected because of the paucity of experimental tools. Nevertheless, CD8$^+$ cells are thought to constitute one of the main effector arms in the immune response and to be responsible for viral clearance and protection on reexposure. Studies of other persistent viruses have shown that, during primary infection, CD8$^+$ cells expand and differentiate. CD8$^+$ cells can be subdivided into functional subsets by use of markers such as CD45RA, the costimulatory molecule CD27 [5], and CCR7 [6]. During acute infection, virus-specific CD8$^+$ cells show a CD45RA$^-$CD27$^+$CCR7$^-$ phenotype and abundantly express perforin and proteases such as granzymes (Grs) [7].

NK cells are also believed to be important in the clearance of herpesvirus infections [8, 9]. Biron et al.

Table 1. Characteristics of the patients.

Patient (sex)	Age at onset of varicella	Admission[a]	Symptoms	Severity score[b]	Therapy
1 (M)	8 months	4	Vesicular rash, pneumonitis, hepatitis, encephalitis	30	Acyclovir, IVIG, clindamycin
2 (M)	10 months	7	Vesicular rash, pneumonitis, hepatitis	25	Acyclovir, IVIG, clindamycin
3 (F)	5 years	5	Vesicular rash, encephalitis, multiorgan failure	30	Acyclovir, IVIG, clindamycin
4[c] (F)	11 months	2	Vesicular rash, pneumonitis, hepatitis	24	Acyclovir, clindamycin
5[c] (F)	11 months	1	Vesicular rash, pneumonitis	16	Acyclovir

NOTE. IVIG, intravenous immunoglobulins.

[a] Days after onset of rash; the no. of lesions remained <500 in all cases.

[b] According to Vazquez et al. [14], mild disease, ⩽7 points; moderately severe disease, 8–15 points; severe disease, ⩾16 points on a scale to assess severity of varicella.

[c] Homozygous twins.

[10] described a female patient with a severe course of VZV infection accompanied by a complete absence of NK cells, which persisted after resolution of the infection. NK cells normally constitute up to 15% of circulating human lymphocytes. The development of NK cells is dependent on cell-to-cell contact between their progenitors and bone-marrow stromal cells, on cytokines such as IL-15, and on growth factors such as the Flt3 ligand and stem-cell factor [11]. Although the precise mechanisms of the activation of NK cells have yet to be resolved, it appears that the regulation of NK cell activity results from a balance between signals from activating and inhibitory receptors on the cell, the latter of which is dominant in steady state [12, 13].

We describe 5 children with a severe course of varicella who had symptoms that included generalized varicella, skin infection, pneumonitis, hepatitis, and encephalitis. None of these children had a history of recurrent infection or known immunodeficiency. Four of the children were admitted to the pediatric intensive care unit (PICU) of the Emma Children's Hospital at the Academic Medical Center. After >4–12 weeks of intensive treatment, 4 of the children recovered, and the fifth child died of multiorgan failure. We studied functions and phenotypes of T and NK cells to investigate the incapability of these children to control the virus adequately.

PATIENTS, MATERIALS, AND METHODS

Patients. Five children with a severe course of varicella were admitted to the Emma Children's Hospital at the Academic Medical Center (index patients). Four of these children were admitted to the PICU because of respiratory and circulatory failure. The cohort included 1 female homozygous set of twins, of which 1 child was admitted to the PICU (patient 4), whereas the other child had a severe, yet not life-threatening, course of varicella (patient 5). The children differed in age and sex (table 1). According to the clinical scoring system of Vazquez et al. [14], rash, character of the lesions, height of fever, and systemic signs and a subjective assessment of the patient's appearance determined the severity of varicella. According to this scoring system, all children were categorized as having "severe disease" caused by VZV (i.e., >16 points; table 1). Treatment consisted of intravenous acyclovir, with or without intravenous immunoglobulins, along with medication for respiratory and circulatory support. Blood samples were obtained from the patients at early (within 10 days) and late (4–6 weeks) time points after the appearance of exanthem.

We included 3 children with varicella who were admitted to the PICU because of respiratory and circulatory failure caused by complications (bacterial superinfections) as control subjects for our index patients. One of these children (4 months old) had <50 vesicles and epiglottis. Blood cultures from this patient were positive for *Haemophilus influenzae* type b (Hib); she had been vaccinated once for Hib in the past. The second child (11 months old) had bilateral lobar pneumonia; blood cultures were positive for *Streptococcus pneumoniae.* The third child (2 years old) was admitted with blood culture–positive septic shock caused by group A β-hemolytic streptococci spreading from impetiginized vesicles. These patients had a rash of <200 vesicles and looked severely ill. All 3 patients had "moderately severe disease" (i.e., 14 points) [14]. A second group of control subjects (diagnosed and monitored at the outpatient care unit) consisted of children with mild varicella (severity score, 6.2; $n = 8$; mean age, 3.8 years) [14]. Blood samples from these control subjects were collected and stored when permitted by parental consent.

Determination of VZV-specific CD4$^+$ cells by intracellular cytokine staining. Peripheral blood mononuclear cells (PBMCs) were isolated by use of standard density-gradient centrifugation techniques with Lymphoprep (Nycomed; Pharma). PBMCs were cryopreserved until use and thawed according to standard procedures. VZV-specific CD4$^+$ cell frequencies were determined as described elsewhere [4]. In brief, PBMCs were stimulated for 6 h with VZV antigen (20 μL/mL; Microbix Biosystems), the final 5 h in the presence of brefeldin A (10 μg/mL; Sigma Chemical), and costimulated with anti-CD28 (2 μg/mL; CLB 15E8; Sanquin) and anti-CD49d (1 μg/mL; BD Biosciences). Next, cells were

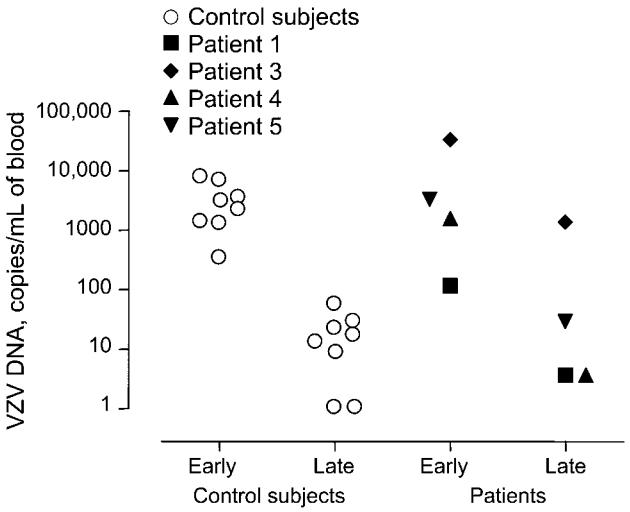

Figure 1. Varicella-zoster virus (VZV) load in blood during the course of varicella. High VZV loads were detected in blood from patient 3 during the early phase of varicella (within 10 days after appearance of the exanthem) and remained high throughout the infection. VZV loads were observed in blood from patients 1, 4, and 5 that were comparable to those in control subjects (pediatric patients with a mild course of varicella). No data were available for patient 2. Differences of VZV loads between the patient groups were not statistically significant. Early, within 10 days after the appearance of the vesicular exanthem. Late, 4–6 weeks after the appearance of exanthem.

incubated with fluorescence-activated cell sorting (FACS) lysing and FACS permeabilization solutions (BD Biosciences). Cells were stained for CD4–peridinin-chlorophyll-protein complex (PerCPCy5.5), IFN-γ–fluorescein isothiocyanate (FITC; both from BD Biosciences), and CD69–allophycocyanin (APC; Caltag Laboratories). The $CD4^+CD69^+IFN\text{-}\gamma^+$ cells were designated as antigen-specific $CD4^+$ cells. Negative controls consisted of stimulation with medium, and positive controls consisted of stimulation with *Staphylococcus aureus* enterotoxin B (Sigma). Percentages of VZV-specific $CD4^+$ cells were corrected for background staining. Analysis of cells was performed by use of a FACSCalibur flow cytometer and CellQuest Pro software (BD Biosciences).

VZV polymerase chain reaction (PCR). Quantitative PCR was performed on blood samples from the patients and control subjects as described elsewhere [15]. The electrochemiluminescence signal was measured by use of an M-8 analyzer (IGEN).

Immunofluorescent staining. PBMCs were washed in PBS that contained 0.01% (wt/vol) NaN_3 and 0.5% (wt/vol) bovine serum albumin. A total of 200,000 PBMCs were incubated with the fluorescent-labeled conjugated monoclonal antibodies CD3-FITC, CD4-PerCP, CD8-PerCP, CD8-APC, CD16–phycoerythrin (PE), CD27-PE, CD45RA-FITC, and CD56-APC (BD Biosciences; concentrations in accordance with the manufacturer's instructions) for 30 min at 4°C.

Cytokine production. PBMCs were stimulated with PMA (2 ng/mL; Sigma) and ionomycin (1 μg/mL; Sigma) for 4.5 h, 3.5 h of which in presence of brefeldin A (10 μg/mL). After stimulation, cells were washed and incubated with FACS lysing and permeabilization solutions (BD Biosciences). Cells were stained with IFN-γ–FITC, IL-2–PE, IL-4–PE, CD4-PerCP, and CD8-APC (BD Biosciences).

5-(and-6)-carboxyfluorescein diacetate succinimidyl ester (CFSE) labeling and cell culture. PBMCs were resuspended in PBS at a final concentration of $5–10 \times 10^6$ cells/mL and labeled with CFSE (2.5 μmol/L; Molecular Probes) for 8 min while being shaken at 37°C. Labeling was stopped by the addition of the Iscove modification of Dulbecco's medium (IMDM) that contained 10% fetal calf serum. Cells were washed and subsequently resuspended in IMDM (10% human pool serum), antibiotics, and 3.57×10^{-4}% (vol/vol) β-mercapto ethanol (Merck). CFSE-

Table 2. NK cells in varicella-zoster virus (VZV)–induced life-threatening varicella, compared with those in varicella complicated by bacterial super- or coinfection.

Infection	Severe VZV disease (n = 5)	Complicated VZV disease (n = 3)[a]	Age of control subjects[b]		P
			<1 year (n = 34)	1–4 years (n = 18)	
Severity score[c]	22	14			
Acute					
NK cells, cells/μL	20 ± 20	510 ± 100			.001
NK cells, % lymphocytes	0.9 ± 0.6	10.2 ± 3.4			.020
Convalescent					
NK cells, cells/μL	230 ± 90		520 ± 320	480 ± 290	.06
NK cells, % lymphocytes	6.5 ± 3.6		10.1 ± 8.1	10.8 ± 3.6	.35

NOTE. Data are mean ± SD.

[a] Patients (n = 3) with epiglottis, pneumococcal pneumonia, and group A β-hemolytic streptococcal septicemia, respectively.

[b] The control subjects were of mixed racial background.

[c] Severity score according to Vazquez et al. [14]: mild disease, ⩽7; moderate, 8–15; and severe, ⩾16.

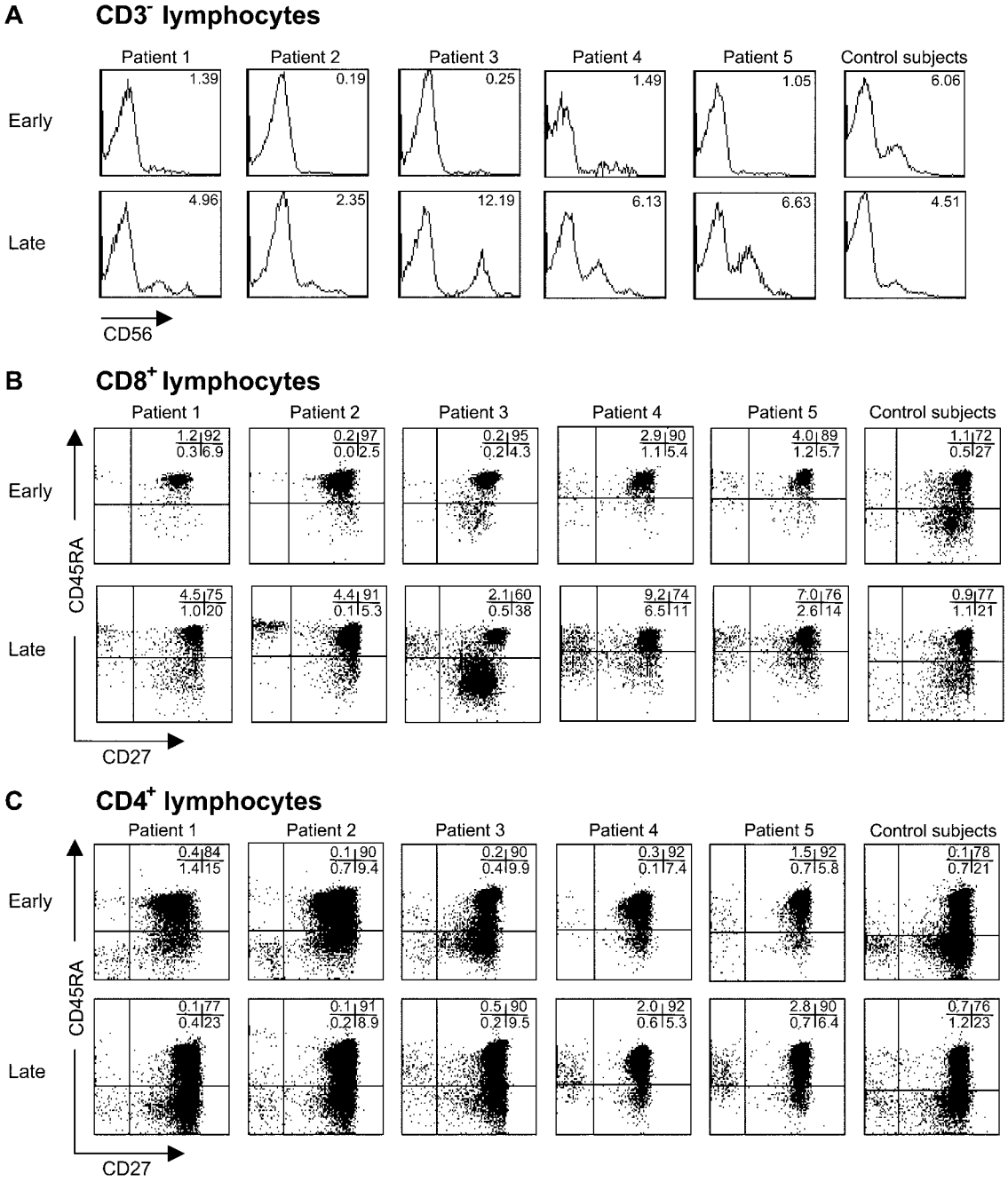

Figure 2. Disturbed pattern of NK cells and primed $CD8^+$ cells in peripheral blood from patients. During the early phase of infection ("early"), NK cells (*A,* gated on $CD3^-$ lymphocytes; nos. indicate the percentage of $CD3^-CD56^+$ cells within the total lymphocyte pool) and primed $CD8^+$ cells (*B,* gated on $CD8^+$ cells) were absent from peripheral blood from patients, despite acute viral infection, whereas these cells appeared in peripheral blood during the late phase of infection ("late"). NK cells and primed (i.e., $CD45RA^-CD27^+$, $CD45RA^-CD27^-$, or $CD45RA^+CD27^-$) $CD8^+$ cells were detectable in peripheral blood at all time points after varicella-zoster virus infection in 1 of the representative pediatric control subjects, who had a mild course of varicella. *C,* Normal distribution of $CD4^+$ cells throughout the infection. For patients, "early" refers to within 10 days and "late" to 4–6 weeks after the appearance of the vesicular exanthem. Because control subjects with mild varicella resolved the infection within 1 week, "early" refers to 0–3 days and "late" to 7–10 days after the appearance of exanthem in these children.

Table 3. T cell subsets in varicella-zoster virus (VZV)–induced life-threatening varicella, compared with varicella complicated by bacterial super- or coinfection.

Cell subset	VZV disease			Age of control subjects <1 year[c]
	Acute severe[a]	Convalescent[a]	Complicated[b]	
$CD4^+$				
Naive ($CD45RA^+CD27^+$)	89.6 ± 3.3	88.4 ± 6.4	78.0 ± 3.5 ($P = .005$)	80.8 ± 5.0 ($P = .003$)
Memory ($CD45RA^-CD27^+$)	9.5 ± 3.5	10.6 ± 7.2	18.7 ± 4.0 ($P = .01$)	16.6 ± 4.7 ($P = .01$)
Effector/memory ($CD27^-$)	1.2 ± 0.5	1.4 ± 1.1	3.3 ± 1.5 ($P = .02$)	2.4 ± 1.4 ($P = .12$)
$CD8^+$				
Naive ($CD45RA^+CD27^+$)	92.6 ± 3.4	75.0 ± 11.0	70.0 ± 5.6 ($P = .0003$)	74.8 ± 14.9 ($P = .009$)
Memory ($CD45RA^-CD27^+$)	5.0 ± 1.6	17.6 ± 12.6	23.0 ± 6.6 ($P = .0008$)	13.9 ± 9.9 ($P = .01$)
Effector/memory ($CD27^-$)	1.8 ± 1.6	4.8 ± 3.6	6.9 ± 3.2 ($P = .0001$)	12.6 ± 8.3 ($P = .09$)

NOTE. Data are mean ± SD.

[a] Index patients ($n = 5$).

[b] Patients ($n = 3$) with epiglottis, pneumococcal pneumonia, and group A β-hemolytic streptococcal septicemia, respectively; *P* values are derived from Student's *t* test of acute severe VZV disease vs. complicated VZV disease.

[c] Age of control subjects <12 months, $n = 16$; mixed racial background; *P* values are derived from Student's *t* test on acute severe VZV disease vs. age of control subjects (<1 year).

labeled cells were cultured in 24-well plates at concentrations of $0.5–1 \times 10^6$ cells/mL in culture medium in the presence or absence of IL-15 (10 ng/mL; R&D Systems) for 5 days.

Gr ELISA. Cell-free plasma samples were collected from heparin-anticoagulated blood from the patients. ELISAs to measure the levels of GrA and GrB were performed as described elsewhere [16].

NK cell cytotoxicity assay. The target cell line K562 was prepared by labeling it with ^{51}Cr (Amersham Pharmacia Biotech) for 1 h at 37°C in 5% CO_2. ^{51}Cr-labeled target cells were incubated in triplicate with NK cells at various effector:target ratios for 4 h at 37°C in 5% CO_2. Spontaneous release was determined by the incubation of labeled target cells with medium and maximal release by incubation with NP40 solution. Supernatants were harvested and counted in a γ-radiation detector. The percentage of specific lysis was calculated from the following formula: percentage specific lysis = [(experimental release − spontaneous release)/(maximal release − spontaneous release)] × 100%.

Statistics. Where applicable, differences in means between groups were determined by Student's *t* test. $P < .05$ was considered to be statistically significant. All analyses were done by use of SPSS (version 11.5; SPSS).

RESULTS

VZV load in blood samples. VZV loads in blood samples from patients 1, 4, and 5, obtained during the early phase of infection, were within the range of loads observed in pediatric patients with a mild to moderate course of varicella (figure 1). Loads decreased during the course of infection to background levels similar to those observed in control subjects after resolution of the infection (figure 1). In contrast, the VZV load in patient 3 was high during the early phase of infection, and, although the load decreased, it remained high throughout the course of infection. VZV loads were not determined in serial blood samples from patient 2. Taken together, the differences between the index patients and the control patients with mild to moderate VZV infection did not reach statistical significance.

Disturbed distribution of $CD8^+$ and NK cells in blood during the early phase of VZV infection. With regard to humoral immunity, immunoglobulin spectra were normal. The patients had developed normal antibody responses to childhood vaccinations (diphtheria–tetanus toxoids–pertussis–polio-Hib at age 2, 3, 4, and 11 months for all patients or mumps-measles-rubella at age 14 months for patient 3). VZV-specific IgM and IgG could be detected in blood from the patients at the time of admission (before treatment with immunoglobulins) and 3–4 months after convalescence (data not shown).

Because T cells and NK cells play a key role in control of VZV infection, the presence and differentiation of these cells were studied. Significantly lower levels of NK cells were found in the 5 index patients, compared with those in the 3 patients admitted to the PICU because of circulatory and respiratory failure caused by bacterial superinfection during acute varicella (table 2; $P = .001$, absolute numbers; $P = .02$ percentages). Furthermore, NK cells were detectable in children with a mild course of varicella (figure 2*A*). The levels of NK cells in the index patients normalized during convalescence (table 2).

Although the index patients had an acute viral infection, the majority of $CD8^+$ cells found during the early phase of infection were of the naive subset (i.e., $CD45RA^+CD27^+$). Significantly lower levels of primed $CD8^+$ cells were detectable in the index patients, compared with those in the control patients in the PICU (table 3). Furthermore, in control patients with a mild course of varicella, $CD8^+$ cells showed a primed phenotype (i.e., $CD45RA^-CD27^+$, $CD45RA^-CD27^-$, or $CD45RA^+CD27^-$) and

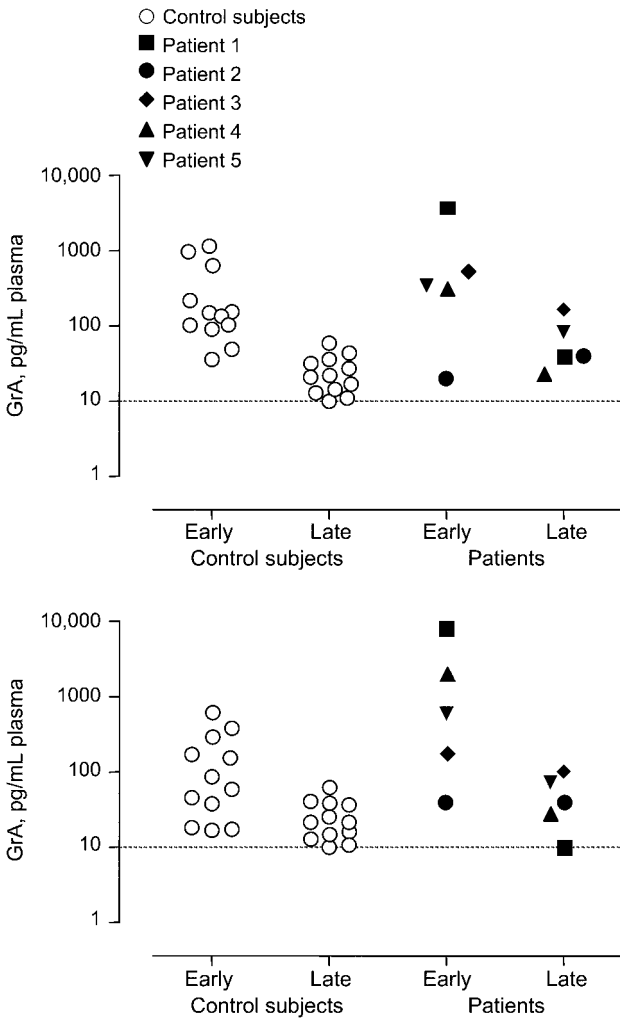

Figure 3. Secretion of granzymes (Gr) A and B in plasma. During the early phase of varicella-zoster virus infection, all patients secreted GrA and GrB in plasma. High levels of these cytolytic proteins were detected in plasma from patient 1, compared with the pediatric control subjects (who had mild varicella). The levels of Gr secretion normalized in these patients during the course of infection. At both the early and the late time point, the differences in Gr concentrations between the patient groups were not statistically significant. Early, within 10 days after the appearance of the vesicular exanthem. Late, 4–6 weeks after the appearance of exanthem. *Dotted horizontal line,* detection limit of ELISA.

were already detectable in peripheral blood within 5 days after the appearance of exanthem (figure 2*B*) [5]. In the index patients, naive $CD4^+$ cells were present during the early phase of infection and seemed to persist, although this distribution of naive and primed $CD4^+$ cells was variable (figure 2*C* and table 3). During the late phase of infection, NK cells (figure 2*A* and table 2) and primed $CD8^+$ cells of the memory and effector phenotypes (figure 2*B* and table 3) appeared in blood from the index patients [5]. Numbers of primed and naive $CD4^+$ cells and of B cells remained stable (table 3 and data not shown). At all time points studied, circulating $CD4^+$ and $CD8^+$ cells were able to secrete IFN-γ, IL-2, and IL-4 on stimulation with PMA/ionomycin (data not shown).

High levels of Gr in plasma. GrA and GrB are produced by cytotoxic T cells and NK cells and are important mediators of the cytolysis of virus-infected cells. The level of GrA in plasma was relatively high during the early phase of infection in patient 1, whereas the level of GrB was relatively high in patients 1 and 4. Gr levels detected in the other patients were within the range of those in pediatric control subjects with mild to moderate varicella (figure 3). Gr levels normalized during the course of infection to levels observed in control subjects after the resolution of VZV infection (data not shown). Because GrA and GrB were detectable during both the early and the late phases of infection, we believe that primed $CD8^+$ cells and NK cells were present throughout the infection and were functional in vivo, even though they were not detectable in blood during the early phase of infection. During early and late infection, the Gr levels between the 2 patient groups did not show any statistically significant difference.

Delayed appearance of VZV-specific $CD4^+$ cells in blood. The VZV-specific immune response was studied by use of a functional assay in which VZV-specific $CD4^+$ cells were detected by the up-regulation of CD69 and the production of IFN-γ after in vitro stimulation with a VZV lysate (VZV antigen) [4]. Only very low frequencies of VZV-specific $CD4^+$ cells could be detected in blood from 3 of the patients during the early phase of infection when we used this assay, whereas these cells were absent from the circulation in the other 2 patients (table 4). Increased frequencies of virus-specific $CD4^+$ cells were detected during the late phase of infection, after treatment with antiviral therapy (table 4). In contrast, these cells were already detectable

Table 4. Varicella-zoster virus (VZV)–specific $CD4^+$ cells.

Patients	Early, %	Late, %
Index		
1	0.02	0.10
2	0.03	0.06
3	0.00	0.05
4	0.02	0.06
5	0.00	0.02
Mild VZV		
Control 1	0.23	0.05
Control 2	0.13	0.03

NOTE. Nos. indicate the percentages of specific interferon-γ^+/$CD69^+$ cells within the $CD4^+$ cell population. From 2 control subjects, serial samples were available at identical time points as those tested in the index patients. Early, within 10 days after the appearance of exanthem; late, 4–6 weeks after the appearance of exanthem.

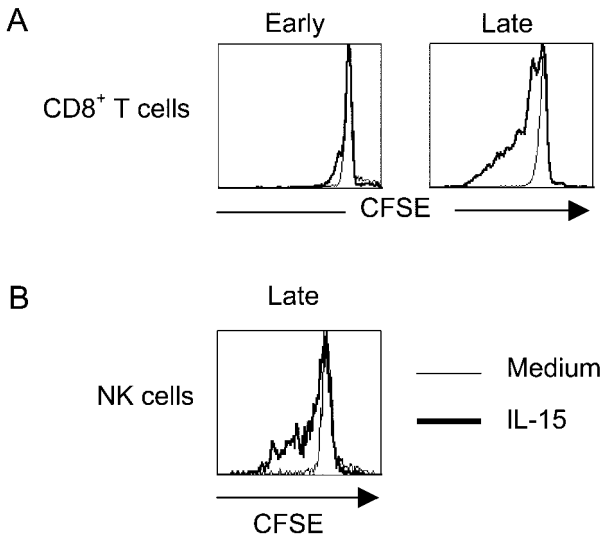

Figure 4. Proliferation of $CD8^+$ cells and NK cells on interleukin (IL)–15 stimulation. *A,* Stimulation with IL-15 resulting in proliferation of $CD8^+$ cells on day 5 of culture *(thick line)* but no proliferation of $CD8^+$ cells cultured in medium only *(thin line).* $CD8^+$ cells in peripheral blood during the late phase of infection ("late") exhibited increased proliferative potential, compared with $CD8^+$ T cells during the early phase of infection ("early"). *B,* Proliferation on stimulation with IL-15 of NK cells that appeared in peripheral blood from patients during the late phase of infection. Data from 1 representative patient (patient 3) are shown. CFSE, 5-(and-6)-carboxyfluorescein diacetate succinimidyl ester.

during the early phase of VZV infection in blood from pediatric patients with a mild course of varicella.

Proper function of the IL-15 receptor. Signaling of IL-15 through the IL-15 receptor is important in the maturation and differentiation of NK and $CD8^+$ cells. $CD8^+$ cells from the patients were able to proliferate on stimulation with IL-15, as was demonstrated by the dilution of CFSE on day 5 of culture, whereas no proliferation was detected when cells were stimulated with medium (figure 4*A*). $CD8^+$ cells obtained during the late phase of infection exhibited higher proliferative capacity on stimulation with IL-15 than did $CD8^+$ cells obtained during the early phase of infection. Furthermore, NK cells (which were only detectable during the late phase of infection) proliferated on stimulation with IL-15 (figure 4*B*).

NK cell cytotoxicity. The cytotoxicity of NK cells appearing in blood obtained from the patients during the late phase of infection was determined by K562 lysis. NK cells from all patients were capable of lysing K562 at levels comparable to NK cells derived from pediatric control subjects (figure 5; data from 1 representative control subject are shown).

DISCUSSION

We have described the immune response of 5 patients with a life-threatening—and, in 1 case, fatal—course of varicella. Most strikingly, NK cells and primed $CD8^+$ cells were nearly absent from the circulation during the early phase of primary VZV infection, whereas these cells could be detected in control patients with varicella who were admitted to the PICU because of bacterial super- or coinfection. The absence of these cells, which appear to be crucial in antiviral defense [7, 8, 17, 18], may have resulted from a primary defect in the development or differentiation of these cells. Several observations in our patients excluded this possibility. First, $CD8^+$ and NK cells from the patients proliferated on stimulation with IL-15, a key factor in the differentiation and homeostasis of these cells [19–24], which excluded defects in IL-15–Rα, IL-2–Rβ, the common γ-chain (CD132), or signaling molecules coupled to this receptor [25]. Second, GrA and GrB were detectable in plasma from the patients during the early phase of infection, which suggests that primed $CD8^+$ cells and NK cells were functionally present. Third, primed $CD8^+$ cells and NK cells reappeared in the circulation during convalescence.

Alternatively, the absence of these cytotoxic cells from the circulation could be the consequence of redistribution to target sites. A major caveat of studies of human viral infections is the limitation of compartments that can be studied, which

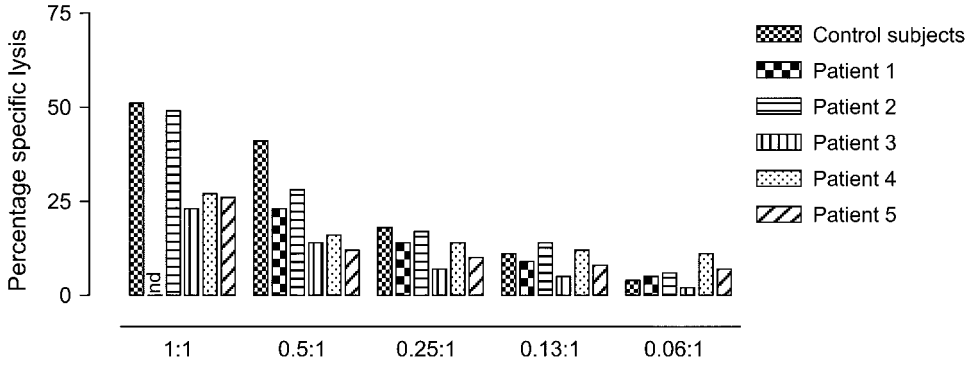

Figure 5. NK cell cytotoxicity. NK cells that reappeared in peripheral blood from the patients during recovery from the infection were capable of lysing the NK-cell–sensitive target K562. ND, not done.

are largely restricted to the circulating pool of immune cells. The majority of NK cells and primed T cells express chemokine receptors, such as CCR5 and CXCR3, whereas they do not express the secondary lymphoid homing receptor CCR7 [6, 7, 26]. This pattern of chemokine receptor expression enables them to migrate from the circulation to inflamed tissue to eliminate virus-infected cells [27–30]. The distribution of virus-specific cells in peripheral blood may therefore differ from that in target sites [31, 32]. Indeed, several studies have suggested that primed cells may be secluded from the circulation during viral infection. Virus-specific T cells have been shown to be present at higher frequencies in nonlymphoid tissue than in lymphoid tissue during acute infection in murine models [31, 33]. A few studies of hepatitis B and C virus infections in humans have shown that the percentages of virus-specific T cells are higher in the liver, which is the major target site of these viruses, than in the circulation [34–36].

As our results show, significantly fewer primed $CD4^+$ cells were observed during the early phase of infection in the index patients. A low number of VZV-specific $CD4^+$ cells appeared in the circulation after intensive antiviral treatment, with an extensive delay, compared with that in patients who had mild varicella. These data are consistent with studies that have shown that, during the acute phase of HIV and symptomatic cytomegalovirus (CMV) infection, virus-specific $CD4^+$ cells are kept out of the circulation and only appear after control of the virus by antiviral therapy [37, 38].

The absence of primed $CD8^+$ cells and NK cells from the circulation during life-threatening varicella in our patients seems, at present, to be unique to VZV, given that, in CMV and HIV infection, primed $CD8^+$ cells are detectable in the circulation, regardless of therapeutic intervention [37, 39]. Whereas the targeting of $CD4^+$ cells by CMV, HIV, and VZV from the circulation probably only involves virus-specific $CD4^+$ cells, all primed $CD8^+$ cells were absent from the circulation during the early phase of severe, but not mild, courses of varicella. We therefore suggest that, in some cases, VZV may be able to seclude primed $CD8^+$ cells and NK cells from the circulation.

The patients described in the present study probably had an overwhelming immune response to VZV that was accompanied by high levels of chemokines at the inflamed sites, which led to the targeting of cytotoxic cells at these sites. Because chemokine receptors that enable migration to target sites are present not only on VZV-specific $CD8^+$ cells but also on the majority of primed $CD8^+$ cells, cytotoxic cells of broad antigenic specificities may be secluded from the circulation. Only after the normalization of the chemokine levels by spontaneous recovery or intervention with antiviral therapy might these cells be enabled to return to the circulation. In addition, the ongoing infection may lead to the exhaustion of primed $CD8^+$ cells and NK cells because of a disturbed balance between the generation of these cells and apoptosis [40–42]. NK cells were absent not only from the circulation during the early phase of infection but also from bone marrow (<100 NK cells/10^5 cells [$n = 3$] vs. mean $\pm$ SD, 2480 $\pm$ 960 NK cells/10^5 cells in bone marrow from pediatric control subjects [$n = 7$]), whereas they could be cultured from $CD34^+$-selected hematopoietic stem cells in vitro for the patients from whom sufficient bone marrow was obtained (patients 1 and 3; H. Spits, Academic Medical Center, Amsterdam, The Netherlands, unpublished observation). Although it has never been described, the infection of NK cells by VZV may induce the apoptosis of these cells. However, the restriction of this phenomenon to only NK cells seems implausible.

Severe courses of varicella are rare in otherwise healthy children. Primary VZV infection during infancy has been defined as one of the risk factors for developing complicated varicella [43], which may partly explain the severity of the disease in 4 of our patients. In addition, infectious viral dose, the duration of antigen exposure, and passive protection by maternal antibodies during infancy may influence the induction of the antiviral immune response and, thereby, the severity of the course of varicella. Furthermore, genetic factors may contribute to the outcome of VZV infection, given that both homozygous twin sisters included in the present study developed a complicated course of varicella.

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