Pediatric HIV-1-infection: perspectives on vaccination strategies and immune reconstitution during long-term antiretroviral therapy
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Varicella vaccination in HIV-1-infected children after immune reconstitution

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Abstract

Context: HIV-1-infected children have an increased risk of severe chickenpox. However, vaccination is not recommended in severely immunocompromised children.

Objective: Can the live-attenuated VZV Oka strain be safely and effectively given to HIV-1-infected children despite previously low CD4+ T cell counts?

Methods: VZV-vaccine was administered twice to fifteen VZV-seronegative HIV-1-infected children when total lymphocyte counts were >700 lymphocytes/µL, and 6 HIV-negative VZV-seronegative siblings. Weekly clinical follow-up and sampling was performed.

Results: None of the children developed any clinical symptom or serious adverse reaction following immunization. VZV-specific T-cell responses increased after vaccination and were comparable in both groups over time. Only nine (60%) of the HIV-1-infected children had VZV-specific antibodies after two immunizations, whereas 100% of the siblings seroconverted. VZV-specific antibodies were lower in HIV-1-infected children after 2 vaccinations than in HIV-negative siblings. Additionally, after wild-type infection with VZV, varicella-specific IgG was lower than in HIV-negative siblings. Age at baseline was negatively correlated with the VZV IgG titer at 6 weeks after the second vaccination in HIV-1-infected children.

Conclusions: VZV vaccination of previously immunocompromised HIV-1-infected children was safe and induced specific immune responses in part of the vaccinated children, suggesting that previously immunocompromised individuals are protected against severe forms of varicella.
Introduction

Varicella is an acute, highly infectious disease caused by varicella zoster virus (VZV). Children generally develop mild disease. After the initial infection VZV establishes latency in the dorsal root ganglia. Reactivation, resulting in zoster, is thought to occur when alterations in the balance between the virus and host factors allow local replication of the virus in the ganglion and axonal transport to the skin [1,2]. Complications of varicella are bacterial super-infection, cerebellitis and encephalitis. VZV is very common in temperate climates, where about 95% of the young adult population has serologic evidence of previous infection [3]. In tropical areas VZV is less common [4]. After migration to colder areas persons are at risk of contracting varicella at older age, which increases the risk of developing complications [5].

Both virus-specific IgG and T cells can be detected after varicella infection [6] as well as after VZV-vaccination of healthy individuals [7]. Virus-specific IgG probably neutralizes free viral particles and therefore helps to counter re-infection upon repeated exposure. This is supported by the observation that early administration of varicella-zoster hyper immune immunoglobulin (VZIG) protects against VZV infection after close contact with a contagious person with active varicella. Both CD4⁺ and CD8⁺ cytotoxic T cells are thought to suppress reactivation from viral latency. Exposure to a person with chickenpox gives a rise in VZV-specific T cells and reduces the risk of zoster later in life [8,9].

Persons with untreated HIV-1-infection show continuous viral replication and subsequent loss of CD4⁺ T cells, which eventually causes an immune deficiency and opportunistic infections as a consequence. VZV infection in these patients can cause severe chickenpox with major morbidity and mortality [10,11]. Even during treatment with HAART, HIV-1-infected adults were found to have an increased risk of reactivation of VZV, causing shingles [12]. In our cohort of HIV-1-infected children we recently observed that during treatment with HAART serologic protection against VZV after natural infection fades over time despite immune reconstitution [13]. Thus, HAART may not normalize the risks of recurrent or reactivating VZV infection in HIV-1-infected patients.

Since 1974 a live-attenuated vaccine against VZV is available [14]. Routine vaccination of all children without a history of varicella is recommended in the US [15]. However, vaccination of children with any known immune deficiency is not recommended, because of the potential of disseminated viral infection. To date VZV immunization of HIV-1-infected children has been restricted to children with stable disease (stage N1 or A1 according to CDC classification [16], with CD4⁺ T-lymphocyte percentages greater than or equal to 25%) [15]. HIV-1-infected patients would benefit most of an effective vaccination, in order to prevent severe forms of primary wild-type VZV infection.

The aim of the study was to evaluate the safety and efficacy of VZV vaccination of HIV-1-infected children irrespective of CDC-category or treatment. To this end, children
were followed intensively for the development of clinical symptoms and both cellular and humoral responses were measured.

Patients and methods

From June 2002 to June 2005 all patients in the Pediatric Amsterdam Cohort on HIV (PEACH) \((n=78)\) were evaluated for inclusion in the study. Inclusion criteria were: HIV-1-seropositive, VZV-seronegative, under the age of 18, lymphocyte count above 700 cells/μL, and without any active disease. Of the included patients all HIV-1-negative household members were also tested for VZV serology. If tested VZV-seronegative, immunization was offered and when consent was given, these family members were also included in the study. Written informed consent was obtained from all patients, controls and caregivers. The medical ethical committee of the Academic Medical Centre approved the research protocol.

Study design:
All VZV-seronegative patients received the VZV vaccine. Study visits and blood samples were planned at 2, 3, 4 and 6 weeks after primary immunization. All patients received a second immunization 3-6 months after the primary immunization, with an evaluation of the serological and cellular responses after 6 weeks.

Vaccine:
Varicella vaccine (Varilrix, GlaxoSmithKline UK, Uxbridge, UK) containing the live-attenuated VZV Oka strain was administered subcutaneously as per the manufacturer’s recommendations for immunocompromised children.

Lymphocytes, T cell subsets
Blood samples were collected and numbers of B cells (CD19⁺), T cells (CD3⁺) and T cell subsets (CD3⁺CD4⁺, CD3⁺CD8⁺) were determined real time by standard FACScan procedures, as described before in detail [3]. In short, 100 μl EDTA anti-coagulated whole blood was incubated with fluorescent label conjugated monoclonal antibodies (mAbs) (concentrations according to manufacturer’s instructions). CD4⁺ T-cell and CD8⁺ T-cell subsets were additionally defined by mAbs specific for CCR5-FITC (BD) and CXCR4-PE (BD) (CD184 and CD195, respectively). The number of activated CD4⁺ and CD8⁺ T cells were determined using monoclonal antibodies against HLA-DR (Becton Dickinson) and CD38 (Coulter Immunology, Paris, France). Analysis of cells was performed using a FACS scan flowcytometer and CellQuest software (BD Biosciences). Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples using standard density gradient centrifugation techniques, after separation of the plasma. Both plasma and PBMCs were stored until further analyses.

Antigen-specific T-cell proliferation assay
In vitro T-cell proliferation to VZV antigen was measured using CFSE (5,6-carboxyfluorescein diacetate succinimidyl ester) (Molecular Probes, Eugene, OR) dye
dilution assay according to the manufacturer’s protocol. Briefly, after thawing, PBMC were resuspended in PBS at a final concentration of 5-10 x 10^6 cells/ml and labeled with 0.5 μM (final concentration) of CFSE for 8 min. at room temperature. Labeling was stopped using human pool serum (Sanquin, Amsterdam, The Netherlands). Cells were washed and subsequently resuspended in RPMI (BioWhittaker, Verviers, Belgium), supplemented with 10% human pool serum and antibiotics (penicillin/streptomycin).

CFSE-labeled PBMCs were cultured for 6 days at 37°C with VZV antigen in a final concentration of 20 μl/ml (Microbix Biosystems, Toronto, Canada), previously defined as the optimal effective dose for lymphocyte stimulation [17]. Cells were stimulated otherwise with CD3 and CD28 mAbs to define T-cell reactivity under optimal lymphocyte proliferation induction at final concentrations of 0.3 μg/mL, and 2 μg/mL, respectively (Sanquin Research, Amsterdam, The Netherlands). At day 6, cells were stained extra-cellularly with fluorochrome-conjugated mAb against CD4 and CD8 (BD). Cells were fixed in Cellfix (BD), and 60,000 up to 300,000 events were acquired using FACScalibur flow cytometer (BD). The Stimulation Index (SI) was calculated by dividing the percentage of proliferating (CFSElow) CD4^+ or CD8^+ T cells after stimulation with VZV by the percentage of proliferation after stimulation without VZV antigen (control stimulation).

**Plasma HIV-1 RNA determination**

Plasma HIV-1 RNA concentration (pVL) was determined using Versant HIV-1 RNA 3.0 (Bayer, Tarrytown, NY, USA). All tests were performed according to the instructions of the manufacturers.

**VZV serology**

Specific IgG to VZV was determined by Vidas immunoassay (Biomerieux, Lyon, France) following the instructions of the manufacturers. The test values of this assay were converted to IU/ml using the conversion factor as determined by Van der Zwet et al [18]. An antibody level of 0.14 IU/mL or more was regarded as positive. Seropositivity was defined by the presence of specific IgG after the age of 18 months to exclude any confounding contribution of maternal antibodies in the very young. Serological tests within 3 months after the administration of blood products were excluded from the analyses.

**Statistical analysis**

Statistical analyses were performed using SPSS for Windows version 11.5 (SPSS, Chicago, USA). All p-values were two-tailed. P-values smaller than 0.05 were considered statistically significant. Continuous data were analyzed using a Mann-Whitney U test. Categorical data were compared with a Fisher’s exact test. Correlation was tested using the Spearman’s correlation test. SI was modeled using a mixed model incorporating repeated measurements. This model handles missing data adequately by estimating the outcome using a ‘first order autoregressive’ structure. Differences in these estimates between different levels of the variable were tested for significance using t-statistics. Age correction for CD4^+ and CD8^+ T cells was done by dividing the counts by the mean of an age matched healthy control group [3].
Results

Baseline characteristics of study population
Of 22 eligible HIV-1-infected children in our pediatric cohort 15 consented to participate. Baseline characteristics before immunization are outlined in Table 1. During vaccination 2 children were not treated with antiretroviral drugs, others were on HAART during a median of 289 weeks. Twelve of 13 patients on HAART (92%) had an undetectable pVL at first vaccination. Median nadir percentage of CD4+ T lymphocytes was 19%, 12 of 15 vaccinated children had a nadir percentage below 25% and 6 even below 15%, having by definition a severe immune deficiency [16].

Six siblings of the HIV-1-infected children had a negative VZV serology. CD4+ T and CD8+ T cell counts were comparable between HIV-1-infected and HIV-negative children (p=1.0 and p=0.3, respectively) (Table 1).

Safety of the vaccine
After vaccination none of the 15 HIV-1-positive children experienced a serious adverse event related to primary or secondary vaccination. The pVL did not change within the 6 weeks after the first or second immunization, irrespective of treatment or detectable pVL at the time of immunization. VZV DNA was only detectable once in whole blood

Table 1: Baseline characteristics of HIV-1-infected children vaccinated against VZV.

<table>
<thead>
<tr>
<th></th>
<th>HIV-1-positive</th>
<th>HIV-negative sibling</th>
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<tbody>
<tr>
<td>Number (n)</td>
<td>15</td>
<td>6</td>
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<tr>
<td>Age, median (IQR)</td>
<td>8.3 (4.3-11.7)</td>
<td>4.6 (1.3-8.3)</td>
</tr>
<tr>
<td>Sex, female</td>
<td></td>
<td></td>
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<tr>
<td>CDC-classification, N*</td>
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</tr>
<tr>
<td>A</td>
<td>2</td>
<td>2</td>
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<td>B</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Children on HAART (n)</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Time since start HAART,</td>
<td>289 (132-337)</td>
<td></td>
</tr>
<tr>
<td>median weeks (IQR)</td>
<td></td>
<td></td>
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<tr>
<td>Plasma HIV-1 RNA, median</td>
<td>1.9 (1.7-3.0)</td>
<td></td>
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<tr>
<td>log copies/mL (IQR)</td>
<td></td>
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<tr>
<td>Baseline</td>
<td></td>
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<tr>
<td>CD4+ T cells, median (IQR), absolute cells/µL</td>
<td>1410 (740-1640)</td>
<td>1550 (1080-2380)</td>
</tr>
<tr>
<td>CD4+ T cell percentage, median (IQR), %L</td>
<td>38 (28-42)</td>
<td>41 (35-49)</td>
</tr>
<tr>
<td>Age adjusted CD4+ T cell ratio, median (IQR)</td>
<td>0.9 (0.7-1.4)</td>
<td>1.0 (0.8-1.3)</td>
</tr>
<tr>
<td>CD8+ T cells, median (IQR), absolute cells/µL</td>
<td>1.170 (950-1550)</td>
<td>1130 (700-1200)</td>
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<tr>
<td>CD4+CCR5+ T cell percentage (IQR)</td>
<td>7 (4-11)</td>
<td>6 (4-14)</td>
</tr>
<tr>
<td>CD4+CXCR4+ T cell percentage (IQR)</td>
<td>76 (74-86)</td>
<td>84 (72-92)</td>
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<tr>
<td>CD8+CCR5+ T cell percentage (IQR)</td>
<td>17 (7-28)</td>
<td>12 (10-24)</td>
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<tr>
<td>CD8+CXCR4+ T cell percentage (IQR)</td>
<td>41 (32-52)</td>
<td>56 (42-74)</td>
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<tr>
<td>Nadir</td>
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<td></td>
</tr>
<tr>
<td>CD4+ T cells, median (IQR), absolute cells/µL</td>
<td>540 (230-910)</td>
<td></td>
</tr>
<tr>
<td>Percentage of CD4+ T cells, median (IQR), %</td>
<td>19 (11-24)</td>
<td></td>
</tr>
<tr>
<td>Age-adjusted CD4+ T cell ratio, median (IQR)</td>
<td>0.38 (0.17-0.56)</td>
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</table>

*Clinical categories as defined by the US Centers for Disease Control and Prevention (CDC) [16].
of a single patient at 2 weeks after the first immunization (100 copies/mL). The patient had no clinical complaints or signs of varicella infection upon physical examination. All cultures for VZV from throat swabs remained negative.

No change in immune activation markers on T cells
Vaccination could induce immune activation of T cells and up regulation of HIV coreceptors on CD4+ T cells, which would lead to more target cells for HIV and potentially resulting in an increase in pVL. During the weeks after vaccination we measured the CD4+ and CD8+ T cell counts and CCR5 and CXCR4 receptors and immune activation as assessed by HLA-DR and CD38. At baseline median percentages of CCR5 and CXCR4 positive CD4+ and CD8+ T cells were comparable between HIV-1-infected and HIV-negative children (p=0.5, p=0.07, p=0.8, p=0.9, respectively) (Table 1). CD4+ and CD8+ T cell count did not change over time after immunization (Figure 1A and B). The number of activated T cells as assessed by HLA-DR and CD38 expression remained unchanged. Expression of both chemokine receptors CCR5 and CXCR4 did not show any change in the weeks after primary and secondary immunization (Figure 1C-F).

Lower humoral response rate of HIV-1-infected children
At 6 weeks after each immunization the VZV-specific serologic response was assessed. After primary vaccination 5 of 15 HIV-1-infected children seroconverted for VZV. Median VZV IgG was 0.1 IU/mL. After booster vaccination 9 of the 15 (60%) children became VZV IgG-positive. In the HIV-negative siblings, the response rate was 3/6 (50%) after primary vaccination and 6/6 (100%) after boosting. Additionally, the anti-VZV IgG titers in HIV-1-infected children after 2 vaccinations against VZV were significantly lower than of the HIV-negative siblings (median 0.2 vs. 4.6 IU/ml; p=0.002) (Figure 2A).

Of the 9 children who seroconverted, 7 were retested at 24 weeks after the second immunization. VZV-specific IgG was no longer detectable in two of the children. Of the 5 VZV-seronegative children who remained seronegative after 2 immunizations, 2 received a third vaccination. Only one of these 2 children seroconverted.

Low VZV-IgG titers after wild-type VZV in HIV-1-infected children
To investigate antibody responses against VZV in natural VZV infection in HIV-infected children, we studied 8 children with clinically evident chickenpox and 4 children with clinical herpes zoster during treatment with HAART (Table 2). Four children (50%) were VZV-IgG positive prior to chickenpox. Only 6 of these 8 children with wild-type varicella were VZV-IgG positive after chickenpox. The titers of anti-VZV IgG after chickenpox in HIV-1-infected children were significantly lower than in HIV-negative siblings after vaccination (median 0.8 vs. 4.6 IU/mL, p=0.04). The titer after chickenpox was not different from the titer after vaccination of HIV-1-infected children (median 0.8 vs. 0.2 IU/mL, p=0.3) (Figure 2B).

The 4 children with herpes zoster were previously tested positive for VZV-IgG. However, one child lost VZV antibodies during HAART, prior to herpes zoster. All 4
Figure 1. CD4+ and CD8+ T cells and chemokine positive T lymphocytes after VZV vaccination. In A, CD4+ T cells. In B, CD8+ T cells. The median cells/μL and interquartile range are shown. In C, CD4+CCR5+ T cells. In D, CD8+CCR5+ T cells. In E, CD4+CXCR4+ T cells. In F, CD8+CXCR4+ T cells. No reaction in T lymphocyte subsets is seen upon vaccination. The median percentage of positive cells and interquartile range are shown.

children were VZV seropositive after herpes zoster. The titer after wild-type zoster in HIV-1-infected (0.4 IU/mL) was also lower than the titer after immunization of HIV-negative siblings, but comparable to immunization of HIV-1-infected children (p=0.02 and p=0.4, respectively).

**VZV-specific T cell responses increase after vaccination in both HIV-infected and HIV-negative siblings**

The cellular immune response upon vaccination was measured after stimulation with VZV antigen using CFSE dye-dilution assay and was expressed as stimulation index (SI). We were able to measure the VZV-specific CD4+ and CD8+ T cell proliferative
**Figure 2.** VZV serology after VZV vaccination. In A, VZV IgG in HIV-1-infected children (square) and HIV-negative children (triangle) immunized against VZV. In B, Comparison of titers 6 weeks after second immunization with VZV Oka strain in HIV-1 infected children and HIV-negative siblings, as well as non-immunized HIV-1 infected children with wild-type (re) infection or reactivation of herpes zoster during HAART. The median international units/mL and interquartile range are shown.

### Table 2. Characteristics of HIV-1 infected children with wild-type VZV and herpes zoster.

<table>
<thead>
<tr>
<th></th>
<th>Wild-type VZV</th>
<th>Herpes zoster</th>
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<tbody>
<tr>
<td><strong>Number (n)</strong></td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td><strong>Age, median (IQR)</strong></td>
<td>4.3 (2.3-4.8)</td>
<td>10.4 (4.0-17.7)</td>
</tr>
<tr>
<td><strong>Sex, female</strong></td>
<td>3 (33%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><strong>CDC-classification, N</strong></td>
<td>2</td>
<td>2</td>
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<tr>
<td><strong>A</strong></td>
<td>2</td>
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<td><strong>B</strong></td>
<td>1</td>
<td>2</td>
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<tr>
<td><strong>C</strong></td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td><strong>Children on HAART (n)</strong></td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td><strong>Time since start HAART, median weeks (IQR)</strong></td>
<td>132 (55-197)</td>
<td>14 (7-220)</td>
</tr>
<tr>
<td><strong>Plasma HIV-1 RNA, median log copies/mL (IQR)</strong></td>
<td>2.7 (2.5-3.5)</td>
<td>2.4 (1.7-4.5)</td>
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<tr>
<td><strong>Baseline</strong></td>
<td></td>
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<tr>
<td><strong>CD4+ T cells, median (IQR), absolute cells/μL</strong></td>
<td>1190 (740-1280)</td>
<td>350 (330-1090)</td>
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<tr>
<td><strong>CD4+ T cell percentage, median (IQR), %</strong></td>
<td>30 (25-39)</td>
<td>12 (9-39)</td>
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<tr>
<td><strong>Age adjusted CD4+ T cell ratio, median (IQR)</strong></td>
<td>0.55 (0.42-0.76)</td>
<td>0.31 (0.23-0.56)</td>
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<tr>
<td><strong>CD8+ T cells, median (IQR), absolute cells/μL</strong></td>
<td>860 (540-2250)</td>
<td>1750 (1090-2110)</td>
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<tr>
<td><strong>Nadir</strong></td>
<td></td>
<td></td>
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<tr>
<td><strong>CD4+ T cells, median (IQR), absolute cells/μL</strong></td>
<td>440 (210-670)</td>
<td>60 (10-410)</td>
</tr>
<tr>
<td><strong>Percentage of CD4+ T cells, median (IQR), %</strong></td>
<td>16 (8-23)</td>
<td>7 (2-15)</td>
</tr>
<tr>
<td><strong>Age-adjusted CD4+ T cell ratio, median (IQR)</strong></td>
<td>0.22 (0.10-0.41)</td>
<td>0.05 (0.01-0.22)</td>
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</table>
Figure 3. Cellular response upon VZV vaccination. In A, representative FACS analyses of CFSE of CD4+ T cells after stimulation with VZV antigen in a HIV-1-infected and a HIV-negative child, before vaccination (upper row), after the first vaccination (middle row), and after the second vaccination (lower row). In B, CD8+ T cell CFSE after stimulation with VZV antigen. Numbers indicate percentages of proliferating (CFSElow) CD4+ or CD8+ T cells after stimulation with medium (left panel) or VZV-antigen (right panel). SI: Stimulation Index. In C, T cell stimulation index upon immunization against VZV. Upper row, CD4+ T cell stimulation index. Lower row, CD8+ T cell stimulation index. Left side weeks after primary vaccination, right side after booster vaccination. Squares indicate HIV-1-infected children, open circles indicate HIV-negative siblings. The median and interquartile range are shown. No difference was found over time in response between HIV-1-infected and HIV-negative children (p=0.5).
responses, separately after 6-day stimulation. Figure 3A clearly shows the occurrence of VZV-specific CD4+ and CD8+ T cell proliferation after vaccination in an HIV-1-infected child (CD4+ SI: 4.3 and CD8+ SI: 3.0) and an HIV-negative sibling (CD4+ SI: 5.8% and CD8+ SI: 3.9%). Also after the second vaccination, VZV-specific proliferation was observed (HIV-1-infected child, CD4+ SI: 8.3 and CD8+ SI: 4.4; HIV-negative sibling: CD4+ SI: 10.3 and CD8+ SI: 1.8). All children showed a peak in SI around 4 weeks after immunization. Median peak CD4+ SI of HIV-1-infected children was 6.9 (IQR 3.0-18.1) and of HIV-negative siblings 3.3 (2.0-8.2) (p=0.2). Median peak CD8+ SI of HIV-1-infected children was 0.7 (IQR 0.4-1.8) and of HIV-negative siblings 1.0 (IQR 0.7-1.4) (p=0.5) (Figure 3B). Also after the second immunization an increase in cellular reactivity was seen. Median CD4+ SI at 6 weeks after the second vaccination was 4.2 in HIV-1-infected children and 3.9 in HIV-negative children. Median CD8+ SI reached 1.5 in HIV-1-infected children and 1.6 in HIV-negative children (Figure 3C).

No difference over time was seen in either CD4+ or CD8+ proliferation between HIV-1-infected children and HIV-negative siblings (interaction term (SI*time) p=0.5 and p=0.7, respectively).

Age at baseline was negatively correlated with the anti-VZV IgG titer at 6 weeks after the second vaccination (r=-0.7, p=0.007), but not with the peak in VZV-specific T-cell response (r=-0.15, p=0.6) or the T-cell response 6 weeks after the second vaccination (r=-0.27, p=0.4). Nadir CD4+ T cell ratio was neither correlated with the T cell response nor with the serologic response or peak of the CD4+ T cell response. We also found no correlation with any of the immunologic outcomes and baseline CD4+ T cell ratio. The T cell response at 6 weeks after the second vaccination was positively correlated with the antibody titer at the same time point (r=0.6, p=0.03). Moreover, none of the three children above 13 years of age seroconverted whereas 9 of 12 children younger than 13 years of age demonstrated seroconversion upon immunization.

Discussion

This study describes the results of VZV vaccination in a single-centre study in VZV-seronegative HIV-1-infected children. The live-attenuated VZV vaccine was well tolerated by both previously immunocompromised HIV-1-infected children treated with HAART, as well as by stable, non-progressing HIV-1-infected children. VZV-vaccination of HIV-1-infected children did not result in any adverse reaction upon vaccination. pVL did not increase for a period of up to 3 months after VZV immunization. Also no strong systemic activation of the immune system was seen in the peripheral blood in reaction to the vaccination, whereas specific T-cell responses against VZV were induced. Although safe, and despite the presence of VZV-specific T cell responses, seroconversion occurred in only 60% of the children after 2 vaccinations.

Current guidelines recommend vaccination of HIV-1-infected children with a well-maintained immune system [16]. However, most children are tested HIV-1-positive at a later stage of the disease. VZV vaccination of 15 HIV-1-infected children in our cohort
and 6 healthy siblings was undertaken under strict surveillance by weekly clinical checks for fever, vesicular lesions and other clinical symptoms that could be associated with VZV infection by the Oka-vaccine strain. No adverse reactions were seen. Out of 72 blood samples one single positive VZV PCR sample was detected at 2 weeks after the first immunization in a child at 100 copies/mL (cut-off at 80 copies/mL). All cultures for VZV from throat swabs remained negative, indicating the lack of transmissibility of the VZV-Oka strain. Therefore, VZV vaccination can be considered safe in HIV-1-infected children during HAART, despite previous immunodeficiency.

High pVL is correlated with rapid CD4+ T cell decline and disease progression [19-21]. HIV enters its target cell by binding to CD4 and one of the chemokine receptors CCR5 and CXCR4 as a co-receptor [22]. Both receptors are up-regulated during activation of the immune system as occurs during acute viral infections like HIV-1-infection but may also occur during vaccination [23]. Therefore it was hypothesized that vaccination of HIV-1-infected individuals may increase T cells susceptible for HIV-1 entry. However, we show that the number of CCR5- or CXCR4-positive cells was stable in the weeks after vaccination. In line herewith pVL did also not increase upon vaccination in any of the children, either with or without antiretroviral treatment. These data suggest that VZV vaccination has no effect on HIV-1 infection.

It has been shown that individuals carrying the CCR5Δ32 gene are more likely to be VZV seronegative, although this correlation has been debated. None of the children vaccinated in our cohort were carriers of this deletion. Therefore, a relation between the CCR5Δ32 gene and serologic response to varicella would not have confounded our results.

Long-term follow-up after vaccination of healthy children showed that a second vaccination 3 months after primary VZV vaccination gave a 3.3-fold lower risk of developing varicella during 10 years follow-up compared to a single vaccination [24]. Current US guidelines recommend vaccination of all immunocompetent healthy children under the age of 13 years with a single dose [15]. Because of lower effectiveness after a single immunization, persons older than 13 years of age are recommended to receive a second dose [15]. In our study we found that none of the three children above 13 years of age seroconverted after 2 doses versus 9 out of the 12 children younger than 13. In line with the findings of healthy children older HIV-1-infected children should be vaccinated more frequently to induce a serologic response.

Early trials of VZV vaccination of healthy immunocompetent children and adults showed high seroconversion rates (up to 100%) after primary vaccination. Serological and cellular responses persisted 3-4 years post-immunization in about 95% [6,24]. Clinical reactions were generally mild, being limited to 5-10% of recipients in the form of a mild generalized rash with fewer than 50 papules and some vesicles. VZV vaccination gave breakthrough varicella during follow-up in 9% of healthy vaccinated individuals after a mean of 3.3 years. Symptoms were generally mild though [25,26]. These results are in
line with our findings in HIV-negative siblings who showed 100% seroconversion after 2 vaccinations.

Children on chemotherapy and/or radiotherapy for malignant disease showed cellular and humoral immune responses after vaccination, however at lower level compared to otherwise healthy individuals [27]. Lymphocyte counts below 700 cells/mm$^3$ resulted in more frequent and severe side effects. This observation underlines the need for a re-established immunity before the vaccine can be administered safely. In our study immune reconstitution was established since HAART was started at months to years prior to immunization and pVL was undetectable in 12 out of 15 vaccinated children. VZV vaccination was also found to be safe after organ transplantsations; seroconversion occurred in 86% of these chronically immunosuppressed children [28]. Seroconversion rates in immunocompromised patients are lower than in healthy children. Also in HIV-1-infected children only 60% had detectable VZV-specific IgGs.

Recent reports suggest reactivation of the latent Oka-strain or re-exposure to wild-type VZV as potential mechanisms to maintain life-long immunity against VZV [8,29]. Recovery of VZV-specific immunologic memory in HIV-1-infected children may require both an increase in the number of functional CD4$^+$ T cells and the re-exposure to wild-type circulating VZV strains [30], which may both occur in HIV-1-infected children upon start of HAART. Our study provides new evidence that HIV-1-infected children remain immunodeficient, even when properly treated with HAART. First, serologic responses to vaccination were significantly less than those observed in HIV-negative siblings or previously described in the literature [6,25]. Second, a relatively high number of wild-type VZV re-infections were observed in VZV-seropositive HIV-infected children, and once re-infected, titers did not steeply rise.

The lack of any clinical symptom after vaccination and only one positive VZV PCR result in blood indicates that the immune system of children during HAART is able to mount an adequate primary cellular immune response upon vaccination. However, in the presence of comparable T-cell reactivity between HIV-1-positive and -negative children, the serologic response was much lower in HIV-1-infected individuals. While the short-term restoration of the immune function seems to largely protect persons from disseminated vaccination induced varicella infection, the functional immune restoration and immunologic fine-tuning is still incomplete, resulting in absent and low VZV-specific IgG. Recently, it was shown that perturbations in B cell responsiveness were found not only to be due to impaired CD4$^+$ T cell help, but also intrinsic to the detrimental changes in the B cell compartment itself [31]. Reduction in pVL was shown to improve B-cell responses upon various stimuli in vitro [32]. However, in our study we show that even during successful treatment with HAART, the B cell function remained disturbed. The absence of serologic memory induction may also explain the increased rate of herpes zoster found in adults during HAART [12].

In adults treated with HAART it was shown that responses upon various immunizations were dependent upon nadir CD4$^+$ T cell count and the number of circulating CD4$^+$CD28$^+$
T cells at the moment of vaccination [33]. In contrast, in our study the cellular response after two vaccinations was neither correlated with age nor with the nadir or baseline CD4+ T cell ratio. This could indicate that children show better recovery and immune reconstitution after start of HAART than adults after severe immunodeficiency.

As we demonstrate in this study, VZV vaccination of HIV-1-infected children is safe during HAART, even in children with previous CDC-C events. Effectiveness is similar to the seroconversion rate of 60% after 2 doses, as was previously reported in 41 HIV-1-infected children with N1 or A1 disease [34]. Repeated vaccination of VZV-seronegative HIV-positive children seems mandatory, since primary and secondary vaccine failures occur frequently. Additional research with more children and longer follow-up is needed to further determine the effectiveness of the vaccination against chickenpox by breakthrough wild-type infection or shingles by reactivated VZV.

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