Early Reconstitution of Immunity and Decreased Severity of Herpes Zoster in Bone Marrow Transplant Recipients Immunized with Inactivated Varicella Vaccine

Rebecca L. Redman, Sonia Nader, Leigh Zerboni, Catherine Liu, Ruby M. Wong, Byron W. Brown, and Ann M. Arvin

Varicella-zoster virus (VZV) causes herpes zoster after bone marrow transplantation (BMT). The immunogenicity of heat-inactivated varicella vaccine and effects on VZV pathogenesis were evaluated in 75 BMT patients randomized to receive vaccine or no intervention. Among 14 patients given a single dose at 1 month after transplantation, the mean (±SE) stimulation index (SI) was 12.20 ± 3.13 compared with 4.83 ± 2.74 (P = .036) in 14 unvaccinated patients, but clinical disease was not altered. Among 24 patients vaccinated at 1, 2, and 3 months, mean SI was 8.43 ± 3.89 versus 2.00 ± 0.33 (P = .014) in 23 unvaccinated patients at 4 months and 8.56 ± 2.81 versus 5.30 ± 2.47 (P = .043) at 5 months. Disease severity associated with VZV reactivation was decreased dramatically in vaccinees given three doses; severity scores were 6.4 ± 1.0 versus 11.8 ± 1.1 (P = .007). This experience with varicella vaccine in BMT patients is the first evidence that active immunization can reduce morbidity due to herpesvirus reactivation in high-risk populations.

Impaired cellular immunity to varicella zoster virus (VZV) in immunocompromised patients and otherwise healthy elderly persons correlates with an increased risk of reactivation of the virus from sites of latency in dorsal root ganglia [1–4]. Clinically, VZV reactivation causes herpes zoster. The incidence of herpes zoster in bone marrow transplant (BMT) recipients ranges from 23% to 50% during the first year, with most episodes occurring within the first 2–10 months [1, 5–11]. This high frequency of VZV reactivation in BMT recipients provided an opportunity to investigate the basic immunobiology of herpesvirus infection by assessing the impact of vaccine-induced reconstitution of virus-specific host responses. T cell recognition of VZV proteins is recovered gradually after BMT [2, 3]. Nevertheless, the reconstitution of VZV immunity is delayed for months and often does not occur until after the patient has experienced an endogenous reexposure to VZV antigens associated with clinical disease or, in some cases, with subclinical reactivation [1–3, 9]. Acyclovir therapy controls most life-threatening VZV complications in BMT recipients, but severe morbidity occurs, especially in patients with cranial nerve involvement, and postherpetic neuralgia is not prevented [12, 13].

The live attenuated varicella vaccine protects against varicella, which is caused by primary VZV infection [14, 15]. Our hypothesis was that immunization with a heat-killed preparation of the varicella vaccine could substitute for the “natural” resensitization caused by VZV reactivation after BMT and that early restoration of immunity would modify the pathogenesis of recurrent VZV disease.

Methods

Study population. Patients scheduled to undergo autologous BMT or peripheral blood stem-cell infusion, or allogeneic or matched unrelated donor BMT, at Stanford University Medical Center, who were 18–49 years old, and who had leukemia or lymphoma were eligible to participate (table 1). Other enrollment criteria included serologic evidence of VZV infection before transplantation, no history of herpes zoster, and no exposures to VZV or other immunizations during the first month after BMT. Standardized preparative regimens were given before BMT. Randomization was done through the Biostatistics Core, Stanford University Bone Marrow Transplant Program. Subjects were stratified by graft type, autologous versus allogeneic, and randomized to receive vaccine or no intervention. A time blocking factor was used to ensure balance of patients between arms of the study across strata. Participants and research physicians were blinded to the randomization process.

Vaccine preparation. Live attenuated varicella vaccine (VARIVAX, lot C-R453; Merck, Rahway, NJ) containing 2900 pfu/0.5 mL was produced and inactivated by heating at 50°C by Merck. The inactivated preparation was reduced in infectious virus content to ≤1.2 pfu/0.5 mL, with a stable viral antigen content of
4.5 U/0.5 mL; it was stored at −70°C, reconstituted with diluent (lot 1291V), and given by subcutaneous injection (0.5 mL/dose).

Study design. In the first protocol, a single dose of vaccine was given 1 month after BMT. The second protocol evaluated a three-dose regimen with vaccination at 1, 2, and 3 months after BMT. Patients were enrolled in the single-dose protocol during 1993 and in the three-dose protocol during 1994–1995. A standard form was used to record symptoms during the first 21 days after vaccination, including temperature, pain, rash, and other events. Acyclovir was administered to patients at the time of herpes simplex virus outbreak, but prophylactic therapy was not given. Inflammatory ganciclovir and intravenous immune globulin were given prophylactically to allogeneic transplant recipients for 3 months after transplantation and to all patients with human cytomegalovirus (HCMV) disease.

Patients were monitored for clinical signs of VZV reactivation for 12 months after BMT. Unusual rashes and varicella or zoster-like lesions were evaluated by the patient’s physician; herpes zoster was diagnosed by the clinical presentation of a localized, vesicular rash with a dermatomal distribution, Tzanck or direct immunofluorescence stain of cells from lesions, or viral culture. Clinical disease was assessed with parameters used to quantify the efficacy of antiviral drugs in patients with herpes zoster [12]. The severity of herpes zoster was scored numerically according to the sum of disease manifestations as follows: fever >37.8°C: no = 0, yes = 1; extent of rash: ≤25 lesions = 1, 26–100 lesions = 2, 101–250 lesions = 3, >250 lesions = 4; persistence of new lesion formation: ≤7 days = 1, 8–14 days = 2, 15–21 days = 3, >22 days = 4; time to complete crusting: ≤7 days = 1, 8–14 days = 2, 15–21 days = 3, >22 days = 4. Cutaneous dissemination was defined as ≥6 lesions outside the primary dermatome. Acute pain, as evaluated by the patient, was scored as follows: none = 0, mild = 1, moderate = 2, severe = 3. The requirement for acyclovir therapy, as prescribed by the patient’s physician, was scored as follows: oral therapy only = 1, oral and intravenous therapy = 2. Postherpetic neuralgia was defined as pain persisting after resolution of cutaneous disease.

Assays for VZV immunity. Participants in the single-dose study were tested for T cell immunity to VZV antigen and for VZV IgG antibodies at 1, 2, 3, and 12 months after BMT; those in the three-dose study were also evaluated at 4 and 5 months. Patients receiving vaccine provided blood samples prior to immunization.

T cell proliferation and VZV IgG antibodies were measured during episodes of herpes zoster and 1–2 months later when possible. T cell recognition of VZV antigen was determined by incubating peripheral blood mononuclear cells (PBMC) (3 × 10⁶ cells/well) with dilutions of VZV-infected cell extract (1:16–1:256), uninfected cell control, or phytohemagglutinin, in RPMI medium with 10% human serum for 5–6 days [16]. Proliferation was detected by tritiated thymidine uptake. The stimulation index (SI) was calculated as the ratio of mean counts per minute in antigen-stimulated wells to mean counts in control wells. The phenotypes of T cells from PBMC cultures stimulated with whole VZV antigen (1:64 dilution) were assessed by cytofluorometry after staining with CD4, CD8, and CD16 monoclonal antibodies (Becton Dickinson, San Jose, CA).

Production of interferon-γ (IFN-γ), interleukin-4 (IL-4), and IL-10 was assessed after stimulation with VZV antigen (1:64 dilution) for 5–6 days when sufficient numbers of PBMC were obtained. Supernatant samples were tested in duplicate for each cytokine. IFN-γ and IL-10 were detected by EIA (Endogen, Cambridge, MA), and IL-4 was assessed by use of an ultrasensitive assay (Cytoscreen; Biosource, Camarillo, CA).

### Table 1. Demographic characteristics of BMT patients participating in evaluation of inactivated varicella vaccine.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Single-dose regimen</th>
<th>3-dose regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vaccinated</td>
<td>Unvaccinated</td>
</tr>
<tr>
<td>No. of patients enrolled</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>No. male</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>No. female</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>No. of patients completing study*</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Mean age (range), years</td>
<td>38 (18–49)</td>
<td>38 (18–49)</td>
</tr>
<tr>
<td>Transplant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autologous BMT/PBSC</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Allogeneic BMT</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Matched, unrelated BMT</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>No. surviving &gt;12 months</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>No. (%) with herpes zoster</td>
<td>5 (38)</td>
<td>5 (36)</td>
</tr>
</tbody>
</table>

* Followed until death or 1 year after transplantation.

NOTE. ALL, acute lymphoblastic leukemia; ANLL, acute nonlymphoblastic leukemia; CML, chronic myelogenous leukemia; HD, Hodgkin’s disease; NHL, non-Hodgkin’s lymphoma. PBSC, peripheral blood stem cell infusion.
Results

Immunogenicity of the single-dose regimen. Patients who were immunized with a single dose of inactivated varicella vaccine given at 1 month after transplantation achieved earlier recovery of cell-mediated immunity to VZV than did unvaccinated patients. The mean (±SE) SI at 3 months after BMT was 12.20 ± 3.13 among vaccinated patients compared with 4.83 ± 2.74 (P = .036) among unvaccinated patients (figure 1).

Among the vaccinated patients, the mean SI at 3 months after BMT was higher for those patients who subsequently had no episodes of herpes zoster during the year following transplantation than for those patients who experienced symptomatic VZV reactivation; the mean SIs were 18.60 ± 3.39 and 5.80 ± 2.60 (P = .065), respectively. In contrast, the mean SIs at 3 months after BMT among unvaccinated patients were equivalent in those who did not develop herpes zoster and in those who did; the mean SIs were 5.63 ± 3.52 and 2.05 ± 0.45 (P = .50), respectively.

Vaccine recipients who developed herpes zoster had evidence of immunologic priming of cell-mediated immunity. The mean T cell proliferation to VZV antigen measured after resolution of acute herpes zoster was 48.2 ± 19.89 in vaccinees compared with 13.5 ± 5.8 (P = .05) in unvaccinated patients. Among the vaccinated patients, the mean SI 1 year after transplantation was 80.86 ± 41.72 in vaccinees (n = 5) who had experienced herpes zoster compared with 7.63 ± 2.14 (P = .021) in vaccinated patients (n = 3) who had not. The mean SI at 1 year was also higher in unvaccinated patients who developed herpes zoster (n = 3) than in those patients who did not (n = 6), but the difference was not significant; the mean SIs were 17.10 ± 5.83 and 5.83 ± 1.66 (P = .07), respectively. At 1 year after BMT, both vaccinated and unvaccinated patients who had not experienced clinical episodes of VZV reactivation showed similar T cell responses to VZV; the mean SI was 7.63 ± 2.14 in vaccinees and 5.83 ± 1.66 (P = .42) in unvaccinated patients.

IgG antibodies to VZV ranged from 1:256 to 1:16,384 and were not significantly different between vaccinated and unvaccinated patients, and sequential titers did not vary >4-fold in individual patients. VZV IgG titers were not predictive of the risk of VZV reactivation. Allogeneic and matched unrelated donor marrow recipients who received intravenous immune globulin had VZV IgG titers equivalent to those of autologous BMT patients.

Clinical efficacy of the single-dose regimen. The incidence of herpes zoster was 38% (5/13) in patients given a single dose of inactivated varicella vaccine and 36% (5/14) in unvaccinated patients. There was no difference in severity of disease caused by VZV reactivation between the vaccinated and unvaccinated patients. The mean severity scores were 11.0 ± 1.8 and 8.4 ± 0.6, respectively (P = .17).

The 5 vaccinated patients who developed herpes zoster during the first year had VZV reactivation 4–7 months after transplantation. The incidence of herpes zoster was 56% among the 9 vaccinated patients who survived for at least 12 months; 4 of 8 autologous transplant patients and the 1 surviving allogeneic transplant patient developed herpes zoster. Four of the 14 unvaccinated patients developed herpes zoster within 2–12 months, and 1 patient had reactivation at 18 months. Of the 11 unvaccinated patients who survived for at least 1 year, 2 of 7 autologous transplant patients and 1 of 4 allogeneic BMT recipients developed herpes zoster during the first year. Among the 16 study participants who survived for 2 years, 1 unvaccinated patient developed herpes zoster between 12 and 24 months.
that BMT recipients could respond to inactivated varicella vaccinees and 77% of unvaccinated patients. A linear correlation significantly higher beginning at 3 months after transplantation unrelated donor marrow survived for 1 year and did not develop vaccinated and unvaccinated groups at 1, 2, 3, 4, 5, and 12 months; IL-10 production to VZV antigen was detected consistently in PBMC cultures from vaccinated and unvaccinated patients at mean concentrations of \( >100 \text{ pg/mL} \) during the first 12 months; IL-10 concentrations did not change significantly in relation to the interval after transplantation. While mean concentrations did not differ among patient groups, production of IFN-\( \gamma \) and IL-10 was significantly higher among patients who had recovered T cell proliferation to VZV antigen. The mean IFN-\( \gamma \) concentration was 158 pg/mL \( \pm 32.83 \) in those with SIs \( \geq 2.0 \) compared with 69 pg/mL \( \pm 24.80 \) \((P = .03)\) in patients with no detectable T cell proliferation to VZV antigen, and the mean IL-10 concentration was 175 pg/mL \( \pm 20.42 \) in patients who had recovered T cell responses compared with 117 pg/mL \( \pm 17.70 \) \((P = .04)\) in those who had not.

After short-term stimulation of PBMC from BMT patients with VZV antigen, the mean percentages of CD4 T cells ranged from 15% to 42%, the percentage of CD8 T cells was 14%–30%, and the percentage of CD16 T cells ranged from 5% to 9%. There were no significant differences in ratios of CD4 to CD8 T cells in cultures of PBMC done at 1, 3, 4, 5, and 12 months after BMT.

At 1 year after transplantation, the mean SIs to VZV antigen were 15.15 \( \pm 4.62 \) in vaccinees and 9.38 \( \pm 4.62 \) in unvaccinated patients \((P = .389)\) (figure 2). Recovery of T cell recognition of VZV antigen (SI \( \geq 2.0 \)) was documented in 83% of vaccinees and 77% of unvaccinated patients. A linear correlation between T cell proliferation to VZV antigen and IFN-\( \gamma \) production was observed at 1 year after BMT \((P < .001; \text{correlation coefficient} = .81)\).

No correlations were observed between VZV IgG titers and the incidence of herpes zoster among participants in the three-dose study. VZV IgG antibody titers were equivalent in vaccinated and unvaccinated patients \((\text{range}, 1.64–1.262,144)\).

Clinical efficacy of the three-dose regimen. The incidence of herpes zoster during the first year after transplantation was 23% \((5/22)\) in vaccinated patients and 22% \((5/23)\) in unvaccinated participants, but the clinical disease among vaccinated patients was markedly less severe \((\text{table 3})\). The mean severity score was 6.4 \( \pm 1.0 \) in vaccinees compared with 11.8 \( \pm 1.1 \) in patients who were not immunized \((P = .007)\).

The interval to disease occurrence was 4–8 months among vaccinated patients. The incidence of herpes zoster was 29% among 17 vaccinees who survived for \( \geq 12 \) months. Eleven of 12 autologous transplant patients survived for at least 1 year; 1 patient developed herpes zoster. Five of 8 vaccinees who received related donor allogeneic marrow survived for 1 year; 4 patients had herpes zoster. One of 2 recipients of matched, unrelated donor marrow survived for 1 year and did not develop herpes zoster.

**Figure 2.** T cell proliferation to VZV antigen in BMT recipients participating in study of 3-dose regimen of inactivated varicella vaccine. ■. Mean stimulation index (SI) \( \pm \text{SE} \) in vaccine recipients tested immediately before immunization at month after transplantation and at 2, 3, 4, 5, and 12 months; ○, responses of unvaccinated patients at same time intervals after transplantation. ▲, Vaccinations. * Statistically significant \((P < .05)\) difference in mean SI between vaccinated and unvaccinated patients.

Assessment of T cell proliferation to VZV antigen showed that BMT recipients could respond to inactivated varicella vaccine given 1 month after transplantation, but the failure of a single dose of vaccine to alter clinical disease provided the rationale for evaluating a multidose regimen.

**Immunogenicity of the three-dose regimen.** Administration of vaccine at 1, 2, and 3 months after transplantation elicited early recovery of T cell responses to VZV (figure 2). The mean SIs to VZV antigen were higher in vaccinated patients by 3 months after BMT, and the differences in mean SIs between vaccinated and unvaccinated patients were significant by 4 and 5 months after transplantation. The mean SI was 8.43 \( \pm 3.89 \) in vaccinees compared with 2.00 \( \pm 0.33 \) \((P = .014)\) in unvaccinated patients at 4 months and 8.56 \( \pm 2.81 \) in vaccinees compared with 5.30 \( \pm 2.47 \) \((P = .043)\) in unvaccinated patients at 5 months after BMT. The mean increase in T cell proliferation at 4 and 5 months after transplantation relative to the baseline response of each patient measured at 1 month was significantly greater among vaccinees \((P = .01 \text{ at 4 months}; P = .02 \text{ at 5 months})\).

Mean IFN-\( \gamma \) concentrations were comparable between the vaccinated and unvaccinated groups at 1, 2, 3, 4, 5, and 12 months after transplantation (table 2). IFN-\( \gamma \) production was significantly higher beginning at 3 months after transplantation in PBMC from both vaccinated and unvaccinated patients compared with concentrations at 1 month. IL-4 production by PBMC stimulated with VZV antigen was not detected in PBMC from vaccinated or unvaccinated patients, which is consistent with the absence of IL-4 responses to VZV antigen in otherwise healthy persons (Redman R, Arvin A, unpublished data).
Table 2. Cytokine production after in vitro stimulation of peripheral blood cells with VZV antigen among BMT recipients given 3-dose regimen of inactivated varicella vaccine or no vaccine.

<table>
<thead>
<tr>
<th>Regimen, cytokine</th>
<th>Interval after bone marrow transplantation (months)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine</td>
<td>Interferon-γ</td>
<td>30 ± 9</td>
<td>54 ± 27</td>
<td>149 ± 84</td>
<td>142 ± 81</td>
<td>121 ± 60</td>
<td>166 ± 71</td>
</tr>
<tr>
<td></td>
<td>Interleukin-10</td>
<td>116 ± 28</td>
<td>125 ± 35</td>
<td>111 ± 45</td>
<td>138 ± 50</td>
<td>121 ± 53</td>
<td>163 ± 65</td>
</tr>
<tr>
<td></td>
<td>No. tested</td>
<td>14</td>
<td>12</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>No vaccine</td>
<td>Interferon-γ</td>
<td>22 ± 6</td>
<td>161 ± 142</td>
<td>141 ± 62</td>
<td>68 ± 19</td>
<td>231 ± 150</td>
<td>314 ± 185</td>
</tr>
<tr>
<td></td>
<td>Interleukin-10</td>
<td>149 ± 37</td>
<td>164 ± 49</td>
<td>150 ± 54</td>
<td>194 ± 50</td>
<td>147 ± 72</td>
<td>216 ± 73</td>
</tr>
<tr>
<td></td>
<td>No. tested</td>
<td>15</td>
<td>8</td>
<td>8</td>
<td>13</td>
<td>8</td>
<td>6</td>
</tr>
</tbody>
</table>

NOTE. Cytokine data are pg/mL (mean ± SE).

The interval to the occurrence of herpes zoster in unvaccinated BMT patients was 2–12 months. The incidence of herpes zoster was 31% in 16 unvaccinated patients who survived for at least 12 months. Two of 8 autologous transplant patients who survived for at least 1 year and 3 of 8 survivors given allogeneic bone marrow from a related donor had herpes zoster. The 1 recipient of matched, unrelated donor marrow died 6 months after BMT without developing herpes zoster.

As shown in table 3, the clinical manifestations of herpes zoster were much less severe in vaccinated patients. The number of cutaneous lesions ranged from 3 to 24 in vaccine recipients, and only 1 patient had fever, which lasted <1 day. Among unvaccinated patients, the number of lesions ranged from 100 to >500, and 3 had fevers for 1, 3, and >7 days, respectively. Among vaccinees, the intensity of acute pain ranged from none to moderate and none of the vaccinated patients developed postherpetic neuralgia, whereas the intensity of acute pain was mild to moderate or severe in unvaccinated patients and 4 had postherpetic neuralgia. One vaccinated patient with a history of gentamicin toxicity and a cavernous sinus mass had Ramsay Hunt syndrome associated with 3 vesicular lesions in the ear that were positive for VZV by direct immunofluorescence. Pathologic evaluation of the mass was inconclusive, and clinical signs improved after surgical severance of the vestibular nerve, but resolution of seventh nerve palsy was incomplete. No other vaccinee required hospitalization, while 3 of 5 unvaccinated patients were admitted for intravenous acyclovir therapy to control VZV reactivation.

Safety and tolerability of inactivated varicella vaccine. No serious adverse effects were observed with the administration

Table 3. Severity of disease caused by reactivation of VZV in BMT recipients given 3-dose regimen of inactivated varicella vaccine or no vaccine.

<table>
<thead>
<tr>
<th>Regimen, patient, BMT</th>
<th>Fever</th>
<th>Location of rash</th>
<th>Total lesions</th>
<th>New lesions</th>
<th>Complete crusting</th>
<th>Acute pain</th>
<th>Postherpetic neuralgia</th>
<th>Acyclovir therapy</th>
<th>Severity score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1, allogeneic</td>
<td>No</td>
<td>Generalized</td>
<td>19</td>
<td>3 weeks</td>
<td>4 weeks</td>
<td>Mild</td>
<td>No</td>
<td>Oral</td>
<td>10</td>
</tr>
<tr>
<td>2, autologous</td>
<td>1 day</td>
<td>L1</td>
<td>24</td>
<td>1 day</td>
<td>1 day</td>
<td>Moderate: 4 days</td>
<td>No</td>
<td>Oral</td>
<td>7</td>
</tr>
<tr>
<td>3, allogeneic</td>
<td>No</td>
<td>T10</td>
<td>≤10</td>
<td>3 days</td>
<td>4 days</td>
<td>Mild: 4 days</td>
<td>No</td>
<td>Oral</td>
<td>5</td>
</tr>
<tr>
<td>4, allogeneic</td>
<td>No</td>
<td>C7</td>
<td>3</td>
<td>2 days</td>
<td>4 days</td>
<td>Mild: 7 days</td>
<td>No</td>
<td>Oral and iv</td>
<td>6</td>
</tr>
<tr>
<td>5, allogeneic</td>
<td>No</td>
<td>C4</td>
<td>10</td>
<td>3 days</td>
<td>3 days</td>
<td>None</td>
<td>No</td>
<td>Oral</td>
<td>4</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.4 ± 1.0</td>
</tr>
<tr>
<td>No vaccine</td>
<td>1, allogeneic</td>
<td>1 day</td>
<td>T12/L1</td>
<td>~300</td>
<td>3 days</td>
<td>8 days</td>
<td>Severe: 1 week; moderate: 1–2 weeks</td>
<td>No</td>
<td>Oral</td>
</tr>
<tr>
<td>2, allogeneic</td>
<td>3 days</td>
<td>T7</td>
<td>~300</td>
<td>9 days</td>
<td>20 days</td>
<td>Severe: 3 weeks</td>
<td>Yes</td>
<td>Oral and iv</td>
<td>15</td>
</tr>
<tr>
<td>3, autologous</td>
<td>No</td>
<td>Trigeminal</td>
<td>~100</td>
<td>5 days</td>
<td>11 days</td>
<td>Moderate: 2 weeks</td>
<td>Yes</td>
<td>Oral and iv</td>
<td>9</td>
</tr>
<tr>
<td>4, allogeneic</td>
<td>No</td>
<td>L1</td>
<td>~500</td>
<td>4 days</td>
<td>7 days</td>
<td>Severe: 2 weeks</td>
<td>Yes</td>
<td>Oral</td>
<td>10</td>
</tr>
<tr>
<td>5, autologous</td>
<td>&gt;7 days</td>
<td>T5</td>
<td>&gt;500</td>
<td>5 days</td>
<td>11 days</td>
<td>Severe: 5 days; mild: 1 week</td>
<td>Yes</td>
<td>Oral and iv</td>
<td>13</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11.8 ± 1.1</td>
</tr>
</tbody>
</table>

NOTE. iv, intravenous.
of 76 doses of inactivated varicella vaccine to 36 BMT patients. Of the 14 patients who received vaccine in the single-dose study, 1 patient was dropped from the study before vaccination because of complications of BMT, 1 patient had a bruise at the injection site, and 1 patient experienced 1 day of pain at the injection site. Of the 24 patients randomized to receive vaccine in the three-dose study, 1 patient was dropped before vaccination for fever of unknown origin, and 1 patient withdrew after receiving two doses of vaccine because of relapse of malignancy. None of 3 patients who died of BMT complications before completing the regimen had adverse reactions to the vaccine. Among patients completing the three-dose series, 1 experienced headache after the first vaccination and 1 had local erythema and swelling after the third injection. Three patients had pain at the injection site: 1 patient experienced concurrent injection site pain and headache after the first vaccination and injection site pain only after the second vaccination; 1 patient experienced injection site pain, muscle ache, and joint pain after the first and third vaccinations; and 1 patient experienced muscle ache and joint pain after the first vaccination and the same symptoms with injection site pain after the third dose.

Discussion

This study is the first demonstration that host responses to a human herpesvirus can be reconstituted by active immunization of persons who have a diminished capacity to preserve latency and that vaccine-induced immunity confers protection from progressive disease due to virus reactivation. The pathogenesis of VZV reactivation from latency in dorsal root ganglia requires induction of viral genes that allow the virus to enter a replicative cycle. When reactivation occurs, immunologic surveillance is predicted to determine whether infection progresses locally and whether symptomatic disease results [17, 18]. Long-term memory immunity to VZV is characterized by helper CD4 T cell responses and by cytotoxicity against virus-infected cells mediated by CD4 and CD8 T cells (reviewed in [19]). In a recent study, Walter et al. [20] documented the importance of cellular immunity for restricting the pathogenicity of HCMV in BMT patients by using adoptive immunotherapy with donor-derived, cloned CD8 T cells specific for HCMV. Our evaluation of inactivated varicella vaccine in BMT patients demonstrates that reconstitution of cellular immunity to VZV can be elicited by active immunization in the immediate posttransplant period. The impact of immune enhancement observed after active immunization of BMT recipients upon recurrent disease establishes the critical role of virus-specific T cell responses in preserving the balance between VZV and the host.

In BMT patients, IFN-γ production was associated with the recovery of T cell proliferation to VZV antigen and increased with time following transplantation. IFN-γ production by T cells stimulated with VZV has been observed in healthy sub-

jects with natural immunity, children receiving primary immunization with varicella vaccine, and naturally immune, elderly adults given varicella vaccine [21–23]. The frequency of T cells that released IFN-γ paralleled the frequency of T cells that proliferated in response to VZV antigen [23]. Human CD4 T cells of the type 1 subgroup (Th1) produce IL-2 and IFN-γ, while CD4 T cells of the type 2 subgroup (Th2) release IL-4, -5, -6, -10, and -13 [24]. Whereas IL-4 production was not detected, IL-10 release by PBMC was observed consistently after BMT and was highest in patients who recovered T cell recognition of VZV antigen, suggesting an antigen-specific enhancement of its production. The reconstitution of the Th1 response to VZV in BMT patients is likely to be particularly important, because local IFN production has been correlated with more rapid resolution of cutaneous lesions and IFN-γ induces clonal expansion of cytotoxic T cells [19]. As observed by Walter et al. [20], cytotoxic T cell function was not sustained well after adoptive transfer of CD8 T cells in BMT patients who lacked CD4 T cells specific for HCMV. In contrast to cellular immunity, VZV IgG antibody titers were not a surrogate marker for risk or severity of herpes zoster in BMT recipients. This observation is consistent with previous evidence that humoral immunity does not correlate with VZV reactivation in immunocompromised patients [25].

Immunization of BMT patients against herpesviruses represents a particular challenge, because disease is usually due to the reactivation of endogenous viruses rather than to new exposures, and most recurrent disease occurs during the first several months after transplantation. Our evaluation of the single-dose regimen of varicella vaccine showed that short-term immunologic enhancement did not result in clinical benefit. In contrast, the three-dose regimen boosted cell-mediated immunity and modified the clinical course of herpes zoster. Optimal reconstitution of immunity to other pathogens, including tetanus, pneumococci, Haemophilus influenzae type b, and polioviruses, has been achieved with multidose regimens after BMT [26–36]. Sustaining the host response by repeated doses is likely to be necessary to preserve clinical efficacy against herpesvirus reactivation while immunosuppressive therapy continues.

Our assessment of the inactivated varicella vaccine showed that a noninfectious, whole virus vaccine induced a functional enhancement of antiviral immunity in immunosuppressed patients. This observation supports the concept that subunit vaccines against other herpesviruses, such as the glycoprotein vaccines that are being developed for herpes simplex virus and HCMV, may modify the risk of recurrent disease in BMT patients or other immunocompromised populations [37, 38]. Recovery of VZV immunity in BMT recipients may be improved further by immunizing donors and recipients before allogeneic transplantation, by vaccinating autologous transplant recipients before and after BMT, or by initiating the vaccine regimen at a shorter interval after transplantation. Vaccination of donors with tetanus and diphtheria toxoids or with H. in-
fluenzae type b conjugate vaccines enhanced the responses of BMT patients to these antigens when combined with immunization of the BMT recipient after transplantation [26, 32]. The administration of tetanus toxoid on the day of transplantation was associated with the detection of T cell proliferation to tetanus toxoid when vaccinated patients emerged from aplasia at an interval of 3 or 4 weeks after BMT, whereas unimmunized patients had no T cell recognition of tetanus antigen [32]. The enhanced T cell immunity in BMT patients who had herpes zoster indicates that these patients have the capacity to respond to a more potent antigenic stimulus. Vaccine strategies that may improve the efficacy of VZV and other herpesvirus vaccines in high-risk patients are the use of newer adjuvants, the incorporation of cytokines that improve Th1 responses, such as IL-12, and the use of novel antigen delivery systems, including sensitized dendritic cells [39, 40].

Despite their immunodeficiencies, BMT patients who were immunized with the three-dose regimen of inactivated varicella vaccine had minimal cutaneous disease and no persistent pain, providing the first evidence that active immunization can modify VZV reactivation as well as primary disease. Susceptibility to herpes zoster and waning cellular immunity to VZV are also associated with advancing age [41]. A prolonged reversal of the age-related decline in memory T cell immunity was demonstrated in adults immunized with live attenuated varicella vaccine, and the heat-inactivated varicella vaccine was also immunogenic in healthy immune adults [21, 41–43]. Our experience in BMT recipients indicates that this enhanced immunity elicited by varicella vaccine in the elderly will limit the pathologic consequences of VZV reactivation and that immunotherapy using vaccines against other herpesviruses is likely to reduce the morbidity caused by these common pathogens in high-risk patients.

Acknowledgments

We acknowledge the assistance of Karl Blume, the staff of the Bone Marrow Transplant Program, Stanford University Medical Center, Fidencio Saldana, and Darlene Jenkins. We thank the patients and their families for their dedicated participation in the study.

References


