Varicella Zoster Virus–Specific Immune Response after Treatment with Sodium Stibogluconate for Cutaneous Leishmaniasis

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Abstract. Sodium stibogluconate has been associated with the reactivation of varicella zoster virus (VZV) in otherwise healthy adults who receive the drug as treatment for cutaneous leishmaniasis. Ten patients receiving daily sodium stibogluconate underwent phlebotomy at baseline and at day 10. Flow cytometry–based immunophenotyping, VZV-specific IgG levels, and lymphocyte proliferative responses and intracellular cytokine secretion to VZV, cytomegalovirus, tetanus toxoid, superantigen, and mitogens were performed at both time points. The absolute number of total leukocytes, total lymphocytes, and lymphocyte subsets decreased overall without predilection for any particular subset of lymphocytes, such that the percentage of the total lymphocyte population for each lymphocyte subset did not change significantly (except for a marginal increase in percentage of cytotoxic T cells). Antibodies to VZV were measured in seven patients before and after treatment, and did not change. Lymphocyte proliferative responses to VZV and other antigens and mitogens did not change from baseline. The mechanism for the increased rate of VZV reactivation after treatment with sodium stibogluconate remains undefined.

INTRODUCTION

Leishmaniasis is a parasitic disease transmitted by the bite of a phlebotomine sandfly, with manifestations of infection ranging from asymptomatic disease to self-healing skin sores to progressive visceral infection and death, depending on the strain of the infecting organism and the immune status of the host. For decades, the standard therapy for most forms of leishmaniasis has been sodium stibogluconate, which is available in the United States as Pentostam® (GlaxoSmithKline, London, United Kingdom).

Sodium stibogluconate, although efficacious, has been associated with numerous toxicities including pancreatitis, hepatitis, myalgias/arthritis and electrocardiographic changes. Varicella zoster virus (VZV) infection can occur in patients treated with sodium stibogluconate and various other antimonial compounds. In a review of patients treated with sodium stibogluconate at one center, incidence of VZV reactivation (shingles) was 3.6%, which was greater than the reported incidence of 0.2% in healthy persons 20–30 years of age. More recently, a patient with concomitant shingles and VZV meningitis after sodium stibogluconate treatment has been reported.

The mechanism for the reactivation of VZV is not completely understood. Presumably, latent VZV can reactivate and produce symptoms when VZV-specific cell-mediated immunity decreases below a critical threshold level. For example, the decrease in VZV-specific cell-mediated immunity that occurs with age is associated with an increased frequency of VZV infection. In a prospective study of eight patients with cutaneous leishmaniasis receiving sodium stibogluconate, a decrease in helper T cells of 306 cells/mm³ or 67% of baseline was reported by day seven of treatment. The investigators surmised that the decrease in T cells occurring with treatment with sodium stibogluconate may have predisposed to VZV reactivation. The current study seeks to expand the findings of this report by examining the antibody- and cell-mediated immune response to VZV that occurs during treatment with sodium stibogluconate.

METHODS

The study was performed under an approved human use protocol, and all study volunteers provided written informed consent. Patients with a confirmed diagnosis of cutaneous leishmaniasis who were scheduled to receive sodium stibogluconate for treatment of leishmaniasis were offered participation in the study. Phlebotomy was performed at baseline and then after 10 daily doses (± one dose) of sodium stibogluconate (20 mg/kg/day, with no upper limit on dose). A total of 32 mL of blood was collected at each time point in serum-separator tubes, heparin tubes, and cell-preparation tubes. Peripheral blood mononuclear cells (PBMCs) were separated from heparinized blood by Ficoll-hypaque gradient centrifugation and cryopreserved.

Quantitative levels of IgG to VZV were measured by enzyme-linked immunosorbent assay according to the manufacturer's instructions (Wampole Laboratories, Princeton, NJ). Phenotypes of T cell subsets and natural killer (NK) cells were determined by flow cytometry with fluorochrome-labeled monoclonal antibodies against the cell surface markers CD3, CD4, CD8, CD45RO, CD25, CD16, and CD56 (all from BD Bioscience, San Jose, CA). Briefly, 100 μL of whole blood was stained with appropriate antibodies at room temperature for 30 minutes. After incubation, erythrocytes were lysed with a fluorescent-activated cell sorter (FACS) lysis solution (BD Bioscience) and T cell subsets and NK cells were analyzed using a FACSCalibur flow cytometer and CellQuest Analysis Software (BD Bioscience).

A lymphocyte proliferation assay (LPA) was used for prestibogluconate and day 10 samples by incubating 1 × 10⁷ PBMCs with VZV lysate at dilutions of 1:100 and 1:200 or control cell lysate prepared in the same way as the VZV lysate at a dilution of 1:100 (University of Colorado Hospital Clinic, Denver, CO). In addition, PBMCs were incubated...
with cytomegalovirus (CMV) lysate at concentrations of 5 and 2.5 µg/mL (Advanced Biotechnologies Inc., Columbia, MD), tetanus toxoid at concentrations of 5 and 2.5 µg/mL (Staten Serum Institute, Copenhagen, Denmark), and the mitogens (Sigma-Aldrich, St. Louis, MO) phytohemagglutinin (PHA, 2 µg/mL), concanavalin A (ConA, 20 µg/mL), and pokeweed mitogen (PWM, 1.25 µg/mL). After three days of incubation with the mitogen and six days with the tested antigens, cells were pulsed with 1 µCi/well of ³H-thymidine for six hours, harvested using the Tomtec, Mach3M (EG&G Wallac, Gaithersburg, MD), and counted in a 1450 microbeta trilux (EG&E Wallac). The data were expressed as a lymphocyte stimulation index (LSI) = PBMC counts per minute (cpm) + antigen/mitogen/PBMC cpm + medium to define an-cellular cytokine secretion (ICS) assay as described previ-

LZ11 Pre-treatment

LZ10 Pre-treatment

LZ07 Pre-treatment

LZ06 Pre-treatment

LZ05 Pre-treatment

LZ04 Pre-treatment

LZ03 Pre-treatment

LZ02 Pre-treatment

LZ01 Pre-treatment

**RESULTS**

A total of 11 adult patients with a diagnosis of cutaneous leishmaniasis requiring treatment with sodium stibogluconate were initially enrolled in the study. One patient was withdrawn from the study for reasons unrelated to the study, leaving 10 subjects for whom data was available. The mean dose of sodium stibogluconate administered to each patient was 1,865 mg (range = 1,336–1,955 mg). No patient developed reactivation of VZV during the study or during six months of follow-up. Compared with baseline, at day 10 the absolute number of total leukocytes decreased by a median of 2,400/mL (P = 0.004), total lymphocytes by 800/mL (P = 0.002), helper T cells by 265/mL (P = 0.002), cytotoxic T cells by 159/mL (P = 0.002), memory T cells by 321/mL (P = 0.002), CD4/CD25 + regulatory T cells by 29/mL (P = 0.006), and NK cells by 54/mL (P = 0.004). The percentage of the total lymphocyte population for each lymphocyte subset did not change significantly except for a marginal increase in percentage of cytotoxic T cells by 0.77% (P = 0.049). Therefore, lymphocyte subset numbers decreased overall without predi-

**TABLE 1**

Vaccinia virus (VZV) T cell responses pre-treatment and post-treatment with sodium stibogluconate in *Leishmania*-infected persons

<table>
<thead>
<tr>
<th>Subject</th>
<th>Time</th>
<th>VZV 1,000</th>
<th>VZV 1,200</th>
<th>Control lysate</th>
<th>% CD4 interferon-γ/interleukin-2-positive T cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>LZ01</td>
<td>Pre-treatment</td>
<td>34</td>
<td>12</td>
<td>1</td>
<td>0.08</td>
</tr>
<tr>
<td>LZ02</td>
<td>Pre-treatment</td>
<td>144</td>
<td>113</td>
<td>1</td>
<td>0.07</td>
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<tr>
<td>LZ03</td>
<td>Pre-treatment</td>
<td>4</td>
<td>6</td>
<td>1</td>
<td>0.04</td>
</tr>
<tr>
<td>LZ04</td>
<td>Pre-treatment</td>
<td>7</td>
<td>5</td>
<td>1</td>
<td>0.02</td>
</tr>
<tr>
<td>LZ05</td>
<td>Pre-treatment</td>
<td>18</td>
<td>10</td>
<td>1</td>
<td>0.04</td>
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<td>LZ06</td>
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<td>12</td>
<td>20</td>
<td>1</td>
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</tr>
<tr>
<td>LZ07</td>
<td>Pre-treatment</td>
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<td>4</td>
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<td>0.03</td>
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<td>5</td>
<td>1</td>
<td>0.51</td>
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<tr>
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<td>6</td>
<td>7</td>
<td>1</td>
<td>0.08</td>
</tr>
<tr>
<td>LZ10</td>
<td>Pre-treatment</td>
<td>8</td>
<td>6</td>
<td>1</td>
<td>0.04</td>
</tr>
<tr>
<td>LZ11</td>
<td>Pre-treatment</td>
<td>40</td>
<td>25</td>
<td>1</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* Positive responses (LSI > 5) are in **bold**. ND = not done.

† Results are shown with background (control lysate) subtracted. Positive cytokine responses (see text) are in **bold**.
The PBMCs from two persons secreted IFN-γ/IL-2 after stimulation with CMV lyse, but the net % IFN-γ/IL-2 positive CD4+ T cells did not change before and after treatment. None of the persons had detectable CD8+ T cell responses to VZV. Responses to the control antigen (SEB) were robust in all persons and ranged from 1% to 8% IFN-γ/IL-2–positive T cells. The average CD4+ and CD8+ SEB response was 3 ± 1.1% and 3.1 ± 1.8% respectively.

For the thawed PBMCs used in the LPA, the mean ± SD viability of the PBMCs was 82% ± 6%. Eight persons had LPA responses to VZV with an LSI up to 44; six of these persons had responses to VZV detected before and after treatment with sodium stibogluconate, and two persons had a positive LPA response to VZV before treatment but not after treatment (Table 1). All persons had robust responses to the three mitogens (PHA, PWM, and ConA). In five of the eight persons, pre-treatment and post-treatment mitogen responses were assessed, and the average LSI was 171 pre-treatment and 166 post-treatment. The LSI pre-treatment and post-treatment were highly correlated, and there was no significant difference between the values (P = 0.79, by paired t test). In three of eight patients, the pre-treatment LSI to the three mitogens was robust, but there were insufficient PBMC available after treatment for testing. In parallel PBMCs from two normal donors were assessed, and the LSI to VZV was < 3. None of the controls or patient samples responded to the control viral lysate preparation; all LSI were < 1. Responses to tetanus and CMV antigen were seen in five of eight persons, and the responses were similar pre-treatment and post-treatment.

**DISCUSSION**

Despite several decades of use, the exact mechanism of action of sodium stibogluconate is unknown, as are the reasons for its side effects. The reactivation of VZV during or shortly after the administration of sodium stibogluconate is well-described, and is presumably caused by suppression of cellular immunity. The current study reports a decrease in CD4+ T cells of 265/mL at day 10 of therapy, which is similar to what is seen in other studies. The decrease of 306/mL reported in a previous study was not observed in this study.

The LPA and ICS analysis of cellular immune responsive nature for the VZV-specific T cells was performed. The LPA response to VZV was positive in one person before treatment but not after treatment. Potentially, VZV-specific T cell numbers may take longer to recover than other antigen-specific T cells or total lymphocytes, leading to increased VZV risk. Alternatively, VZV may be more likely to take advantage of specific cellular immune suppression than other potential infectious agents, resulting in the cases of VZV reactivation seen after sodium stibogluconate treatment.

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**References**

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