Primary Induction of Human CD8+ Cytotoxic T Lymphocytes and Interferon-γ–Producing T Cells after Smallpox Vaccination

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CONCISE COMMUNICATION

This study measured the ability of a standard smallpox vaccine, given by scarification (by bifurcated needle), to induce primary human vaccinia virus–specific cytotoxic and interferon (IFN)–γ–producing T lymphocyte responses. Because protection against smallpox may be mediated in part by T cell memory responses induced by vaccination, an analysis of the induction of primary human cytotoxic T lymphocytes (CTL) and IFN-γ–producing T cell responses was performed. Although smallpox is no longer an epidemic threat under natural conditions, vaccination is still recommended for persons working with vaccinia viruses in the laboratory and for those who may be at risk from the potential use of smallpox virus as a bioterrorism agent. The results demonstrate that smallpox vaccine given by bifurcated needle induces strong vaccinia virus–specific CD8+ CTL and IFN-γ–producing T cell responses and provide baseline information useful for planning the immunologic assessment of future smallpox vaccines.

Smallpox vaccines produced from the lymphatic fluid of bovines infected with vaccinia virus successfully induced long-term protection against smallpox, and vaccine use eventually eliminated the circulation of smallpox in the world. Vaccine-induced immunity resulted in long-term protection against smallpox [1, 2]. The vaccines were administered by bifurcated needles, and the development of a central vesicle at the injection site, surrounded by erythema, was the hallmark of successful primary vaccination in a nonimmune host. This was associated with regional lymph node swelling and mild systemic reactions. When a second dose of vaccine was administered later, a more rapid induction of local redness and swelling occurred at the vaccine site but resolved without the development of a vesicle and with fewer systemic symptoms [3, 4].

The immune responses to smallpox vaccination were analyzed almost exclusively by using serum samples to measure antibodies. Most studies demonstrated that neutralizing antibodies were long lived, and detectable levels usually correlated with protection against smallpox. The passive transfer of immune serum protected against challenge with mouse poxvirus, supporting these observations [5]. Little is known about the induction of human vaccinia virus–specific CD4+ and CD8+ cytotoxic T lymphocytes (CTL) following vaccination. A limited study of vaccinia antigen–induced lymphoproliferation was reported after vaccination [4], and 3 of 6 vaccinated persons had CTL responses when tested 12–30 months after vaccination [6].

It has been assumed that T cell immune responses are important in the protection and recovery from smallpox for several reasons. Children with congenital T cell immunodeficiency disorders [7, 8] and adults with acquired T cell immunodeficiency due to human immunodeficiency virus type 1 infection [9] had serious and at times fatal infections when vaccinated, whereas children with agammaglobulinemia did not have these vaccination complications [7]. Persons also were protected against natural challenge with smallpox if they were immune, as evidenced by a secondary local reaction to vaccination. In addition, the earlier localized erythema and swelling seen after secondary vaccination appeared to reflect delayed type hypersensitivity, which prevented extensive vaccinia virus replication locally and vesicle development. This “secondary take” correlated with protection against smallpox in the field.

Here we describe the primary induction of vaccinia virus–specific CD8+ CTL in vaccinia virus–naïve adults by a standard smallpox vaccine. Results of the clinical reactivity and antibody responses and a summary of the lymphoproliferative responses in these subjects have been published elsewhere [10]. In the present study, we analyzed the induction of vaccinia virus–
specific cytotoxic interferon (IFN)–γ–producing T cells and lymphoproliferative responses in a randomly selected subset of peripheral blood mononuclear cell (PBMC) samples obtained from volunteers given the vaccine.

Materials and Methods

Vaccines, study subjects, and study design. Vaccines, study subjects, and study design have been described elsewhere [10]. Eight subjects in the standard vaccine group, all of whom developed “primary take” reactions, were randomly selected for detailed testing in CTL assays and for precursor-frequency ELISPOT assays, to quantitate the number of vaccinia virus–specific IFN-γ–producing T cells.

Collection and preparation of blood samples and T cell assays. Approximately 60 mL of venous blood was obtained from each volunteer just before vaccination (day 0) and 27 days after vaccination. Vacutainer CPT tubes (Becton Dickinson) with sodium citrate were labeled for identification, held at room temperature for 1 hour, inactivated, and stored at −70°C until use. Cryopreserved PBMC from days 0 and 27 were thawed, washed once, and counted; 5 × 10^6 cells from the day 0 bleed were pelleted and infected with vaccinia virus (NYCBH strain; Wyeth) grown in CV-1 cells (American Type Culture Collection) [11], at an MOI of 10, for 60 min. Virus-infected stimulators were washed once and added to 4 × 10^6 cells from the day 0 and day 27 samples in a 6-well plate. The culture medium was AIM V serum (ABI). Effector cells were incubated for 6 days at 37°C in 5% CO2 and 95% humidity. The cytotoxic assays and lymphoproliferation assays were done with live vaccinia virus stimulation, as described elsewhere [11, 12].

Single-cell ELISPOT assay. Quantitation of IFN-γ–producing T cells was done as described elsewhere [13], except that vaccinia virus (NYCBH strain) was added to wells containing 2–3 × 10^5 cells/well.

Results

Table 1 shows the results, with the PBMC samples, for the smallpox vaccine recipients, all of whom developed primary take reactions at the injection site. Seven of 8 vaccine recipients had strong vaccinia virus–specific CTL responses and 8 of 8 had increases (7 strong and 1 moderate) in the number of vaccinia virus–specific IFN-γ–producing T cells after vaccination. Similarly, all 8 vaccine recipients had increased lymphoproliferative responses, and all had neutralizing antibody responses, most of which were at very high titers.

The virus-stimulated bulk-cultured cells of other vaccine recipients in this clinical study, whose effector cells lysed virus-infected autologous cells on day 6 after stimulation with live virus, were treated with monoclonal antibody to CD4+ or CD8+ cells and complement. The results demonstrated that this vaccinia virus–specific cytotoxic activity was depleted by treatment with anti-CD8+ plus complement but not by anti-CD4+ plus complement. The percentage of specific immune lysis was reduced by 63.7%–100% with anti-CD8+ and by 0–17.2% with anti-CD4+.

In addition, we found that, after stimulation of PBMC with live vaccinia virus, the vaccinia virus–specific IFN-γ–producing T cells were predominantly CD8+ (data not shown).

Table 1. Induction of vaccinia virus–specific cytotoxic T lymphocytes (CTL) and interferon (IFN)–γ–producing T cells in vaccine recipients.

<table>
<thead>
<tr>
<th>Vaccine recipient</th>
<th>Day 0</th>
<th>Day 27</th>
<th>Day 0</th>
<th>Day 27</th>
<th>Day 0</th>
<th>Day 27</th>
<th>Day 0</th>
<th>Day 27</th>
<th>Before</th>
<th>Day 27</th>
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<tr>
<td>1</td>
<td>0</td>
<td>57.8</td>
<td>1</td>
<td>180</td>
<td>1</td>
<td>23.9</td>
<td>5</td>
<td>664</td>
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<td></td>
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<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>79</td>
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<td>3</td>
<td>46</td>
<td></td>
<td></td>
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<tr>
<td>3</td>
<td>0</td>
<td>61.8</td>
<td>0</td>
<td>228</td>
<td>1</td>
<td>12.9</td>
<td>5</td>
<td>227</td>
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<td>0</td>
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<td>3</td>
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<tr>
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<td>0</td>
<td>68</td>
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<td>7</td>
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<td>6</td>
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<tr>
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<td>11</td>
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<td>30.5</td>
<td>3</td>
<td>286</td>
<td></td>
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</tr>
</tbody>
</table>

NOTE. E:T, effector-to-target cell ratio; NT, not tested; PRNT, plaque-reduction neutralization titer (as in [10]); SI, stimulation index; SIL, specific immune lysis.

All recipients of this vaccine were positive for pox lesions, including a randomly selected subset.

Lysis of uninfected cells, if present, was subtracted to give vaccinia-specific lysis.

Level of detection is 1 IFN-γ–positive T cell/300,000 T cells.

The SI was calculated as mean counts per minute of [3H]thymidine incorporation after stimulation with live virus/clean walls per minute in medium alone.
Discussion

These results convincingly demonstrate the induction of strong vaccinia virus–specific CTL responses and increases in IFN-γ–producing T cells in persons who received primary vaccination with a licensed smallpox vaccine administered by the standard scarification method. The results confirm and extend some of the findings of an earlier study in which 2 of the 3 primary vaccinations produced CTL responses [6]. These results also show that the number of vaccinia virus–specific IFN-γ–producing cells significantly increase after vaccination.

The induction of vaccinia virus–specific T cell responses was assessed in response to live vaccinia virus stimulation in bulk-culture CTL assays and by increases in the number of IFN-γ–producing T cells in ELISPOT and lymphoproliferation assays. The results show that standard vaccination induces virus-specific immune responses and that 7 of 8 vaccine recipients responded with the generation of very strong T cell responses, detected in all 3 assays, that were associated with high levels of neutralizing antibody. One vaccine recipient had detectable but lower levels of antibody and proliferative responses without a detectable CTL response.

In the future, it will be important to perform these assays for recipients of the tissue culture–derived smallpox vaccine administered by scarification, which may be required for the induction of optimal immune responses. These assays also should be done with persons given a second dose of standard vaccine as a challenge, which should be followed by a “secondary take,” or delayed type immune reaction at the vaccination site, in a few days and without the later development of a pox lesion at the site. A secondary take to vaccinia correlates with protection against smallpox. It will be important to define the immune parameters of this protection mediated by vaccination.

These results also have implications for the use of vaccinia viruses as a recombinant vector for experimental vaccines. Vaccinia viruses are powerful vectors with which to express foreign genes and have been invaluable reagents for detecting human virus-specific CD8+ CTL responses [14]. The results of this study, as well as earlier studies [11, 12], suggest that the replication of substantial amounts of vaccinia virus within pox lesions at the injection site may be required for the induction of CTL memory responses in recipients of standard smallpox vaccine and optimal induction of virus-specific IFN-γ–producing T cells. This also may apply to the administration of a recombinant vaccinia virus when administered to vaccinia virus–immune persons [15] and may apply to more-attenuated vaccinia viruses designed, for safety reasons, to not create pox lesions. Because the ability to immunize with a recombinant vaccinia virus expressing a novel foreign gene appears to be decreased substantially by the prior administration of vaccinia virus, the use of such vaccines potentially may be limited to vaccinia virus–naïve persons.

Acknowledgments

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References