Kinetics of Serum Cytokines after Primary or Repeat Vaccination with the Smallpox Vaccine

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Background. The smallpox vaccine is associated with more serious adverse events than any other live attenuated vaccine in use today. Although studies have examined serum cytokine levels in primary vaccine recipients at 1 and 3–5 weeks after vaccination with the smallpox vaccine, serial measurements have not been performed, and studies in revaccinated subjects have not been conducted.

Methods. We analyzed cytokine responses in both primary vaccine recipients and revaccinated subjects every other day for 2 weeks after vaccination.

Results. Primary vaccine recipients had maximal levels of granulocyte–colony-stimulating factor on days 6–7 after vaccination; peak levels of tumor necrosis factor (TNF)–α, soluble TNF receptor 1, interferon (IFN)–γ, IFN-inducible protein–10 (IP-10), interleukin (IL)–6, and tissue inhibitor of metalloproteinases–1 on days 8–9 after vaccination; peak levels of soluble TNF receptor 2 and monokine induced by IFN-γ (MIG) on days 10–11 after vaccination; and peak levels of granulocyte-macrophage–colony-stimulating factor on days 12–13 after vaccination. Primary vaccine recipients were significantly more likely to have higher peak levels of IFN-γ, IP-10, and MIG after vaccination than were revaccinated subjects. Primary vaccine recipients were significantly more likely to have fatigue, lymphadenopathy, and headache, as well as a longer duration of these symptoms and more hours missed from work, compared with revaccinated subjects.

Conclusions. The increased frequency and duration of symptoms observed in primary vaccine recipients, compared with revaccinated subjects, paralleled the increases in serum cytokine levels in these individuals.

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Primary vaccination with the smallpox vaccine usually results in a papular lesion with surrounding erythema ∼3–5 days later at the site of inoculation; this is followed 2–3 days later by development of a vesicle and then a pustule [1]. The pustule is surrounded by erythema and induration and reaches its maximum size at 8–12 days after vaccination. A scab begins to form, which separates at 14–21 days. A low-grade fever was present in ∼9% of adult primary vaccine recipients at 7–9 days after vaccination with the Dryvax smallpox vaccine and in 5% of such vaccine recipients at 10–12 days after vaccination [2]. At 7–9 days after vaccination, myalgias were noted in 50% of primary vaccine recipients, fatigue in 48%, headache in 41%, axillary lymphadenopathy in 30%, chills in 18%, and nausea in 14%. Approximately 36% of subjects were sufficiently uncomfortable after vaccination that they missed school, work, or outside activities or had difficulty sleeping. In a second study using the Aventis Pasteur smallpox vaccine in vaccinia-naive subjects, fatigue occurred in 48%, headache in 41%, regional lymphadenopathy in 30%, chills in 18%, and nausea in 14%. Approximately 36% of subjects were sufficiently uncomfortable after vaccination that they missed school, work, or outside activities or had difficulty sleeping. In a second study using the Aventis Pasteur smallpox vaccine in vaccinia-naive subjects, fatigue occurred in 79% of vaccine recipients, myalgias in 78%, headache in 75%, axillary lymphadenopathy in 62%, chills in 48%, nausea in 38%, missed activities in 25%, and fever in 22% during the first 2 weeks after vaccination [3].
In contrast to primary vaccine recipients, persons who are revaccinated with the smallpox vaccine have a more rapid sequence of events after vaccination [1]. Erythema develops in 1–2 days, and the papule and pustule form more rapidly than in vaccinia-naive persons. Although persons with some residual cellular immunity to the vaccine may develop a vesicle that may or may not form a pustule, others with substantial prior immunity may not develop a vesicle or pustule. In one study using the Dryvax smallpox vaccine, fever occurred less frequently in revaccinated adults (9%) than in primary vaccine recipients (60%), but there was no significant difference in other signs and symptoms between the 2 groups [4]. Lymphadenopathy was present in 27% of revaccinated adults versus 50% of primary vaccine recipients at 6–8 days after vaccination. The study was limited by having only 10 subjects in the primary vaccine group. Another study comparing the Dryvax smallpox vaccine in vaccinia-naive adults versus vaccinia-naive adults showed that axillary adenopathy occurred in 86%, fatigue in 66%, headache in 62%, and nausea in 24% of primary vaccine recipients, whereas adenopathy was present in 36%, fatigue in 38%, headache in 28%, and nausea in 8% of revaccinated subjects [5].

Systemic adverse events occurring after smallpox vaccination have been shown to be associated with elevated serum levels of cytokines in primary smallpox vaccine recipients. Rock et al [6] reported increased levels of interferon (IFN–γ), interleukin (IL)–10, and tumor necrosis factor (TNF–α) after vaccination with the Aventis Pasteur smallpox vaccine, but they reported no differences in IL-2, IL-4, and IL-5 levels. The authors found that persons who developed ≥1 adverse event (localized or generalized rash, lymphadenopathy, or fever) after vaccination had higher levels of IFN–γ, IL-2, IL-5, IL-10, and TNF–α after vaccination than at baseline. McKinney et al [7] found that serum levels of 6 cytokines (granulocyte colony-stimulating factor [G-CSF], stem cell factor [SCF], monokine induced by IFN–γ [MIG], intercellular adhesion molecule–1 [ICAM-1], eotaxin, and tissue inhibitor of metalloproteinases–2 [TIMP-2]) helped to distinguish individuals with or without adverse events after vaccination. These studies compared cytokine levels before vaccination with levels noted at a single time point at 6–9 days after vaccination in primary vaccine recipients. In the present study, we compared cytokine responses in primary vaccine recipients with those in revaccinated persons over multiple time points shortly after vaccination, and we correlated them with development of symptoms after vaccination.

SUBJECTS AND METHODS

Research subjects. Serial blood samples were obtained from 42 consecutive employees at the National Institutes of Health.
who were vaccinated with smallpox vaccine from March 2003 to September 2008. Of the 27 subjects who were primary vaccine recipients, 21 received Dryvax and 6 received ACAM2000 (a plaque-purified clone of Dryvax grown in cell culture). A total of 15 other persons who were previously vaccinated were revaccinated, and all 15 received Dryvax. Healthy employees who were ≥18 years of age were vaccinated as a routine part of their employment, either because they were laboratory workers who would be exposed to recombinant vaccinia virus or because they were health care workers who might be first responders in the event of a smallpox outbreak. The protocol was approved by the internal review board of the National Institute of Allergy and Infectious Diseases, and all patients provided written consent. Samples were obtained before and every other day after vaccination for the first 2 weeks of the study (days 2–4, 5–6, 7–8, 9–10, 11–12, 13–14, and 14–15) and at 1 month after vaccination. Samples were not obtained on weekends. Serum was stored in the vapor phase of liquid nitrogen until use.

**Cytokine analyses.** Serum cytokine levels were assayed in 2 primary vaccine recipients and 2 revaccinated persons who were among the first 13 subjects with the most prominent systemic symptoms (including fever and lymphadenopathy), by use of the Pierce SearchLight Protein Array multiplexed sandwich–enzyme linked-immunosorbent assay system (ThermoFischer). At each time point, the following cytokines were assessed in each of the 4 subjects: IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-6R, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12p40, IL-12p70, IL-13, IL-15 to IL-18, IFN-α, IFN-γ, MIG, IFN-inducible protein–10 (IP-10), TNF-α, soluble TNF receptor 1 (sTNFR1), soluble TNF receptor 2 (sTNFR2), eotaxin, granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage inflammatory protein–1α (MIP-1α), MIP-1β, MIP-3α, MIP–3β, RANTES, Exodus, macrophage-derived chemokine, I-309, monocyte chemotactic protein (MCP) 1 to MCP4, thymus- and activation-regulated chemokine,

### Table 1. Significance of the Difference in Symptoms in Primary Vaccine Recipients and Revaccinated Subjects

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Frequency of symptoms</th>
<th>Duration of symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Headache</td>
<td>0.004&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Myalgia</td>
<td>0.163</td>
<td>0.027</td>
</tr>
<tr>
<td>Pruritis</td>
<td>0.31</td>
<td>0.066</td>
</tr>
<tr>
<td>Loss of appetite</td>
<td>0.27</td>
<td>0.054</td>
</tr>
<tr>
<td>Chills</td>
<td>0.12</td>
<td>0.006&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fever</td>
<td>1.0</td>
<td>0.01</td>
</tr>
<tr>
<td>Time off from work</td>
<td>0.15</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

<sup>a</sup> For comparison of primary vaccine recipients with revaccinated subjects.

<sup>b</sup> P<0.01.

**Figure 2.** Percentage of vaccine recipients with a >1.5-fold increase in cytokine levels (from baseline) after receipt of the smallpox vaccine. Serum levels of interferon (IFN–γ), IFN-inducible protein–10 (IP-10), monokine induced by IFN-γ (MIG), tumor necrosis factor (TNF–α), soluble TNF receptor 1 (sTNFR1), soluble TNF receptor 2 (sTNFR2), interleukin (IL–6), and granulocyte colony-stimulating factor (G-CSF) (A), and levels of granulocyte-macrophage colony-stimulating factor (GM-CSF), tissue inhibitor of metalloproteinases–1 (TIMP-1), tissue inhibitor of metalloproteinases–2 (TIMP-2), stem cell factor (SCF), eotaxin, and soluble intercellular adhesion molecule–1 (sICAM-1) (B) are shown.

Based on the cytokines that showed the most prominent increase from baseline, the following cytokines were chosen for testing by enzyme-linked immunosorbent assay in all subsequent subjects: TNF-α, IFN-γ, IL-6, GM-CSF (Endogen-Thermo), MIG (CXCL9), sTNFR1 (TNFRSF1A), sTNFR2 (TNFRSF1B), sICAM-1 (CD54), TIMP-1 (R & D Systems), eotaxin, and IP-10 (Biosource). In addition, on the basis of recent literature [7], G-CSF, SCF, and TIMP-2 (R & D Systems) were also tested in all subjects.

**Statistical analyses.** Fisher’s exact test was used to assess the association between the percentage of vaccine recipients with symptoms for primary vaccine recipients versus revaccinated subjects. This test was also used to evaluate the association between the percentage of vaccine recipients with >1.5-fold increases in cytokine levels (ie, the peak cytokine level divided by the level noted at day 0) in primary vaccine recipients versus revaccinated subjects. The duration or severity of symptoms was aggregated, and the Poisson model and maximum likelihood ratio statistic were used to test the difference between primary vaccine recipients and revaccinated subjects. The Wilcoxon statistic was used to assess the differences in the peak levels of cytokines in primary vaccine recipients versus revaccinated subjects.

To test for an association between cytokine levels and the presence of symptoms over time, we fit a logistic regression model for each symptom. The correlation induced by having symptom outcomes on multiple days for each subject was modeled by use of generalized estimating equations. In the logistic regression model, the response variable is the presence of a symptom (with “1” denoting yes and “0” denoting no). The explanatory variables include log10 (cytokine levels + 0.5), age, sex, and revaccination indicator (with “1” denoting a revaccinated subject and “0” denoting a primary vaccine recipient). (To avoid taking the log of 0, 0.5 was added to the cytokine level value before converting to log values.) Fever and time off from work were not assessed because of the very small number of events. To address the multiple tests that were conducted, only P values <0.01 were deemed to be statistically significant for all of the analyses performed.

**RESULTS**

**Higher frequency of symptoms and prolonged duration of symptoms after vaccination among primary vaccine recipients than among revaccinated subjects.** Healthy National Institutes of Health employees who were 21–64 years of age received the smallpox vaccine when it was indicated for their employment. The mean age of the subjects was 35 years; 26 were women and 16 were men. The mean age of the 27 primary vaccine recipients was 27 years (range, 22–33 years), and that of the 15 revaccinated subjects was 45 years (range, 21–64 years). A total of 63% of primary vaccine recipients were female, and 60% of revaccinated subjects were female. For revaccinated subjects, the mean time since receipt of the previous smallpox vaccine was 33 years (range, 11–53 years), and the mean number of previous smallpox vaccinations received by these subjects was 1.3 (range, 1–3 vaccinations). Of the revaccinated subjects, 73% had received the smallpox vaccine once before revaccination, 20% had been vaccinated twice before revaccination in this study, and 7% had been vaccinated 3 times before revaccination in this study.

Among primary vaccine recipients, the most frequent symptoms were fatigue (frequency, 96%), lymphadenopathy (89%), headache (70%), and myalgias (63%); other symptoms were
Serum cytokine levels over time in primary vaccine recipients and revaccinated subjects receiving the smallpox vaccine. Serum levels of interferon (IFN)-γ (A), IFN-inducible protein–10 (IP-10) (B), monokine induced by IFN-γ (MIG) (C), tumor necrosis factor (TNF)-α (D), soluble TNF receptor 1 (sTNFR1) (E), soluble TNF receptor 2 (sTNFR2) (F), interleukin (IL)–6 (G), granulocyte colony-stimulating factor (G-CSF) (H), and granulocyte-macrophage colony-stimulating factor (GM-CSF) (I) are shown.

Figure 3. Serum cytokine levels over time in primary vaccine recipients and revaccinated subjects receiving the smallpox vaccine. Serum levels of interferon (IFN)–γ (A), IFN-inducible protein–10 (IP-10) (B), monokine induced by IFN-γ (MIG) (C), tumor necrosis factor (TNF)–α (D), soluble TNF receptor 1 (sTNFR1) (E), soluble TNF receptor 2 (sTNFR2) (F), interleukin (IL)–6 (G), granulocyte colony-stimulating factor (G-CSF) (H), and granulocyte-macrophage colony-stimulating factor (GM-CSF) (I) are shown.

noted in <50% of these vaccine recipients (Figure 1A). Fever (defined as body temperature ≥38.2°C) was noted in 7% of primary vaccine recipients, and 22% took time off from work. Symptoms were much less common in revaccinated subjects, with fatigue noted in 33% of revaccinated subjects, lymphadenopathy in 33%, and myalgias in 33%; none of the revaccinated subjects took time off from work.

Primary vaccine recipients were significantly more likely to have fatigue, lymphadenopathy, and headache than were revaccinated subjects (Table 1). The differences in the frequency of other symptoms did not reach statistical significance.

We measured the mean duration of symptoms on the basis of the number of clinic visits (which occurred every other day for the first 2 weeks, excluding weekends); diary card records were often incomplete and were less accurate. Primary vaccine recipients generally had a longer duration of symptoms than did revaccinated subjects (Figure 1B). Lymphadenopathy persisted for a mean of 2.3 clinical visit–days, fatigue for 2.0 visit–days, headache for 1.5 visit–days, and myalgias for 1 visit–day in primary vaccine recipients. Primary vaccine recipients took time off from work for a mean of 1.8 h after vaccination. In contrast, none of the revaccinated subjects had symptoms that persisted for a mean of ≥1 clinic visit–days, and none took time off from work after vaccination.

Primary vaccine recipients had a significantly longer duration of fatigue, lymphadenopathy, headache, chills, and time...
off from work than did persons who had been revaccinated with the smallpox vaccine (Table 1).

**Significantly greater likelihood of increased levels of sTNFR2 and G-CSF after vaccination in primary vaccine recipients versus revaccinated subjects.** Serum cytokine levels were measured before vaccination and every other day after vaccination for 2 weeks. More than 50% of primary vaccine recipients had >1.5-fold increases in serum levels of IFN-γ, IP-10, MIG, and G-CSF, whereas 40%–50% of vaccine recipients had >1.5-fold increases in serum levels of TNF-α, sTNFR-2, and IL-6 (Figure 2).

Primary vaccine recipients were more likely to have elevations in serum cytokine levels than were revaccinated subjects (Figure 2). More than 75% of primary vaccine recipients had >1.5-fold increases in levels of IFN-γ, IP-10, MIG, and G-CSF. A total of 50%-60% had >1.5-fold increases in levels of TNF-α, sTNFR-2, and IL-6, whereas 20%-30% had >1.5-fold increases in levels of sTNFR1 and GM-CSF. In contrast, among revaccinated subjects, the only cytokine level that increased by >1.5-fold in >50% of patients was MIG, and 40%-47% of revaccinated subjects had >1.5-fold increases in levels of IFN-γ, IP-10, and TNF-α levels. Increases of >1.5-fold in IL-6, G-CSF, sTNFR1, and sTNFR2 levels were noted in 33%, 27%, 13%, and 13% of revaccinated subjects, respectively. The differences between primary vaccine recipients and revaccinated persons for cytokine level increases of >1.5-fold were significant for sTNFR2 and G-CSF (Table 2). The level of each of these cytokines was higher in primary vaccine recipients than in revaccinated subjects.

The GM-CSF level was elevated >1.5-fold in 20% of primary vaccine recipients and 20% of revaccinated subjects. TIMP-1, TIMP-2, SCF, eotaxin, and sICAM-1 levels were elevated >1.5-fold in <10% of primary vaccine recipients, and surprisingly, they were elevated >1.5-fold in a higher percentage (13%-27%) of revaccinated subjects.

**Time of development of peak cytokine levels in primary vaccine recipients.** We measured serum cytokine levels every other day for 2 weeks after vaccination (Figure 3). In primary vaccine recipients, cytokine levels typically began to increase 4–5 days after vaccination, and they reached peak levels at 8–11 days. In primary vaccine recipients, G-CSF levels peaked at 6–7 days after vaccination, and TNF-α, sTNFR1, IFN-γ, IP-10, IL-6, and TIMP-1 levels (data not shown) peaked at 8–9 days after vaccination. sTNFR2 and MIG levels peaked at 10–11 days after vaccination, whereas GM-CSF levels peaked at 12–13 days after vaccination. Little change was noted in levels of TIMP-2, SCF, eotaxin, and sICAM-1 in primary vaccine recipients (data not shown). In contrast to cytokine levels in primary vaccine recipients, cytokine levels in revaccinated subjects generally showed little or no change, with the possible exception of the IL-6 level, which fluctuated widely for the first 9 days (Figure 3). The difference in the peak cytokine levels of primary vaccine recipients and revaccinated subjects was significant for IFN-γ, IP-10, and MIG (Table 2).

**Correlation of increases in levels of several serum cytokines with symptoms over time.** For all of the subjects, we tested for an association between the serum cytokine level and the percentage of vaccine recipients with symptoms, regardless of whether the subjects were primary vaccine recipients or revaccinated subjects. Levels of several cytokines showed a significant (P < 0.01) correlation with certain symptoms over time (Table 3). For the 4 serum cytokines that showed a significant correlation with ≥2 symptoms, the levels of cytokines over time correlated with the percentage of vaccine recipients with symptoms (Figure 4).

**DISCUSSION**

We found that serum levels of several cytokines, particularly those involving the TNF-α and IFN-γ signaling pathways, are increased after primary vaccination with the smallpox vaccine, and a higher frequency of primary vaccine recipients had elevated serum cytokines than did revaccinated persons. The elevation in serum cytokine levels paralleled the increase in symptoms after vaccination in primary vaccine recipients, compared with revaccinated subjects.

Primary vaccine recipients tended to be younger (mean age, 27 years) than revaccinated persons (mean age, 45 years), and
Figure 4. Serum cytokine levels and symptoms over time for all vaccine recipients receiving the smallpox vaccine. Percentages of patients with given symptoms (A) and serum levels of interferon (IFN)-γ (B), IFN-inducible protein–10 (IP-10) (C), monokine induced by IFN-γ (MIG) (D), and granulocyte colony-stimulating factor (G-CSF) (E) on different days are shown.
it was possible that the more robust cytokine response to vaccination in the primary vaccine recipients was a result of their younger age. However, comparison of the 3 revaccinated persons whose ages were in the range of the age of the primary vaccine recipients indicated that their cytokine responses were no more robust after vaccination than those of the other revaccinated persons (data not shown).

Previous studies reported an increase in systemic adverse events in persons with elevated levels of IFN-γ, TNF-α, IL-2, IL-5, and IL-10 [6]. A follow-up study found that serum levels of G-CSF, SCF, MIG, ICAM-1, eotaxin, and TIMP-2 separated individuals with adverse events from those without adverse events after smallpox vaccination [7]. We found that primary vaccine recipients were significantly more likely to have elevated levels of sTNFR2 and G-CSF than were revaccinated persons. Therefore, sTNFR2 and G-CSF seem to correlate with the likelihood of having more symptoms after vaccination in these studies.

We found that serum levels of IFN-γ, MIG, and IP10 were increased after vaccination with the smallpox vaccine. IFN-γ regulates activity of macrophages, monocytes, and natural killer (NK) cells; induces major histocompatibility complex (MHC) class II expression; and increases T helper 1 (Th1) cell maturation. IFN-γ induces expression of IP-10 and MIG, which recruit NK and T cells to sites of inflammation. The importance of IFN-γ, MIG, and IP-10 in controlling poxvirus infections has been demonstrated in several studies. IFN-γ-deficient mice are more susceptible to certain poxviruses [8] and IFN-γ receptor knockout mice have more severe infection with vaccinia virus than do wild-type mice [9]. Intranasal administration of IFN-γ improves survival of mice inoculated with vaccinia virus [10]. Recombinant vaccinia virus encoding IFN-γ is less virulent in mice [11]. Vaccinia virus expressing MIG or Crg-2 (the murine homolog of IP-10) is attenuated in mice, and anti–IFN-γ antibody increases the titer of the recombinant vaccinia viruses, but not wild-type virus [12].

The importance of IFN-γ for vaccinia virus infection is demonstrated by the observation that the virus has developed several mechanisms to block the effect of the cytokine. Vaccinia virus encodes the B8R protein that has sequence homology to the extracellular domain of IFN-γ receptors, binds to IFN-γ, and inhibits the antiviral activity of IFN-γ [13]. The vaccinia virus IFN-γ receptor B8R protein has a low affinity for binding mouse IFN-γ, and therefore it was not unexpected that deletion of the B8R gene from the virus did not affect virulence in mice [14]. However, another study using a different strain of mice and a different B8R mutant found that vaccinia virus with a B8R deletion was reduced for virulence in mice, suggesting that B8R might interact with other immunomodulatory proteins [15]. Deletion of the B8R homolog in ectromelia virus, a mouse poxvirus, reduced the virulence of the virus and was associated with higher levels of IFN-γ and enhanced cell-mediated immunity to the virus [16]. The vaccinia virus double-stranded RNA–binding protein, E3, inhibits the activity of several IFNs, including IFN-γ [17]. The vaccinia virus IL-18–binding protein (c12L) inhibits IFN-γ production, inhibits NK cell and vaccinia virus-specific T cell cytotoxicity, and enhances virulence of vaccinia virus [18]. Vaccinia virus contains a phosphatase (VH1) that dephosphorylates Stat1 and blocks IFN-γ signaling [19].

We found that serum levels of TNF-α, sTNFR-1, and sTNFR-2 were increased after vaccination with vaccinia virus. TNF-α increases the cytotoxicity of leukocytes and enhances NK cell activity. sTNFR1 regulates TNF-α, and in some studies, it gives a better indication of TNF-α biological activity than TNF-α, which has a very short half-life in the circulation [20]. Several studies have demonstrated the importance of TNF-α in controlling infection with vaccinia virus. Treatment of mice with TNF-α results in reduced replication of virus in the ovaries [21]. Recombinant vaccinia virus encoding TNF-α is less virulent than parental virus in mice [22]. Mice deficient for TNF receptors are more susceptible to certain poxviruses [23]. The vaccinia virus E3L protein inhibits expression of TNF-α [24]. Some strains of vaccinia virus contain crmE, which encodes both cell surface and soluble TNF receptors that inhibit the activity of TNF; virus lacking the TNF receptor is attenuated [25]. Certain strains of vaccinia virus encode crmC, which encodes a soluble TNF receptor that binds to TNF receptor and blocks binding of TNF to host cells [26]. Vaccinia virus encodes a serpin that inhibits the IL-1β enzyme and protects cells from TNF-induced apoptosis [27].

Serum levels of G-CSF and IL-6 were also increased after vaccination with vaccinia virus. G-CSF enhances granulocyte production and function. IL-6 induces B cell and T cell differentiation and growth. IL-6–deficient mice have impaired cytotoxic T cell activity against vaccinia virus [28] and impaired IgA responses to the virus [29]. The vaccinia virus E3L protein inhibits expression of IL-6 [24].

Although serum levels of eotaxin were increased after vaccination with the smallpox vaccine, primary vaccine recipients were less likely to have increased levels of eotaxin than were revaccinated persons. Eotaxin recruits eosinophils and basophils to sites of inflammation. Vaccinia virus CC chemokine–binding protein reduces eosinophil infiltration in the skin of guinea pigs that is induced by eotaxin [30]. Eosinophils were the prominent inflammatory cells in the heart of 2 patients with vaccinia-associated myocarditis [31]. The reason why a higher proportion of revaccinated subjects had elevated levels of eotaxin, compared with primary vaccine recipients, is unknown, although it is interesting to note that cardiac adverse events associated with smallpox vaccination were reported more often in revaccinated subjects than in primary vaccine recipients [32].
Our finding that primary vaccine recipients are significantly more likely than revaccinated patients to have symptoms and increased levels of cytokines after vaccination parallels other observations that severe adverse events (including postvaccinia encephalitis, eczema vaccinatum, and generalized vaccinia) occurred 10 times more often in primary vaccine recipients than in revaccinated subjects [33]. These results, as well as the observation that elevated serum cytokine levels are correlated with more serious adverse events after vaccination [6, 7], suggest that cytokines may have a role in the development of serious adverse events after vaccination, and that persons with marked cytokine responses to antigenic stimulation might be more susceptible to such events.

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References