Immunosuppressive Treatments Reduce Long-Term Immunity to Smallpox among Patients with Breast Cancer

Itay Wiser,1,5 Nadav Orr,1,5 Bella Kaufman,1,5 Shlomo Segev,1,5 Zehava Smetana,4 Ania Bialik,1,5 Nava Epstein,1,5 Ella Mendelson,1,5 Raphael Catane,2,5 and Dani Cohen1,5

1Department of Epidemiology and Preventive Medicine, School of Public Health, 2Oncology Institute and 3Infectious Diseases Unit, Sheba Medical Center, 4Central Virology Laboratory, Public Health Services, Ministry of Health, Tel Hashomer, 5Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Background. Mass vaccination is the principal preventive measure against a smallpox outbreak after an act of bioterrorism. Vaccination of subjects who received immunosuppressive therapies is problematic because of smallpox vaccine reactogenicity. Moreover, long-term immunity to vaccinia might be affected.

Objective. The objective of the study was to examine the effect of cytotoxic chemotherapy on long-term immunity to vaccinia.

Methods. In a case-control study, 67 patients with breast cancer who received cytotoxic chemotherapy and who were disease free for at least 1 year were matched with healthy controls according to age, sex, and the number of smallpox vaccinations received. Markers of immunity to smallpox were examined. Forty-one patients with breast cancer who did not receive chemotherapy were used to assess the affect of cancer and radiotherapy on immunity to smallpox.

Results. Patients with breast cancer who received chemotherapy had lower levels of vaccinia total immunoglobulin G and immunoglobulin G1 (expressed as enzyme-linked immunosorbent assay units per milliliter), neutralizing antibodies, vaccinia:memory B cell ratio (expressed as a percentage), and interferon-γ level (expressed as picograms per milliliter), compared with healthy control individuals.

Conclusions. Immunity to smallpox is reduced after receipt of chemotherapy for breast cancer. This finding should be considered when planning smallpox vaccination campaigns. The effect of immunosuppressive treatments on persistence of immunity should be tested with respect to additional vaccines or natural infections.

In 1980, the World Health Organization declared smallpox disease eradicated after a successful vaccination campaign using the live attenuated vaccinia vaccine [1]. Since that time, most countries have discontinued their smallpox vaccination programs, with the exception of immunization programs for laboratory and military personnel. Today, the estimated percentage of the population that is vaccinated against smallpox is ∼50% [2].

The threat of variola virus dissemination in a bioterrorist attack emerged together with the rise of global terror. Various countries have reassessed the immune status of their populations against smallpox and have prepared strategic plans of mass vaccination against smallpox in case of reemergence of the disease as a result of bioterrorism, and some countries have even immunized first responders [3].

Reassessment of the attack and case-fatality rates for smallpox among previously vaccinated subjects involved in large outbreaks of the disease in the prereradication era showed that protection against severe disease may extend for many decades after a single vaccination is given, and protection against death from smallpox may even be lifelong for the majority of vaccine recipients [4]. These epidemiological and clinical observations were supported by immunological data revealing a wide range of markers of humoral and cellular immunity to vaccinia that persist for decades and, even, for life after vacci-
Table 1. Characteristics of Cases and Healthy Controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Casesa (n = 67)</th>
<th>Controlsb (n = 67)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, average ± SD, years</td>
<td>49.54 ± 9.5</td>
<td>49.71 ± 9.1</td>
</tr>
<tr>
<td>Vaccinia vaccination, median (range), no.</td>
<td>3 (1–5)</td>
<td>3 (1–5)</td>
</tr>
<tr>
<td>Time since last vaccination, average ± SD, years</td>
<td>34.11 ± 11.4</td>
<td>32.75 ± 10.9</td>
</tr>
<tr>
<td>Geographic origin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Israel</td>
<td>41 (61)</td>
<td>52 (77)</td>
</tr>
<tr>
<td>East Europe</td>
<td>10 (15)</td>
<td>6 (9)</td>
</tr>
<tr>
<td>West Europe</td>
<td>2 (3)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Middle Eastc</td>
<td>11 (16)</td>
<td>4 (6)</td>
</tr>
<tr>
<td>America</td>
<td>3 (5)</td>
<td>3 (4)</td>
</tr>
</tbody>
</table>

NOTE. Data are the no. (%) of study subjects, unless otherwise indicated. SD, standard deviation.

a Women with breast cancer who were receiving chemotherapy.

b Healthy individuals.

c Except Israel.

nation. These markers included vaccinia-specific total immunoglobulin (Ig) G [5, 6], vaccinia-specific neutralizing antibodies [7], vaccinia-specific memory B cells [8], and vaccinia-specific memory T cells [6, 8–11].

Although these findings refer to the general healthy population of vaccine recipients, it is not known whether acquired vaccine-induced immunity to smallpox would not be deteriorated by various immunosuppressive medical interventions (chemotherapy, transplantation, etc.) received at different time intervals after childhood vaccination against smallpox. In addition, human immunodeficiency virus (HIV) infection, which emerged since the beginning of the 1980s, may significantly contribute to the increase in the number of immunosuppressed individuals previously vaccinated against the disease.

Cytotoxic chemotherapy is widely used as a standard treatment for oncologic and autoimmune diseases. Although the immediate effect of these chemotherapeutic agents on the immune system has been well described elsewhere [12], their long-term effect on immunity to infectious diseases has been much less investigated. Several studies found permanent damage to immune memory against infectious diseases after myeloablative chemotherapy and bone marrow transplantation [13–17]. Cytotoxic chemotherapy used for breast cancer treatment was also found to have a long-term effect on immune memory against infectious diseases [18, 19]. To the best of our knowledge, no previous study dealt with the affect of chemotherapy on the immune response to vaccinia.

In the present study, we examined the effect of cytotoxic chemotherapy on long-term immune memory against vaccinia among patients with breast cancer who received vaccination against smallpox in childhood. This question is of great importance because the current prevalence of cancer in the general population is ~3.6% [20]. Moreover, revaccination against smallpox is contraindicated for those with immune-compromising conditions, such as patients receiving cytotoxic chemotherapy, those who have just undergone organ transplantation, and HIV-infected patients. Reports of erroneous inoculation of smallpox vaccine in these cases resulted in severe to life-threatening complications [21, 22].

METHODS

Study Design and Population

The study was conducted as a retrospective case-control study. The cases were 67 female patients with breast cancer (age, 30–70 years) who had a prior smallpox vaccination record; who received a chemotherapy protocol that included doxorubicin, cyclophosphamide, 5-flourouracil, and/or paclitaxel; and who stayed in complete remission for at least 1 year. The 67 controls were healthy subjects who were matched to cases according to age, sex, and the number of smallpox vaccinations received and who had no history of iatrogenic or natural immunosuppression. Patients with breast cancer who did not receive chemotherapy and who met the remainder of the inclusion criteria were included in an additional control group, to determine a potential independent immunosuppressive effect of radiotherapy and/or illness alone.

All study participants were recruited from the Oncology Institute and Institute of Medical Screening and Assessment, Sheba Medical Center, Tel Hashomer, Israel. The study was approved by the Sheba Medical Center institutional review board. All the participants in the study provided signed informed consent and completed a questionnaire regarding their medical and immunization history.

Laboratory Procedures

Cell and serum separation. Serum samples (10 mL) and blood samples (40 mL) were obtained using ethylenediaminetetraacetic acid tubes (BD). Peripheral blood mononuclear cells (PBMCs) were separated from fresh blood by use of Vacutain-
er cell preparation tubes (BD) and then were resuspended in R-10 solution: RPMI 1640 plus 10% fetal calf serum (FCS) supplemented with penicillin, streptomycin, and L-glutamine. Fresh cells were used in all assays. Serum was separated using serum separation tubes. Fresh cells were used in all assays. Serum was separated using serum separation tubes.

**Measurement of serum vaccinia total immunoglobulin G and immunoglobulin G1–G3 subclasses.** This assay was performed using enzyme-linked immunosorbent assay (ELISA), as described elsewhere [23]. In brief, microtiter 96-well plates are coated with 15 μg/mL β-propiolactone–inactivated crude vaccinia antigen (IHD-J strain; 50 μL/well) (Omrix Lab), buffered with NaHCO3 buffer (50 mmol/L; pH 9.6), and then blocked with Tris buffer (50 mmol/L Tris [pH 7.6], 142 mmol/L sodium chloride, 0.05% sodium azide, 0.05% Tween 20, and 2% bovine serum albumin). Serial 2-fold dilutions of the tested serum (100 μL) were incubated in the plates for 2 h at 37°C. Plates were developed with alkaline phosphatase–conjugated rabbit anti–human IgG, followed by p-nitrophenyl phosphate as substrate (both from Sigma). Finally, absorbance was measured at 405 nm and calculated to be expressed as the number of ELISA units per milliliter.

For measurement of vaccinia IgG subclasses 1–3, a specific conjugate (KPL) was used for each subclass. Serum samples were tested at dilutions of 1:300 and 1:1200. Results for IgG subclasses 1–3 were calculated and expressed as the number of ELISA units per milliliter.

**Total IgG antibody avidity test.** Antibody avidity was measured using an elution ELISA with the chaotrope thiocyanate, as described elsewhere [24], and was modified for the vaccinia ELISA IgG assay. In brief, serum samples were diluted to achieve an optical density (OD) value of ∼1.0 and then incubated on an antigen-coated plate for 1 h at 37°C. The plates were washed, and ammonium thiocyanate that had been diluted in the serum buffer at various concentrations from 0 to 4 mol/L was added to the wells. After 15 min at room temperature, the plates were washed, and alkaline phosphatase–conjugated rabbit anti–human IgG, followed by p-nitrophenyl phosphate as substrate, was added. The absorbance was then read at 450 nm. The percentage reduction of absorbance in the presence of ammonium thiocyanate, compared with that in serum with no chaotrope, was plotted against the thiocyanate concentration. An avidity index (AI) was generated by plotting the log of the percentage reduction against the thiocyanate concentration and calculating the amount of thiocyanate required to reduce the absorbance in a given serum by 50%. Vaccinia IgG antibody avidity was not examined in serum samples with antibody levels <30 OmriU/mL.

**T cell interferon-γ secretion test.** We used the method described by Samandari et al. [25], with some modifications. In brief, fresh PBMCs at a density of 1.5 × 10⁶ cells/mL were re-suspended in AIM-V medium (Invitrogen) together with 5 μL/mL of crude inactivated vaccinia antigen (IHD-J strain [β-propiolactone–inactivated], pfu/mL; Omrix) for 72 h in 5% CO₂ and a 37°C humidified chamber (Forma Scientific). Phytohemagglutinin A (PHA) and AIM-V alone were used as positive and negative controls. Supernatant was collected and evaluated for interferon (IFN)–γ concentrations by use of an ELISA commercial kit (R&D Systems). The amount of IFN-γ that was secreted was determined by subtracting the negative control value from the average level. The lower limit of detection was 7.8 pg/mL.

**Vaccinia-specific memory B cell assay.** This method was adopted from Crotty et al [26], with minor variations. In brief, PBMCs were plated in 24-well dishes (5 × 10⁶ cells/well) in RPMI 10 supplemented with a mix of polyclonal mitogens: 1:100,000 pokeweed mitogen extract (Sigma-Aldrich), 3 μg/mL phosphotheioated CpG ODN-2006 (Sigma-Aldrich), 1:1000 of 50 mmol/L β-mercaptopethanol and 1:10,000 Staphylococcus aureus Cowan (Sigma-Aldrich). Ten wells were cultured per individual, 2 of them without mitogens (media only). Cells were cultured for 6 days at 37°C in 6%–8% CO₂. In preparation for the enzyme-linked immunospot (ELISPOT) assay, 96-well filter plates (MAHA N4510; Millipore) were coated overnight with crude inactivated vaccinia antigen (IHD-J strain [β-propiolactone–inactivated], 10⁷ pfu/mL; Omrix). To detect all IgG-se-
Vaccinia-neutralizing antibodies assay. We used the method previously described by Somekh et al [27]. In brief, samples of 100 μL of 2-fold dilutions of serum were mixed with 100 μL of 100 TCD50 (50% tissue culture infective dose) of vaccinia virus (IHD-J strain). Residual vaccinia virus was detected on Vero cells. Neutralization titers were defined as the highest dilution of serum that inhibited the cytopathic effect by at least 50%.

Data Validation
The reproducibility of the assays was documented by retesting of 10% of samples. For all the assays employed, the coefficient of variance was <15%. Blood samples obtained from 10 vaccinia-naive subjects were found to be negative for all the immune markers examined.

Statistical Analysis
Statistical analysis was performed using SPSS software (version 13; SPSS Technologies). Subject matching was performed by the SAS software using a matching algorithm described elsewhere [28]. Comparisons of continuous variables between the study groups were performed using paired t test. The Wilcoxon rank test was used for nonparametric variables. Differences in categorical variables were compared using the χ² test. Multiple group comparisons were performed using repeated-measures one-way analysis of variance and Tukey’s posttest. Nonparametric multiple group comparisons were performed using Friedman and Dunn’s posttests.

RESULTS
Sixty-seven female patients with breast cancer who received chemotherapy and 67 healthy controls were enrolled in the study. Table 1 presents the characteristics of the 2 study groups and the extent of matching for age (average ± standard deviation [SD], 49.5 ± 9.5 years vs 49.7 ± 9.1 years) and number49.5 49.7 of prior vaccinia vaccinations (3 vs 3). No statistically significant differences were found in the distribution of the 2 groups according to the country of origin of the participants.

The disease and treatment characteristics of the patients with breast cancer are presented in Table 2. The mean number of years since receipt of the last breast cancer treatment was 2.6 years (range, 1–7 years). In this group, the breast cancer stage was mostly 2–3 (~80% of the group), 84% of the women received radiation therapy, 16% of the patients had neutropenia during chemotherapy treatment, 100% of the women went through surgery (lumpectomy or mastectomy), and 42% of the women received granulocyte colony-stimulating factor during chemotherapy treatment (mainly as part of a “dose-dense” protocol).

Cases had significantly lower levels of vaccinia-specific total IgG and IgG1 (mean level, 84 vs 130 ELISA U/mL [P = .02] and 37.6 vs 23.4 ELISA U/mL [P = .02]) and vaccinia-specific neutralizing antibodies (PRNT50) (mean level, 14 vs 22 ELISA U/mL; P = .015), compared with the healthy controls (Table
Immunity to Smallpox after Chemotherapy

Table 4. Subgroup Analysis of the Characteristics Noted at Baseline for Cases and Controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cases a (n = 41)</th>
<th>Controls b (n = 41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, average ± SD, years</td>
<td>56.23 ± 7.1</td>
<td>54.82 ± 7.1</td>
</tr>
<tr>
<td>Vaccinia vaccination, median (range), no.</td>
<td>2 (1–4)</td>
<td>3 (1–5)</td>
</tr>
<tr>
<td>Time since last vaccination, average ± SD, years</td>
<td>41.66 ± 8.1</td>
<td>38.10 ± 9.1</td>
</tr>
<tr>
<td>Geographic origin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Israel</td>
<td>23 (56.1)</td>
<td>30 (73.2)</td>
</tr>
<tr>
<td>East Europe</td>
<td>8 (19.5)</td>
<td>5 (12.2)</td>
</tr>
<tr>
<td>West Europe</td>
<td>2 (4.9)</td>
<td>1 (2.4)</td>
</tr>
<tr>
<td>Middle East c</td>
<td>6 (14.6)</td>
<td>3 (7.3)</td>
</tr>
<tr>
<td>America</td>
<td>2 (4.9)</td>
<td>2 (4.9)</td>
</tr>
<tr>
<td>Cancer stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>17 (41.5)</td>
<td>…</td>
</tr>
<tr>
<td>2</td>
<td>16 (39.0)</td>
<td>…</td>
</tr>
<tr>
<td>3</td>
<td>7 (17.1)</td>
<td>…</td>
</tr>
<tr>
<td>4</td>
<td>1 (2.4)</td>
<td>…</td>
</tr>
<tr>
<td>Received hormonal therapy</td>
<td>38 (92.7)</td>
<td>…</td>
</tr>
</tbody>
</table>

NOTE. Data are the no. (%) of subjects, unless otherwise indicated. No significant differences were observed between the subgroups. Differences between parametric variables were assessed using an analysis of variance test; ordinal variables, by Friedman test; and nominal variables, by χ² test. ELISA, enzyme-linked immunosorbent assay; SD, standard deviation.

a Women with breast cancer who were not receiving chemotherapy.

b Healthy women.

c Except Israel.

3). The vaccinia-specific memory B cell ratio (expressed as a percentage) was 3.5% among cases, compared with 4.75% among controls (P < .001), and the IFN-γ levels among cases were lower than those among controls (25.4 vs 104.4 pg/mL; P < .001).

We found that patients who had neutropenia in the chemotherapy group had lower vaccinia-specific memory B cell ratios (3.8% vs 1.9%; P = .01).

An additional group of patients was enrolled in the study to examine the effect of cancer and/or radiotherapy alone (without chemotherapy) on markers of long-term immunity to vaccinia. The patients in this group were significantly older than those belonging to the chemotherapy group (56 vs 50 years; P < .05). They received fewer smallpox vaccinations during their lifetime (median number of vaccinations received, 2 vs 3; P < .05), and the time since their last vaccination was longer (42 vs 33 years; P < .05). They also had a significantly lower cancer stage, compared with patients in the chemotherapy group (P < .05). The percentage of patients receiving radiotherapy treatments was similar in the chemotherapy and no-chemotherapy groups (83% vs 88%). The no-chemotherapy group was then matched, for age and number of vaccinia vaccinations received, to 41 healthy controls in the group of healthy controls (n = 67) who were initially enrolled in the study (Table 4). No differences in immune markers level were found between the groups (Table 5).

DISCUSSION

The purpose of the present study was to examine the effect of cytotoxic chemotherapy on long-term immunity to smallpox by comparing a broad spectrum of markers of immune memory. The results clearly show that the panel of markers of long-term immunity is significantly lower in past chemotherapy patients than in healthy matched controls.

Traditionally, clinical “take” served as a proven and effective marker for successful vaccination during the eradication period. For primary vaccine recipients, the “take rates” after immunization were usually >95%. [7, 29]. The take rate for revaccinated individuals had a more variable range, depending on the time since the last vaccination, as well as the vaccine strain and its concentration [7, 23, 30, 31]. There is some evidence for a causal relationship between the neutralizing antibody titer and protection, either in preventing the disease or in attenuation of its severity [5].

After the eradication era, several studies have proposed additional surrogate immunological markers for protective immunity, such as a specific antibody reaction measured by ELISA, memory B cell response [8], T cell in vitro response by IFN-
Some of these markers correlated with protection against challenge in animal studies [35–40]. However, to date, no laboratory marker has been established as a “reference standard” for protective immunity, and no validated thresholds of protection are available. The laboratory immune markers selected for comparison in the present study encompass the various immune memory paths of both the humoral and cellular immune systems. For the humoral arm, our results revealed the presence of detectable levels of immune memory markers to smallpox in all study participants for IgG, in 90% of the study participants for neutralizing antibody activity, and in all study participants for B memory cell activity against vaccinia antigen. For the cellular arm, ~80% of all study participants demonstrated IFN-γ secretion in response to vaccinia antigen. These findings are compatible with current available data on long-term immunity to smallpox in the general population after routine vaccination [5, 6, 8, 10, 23, 41]. However, quantitatively, cases had significantly lower levels of humoral and cellular markers of immunity to smallpox, such as total IgG, IgG subclasses, neutralizing antibody activity, IFN-γ, and B memory cell activity against vaccinia antigen. The clinical implication of these findings cannot be well estimated without a clear laboratory threshold for smallpox immunity. However, because the differences cross both arms of the immune system, we can infer a significant damage to smallpox immune status.

Table 5. Subgroup Comparison of Markers of Immunity to Vaccinia between Cases and Controls

<table>
<thead>
<tr>
<th>Immune marker</th>
<th>Cases (n = 41)</th>
<th>Controls (n = 41)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total IgG level, mean (95% CI), ELISA U/mL</td>
<td>93.5 (77.0–110.1)</td>
<td>102.5 (76–129.1)</td>
<td>NS</td>
</tr>
<tr>
<td>IgG1 level, mean (95% CI), ELISA U/mL</td>
<td>22.2 (17.7–26.7)</td>
<td>32 (22.8–41.2)</td>
<td>NS</td>
</tr>
<tr>
<td>IgG2 level, mean (95% CI), ELISA U/mL</td>
<td>79.9 (60.0–100.0)</td>
<td>72.2 (69–85.5)</td>
<td>NS</td>
</tr>
<tr>
<td>IgG3 level, mean (95% CI), ELISA U/mL</td>
<td>6.2 (4.3–8.1)</td>
<td>8.2 (3.43–13.0)</td>
<td>NS</td>
</tr>
<tr>
<td>PRNT50 level, mean (95% CI), ELISA U/mL</td>
<td>14.4 (9.9–18.9)</td>
<td>19.4 (11.7–22.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Avidity index</td>
<td>2.22 (2.06–2.38)</td>
<td>2.48 (2.29–2.68)</td>
<td>NS</td>
</tr>
<tr>
<td>IFN-γ secretion</td>
<td>36.3 (12.2–60.4)</td>
<td>73.8 (24.0–123.7)</td>
<td>NS</td>
</tr>
<tr>
<td>B cell ratio, mean (95% CI), %</td>
<td>4.21 (3.98–5.53)</td>
<td>4.76 (3.53–4.9)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NOTE. CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; IFN, interferon; IgG, immunoglobulin G; NS, not significant.

* All immune markers relate to vaccinia virus.
* Women with breast cancer who were not receiving chemotherapy.
* Healthy women.
* Differences between groups were assessed by paired t test, for parametric variables, and Wilcoxon test, for non-parametric variables. P < .05 was considered to denote statistical significance.

γ ELISPOT [8, 30, 32], or intracellular cytokine staining [6, 33, 34]. Some of these markers correlated with protection against challenge in animal studies [35–40]. However, to date, no laboratory marker has been established as a “reference standard” for protective immunity, and no validated thresholds of protection are available. The laboratory immune markers selected for comparison in the present study encompass the various immune memory paths of both the humoral and cellular immune systems. For the humoral arm, our results revealed the presence of detectable levels of immune memory markers to smallpox in all study participants for IgG, in 90% of the study participants for neutralizing antibody activity, and in all study participants for B memory cell activity against vaccinia antigen. For the cellular arm, ~80% of all study participants demonstrated IFN-γ secretion in response to vaccinia antigen. These findings are compatible with current available data on long-term immunity to smallpox in the general population after routine vaccination [5, 6, 8, 10, 23, 41]. However, quantitatively, cases had significantly lower levels of humoral and cellular markers of immunity to smallpox, such as total IgG, IgG subclasses, neutralizing antibody activity, IFN-γ, and B memory cell activity against vaccinia antigen. The clinical implication of these findings cannot be well estimated without a clear laboratory threshold for smallpox immunity. However, because the differences cross both arms of the immune system, we can infer a significant damage to smallpox immune status.

In this study, cases and (healthy) controls were matched for age, sex, and years since the last vaccination, to prevent confounding of the different immune marker levels examined. Furthermore, cases received a standard chemotherapy protocol with minimal variations, allowing for a homogenous approach to its effect on immunity to smallpox. Still, our study has several limitations. The number of smallpox vaccinations was extrapolated from self-reports, as were the number of vaccination scars and demographic data. Although the risk of recall bias exists, it is nondifferential, and therefore it is not expected to affect the study findings. According to the study design, we drew just one point comparison between the immune status and smallpox in cases and controls without following the immunity dynamics in the 2 study groups. The study included pre- and post-menopausal patients with potential different responses to chemotherapy. We assume, however, that matching by age avoided confounding by this extent of heterogeneity.

We attempted to discover whether the contribution to the decrease in immunologic memory among cases was due exclusively to chemotherapy. For this purpose, we enrolled in the study those patients with breast cancer who did not receive chemotherapy treatments but only radiotherapy after surgery. It turned out that these patients were different from those who received chemotherapy with respect to their age (they were older) and the time since the last smallpox vaccination received, and also had a lower cancer stage. To control for these different characteristics, we compared the immunological parameters of the specific group of patients with breast cancer without chemotherapy with controls matched by age and number of vaccinations received. We found no significant differences between cases with no chemotherapy treatment and their matched healthy controls. These results support our assumption that cytotoxic chemotherapy is the major contributor to the decrease of long-term immunity to smallpox vaccine. Another finding that corroborates this assumption is the negative association between neutropenia, a known event resulting from cytotoxic chemotherapy, and the vaccinia antigen–specific B cell ratio.
The prevalence of contraindication to smallpox vaccination resulting from immunosuppression has steeply increased in recent decades. Approximately 10 million people in the United States are at risk for severe complications resulting from smallpox vaccination, including 184,000 solid-organ transplant recipients, 850,000 HIV-positive individuals, and 8.5 million patients with cancer [20].

Smallpox vaccine is known for its relatively high rate of systemic reactogenicity and serious adverse events in immunocompromised subjects or those who have skin diseases [7]. Significantly more attenuated vaccinia vaccine candidates, such as MVA and Lc16m, showed lower reactogenicity in clinical trials [42] and could be suitable for administration to immunocompromised groups, if needed. MVA, which is a replication deficient vaccinia virus developed in Germany during the 1970s, demonstrated a protective effect in healthy and immunocompromised animal challenge models [36, 38, 39, 43–45], and they showed good immunogenicity and a lower rate of adverse events in humans, compared with traditional vaccines [42].

In light of the results of this study, we would recommend that subjects who received cytotoxic chemotherapy in the past 4 years be revaccinated and given the less reactogenic versions of the smallpox vaccine in the event that a mass smallpox vaccination campaign is initiated [42].

The results of the present study indicating that cytotoxic chemotherapy damages long-term immune memory against vaccinia correspond to data reported from other studies showing a significant decrease in seropositivity or antibody protective level rates to various infectious disease agents in children treated for pediatric malignancies [17]. To our knowledge, no evidence of a higher rate of infectious diseases morbidity in patients who recovered from breast cancer–related chemotherapy treatment has been published to date. Further epidemiological studies that assess patterns of infectious diseases in past cytotoxic chemotherapy patients are needed. Moreover, the morbidity data should be correlated to immune memory markers, such as those used in this study.

**References**


