Clinical Reactogenicity of Intradermal Bacille Calmette-Guérin Vaccination

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Clinical, microbiological, and immunologic responses were evaluated in volunteers vaccinated intradermally with bacille Calmette-Guérin (BCG). Most volunteers (98%) developed ulcerative lesions that drained for a mean ± SE of 4.3 ± 0.29 weeks. Mycobacterial DNA was detected by a polymerase chain reaction–based amplification technique in biopsy specimens from BCG ulcers 2 weeks after vaccination and in blood specimens 3 days after vaccination. Mycobacteria were cultured from ulcer drainage 2 months after vaccination, demonstrating a prolonged potential risk of contact spread of the vaccine strain. The duration of ulcer drainage was inversely correlated with prevaccination lymphoproliferative (r = −0.515; P < .002) and interferon γ (r = −0.841; P < .002) responses specific to mycobacteria and directly correlated with postvaccination increases in lymphoproliferative (r = 0.498; P < .002) and interferon γ (r = 0.688; P < .02) responses specific to mycobacteria. These results demonstrate the clinical reactogenicity of BCG and the potential risk of contact spread of the vaccine strain and suggest that clinical reactogenicity is a trade-off for the induction of protective mycobacterial immunity.

BCG has been used as a vaccine in humans for >75 years, and >3 billion doses have been administered (reviewed in [1]). BCG is a live-attenuated form of Mycobacterium bovis that can be associated with local tissue reactions in immunocompetent individuals and serious disseminated vaccine complications in immunocompromised individuals (detailed analyses of adverse reactions to BCG were presented by Lotte et al. in 1984 [2]). However, this vaccine is considered safe enough for the World Health Organization to recommend that all children in areas where tuberculosis is highly endemic be vaccinated with BCG during infancy [3]. Over the last several decades, attempts have been made to minimize the adverse reactions associated with BCG vaccination. Cutaneous vaccination has evolved to become the most commonly used method of BCG administration and is believed to minimize toxicity and maximize induction of delayed-type hypersensitivity to PPD of tuberculin. Cutaneous vaccinations also minimize the cfu dose of BCG required to induce positive PPD responses, a practical concern for mass production and community vaccine coverage. Intradermal BCG vaccination, involving the injection of viable vaccine into a dermal wheal, and percutaneous BCG vaccination, involving the adsorption of surface-applied vaccine through multiple tiny needle points, are the most common forms of BCG vaccination in use worldwide.

See editorial response by Ernst on pages 791–3.

The clinical features associated with cutaneous BCG vaccination are not widely known in the United States, and the relationships between the reactogenicity and immunogenicity of these vaccinations have not been well characterized. We have previously demonstrated an association between reactogenicity and immunogenicity in comparisons of intradermal and percutaneous BCG vaccinations [4]. We now describe the detailed reactogenicity associated with intradermal BCG vaccination and present further evidence for correlations between BCG reactogenicity and vaccine-induced immunity.

Patients and Methods

Selection of volunteers, BCG vaccination, and clinical follow-up. The study protocols were approved by the Institutional Review Board, Saint Louis University (St. Louis). Volunteers were healthy individuals aged 18 to 50 years who had negative reactions (0 mm of induration) to a 5-TU PPD skin test and negative HIV serology at the time of enrollment. Despite open enrollment and active recruitment of minority groups, all but two of the volunteers were Caucasian.

Volunteers were recruited into three separate BCG vaccination trials conducted between 1993 and 1996. The first trial involved 12 volunteers in an open-label study of Connaught BCG (Connaught Laboratories, Swiftwater, PA) administered
intraderrmally. The second trial included 36 volunteers who were randomized to receive Connaught BCG or Tice BCG (Perimmune, Rockville, MD) intradermally. A third trial involved 60 volunteers randomized to receive either intradermal or percutaneous vaccination with Tice BCG and has been partially described previously [4]. To increase the statistical power of Spearman’s rank-order test of correlations between reactogenicity and immunogenicity, BCG-vaccinated individuals from all three vaccine trials were included for analysis. All other results reported herein were based only on findings for the intradermal BCG–vaccinated individuals enrolled in our first two vaccine trials.

Intradermal BCG vaccinations (2–3 × 10⁶ cfu) were given over the left deltoid area in 100 μL of saline. Percutaneous immunization was done by spreading 0.3 mL of the BCG preparation (3–4 × 10⁶ CFU/mL) on the surface of intact skin over the left deltoid area followed by superficial perforation of the epidermis through the vaccine suspension with a multipronged device supplied by the manufacturer (Perimmune).

Volunteers were clinically assessed on days 3, 7, 14, 28, and 56 after vaccination in the Vaccine Center Clinic, Saint Louis University. Measurements of papules, pustules, and ulcerations (in millimeters) were recorded at each visit. Oral temperatures, local erythema, local tenderness, and regional lymph nodes also were assessed at each visit. In addition, volunteers were questioned about the occurrence of systemic symptoms and whether they had missed any work because of adverse reactions associated with their BCG lesions. Measurements of PPD-specific delayed-type hypersensitivity 2 months after BCG vaccination were done with either apilsol (Parke-Davis, Morris Plains, NJ) or tubersol (Connaught Laboratories) forms of tuberculin. Five tuberculin units of Tween-stabilized tuberculin was injected intradermally, resulting in ~10 mm of wheal formation. Experienced personnel applied the PPD tests and read the millimeters of induration at 48–72 hours with the ballpoint pen technique [5].

Skin biopsies. Seven volunteers vaccinated intradermally with BCG were randomly selected for study with serial punch biopsies done on days 7, 14, and 28 after vaccination. Two-millimeter punch biopsy specimens were obtained from skin immediately adjacent to the vaccination sites. The tissue specimens were formalin-fixed and paraffin-embedded, and tissue sections were stained with hematoxylin-eosin stain for microscopic examination. Other tissue sections stained with Ziehl-Neelsen stain were examined for acid-fast bacilli. Some tissue sections were processed as described below for use in detection of DNA specific to mycobacteria.

Detection of mycobacteria by PCR analysis and cultures. DNA from tissue and blood specimens was extracted and amplified by PCR analysis with some modifications of a previously reported protocol [6]. DNA samples from formalin-fixed, paraffin-embedded biopsy tissue sections were extracted with xlyenes and washed with ethanol. Blood specimens were collected in EDTA-containing tubes, and cellular pellets were washed with Tris-EDTA buffer, followed by extraction with xlyenes and washing with ethanol. These ethanol-washed pellets were suspended in proteinase K digestion buffer and incubated for 3 hours at 55°C before phenol-chloroform extraction and ethanol precipitation. After suspension in ultrapure water, aliquots of these samples were used as DNA templates in PCR reactions with primers specific to a 285-bp product unique to the Mycobacterium tuberculosis complex [6]. Similar reactions were set up with primers specific to the human β-globin gene as controls for DNA extraction.

The amplification protocol included 40 cycles of 94°C for 15 seconds, 70°C for 15 seconds, and 72°C for 15 seconds, after an initial cycle of 94°C for 2 minutes; 25 mM MgCl₂ was used in PCR reactions with DNA extracted from tissue samples, and 20 mM MgCl₂ was used in PCR reactions with DNA extracted from blood samples. The PCR products were dot-botted onto Nytran membranes and hybridized with [γ-³²P] end-labeled oligonucleotide probes specific to an internal sequence present in the 285-bp product unique to the M. tuberculosis complex [7]. Hybridization was visualized by exposing radiolabeled blots to a phosphor screen and then was analyzed on a 425B phosphorimager (Molecular Dynamics, Sunnyvale, CA). PCR products also were run on 2% agarose gels, and Southern blotting was performed as previously described [7] to confirm the specificity of the probe hybridization for the 285-bp sequence unique to the M. tuberculosis complex.

Swab specimens of ulcer drainage were inoculated into Septi-Chek AFB mycobacterial culture systems (Becton Dickinson Microbiology Systems, Cockeysville, MD). Blood samples were collected into ISOSTAT isolator tubes (Wampole Laboratories, Cranbury, NJ), processed by lysis centrifugation, and then inoculated into Septi-Chek AFB mycobacterial culture systems. All cultures were incubated at 37°C with 5% CO₂ for 8 weeks before final reporting of the results. Positive cultures were subcultured onto Löwenstein-Jensen agar slants for biochemical analysis.

Antigen preparations for in vitro stimulation of peripheral blood mononuclear cells (PBMCs). Whole-cell lysates of the Erdman strain of M. tuberculosis were obtained from John T. Belisle (Colorado State University, Fort Collins) under the terms of the National Institutes of Health Mycobacterial Reagent contract NO1-AI-25147. These lysates were prepared from midlogarithmic phase cultures grown on glycerol-alanine salts broth. After washing with PBS, mycobacteria were heat-killed (80°C for 1 hour) and then disrupted by a bead vortex before passage through 0.2-μm filters.

Measurement of lymphoproliferative responses in PBMCs. The kinetics of the proliferation assay and the dose response were optimized in earlier studies [4, 8]. Density gradient purified PBMCs freshly harvested from BCG recipients 2 months after vaccination were diluted to 1 × 10⁶ cells/100 μL in medium (RPMI supplemented with 10% pooled human AB serum, penicillin, streptomycin, glutamine, and β-mercaptoethanol) and incubated in triplicate wells of 96-well flat-bottomed plates.
for 6 days in the presence of optimal doses of \( M. \) \( tuberculosis \) lysate (2 \( \mu g/mL \)) or medium alone at 37°C with 5% CO\(_2\). On day 6, cultures were pulsed with 0.5 \( \mu Ci \) of tritiated thymidine and further incubated overnight before measuring cell-associated radioactivity.

Measurement of IFN-\( \gamma \) responses. IFN-\( \gamma \) responses were measured as described previously [4, 8]. Briefly, PBMCs were incubated on tissue culture plates \((1-4 \times 10^6 \text{ cells/mL})\) with medium alone or \( M. \) \( tuberculosis \) lysate (2 \( \mu g/mL \)) for 6 days at 37°C with 5% CO\(_2\). Supernatants were collected and stored at \(-70^\circ C\). IFN-\( \gamma \) was detected in culture supernatants by ELISA with use of the cytokine-specific antibody pair 400-45/6 mouse IgG1 antibody to hIFN-\( \gamma \) (Chemicon, Temecula, CA) and P-700 rabbit antibody to hIFN-\( \gamma \) (Endogen, Boston).

Statistical analyses. All data analyses were performed with use of Statistica (StatSoft, Tulsa, OK). Spearman’s rank-order test was used to study correlations between independent variables.

Results

PPD responses. The mean PPD response \( \pm SE \) for all 48 intradermal BCG-vaccinated volunteers enrolled into the first two vaccine trials was \( 9.0 \pm 1.0 \text{ mm} \), and 72% of these individuals had a PPD response of \( \geq 5 \text{ mm} \). PPD responses in the intradermal BCG recipients recruited into our intradermal vs. percutaneous BCG vaccine trial were similar and have been reported previously [4].

Clinical reactogenicity. All 48 volunteers vaccinated intradermally with either the Connaught strain or the Tice strain of BCG developed an indurated papule or pustule at the immunization site, and all but one of these lesions ulcerated and drained for a mean duration of 4.3 weeks (table 1). No significant differences were detected in comparisons of the clinical reactogenicity associated with the two different BCG vaccine strains. No temperatures of \( >101^\circ F \) or other systemic symptoms were recorded. All immunization sites healed by 2 months after vaccination. None of the volunteers reported any limitations in their normal working schedules that were related to the local vaccine lesions. As reported previously, only one of 30 percutaneously vaccinated volunteers developed ulceration at the vaccination site [4].

Histopathology of vaccine lesions. Lymphocytic infiltrations with granuloma formation were observed as early as 7 days after BCG vaccination and persisted for 1 month after vaccination. The typical progression seen in tissue sections 14

<table>
<thead>
<tr>
<th>Reaction</th>
<th>No. (% of volunteers with reaction)</th>
<th>Size (mm)</th>
<th>Duration (w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papule or pustule</td>
<td>48 (100)</td>
<td>7.7 ± 0.42</td>
<td>8.0 (4-16)</td>
</tr>
<tr>
<td>Ulcer</td>
<td>47 (98)</td>
<td>4.9 ± 0.33</td>
<td>4.0 (0-11)</td>
</tr>
</tbody>
</table>

NOTE. Other than the reactions shown, only a few cases of self-limited regional lymphadenopathy occurred. All ulcerations eventually healed without further complications.
Table 2. Presence of mycobacteria in local tissue specimens and blood samples after BCG vaccination of volunteers.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>1 w</th>
<th>2 w</th>
<th>1 mo</th>
<th>2 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer drainage</td>
<td>2/2</td>
<td>ND</td>
<td>13/17</td>
<td>ND</td>
</tr>
<tr>
<td>Skin biopsy sample</td>
<td>ND</td>
<td>1/5</td>
<td>ND</td>
<td>6/7</td>
</tr>
<tr>
<td>Blood</td>
<td>0/36</td>
<td>3/36</td>
<td>0/36</td>
<td>0/36</td>
</tr>
</tbody>
</table>

NOTE. Data are no. of positive samples/total no. of samples tested. ND = not done.

* No. cultures positive/no. cultures. Swabs of ulcer drainage were directly cultured on 7H10 agar. Blood was treated with lysis centrifugation and then cultured on 7H10 media.

² No. PCR assays positive/no. PCR assays. DNA extracted from formalin-fixed skin tissue specimens obtained from BCG lesions and DNA extracted from whole blood were amplified by using PCR primers specific to a sequence unique to the Mycobacterium tuberculosis complex.

and 30 days after vaccination (from inflammatory infiltrates composed primarily of epithelioid macrophages and lymphocytes to well-formed noncaseating granulomatous reactions) is shown in figure 1. Acid-fast stains of all tissue sections were uniformly negative.

Prolonged shedding of viable BCG. We studied ulcer drainage, skin biopsy samples, and blood samples for evidence of mycobacteria by culture and with a PCR-based detection system (table 2). Cultures of swabs of the drainage from vaccine ulcers on more than one-half of the volunteers revealed that viable BCG was shed for at least 1 month. Ulcer drainage from one volunteer still had viable BCG present 2 months after vaccination. PCR analyses of biopsy specimens obtained from skin immediately adjacent to vaccine lesions demonstrated the presence of mycobacterial DNA in most specimens 2 weeks after vaccination. PCR analyses of similar tissue specimens obtained from four volunteers 1 month after vaccination were all negative.

PCR amplification detected circulating mycobacteria in blood samples from three volunteers 3 days after vaccination but never 2, 4, or 8 weeks later. A Southern blot of the amplified products from these blood specimens is shown in figure 2. The 285-bp amplimer specific to the M. tuberculosis complex was clearly detected, thus demonstrating the specificity of the PCR reactions. Cultures of lysis centrifugation–processed blood were negative for mycobacteria at all times studied, including 3 days after vaccination.

Correlations of reactogenicity with immunogenicity. Previously, we reported results of in vitro studies of cell-mediated immune responses specific to mycobacteria in these BCG-vaccinated volunteers [4, 8]. Now, we present the results of Spearman’s rank-order test of correlations between these in vitro immune parameters and local BCG reactogenicity in all BCG vaccine recipients who developed a positive PPD response after vaccination (induration of ≥5 mm 48–72 hours after intradermal injection of 5 TU of tuberculin). The baseline lymphoproliferative reactivities with mycobacterial cell lysates and the durations of ulcer drainage after vaccination for 37 BCG vaccine recipients are compared in figure 3. The higher the baseline lymphoproliferative reactivity the shorter the duration of ulceration ($r = -0.515; P < .002$). In contrast with these results, the fold increases after vaccination in the lymphoproliferative reactivity specific to mycobacteria were directly correlated with the length of ulcer duration ($r = 0.498; P < .002$). It can be seen in figure 4 that the fold increases in the lymphoproliferative reactivity specific to mycobacteria were ~2 logs higher in volunteers with draining ulcerations lasting 4–6 weeks than the levels detected in volunteers with ulcerations lasting ≤1 week.

Similar relationships between the duration of ulcer drainage and IFN-γ responses specific to mycobacteria were observed. The baseline levels of IFN-γ responses detected before vacci-
draining BCG vaccination sites and advise appropriate precautions to minimize the risks to immunosuppressed contacts.

The other major finding in these studies was that the clinical reactogenicity associated with intradermal BCG vaccination was correlated with immunity specific to mycobacteria. As pointed out above, ulcerative drainage from vaccine lesions contained viable replicating BCG. Direct correlations between the duration of ulcer drainage (relative duration of BCG replication) and lymphoproliferative and IFN-γ responses specific to mycobacteria after vaccination were detected (figure 4 and Results). IFN-γ is known to be a key cytokine in the induction of effector functions important for the control of intracellular pathogens, including mycobacteria causing human infections [17–22]. These results suggest that the optimal induction of protective mycobacterial immunity by BCG vaccination may require considerable in situ replication of the vaccine strain and the associated local tissue effects. The significant inverse correlations detected between ulcer duration and prevaccination lymphoproliferative and IFN-γ responses (figure 3 and Results) suggest that these baseline immune reactivities were protective against replication of the vaccine strain.

In summary, the clinical reactogenicity of intradermal BCG vaccination may be a trade-off for the induction of at least partially protective immunity. It remains to be determined whether effective vaccines with less clinical toxicity can be developed to prevent a disease like tuberculosis that largely occurs in the face of immunosuppression. Persons with draining BCG ulcers should be advised to cover their ulcerative sites with bandages and dispose of these bandages with proper bio-

Discussion

Our results are consistent with other reports demonstrating a very high incidence of ulcerative lesions at the immunization sites after intradermal BCG vaccination [2, 9]. We also found that these granulomatous ulcerative lesions drained for about 1 month in most cases and that the ulcer drainage contained viable BCG that could be a source for environmental contact spread of the vaccine strain. Clinically significant problems associated with contact spread of BCG to healthy persons would be unlikely. However, contact spread of BCG to patients with AIDS and other immunosuppressed persons could lead to serious consequences. Many reports have documented fatal disseminated disease secondary to BCG infection in immunosuppressed individuals (reviewed in [10]). In addition, significant clinical complications have been reported after accidental contact spread of BCG vaccine [11–16]. Health care workers need to be aware of the probability of viable mycobacteria in

![Figure 3](image-url)  
**Figure 3.** Inverse correlation between duration of BCG ulcers and prevaccination lymphoproliferative responses specific to mycobacteria in BCG-vaccinated volunteers. Ulcer duration was determined from the vaccine clinic records. Peripheral blood mononuclear cells obtained from volunteers before vaccination were stimulated in vitro with *Mycobacterium tuberculosis* lysates, and on day 6 the cultures were pulsed with tritiated thymidine and further incubated overnight before the cell-associated radioactivity was measured. Spearman’s rank-order test of correlation demonstrated a significant negative correlation ($r = -0.515; P < .002$) between ulcer duration and baseline lymphoproliferation specific to mycobacteria in the 37 BCG recipients for whom results were available for this analysis. Note the logarithmic scale for prevaccination proliferative responses specific to mycobacteria. $dpm =$ disintegrations per minute.

![Figure 4](image-url)  
**Figure 4.** Direct correlation between BCG reactogenicity and immunogenicity. Durations of ulcers and lymphoproliferative responses specific to mycobacteria were studied as described in figure 3. The fold increase in lymphoproliferation was calculated by dividing the responses in disintegrations per minute ($dpm$) measured 2 months after BCG vaccination by the $dpm$ responses measured before vaccination. Spearman’s rank-order test of correlation demonstrated a significant positive correlation ($r = 0.498; P < .002$) between ulcer duration and fold increases in lymphoproliferation specific to mycobacteria in the 37 BCG vaccine recipients for whom results were available for this analysis. Note the logarithmic scale for fold increases in responses specific to mycobacteria.
safety concern if they have close contact with immunosuppressed individuals. These simple measures should prevent contact spread of BCG infection to immunosuppressed individuals at risk for disseminated BCG disease.

Acknowledgments

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References