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Ineffectiveness and Toxicity of BCG Vaccine for the Prevention of Recurrent Genital Herpes

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One hundred fifty-five patients with genital herpes were enrolled in a double-blind, placebo-controlled trial comparing 0.1 ml of intradermal BCG vaccine with placebo for the prevention of recurrent episodes of genital herpes. The mean rate or recurrence over 9 months of prospective follow-up was 0.528 recurrences per month in BCG recipients compared with 0.392 recurrences per month in placebo recipients (not significant). The BCG vaccine also failed to influence the duration of lesions in the first recurrent episode of genital herpes after vaccination. Six patients were given a second inoculation of BCG vaccine, and persistent cutaneous granulomas were noted in three of these six patients. Intradermal inoculation with BCG does not appear to affect the natural history of genital herpes, and repeated inoculations can be toxic.

The major concern of patients with genital herpes simplex virus (HSV) infection is the frequent recurrence of local genital lesions and symptoms and the risk of transmission to sexual partners and neonates (10, 15, 17). HSV has been shown to cause latent infection in sensory ganglion cells, but the pathogenesis of recurrent infection remains undefined (4). Patients with disorders of cell-mediated immunity have been shown to develop unusually severe primary and recurrent mucocutaneous HSV infections and to shed HSV in oral and genital sites more frequently and longer than patients with normal cellular immune responses (14). Vaccination with bacillus Calmette-Guerin (BCG), an attenuated strain of *Mycobacterium bovis*, produces nonspecific activation of macrophages (8, 11) and, in combination with anti-HSV type 2 antibody, has been shown to protect mice from vaginitis, posterior paralysis, and encephalitis after intravaginal challenge with HSV type 2 (3). Previous uncontrolled studies in patients with recurrent genital HSV infections have described variable results in the reduction in frequency of episodes of genital herpes after the receipt of BCG vaccine (1, 7). This study was undertaken to assess in a double-blind, randomized, placebo-controlled trial the effect of BCG vaccination upon the rate of recurrence as well as the rate of healing of the next episode of genital HSV infection after inoculation.

MATERIALS AND METHODS

Study population. Patients were enrolled at the Harborview Medical Center Herpes Research Clinic, Seattle, Wash. All patients had culture-proven symptomatic genital herpes before enrollment in the study and were in good general health. All women were not pregnant and were practicing an adequate method of contraception.

Study design. After informed consent was obtained, a standardized medical history was administered, a physical exam was performed, and intradermal skin tests with purified protein derivative (5 tuberculin units), mumps antigen (Lederle Laboratories, Pearl River, N.Y.), *Candida* sp. antigen (Hollister Stier Co., Spokane, Wash.), *Trichophyton* sp. antigen (Hollister Stier Co.), and SK-SD (10 to 40 U; Lederle) were applied. Patients returned at 48 h for reading of skin tests. Those with at least one positive skin test to mumps, *Candida* sp. *Trichophyton* sp., or SK-SD and in whom the Mantoux tests had induration of less than 5 mm in diameter were then randomized into the study. For randomization, patients were stratified by sex and by whether they had a history of multiple prior episodes of genital herpes (recurrent infection) or only one prior episode (post-first episode infection) into receipt of vaccine or placebo (*Candida* sp. skin test antigen). In addition, women were also stratified as to whether or not they were using oral contraceptives.

At the initial treatment visit, 0.1 ml of BCG or 0.1 ml of *Candida* sp. skin test antigen was administered intradermally. All vaccinations were performed between recurrent episodes of disease by a clinician who was not involved in the subsequent prospective follow-up of the patient. *Candida* sp. skin test antigen was selected so that all patients would experience some local reaction after vaccination, and all patients were instructed that the vaccination response would vary from mild erythema at the injection site to the development of an indurated papule or scab. The vaccine was administered in the deltoid area in 104 patients and in the thigh area, in an attempt to accentuate the regional immune response, in 51 patients. BCG vaccine was prepared by Glaxo Pharmaceuticals (lot no. 514) and was supplied as a gift from the Eli Lilly Co., Indianapolis, Ind. (courtesy of Richard Griffith). Titration of the vaccine on Lowenstein medium at the beginning and midportion of the study revealed 2×10^6 viable bacilli per ml of vaccine.

After inoculation, patients were seen at scheduled follow-up visits at 6 weeks and at 3, 6, 9, and 12 months and also during recurrences of genital herpes. At each follow-up visit they completed a standardized interview regarding recurrences of herpes and possible adverse reactions to BCG. In addition, at the initial and routine visits, blood was obtained for a complete blood cell count and leukocyte count differential, alanine amino transferase, alkaline phosphatase, and

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TABLE 1. Demographic and clinical characteristics of the study population

Characteristic	BCG recipients (n = 83)	Placebo recipients (n = 72)
No. of men	40	32
No. of women	43	40
Mean age (yr)	27.3 (±6.8)[a]	27.0 (±4.9)
No. of whites	82	70
No. married	11	11
No. of women using oral contraceptives	17	23
No. with arm injection	53	51
No. with thigh injection	30	21
History of oral herpes	21	20
No. enrolled after first episode of genital herpes	12	9
No. enrolled with history of recurrent genital herpes	71	63
Mean no. of months since first episode (patients with history of recurrences)	26.8 (±28)	23.8 (±22.9)
No. of prior episodes of genital herpes (patients with history of recurrences)	10.4 (±9.5)	13.2 (±11.6)
Recurrence rate (recurrences per 30 days) before enrollment (patients with history of recurrences)	0.608 (±0.412)	0.747 (±0.502)

[a] Numbers within parentheses indicate standard deviations.

bilirubin. During recurrences of disease, a genital examination was performed, and a standardized interview evaluating the severity and duration of local and systemic symptoms was conducted. All suspicious lesions were cultured for HSV. Skin tests, including reapplication of the purified protein derivative tests, were repeated at the 3-month visit. The mean number of visits during the course of this study was 6.52 in BCG recipients and 6.23 in placebo recipients.

After completion of 1 year of follow-up, 19 patients who felt that they had benefited from the original inoculation were revaccinated with either drug or placebo according to their original randomization schedule. Thirteen were revaccinated with *Candida* sp. antigen, and six received BCG vaccine, with 0.5 ml given subcutaneously in each thigh. Follow-up over the subsequent 6 months was similar to that described for the first year.

Laboratory methods. Viral isolation procedures were performed as previously described (16). Herpes simplex virus was identified by typical cytopathic effect in diploid fibroblast cell cultures. The first genital HSV isolate was typed by an indirect immunoperoxidase technique (6).

Statistical analyses. Demographic characteristics of BCG and placebo recipients were compared with use of the chi-square statistic for discrete variables (such as percent married) and the two-sample t-test for continuous variables (such as age). Comparison of days from prior recurrence to vaccination and days from vaccination to next recurrence was accomplished with the log-rank (Mantel-Cox) test. The Wilcoxon test was used to assess treatment effects on rate of recurrence, number of lesions, and duration of itching, pain, and lesions for the first episode after treatment. Paired Wilcoxon statistics were used for pre- versus postvaccination comparisons. Data after revaccination for the 19 patients who received second courses of therapy were analyzed separately.

RESULTS

One hundred fifty-five persons with culture-proven genital HSV infection were enrolled in the study (Table 1); 72 men and 83 were women, of whom 40 used oral contraceptives. The mean age of patients was 27.2 years; 99% were Caucasian, and 14% were married. Twenty-one had a history of only one prior episode of genital HSV, whereas 134 had had recurrent episodes. The mean rate or recurrence in the latter group before the onset of the study was 0.608 episodes per month for men and 0.699 episodes per month for women.

Eighty-three patients, 40 men and 43 women, received injections of BCG, 53 in the arm and 30 in the thigh. Thirty-nine BCG recipients responded to vaccination by forming a scab at the inoculation site (32 patients), converting their PPD reading to positive (≥5-mm induration at 3 months after vaccination (4 patients), or both (3 patients).

Effect of BCG on subsequent rate of recurrence. The mean duration of follow-up was 296 days (±114 days, standard deviation) in BCG recipients and 291 days (±110 days) in placebo recipients (Table 2). The median time to the first recurrence after vaccination was 30 days in BCG recipients compared with 43 days in placebo recipients. The median time from the last recurrence before enrollment until vaccination, however, was significantly longer in BCG recipients (32 days) than in placebo recipients (19 days); therefore, the total time from the last episode pre-inoculation until the first episode post-inoculation was identical between the two groups (62 days) (Table 2). The mean rate of recurrence during the study period was 0.528 recurrences per 30-day period in BCG recipients compared with a rate of 0.392 recurrences per 30-day period for placebo recipients (P = 0.08).

TABLE 2. Effect of BCG on subsequent recurrence rate

Parameter	BCG recipients (n = 83)	Placebo recipients (n = 72)
Median days from prior recurrence to vaccination	32[a]	19
Median days to first recurrence after vaccination	30[b]	43
Mean days followed after vaccinations	296	291
Mean no. of recurrences per 30 days after vaccination (all patients)	0.528	0.392
Mean no. of recurrences per 30 days before vaccination (patients with history of recurrences)	0.608	0.747
Mean no. of recurrences per 30 days after vaccination (patients with history of recurrences)	0.532[c] (n = 71)	0.357 (n = 63)
Mean no. of recurrences per 30 days after vaccination (patients vaccinated after their initial episode of disease)	0.499 (n = 12)	0.638 (n = 9)
Mean no. of recurrences per 30 days after arm vaccination (all patients)	0.441	0.372
Mean no. of recurrences per 30 days after thigh vaccination (all patients)	0.687[b]	0.441

[a] P <0.01, for comparison between BCG and placebo recipients.
[b] P <0.05, for comparison between BCG and placebo recipients.
[c] P <0.002 for comparisn or change from pre- to posttreatment recurrence rates in BCG recipients versus placebo recipients.

significant differences in recurrence rates were noted between BCG and placebo recipients, between men and women, between those who received vaccine after their first episode of disease or after multiple recurrences of disease, between those BCG recipients who formed scabs or had purified protein derivative conversions and those who did not, or between women who did and did not use oral contraceptives. In BCG recipients there was a trend toward a lower rate of recurrence in those inoculated in the deltoid area than in those who received thigh inoculations, 0.441 versus 0.687 ($P = 0.06$), respectively. Among patients with a history of recurrent episodes of genital HSV infection, the mean rates of recurrence before and after vaccination were 0.608 and 0.532, respectively, for BCG recipients, versus 0.747 and 0.357, respectively, for placebo recipients. The change in the mean rate of recurrence from pre- to postvaccination was significantly less in BCG recipients than in placebo recipients ($P < 0.001$). Fifteen of the 19 patients (5 BCG recipients and 10 placebo recipients) who were revaccinated after 1 year were followed for a median of an additional 14.7 months. The mean rate of recurrence during the second year was 0.962 recurrences per 30-day period in the BCG recipients and 0.446 recurrences per 30-day period in the placebo recipients (not significant).

Effect of BCG on manifestations of the first episode post-inoculation. To evaluate the effect of BCG vaccine on manifestations of recurrent episodes of disease, the signs and symptoms of the first clinical episode after vaccination were compared for BCG and placebo recipients (Table 3). In men, no significant differences in the mean duration of itching or pain or in the mean number or duration of lesions were noted between BCG and placebo recipients. In women, although there were no significant differences between BCG and placebo recipients in mean number or duration of lesions, BCG-treated patients did report a significantly shorter duration or itching, 1.2 versus 4.6 days ($P < 0.001$), and pain, 2.1 versus 4.5 days ($P = 0.01$), than did their placebo-treated counterparts. The durations of the recurrent episodes immediately before and after therapy were also evaluated. Among BCG recipients ($n = 48$), the mean duration of lesions in the episode before enrollment was 8.1 days compared with 7.0 days for the first episode after vaccination (not significant). Among placebo recipients ($n = 42$), the mean durations were 6.6 and 6.8 days, respectively (not significant).

Adverse effects. No significant adverse effects of BCG vaccine were noted in those who received single arm or thigh inoculation. Three of six persons who received a second BCG vaccination, however, developed large draining granulomas at or near the site of inoculation in the thigh, with inguinal lymphadenopathy. These lesions persisted for several weeks to months, and one patient required reconstructive surgery for cosmetic reasons. No significant hematological or hepatic abnormalities could be ascribed to either initial or recurrent BCG vaccination.

TABLE 3. Effect of BCG on first episode posttreatment

Sex of patients	Treatment	Mean no. of days (n)			Mean no. of lesions (n)
		Itching[a]	Pain[a]	Lesions	
Male	BCG	1.8 (33)	1.9 (32)	8.2 (33)	6.3 (27)
	Placebo	1.9 (23)	1.0 (22)	7.2 (21)	5.8 (22)
Female	BCG	1.2 (25)[b]	2.1 (25)[c]	5.6 (30)	2.5 (27)
	Placebo	4.6 (25)	4.5 (26)	6.1 (29)	3.0 (24)

[a] Analysis was limited to those with itching or pain present at the onset of the episode.
[b] $P < 0.001$, for difference between BCG and placebo recipients.
[c] $P = 0.01$, for difference between BCG and placebo recipients.

DISCUSSION

Our results indicate that BCG vaccination does not increase the time to subsequent clinical recurrence or decrease the rate of recurrence of genital HSV infection. This lack of efficacy was observed with both proximal-site (thigh) and distal-site (arm) inoculation. Moreover, three of the six patients whom we treated with a second course of BCG developed cutaneous granulomas that were both painful and disfiguring. Although such lesions have become an expected result of BCG immunotherapy of malignant disease (2, 5), the chronic draining lesions were felt to be an unacceptable toxicity of therapy by all three of the affected patients.

The one statistically significant benefit of BCG vaccination that we observed was a reduction in the reported duration of pain and itching associated with the first recurrence after inoculation in women. These data may suggest that nonspecific stimulation of cell-mediated immunity is important in reducing the severity of disease even if it is not effective in preventing reactivation from the latent state. However, the fact that we were unable to note such an effect in men and that no significant reduction in the duration of the episode was achieved suggests that this positive effect, if real, is of limited clinical significance. Alternatively, it may be that, in the assessment of multiple clinical parameters, the effects of BCG on the duration of pain and itching occurred by chance alone.

One of the more interesting outcomes of this trial was the observation that, in the placebo group, the rate of prospectively recorded recurrences, 0.357 recurrences per 30 days, was significantly less than the rate reported before enrollment in the study, 0.747 recurrences per 30 days ($P < 0.001$). Although these data suggest a reduction in the frequency of recurrent genital herpes over time, our population included a selected group of patients with frequent recurrences whose prestudy recurrence rate was determined from patient history. The observed differences, therefore, may have been due to patient selection, i.e., greater likelihood of volunteering for the study in those patients who had by chance been temporarily experiencing more frequent recurrences or retrospective overestimation of past recurrences. To chart more accurately the long-term natural history of the disease, prospective follow-up of a large number of patients being followed from the first episode of disease over time is needed. Our results, however, indicate that studies evaluating the rate of recurrence of genital HSV infection must consider the potential for varying or decreasing rates of recurrence over time. As such, comparing recurrence rates in any one individual before and after therapy may only reflect the natural history of infection rather than the effect of therapy on the long-term course of disease.

There are several possible explanations for our inability to demonstrate a beneficial effect of BCG on the rate of recurrence of genital HSV infection. Our dose and preparation of BCG may not have been immunologically optimal. Previous studies have indicated that the immunogenicity of BCG as measured by lymphocyte uptake of tritiated thymidine in lymph nodes is dependent upon the viability, dose of inoculation, and virulence of the strain of BCG (13). When compared with other strains of BCG, the Glaxo strain used in our study induced a 25% lower peak response ([^{3}H]thymidine uptake at 16 days) than did Montreal, Phipps, and Tice strains and 50% lower than did the Pasteur

strain (13). Each of the two previous uncontrolled studies of BCG and genital herpes in humans used Glaxo vaccine (1, 7). Anderson et al. (1) used a dose identical to that employed in our study, 0.1 ml of intradermal vaccine, whereas Bierman et al. (7) used multiple 1.0-ml doses. Cutaneous responses to BCG vaccine in our study as measured by purified protein derivative conversion or development of a scab at the vaccination site occurred in only 39 of our 83 patients, significantly less than that reported by Anderson and Bierman. Although multiple doses of BCG may have produced more durable immunity as measured by skin test conversion, the toxicity that we encountered with sequential doses of the vaccine made this approach unaceptable to our patients.

Alternatively, BCG vaccination may actually have had a deleterious effect on the subsequent course of recurrent genital herpes. Studies in mice have indicated that BCG vaccination can induce in vitro suppression of spleen cell responses to mitogens and alloantigens (9, 11, 12) and, in mice lacking HSV antibody, can enhance neurovirulence of HSV-2 (3). Our results indicated trends toward higher prospectively observed rates of recurrence in BCG over placebo recipients, proximally over distally vaccinated BCG recipients, and revaccinated over singly vaccinated BCG recipients, which may suggest that the effect of BCG on host cell-mediated immune response to recurrent genital HSV infection is suppressive rather than enhancing.

In summary, this double-blind, placebo-controlled trial suggests no significant clinical benefit in reducing the rate of recurrence of genital herpes infection by intradermal inoculation with one 0.1-ml dose of Glaxo strain BCG vaccine. Repeated doses of vaccine were associated with unacceptable side effects. Further studies of immunotherapy for HSV infections will necessitate more acceptable therapeutic ratios and may require more specific immunomodulation of the cell-mediated immune system, especially with respect to increasing the antigen-specific immune responses.

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LITERATURE CITED

1. **Anderson, F. D., R. N. Ushijima, and C. L. Larson.** 1974. Recurrent herpes genitalis: treatment with *mycobacterium bovis* (BCG). J. Obstet. Gynecol. 43:797–804.
2. **Aungst, C. W., J. E. Sollal, and B. V. C. Jager.** 1975. Complications of BCG vaccinations in neoplastic disease. Ann. Intern. Med. 82:666–669.
3. **Baker, M. B., C. L. Larson, R. N. Ushijima, and F. D. Anderson.** 1974. Resistance of female mice to vaginal infection induced by herpesvirus hominis type 2: effects of immunization with *mycobacterium* bovis, intravenous injection of specific *Herpesvirus hominis* type 2 antiserum, and a combination of these procedures. Infect. Immun. 10:1230–1234.
4. **Barringer, J. R.** 1981. Latency of herpes simplex and varicella zoster viruses in the nervous system, p. 202–205. *In* A. J. Nahmias, W. R. Dowdle, and R. F. Schinazi (ed.), The human herpesvirus. Elsevier-North Holland Publishing Co., New York.
5. **Bast, R. C., B. Zbar, T. Borsos, and H. H. Rapp.** 1974. BCG and cancer. N. Engl. J. Med. 290:1413–1420, 1458–1469.
6. **Benjamin, D. R.** 1977. Use or immunoperoxidase for rapid diagnosis of mucocutaneous herpes simplex virus infection. J. Clin. Microbiol. 6:571–573.
7. **Bierman, S. M.** 1976. BCG immunoprophylaxis of recurrent herpes genitalis. Arch. Dermatol. 112:1410–1415.
8. **Biozzi, G., C. Stiffel, B. N. Halpern, and D. Mouton.** 1960. Recherches sur le mecanisme de l'immunite non specifique produite par les mycobacteries. Rev. Franc. Etudes Clin. Biol. 5:876–890.
9. **Brown, C. A., I. N. Brown, and V. S. Sljivic.** 1979. Suppressed or enhanced antibody responses *in vitro* after BCG treatment of mice: importance of BCG viability. Immunology 38:481–488.
10. **Corey, L., H. G. Adams, Z. A. Brown, and K. K. Holmes.** 1983. Genital herpes simplex virus infection: clinical manifestations, course and complications. Ann. Intern. Med. 98:958–972.
11. **Florentin, I., R. Huchet, M. Bruley-Rosset, O. Halle-Pannenko, and G. Mathe.** 1976. Studies on the mechanism of action of BCG. Cancer Immunol. Immunother. 1:31–39.
12. **Klimpel, G. R., and C. S. Henney.** 1978. BCG-induced suppressor cells: demonstration of a macrophage-like suppressor cell that inhibits cytotoxic T cell generation *in vitro*. J. Immunol. 120:563–569.
13. **Mackaness, G. S., D. J. Auclair, and P. H. Langrange.** 1973. Immunopotentiation with BCG. Immune response to different strains and preparations. J. Natl. Cancer Inst. 51:1655–1663.
14. **Muller, S. A., E. C. Herrmann, and R. K. Winkelmann.** 1972. Herpes simplex infections in hematologic malignancies. Am. J. Med. 52:102–114.
15. **Reeves, W. C., L. Corey, H. G. Adams, L. A. Vontver, and K. K. Holmes.** 1981. Risk or recurrence after first episodes of genital herpes: relation to HSV type and antibody response. N. Engl. J. Med. 305:315–319.
16. **Wentworth, B. B., P. Bonin, K. K. Holmes, and E. R. Alexander.** 1973. Isolation of viruses, bacteria and other organisms from venereal disease clinic patients: methodology and problems associated with multiple isolation. Health Lab. Sci. 10:75–81.
17. **Whitley, R. J., A. J. Nahmias, A. M. Visintine, C. L. Fleming, and C. A. Alford.** 1980. The natural history of herpes simplex virus infection of mother and newborn. Pediatrics 66:489–494.