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Effect of Macrophage Activation on Infection with *Treponema pallidum*

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Infection of rabbits with *Treponema pallidum* induces nonspecific acquired cellular resistance (ACR) to *Listeria monocytogenes*. This resistance can be adoptively transferred using thymus-dependent lymphocytes. Since infections that induce ACR are usually brought under control by cellular mechanisms, we sought to determine whether induction of ACR in rabbits stimulates resistance to challenge with *T. pallidum*. When BCG-infected rabbits which suppressed the growth of *Listeria* were challenged intravenously with *T. pallidum*, lesions appeared at the same time and progressed in a fashion similar to that in non-BCG-infected controls. There was a tendency for syphilitic lesions to disseminate more widely in BCG-infected animals and for the lesions to necrose more rapidly in controls. *T. pallidum* may resist phagocytosis by macrophages, as has been suggested previously, or macrophages may fail to be activated locally in the dermis. Although syphilitic infection appears to stimulate ACR, activation of macrophages may not contribute significantly to the ability of the host to suppress *T. pallidum*.

Infections caused by microorganisms that stimulate acquired cellular resistance (ACR) activate macrophages (3, 10, 13) which inhibit the growth of the homologous organism (3, 13) as well as antigenically unrelated ones (1, 3, 8, 17). Suppression of the growth of *Listeria monocytogenes* has been used to monitor the onset and waning of macrophage activation (3, 10, 17). We have recently shown that rabbits infected with *Treponema pallidum* can suppress the growth of *Listeria* (17). The enhanced listericidal activity was thought to result from nonspecific induction of ACR. However, evidence was not presented to show that ACR plays a role in infection-induced immunity to *T. pallidum*.

The purpose of the present investigation was to determine whether vaccination with BCG, a known inducer of macrophage activation (3, 10, 17), enhances resistance to infection with *T. pallidum*.

MATERIAL AND METHODS

Animals. Outbred New Zealand white male rabbits weighing 2 to 3 kg were housed individually in stainless-steel cages at an ambient temperature of 18 C, a condition which facilitates the development of syphilitic lesions (25). Rabbits with a positive Venereal Disease Research Laboratory reaction were excluded because of the possibility that this resulted from a subclinical infection with *T. cuniculi*.

Organisms. *T. pallidum* (Nichols strain) was maintained by intratesticular passage in rabbits. Inflamed testes were removed aseptically, minced in sterile saline, and ground with sand using a mortar and pestle. After centrifugation at 270 × *g* for 3 min to remove cellular debris, the number of treponemes in the supernatant was determined by dark-field microscopy. Rabbits used in this investigation were infected intravenously (i.v.) with 10^7 motile *T. pallidum*, or intradermally (i.d.) with graded doses ranging from 10^3 to 10^6 motile organisms.

Listeria monocytogenes. *Listeria* was isolated from a patient with meningitis and passed three times in rabbits. Preparation and storage of a standard inoculum for challenge has been described previously (18). Rabbits were challenged i.v. with 3×10^8 organisms, and 30 to 32 h later they were asphyxiated with carbon dioxide. The livers and spleens were removed aseptically and homogenized; dilutions were made, and aliquots were plated on brain heart infusion agar. Colonies were counted after 24 h of incubation at 35 C.

BCG. Strain BCG-Tice [lot IL 74 (s) 11] was obtained from the Research Foundation, University of Illinois, Chicago, Ill. Rabbits were vaccinated i.v. with 3×10^7 viable BCG organisms. The number of viable BCG was verified by serially diluting the inoculum after vaccination and plating aliquots on medium which contained Middlebrook enrichment. Plates were incubated in a CO_2-enriched environment at 35 C for 2 weeks before colonies were counted.

Methods for evaluating the influence of BCG

vaccination on syphilitic infection. Rabbits that are shaved and infected i.v. with 10^7 viable *T. pallidum* develop disseminated cutaneous lesions 17 to 21 days after infection (18, 25). The cutaneous lesions enlarge and become necrotic during the following 7- to 14-day period and thereafter regress. Orchitis is present transiently, appearing 24 to 28 days after i.v. challenge and spontaneously regressing within 1 to 3 days. The course of syphilitic infection was observed by daily examination of each rabbit. Rabbits were shaved two to three times weekly throughout the time of observation. The time that lesions first appeared was recorded, and the size and appearance of cutaneous lesions was noted. In addition, in the final two experiments photographs of every rabbit were taken once or twice each week. At the end of each study the photographs were coded and graded by the investigators for the severity of lesions and the extent of dissemination. A numbering system was devised, assigning the highest number to the animal with the severest lesions and the lowest to the one with least severe lesions. The code was then broken, and results were tabulated for each experimental group.

Syphilis-immune rabbit serum (SIRS). Rabbits were infected i.v. with 10^7 *T. pallidum*, and disseminated syphilitic lesions were allowed to develop and regress spontaneously. These rabbits were shown to be immune to rechallenge i.v. with *T. pallidum*. SIRS from several such rabbits was pooled and heated for 30 min at 56 C.

Randomization of animals. In each phase of these studies, rabbits were randomly selected for vaccination, challenge with *Listeria*, infection with *T. pallidum*, and sacrifice. Each experimental group contained four rabbits.

Statistical analysis. The analysis of variance was used. Fisher's least significant difference test (21) was used to examine pairs of means when a significant F ratio indicated reliable mean differences. The alpha level was set at 0.05 prior to the initiation of the experiments.

RESULTS

The fate of Listeria in rabbits vaccinated with BCG. The purpose of this experiment was to determine whether BCG induces a period of enhanced listericidal activity sufficient to cover the incubation and development of disseminated syphilitic lesions (17 to 21 days). Ninety-two tuberculin-negative rabbits were randomly divided into two groups of which forty-six were vaccinated i.v. with 3×10^7 viable BCG. At weekly intervals three vaccinated and three control rabbits were challenged i.v. with 3×10^8 *Listeria*. After 30 to 32 h the rabbits were sacrificed, and the number of *Listeria* in the livers and spleen was determined. Enhanced listericidal activity was detected in the livers and spleens of BCG-vaccinated rabbits from 3 to 7 weeks ($P < 0.001$) after vaccination (Fig. 1 and 2). The period of maximum listericidal activity occurred 3 or 4 weeks after vaccination and thereafter waned rapidly. A second injection of 10^7 BCG i.v. prolonged the period of maximum listericidal activity to 5 to 6 weeks (Fig. 1 and 2), a period sufficient to span the

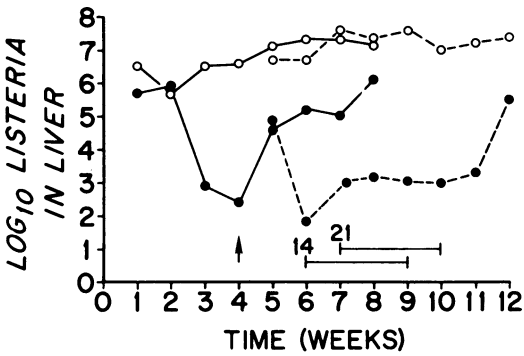

FIG. 1. *Growth of Listeria in the livers of control rabbits* (○——○) *and rabbits vaccinated with BCG* (●——●). *At weekly intervals three control and three BCG-vaccinated rabbits were challenged with Listeria and sacrificed 30 to 32 h later. The time of the second injection of BCG is indicated by an arrow. The growth of Listeria in the livers of rabbits that received a second injection of BCG and uninfected controls is denoted by* (●——●) *and* (○——○), *respectively. The bar lines* (|——|) *denote the 4-week periods that would allow for evolution of syphilitic lesions after challenge on day 14 or 21 after the second BCG injection. The standard error associated with each mean was 0.37.*

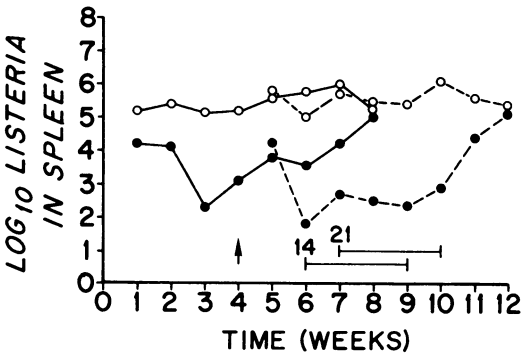

FIG. 2. *Growth of Listeria in the spleens of control rabbits* (○——○) *and rabbits vaccinated with BCG* (●——●). *At weekly intervals three control and three BCG-vaccinated rabbits were challenged with Listeria and sacrificed 30 to 32 h later. The time of the second injection of BCG is indicated by an arrow. The growth of Listeria in the spleens of rabbits that received a second injection of BCG and uninfected controls is denoted by* (●——●) *and* (○——○), *respectively. The bar lines* (|——|) *denote the 4-week periods that would allow for evolution of syphilitic lesions after challenge on day 14 or 21 after the second BCG injection. The standard error associated with each mean was 0.32.*

time of incubation and development of syphilitic lesions following i.v. injection with *T. pallidum*.

The influence of BCG vaccination on syphilitic infection. The purpose of this experiment was to determine whether BCG-vaccinated rabbits were resistant to infection with *T. pallidum* during the period of enhanced listericidal activity. Four rabbits were challenged i.v. with 10^7 *T. pallidum* 14 to 21 days after having received a second injection of BCG. Four control rabbits that had not been vaccinated with BCG also received *T. pallidum* i.v. In the initial three experiments no differences were thought to have been present in the time of appearance of disseminated lesions, their number, or their progression. In the final two experiments, in which photographs were available for comparison at the end of the study, syphilitic lesions appeared at the same time in BCG-infected and control animals but subsequently seemed to disseminate more widely in those that had received BCG (Fig. 3). Interestingly, as the lesions progressed syphilitic lesions appeared to be more necrotic in control rabbits (Fig. 4). Orchitis developed at the same time in BCG-infected and control rabbits. After i.d. challenge chancres appeared at the same time in both groups of rabbits (Fig. 5) and appeared to be equally severe in both groups.

The interaction between macrophage activation and humoral factors. Because of the possibility that acquired cellular resistance plays a role in suppressing *T. pallidum* only in the presence of humoral factors, two additional sets of experiments were carried out. *T. pallidum* was incubated at 37 C for 50 min in heat-inactivated SIRS; loss of viability was not detected by dark-field microscopy. An inoculum of 10^7 treponemes was injected i.v. into rabbits that had received two injections of BCG. Control rabbits that had not been vaccinated with BCG were injected i.v. with 10^7 *T. pallidum* that had or had not been incubated in SIRS. The course of syphilitic infection was identical in all three groups. In the second set of experiments 10 ml of SIRS was injected i.v. into four BCG-vaccinated and four control rabbits. Twenty-four hours later all eight animals were challenged i.v. with 10^7 *T. pallidum*. In two separate experiments no differences were observed in the progression of syphilitic lesions in control versus the BCG-infected group.

DISCUSSION

The nature of the immune response to infection with *T. pallidum* has not been elucidated. Although a number of observations have favored a role for cell-mediated immunity (18, 24, 26, 27), the only data which directly implicate it are those which show that delayed hypersensitivity to treponemal antigens appears late in secondary syphilis and is regularly present in latent and tertiary infections (4, 11, 23). We have shown that syphilitic infection stimulates nonspecific resistance to *Listeria* (18) and that this resistance can be adoptively transferred using lymphocytes (19, 20). Prior exposure of syphilitic donor lymphocytes to anti-thymus serum abrogates this response (20). These observations are significant in that, almost without exception, infections that induce ACR such as tuberculosis, listerosis and brucellosis are also brought under control by cellular mechanisms (3, 5, 7, 13, 16). The purpose of this present investigation was to determine whether induction of ACR increases resistance to infection with *T. pallidum*.

Our results show that vaccination with BCG induced ACR in rabbits as determined by enhanced ability to suppress the growth of *Listeria* in the livers and spleens. Rabbits challenged with *T. pallidum* during this period of ACR developed disseminated cutaneous lesions and orchitis at the same time as nonvaccinated controls. Enhanced resistance to syphilis was not detected in BCG-infected animals. In fact, in two experiments syphilitic lesions actually appeared to disseminate more widely in BCG-infected rabbits than in controls. The tendency for syphilitic lesions to become more necrotic in control rabbits may have reflected the ability of these animals to mount a more intense local immune response. Because of the possibility that activated macrophages are effective only in the presence of humoral factors, we incubated *T. pallidum* with immune rabbit serum before i.v. injection into BCG-vaccinated rabbits, or injected immune serum into BCG-vaccinated rabbits just before infecting them with *T. pallidum;* these maneuvers did not alter the course of syphilitic infection. These results suggest that even though ACR is detected in rabbits that are actively infected with *T. pallidum* (18), cellular mechanisms may not be the primary means by which rabbits develop infection-immunity to *T. pallidum*.

One interpretation of these experiments may be, as has been previously suggested (24, 27), that *T. pallidum* is resistant to phagocytosis even in the presence of immune serum. Although we have demonstrated that macrophages phagocytize avirulent treponemes in vitro (12), conflicting observations have been made on whether virulent *T. pallidum* are

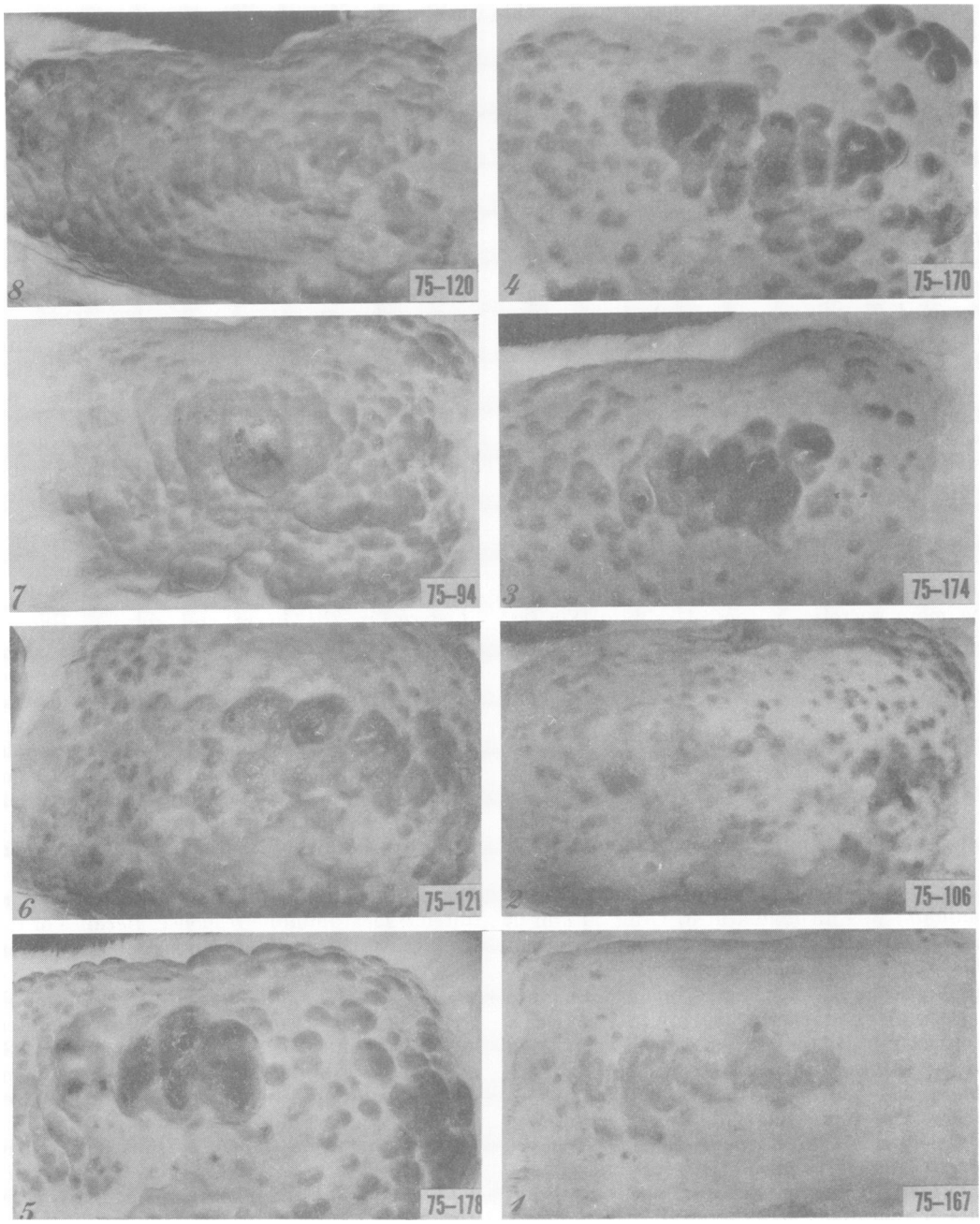

FIG. 3. *Photographs of all eight rabbits from one experiment, taken 26 days after i.v. challenge with T. pallidum, were coded and graded in decreasing order of severity of dissemination of syphilitic lesions. The rabbit with the most widespread lesions received a grade of 8, the next most severe 7, etc. BCG-infected animals (75-121, 75-120, 75-94, and 75-106) had received two injections of BCG 14 days apart; control animals (75-170, 75-178, 75-174, and 75-167) received no BCG. Scoring based on these photographs was 26 for BCG-infected and 10 for control rabbits. This result suggested greater dissemination of syphilitic lesions in BCG-infected animals.*

phagocytized or merely have the ability to penetrate cells in vivo (2, 9, 14, 22). In any case, the role of phagocytosis by macrophages in vivo remain unclear. The failure of infection with BCG to protect rabbits against challenge with *T. pallidum* may suggest that activation of

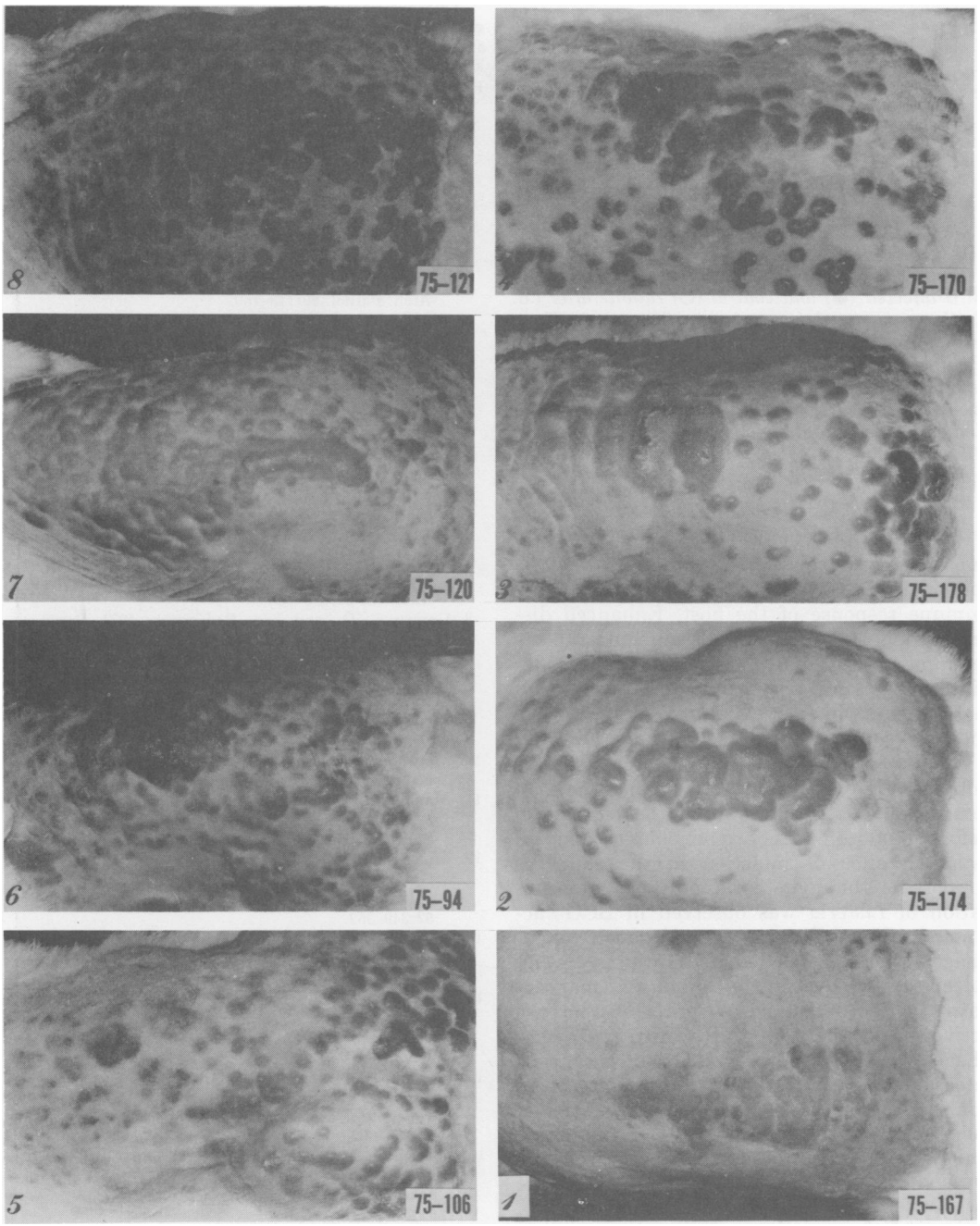

FIG. 4. *The same rabbits were photographed 5 days later (31 days after i.v. challenge with T. pallidum) and again arranged in decreasing order of dissemination of lesions. The order has now changed slightly. Scoring based on these photographs were 23 and 13, respectively, for BCG-infected and control animals, again suggesting more widespread dissemination in BCG-infected rabbits. This figure also shows that necrosis was prominent only in three rabbits, all of which were controls.*

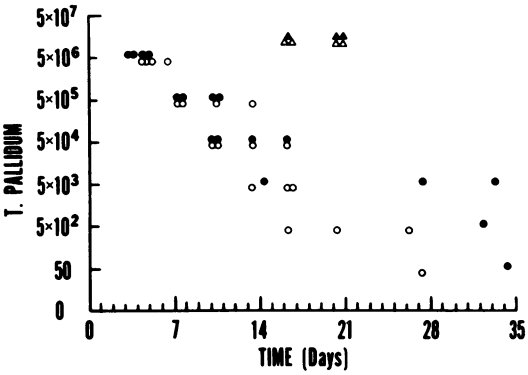

FIG. 5. *The time of appearance of chancres in BCG-infected (●) and control (○) rabbits after i.d. challenge is noted for each inoculum used. These results from one study are interpreted as showing no significant differences between the two groups of animals, a degree of spread appearing only in the lower inocula. The time that disseminated lesions first appeared in rabbits following i.v. challenge with 10^7 T. pallidum is noted for BCG-infected (▲) and control (△) animals.*

macrophages during syphilitic infection may be coincidental, having no important role in the immune response of the host. Enhanced dissemination of lesions in BCG-infected rabbits remains entirely unexplained. Generalized debilitation was not responsible because BCG-infected rabbits in each experimental group were shown to suppress the growth of *Listeria*.

Another possibility is that macrophages are not activated sufficiently to enable them to remove 10^7 *T. pallidum* from the circulation before small numbers of treponemes escape and establish sites of infection in the skin. As support for this hypothesis, only partial eradication of *Listeria* was observed in BCG-vaccinated rabbits (Fig. 1 and 2). Although macrophage activation was detected in the livers and spleens of BCG-vaccinated rabbits, it may not have been accompanied by sufficiently intense activation of dermal and testicular macrophages to retard the progression of cutaneous infection or orchitis due to *T. pallidum*. An analogous situation exists in the case of dermal infection with staphylococci (6) or fungi (15) which are known to infect the skin even in the presence of demonstrated systemic levels of resistance. If, as suggested by Dannenberg (5), activation of macrophages in the skin parallels activation in liver and spleen, this would be further evidence that activation of macrophages does not prevent the evolution of syphilitic lesions.

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