

Response of Syphilitic Rabbits to Reinfection with Homologous and Heterologous *Treponema pallidum* Strains

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Rabbits infected intradermally with 10^3 *Treponema pallidum* (Melbourne 1) cells were examined for their susceptibility to reinfection with 10^2 *T. pallidum* cells (homologous or heterologous strains) at various intervals after the initial infection. At 2.5 weeks after infection, the rabbits were extremely sensitive to reinfection and developed syphilitic lesions significantly faster (i.e., shorter latent periods) than control rabbits that had not received the initial infection. This phenomenon may represent a state of immunosuppression or hypersensitivity in the infected rabbits. Whatever its etiology (at present unknown), it was a transient state since at 5 weeks after infection the rabbits were no longer different from control rabbits in their susceptibility to reinfection. They showed neither immunity (i.e., longer latent periods) nor immunosuppression or hypersensitivity (shorter latent periods) upon reinfection. At 6.5 weeks after infection, two of the three experimental rabbits were fully immune (no lesions upon reinfection), whereas the other rabbit exhibited immunosuppression or hypersensitivity upon reinfection. At 7.5 and 10 weeks after infection, all of the experimental rabbits were immune to reinfection. We conclude that syphilitic rabbits show a biphasic response to reinfection, consisting of an early phase of enhanced sensitivity to *T. pallidum* and a later phase of immunity to *T. pallidum*.

Many early studies of the immunology of experimental rabbit syphilis employed reinoculation with *Treponema pallidum* to show resistance or susceptibility to superinfection. In the first few weeks, the degree of resistance was directly related to the duration of syphilitic infection before superinfection (7, 25). Chesney (7) found that rabbits were readily superinfected up to the appearance of the first lesion but became resistant to challenge 6 to 8 weeks after initial infection. The immunity they developed was more effective against homologous strains of *T. pallidum* than heterologous strains.

Since these early studies, a pattern of intradermal inoculation has been used (24) to provide a more accurate determination of the rate of multiplication of the challenge *T. pallidum*, measured by the latent period of the experimental infection. The latent periods (the time between challenge with *T. pallidum* and first appearance of the challenge syphilitic lesions) can be used to compare the immune status of the control and infected or immunized rabbits (25).

Although evidence suggests that rabbits infected with *T. pallidum* produce a normal proliferative type of immune response (1, 2, 15, 16, 21), they are unable to completely destroy the invading bacteria (8, 22). The result is a latent infection. It has been postulated that latency results from immunosuppression (10). Fitzgerald and Johnson (11) showed that injection of sterile fluid from syphilitic rabbit testes into syphilitic rabbits with dermal lesions that were at the healing stage reactivated the lesions; this may be interpreted as immunosuppression of the healing response of the host, leading to renewed growth of *T. pallidum* in vivo.

There are many reports of immunosuppression during infection of rabbits with *T. pallidum* as demonstrated by in vitro assays of immune function. Lymphocytes from syphilitic rabbits showed decreased responsiveness to mitogens and treponemal antigens (18-20, 28); serum from syphilitic

infected rabbits inhibited in vitro transformation of rabbit lymphocytes by mitogens (17, 27, 29), and a mucopolysaccharide-like material present in *T. pallidum*-infected testes suppressed the response of normal lymphocytes to concanavalin A (5). Aberrant antibody responses in syphilitic rabbits to heterologous antigens may also represent altered immunocompetence (3, 4).

The purpose of our current investigation was to detect temporal changes in susceptibility to reinfection after an initial infection with *T. pallidum*. Were syphilis a normal bacterial infection, one might expect a gradual onset of immunity to reinfection, with gradually increasing latent periods for the reinfection lesions. Complete immunity would become manifest when reinfection did not lead to syphilitic lesions. On the other hand, if syphilis were an atypical bacterial infection, one might expect some aberration in the development of immunity, which may reflect the establishment of a latent and chronic infection. Syphilis in rabbits and humans is a chronic infection. An aberration was detected in our study, at 2.5 weeks after initial infection, when lesions with a very short latent period occurred after reinfection with *T. pallidum*.

MATERIALS AND METHODS

Rabbits. Adult male rabbits were maintained between 16 and 19°C and fed antibiotic-free food ad libitum.

Bacteria. Two strains of *T. pallidum* were used. The Melbourne 1 strain was isolated in 1977 (13) from a human anal lesion. The Nichols strain was obtained from M. Garner of the Institute of Clinical Pathology and Medical Research, Westmead, New South Wales. Both strains were maintained by intratesticular growth in rabbits. Suspensions of *T. pallidum* were obtained from rabbit testicular syphilomas after inoculation with 8×10^7 virulent organisms. The treponemes were harvested anaerobically (30) 8 to 12 days after testicular inoculation, when orchitis appeared to be fully developed. The testes were minced aseptically in the maintenance medium of Graves et al. (14) as modified by Steiner et al. (23). The concentration of the eluted treponemes was deter-

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mined in a bacterial counting chamber (Weber and Sons, Lancing, United Kingdom), using dark-field microscopy, and adjusted to 10^4 *T. pallidum* cells per ml in maintenance medium.

Initial infection of rabbits with *T. pallidum*. The hair on the lower part of the backs of normal male rabbits was removed. *T. pallidum* (Melbourne 1) ($10^3/0.1$ ml) was injected intradermally at one site only, located 15 cm anterior to the base of the tail. Control rabbits were similarly inoculated, using heat-killed (56°C ; 5 min) *T. pallidum*.

Challenge infection of normal and previously infected rabbits with *T. pallidum*. Each rabbit of a group of three was challenged with *T. pallidum* 2.5, 5.0, 6.5, 7.5, or 10.0 weeks after initial infection with *T. pallidum*. A large area of the back of each rabbit was clipped free of hair, and a grid of 16 squares (ca. 12 by 12 cm) was drawn on the skin surface. The nearest challenge sites were located ca. 5 cm anterior to the original *T. pallidum* infection site. Each challenged rabbit received 16 intradermal injections of 0.1 ml, 8 of *T. pallidum* (Nichols) (10^2 cells) and 8 of *T. pallidum* (Melbourne 1) (10^2 cells). Both strains were in suspensions obtained from fresh testicular syphilomas diluted appropriately in maintenance medium.

Control rabbits, previously inoculated with heat-killed *T. pallidum* (Melbourne) (10^3 cells, intradermally) were also challenged with *T. pallidum* (Nichols and Melbourne 1) 2.5 weeks thereafter.

Control rabbits, previously uninfected, were challenged at 2.5, 5.0, 6.5, 7.5, and 10 weeks after the initial infection of the experimental rabbits, using the same intradermal inoculation pattern. Rabbits were observed daily for lesion development. All rabbit groups (control and experimental) consisted of three adult male rabbits selected at random.

Determination of lesion formation. Lesion appearance was judged solely on the development of induration in the skin at the inoculation site. The latent period was defined as the number of days taken for inoculation sites to develop detectable indurations. A rabbit was considered to be immune if a lesion did not develop within 35 days of challenge.

Latent periods of infection doses for *T. pallidum* (Melbourne 1). Defined infecting doses (10^6 to 10 cells) of freshly isolated (hence fully virulent) *T. pallidum* were used to inoculate three normal rabbits, each at eight different intradermal sites. Lesion development was monitored daily, and a semi-log linear relationship was established between infecting dose and latent period of infection.

Statistics. Results were compared statistically with either the chi-square test or Student's *t* test (6).

RESULTS

Relationship between infection dose of *T. pallidum* (Melbourne 1) and latent period of infection. The semi-log relationship was linear within the range of infecting doses tested (10^6 to 10 cells), although inocula of 10^2 or fewer cells did not consistently produce syphilis lesions (Fig. 1). However, the 50% infective dose was below 10 treponemes for the Melbourne 1 strain.

Appearance of syphilitic lesions at inoculation sites of control and previously infected rabbits. Rabbits were initially infected with 10^3 *T. pallidum* (Melbourne 1) cells. At 2.5, 5.0, 6.5, 7.5, or 10.0 weeks after initial infection, the test rabbits were challenged with eight separate doses each of Melbourne 1 and Nichols strains, each containing 10^2 *T. pallidum* cells. The responses of these test rabbits were compared with those of uninfected rabbits challenged at the same time with inocula from the same suspensions of *T.*

TABLE 1. Appearance of syphilitic lesions at inoculation sites in control and previously infected rabbits after superinfection with *T. pallidum* at various times after initial infection

Challenge strain	Wks ^a	Previously infected rabbits ^b	Control rabbits ^c	χ^2 ^d
Melbourne 1	2.5	22/24	20/24	NS ^e
	5.0	24/24	24/24	NS
	6.5	8/24	15/24	$P < 0.05$
	7.5	0/24	24/24	$P < 0.0001$
	10.0	0/24	24/24	$P < 0.0001$
Nichols	2.5	24/24	24/24	NS
	5.0	24/24	24/24	NS
	6.5	8/24	24/24	$P < 0.0001$
	7.5	0/24	24/24	$P < 0.0001$
	10.0	0/24	24/24	$P < 0.0001$

^a Weeks after initial infection of previously infected rabbits before challenge infection.

^b Rabbits infected with *T. pallidum* (Melbourne 1) for various periods before challenge. Number of challenge sites developing indurated syphilitic lesions/number of challenge sites injected with *T. pallidum*.

^c Uninfected control rabbits challenged at the same time as the previously infected rabbits with identical inocula. Number of challenge sites developing indurated syphilitic lesions/number of challenge sites injected with *T. pallidum*.

^d Chi-square test of difference between control and previously infected rabbits.

^e NS. No significant difference.

pallidum. The lesion-site responses of these rabbits to challenge with Melbourne 1 and Nichols strains are shown in Table 1.

Infected rabbits that were challenged 2.5 or 5.0 weeks after initial infection developed lesions with an incidence not significantly different from that of uninfected controls. Rabbits challenged 6.5 weeks after initial infection developed fewer lesions than control animals ($P < 0.05$ [Melbourne 1

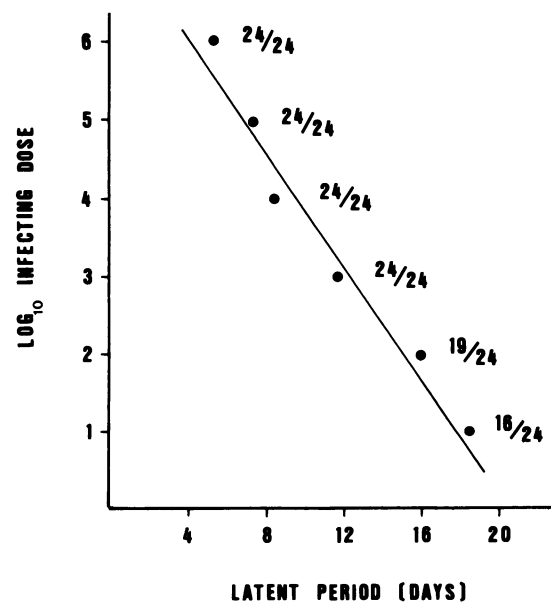


FIG. 1. Latent periods of syphilis lesions in rabbits infected intradermally with various numbers of *T. pallidum* (Melbourne 1). The latent period is the time between inoculation of *T. pallidum* and the first appearance of the lesions, as detected by induration in the skin. The fraction represents: number of inoculation sites that developed into syphilis lesions/total number of inoculation sites for a given inoculum size.

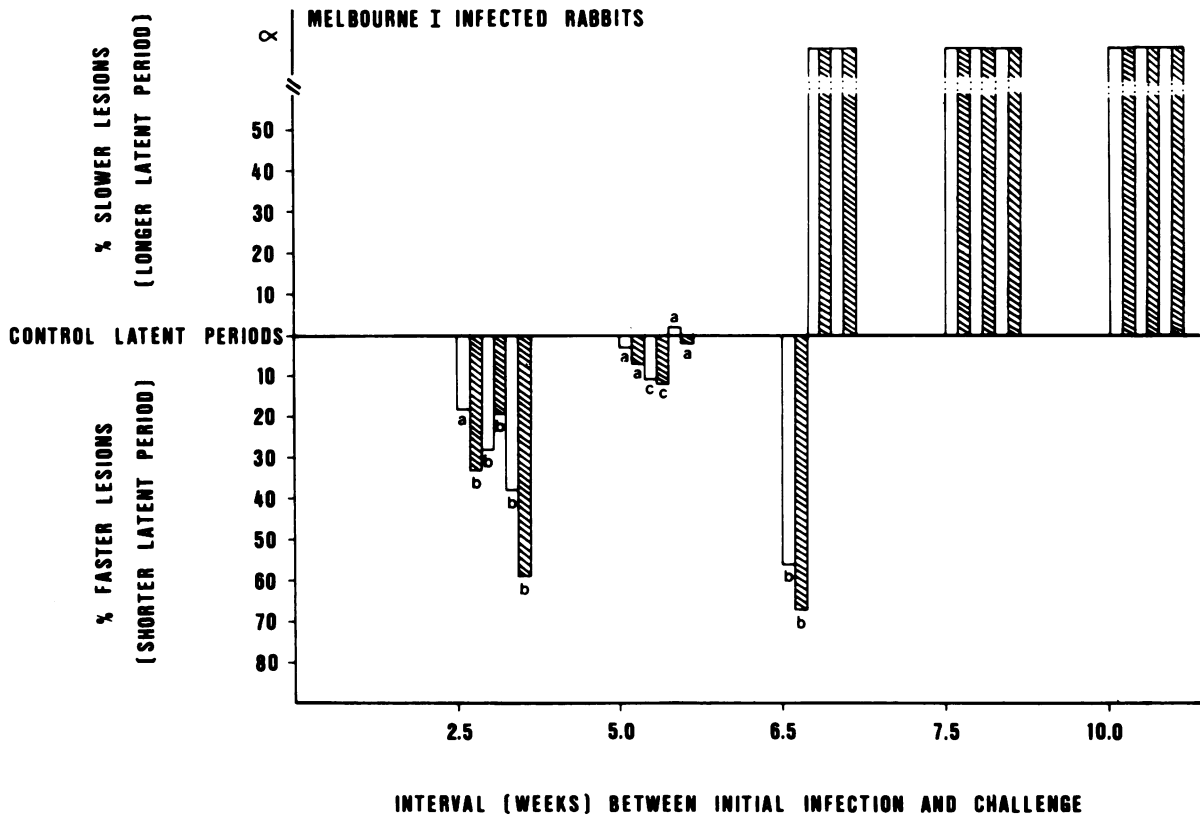


FIG. 2. Latent periods of syphilitic lesions arising from superinfection of rabbits at various times after initial infection with *T. pallidum*. Latent periods are compared with those in control rabbits (control latent periods) similarly challenged but not previously infected with *T. pallidum*. The initial infection was with 10^3 *T. pallidum* (Melbourne 1) cells (test rabbits only), and the challenge infection of both test and control rabbits was with eight intradermal injections of 10^2 *T. pallidum* cells, either Melbourne 1 strain (homologous challenge) (□), or Nichols strain (heterologous challenge) (▨). The latent periods in test animals are expressed as percent shorter or longer latent periods than controls, according to the formula: percent control latent period = $1 - (\text{mean test latent period} / \text{mean control latent period}) \times 100$. a, Difference in latent periods not significant; b, difference in latent periods significant ($P < 0.001$); c, difference in latent periods significant ($P < 0.025$); ∞, infinite latent period, i.e., no infection observed, rabbit immune to superinfection (no lesions detected within 35 days of superinfection).

challenge] and $P < 0.001$ [Nichols challenge]), and rabbits challenged 7.5 or 10.0 weeks after initial infection did not develop any lesions.

These results showed that a *T. pallidum* (Melbourne 1) infection of 6.5 weeks duration induced immunity in two of three rabbits to low-dose challenge (10^2 *T. pallidum* cells per site), whereas infection of 7.5 or 10.0 weeks duration induced immunity in all six rabbits tested.

The control rabbits inoculated with 10^3 *T. pallidum* (Melbourne 1) cells (heat killed) showed no immunity when challenged after 2.5 weeks with *T. pallidum* (Melbourne 1 or Nichols) (data not shown).

Latent periods of syphilitic lesions in control and previously infected rabbits after superinfection with *T. pallidum*. The latent periods of lesions after challenge with homologous (Melbourne 1) or heterologous (Nichols) *T. pallidum* were determined in previously infected rabbits. Results were expressed as the percent difference from the latent period in the previously uninfected control rabbits. When all inoculation sites did not develop into lesions (e.g., inocula of 10 and 10^2 cells), the latent periods of the lesions that did develop were averaged. The results are shown in Fig. 2.

Two of three rabbits challenged 2.5 weeks after initial infection with homologous *T. pallidum* developed lesions significantly faster than controls (for both, $P < 0.001$). The

third rabbit in this group was not significantly different from the controls due to the large variation in latent periods of the individual lesions. All of the three rabbits challenged at 2.5 weeks with heterologous *T. pallidum* (Nichols) developed lesions significantly faster than uninfected controls (for all, $P < 0.001$).

Two of three previously infected rabbits challenged 5.0 weeks after infection produced lesions with mean latent periods that were not significantly different from those of the uninfected controls. The third rabbit developed lesions marginally faster than controls after both homologous and heterologous challenge (for both, $P < 0.025$).

Of the three rabbits challenged 6.5 weeks after initial infection, two were immune to low-dose challenge with both homologous and heterologous strains, but one rabbit developed lesions significantly faster than the controls. The mean latent periods of the lesions in this rabbit were very short, 9.4 days for Melbourne 1 challenge and 7.2 days for Nichols challenge, compared with the respective control rabbits, with mean lesion latent periods of 21.5 and 22.5 days, respectively.

Rabbits challenged 7.5 or 10.0 weeks after initial infection were immune to the low challenge doses used.

The control rabbits inoculated with 10^3 *T. pallidum* (Melbourne 1) cells (heat killed), when challenged 2.5 weeks later

with Melbourne 1 and Nichols strains, developed lesions with latent periods that were not significantly different from those of the control rabbits that had not been previously infected with *T. pallidum* (data not shown).

DISCUSSION

It has been reported that rabbits infected with *T. pallidum* become resistant to treponemal challenge 3 months after primary infection (25). We observed partial immunity to low-dose challenge as early as 6.5 weeks after initial infection and total resistance to low-dose challenge by 7.5 weeks after primary infection.

However, at 2.5 weeks after initial infection, we detected an accelerated response to reinfection with both strains which we have called immunosuppression-hypersensitivity. At present, the etiology of this phenomenon is not understood. The phenomenon was transient, since it disappeared by 5 weeks after initial infection, although one rabbit did manifest it at 6.5 weeks after initial infection.

Accelerated responses to superinfection with *T. pallidum* were observed in an early study (31), and more recently, Fitzgerald (9) reported accelerated dermal lesions in response to infection with small numbers of *T. pallidum* when the same rabbits were also infected with larger numbers of the organism. Accelerated lesions in response to *T. pallidum* challenge were also reported in rabbits previously infected with *T. paraluis-cuniculi* (12).

It is not known whether accelerated syphilitic lesions indicate immunosuppression of the host leading to increased multiplication of the treponemes or increased hypersensitivity after the previous treponemal infection. We have shown a temporal relationship between accelerated lesions and the duration of initial syphilitic infection. Rabbits are more likely to develop lesions faster when challenged soon after the initial infection, at times corresponding to the periods after infection reported for immunosuppression, as measured by *in vitro* assays for immune function (18, 20, 28).

Reports on immunosuppression during syphilitic infection are equivocal; some investigators were unable to detect altered immunocompetence (21), whereas others demonstrated aberrations in both cell-mediated (19) and humoral (4) responses.

We favor the view that the accelerated dermal lesions probably reflect increased hypersensitivity to treponemal antigens, leading to a more rapid cellular infiltration into inoculation sites. However, this hypersensitivity may coexist with a state of immunosuppression, leading to more rapid growth of *T. pallidum* at the 2.5-week reinfection sites. Both phenomena could be mediated by a local increase in Ts (suppressor) lymphocytes at an early stage of the infection.

Accelerated dermal responses to tuberculo-protein (PPD) occur in guinea pigs with increased immunity to this antigen of *Mycobacterium tuberculosis* (26). However, in contrast to tuberculosis, expression of this postulated hypersensitivity in syphilis must be short-lived because infected rabbits challenged 5.0 weeks after initial infection responded in a manner similar to that of previously uninfected rabbits.

The temporal relationship between faster lesions and period of infection reported here was investigated in rabbits initially infected with Melbourne 1 strain organisms. When rabbits initially infected with *T. pallidum* (Nichols) were investigated, accelerated lesions were also observed, but the phenomenon was not well ordered, with considerable variation between individual rabbits (data not shown).

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