Rate of Clearance of Virulent *Treponema pallidum* (Nichols) from the Blood Stream of Normal, *Mycobacterium bovis* BCG-Treated, and Immune Syphilitic Rabbits

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The rate of clearance of virulent *Treponema pallidum* (Nichols) from the blood stream of normal rabbits and rabbits previously treated with *Mycobacterium bovis* BCG was similar, there being treponemes still circulating 8 h after intravenous inoculation. In contrast, immune syphilitic rabbits cleared the virulent treponemes within 1 to 2 hours. Rabbits with passive humoral immunity to *T. pallidum* (after the transfer of 70 ml of immune serum) showed a similar clearance rate to that of the immune rabbits. Rabbits previously treated with BCG and with passive humoral immunity did not show a synergistic enhanced clearance rate, it being similar to that of immune rabbits.

Immunity in experimental rabbit syphilis appears to be partly antibody mediated, since the transfer of passive humoral immunity before or during challenge with *Treponema pallidum* results in amelioration of the infection. Cell-mediated immunity is also involved as shown by various in vitro tests of lymphocyte function and other evidence, although a period of T-cell immunosuppression appears to be an important stage in the pathogenesis of syphilis. These matters have been well reviewed (11, 15, 17).

This experiment was carried out to compare the first 8 h of syphilis infection in rabbits with different levels of immunity to *T. pallidum*, after inoculation of virulent *T. pallidum* (Nichols) by the intravenous route. The rate of clearance of the treponemes was measured by taking blood samples from the marginal ear vein at 3, 16, and 32 min and 2, 4, 6, and 8 h after inoculation and immediately reinoculating the blood intradermally into the shaved back of normal ("indicator") rabbits. No anticoagulant was used, and only 1 or 2 min elapsed between taking the blood (0.5 ml) and the inoculation of four replicate 0.1-ml samples into the indicator rabbits. The presence of virulent *T. pallidum* in the blood of the "donor" rabbits was shown by the subsequent development of dermal syphilitic lesions in the indicator rabbits at the sites of inoculation.

Five groups of donor rabbits were used (Table 1); (i) normal rabbits, to establish a baseline clearance rate; (ii) the BCG-treated rabbits, to determine the effect of activated liver and spleen macrophages (8) on the clearance rate; (iii) rabbits with passive humoral immunity, to examine the effect of circulating humoral factors (probably mainly antibody) on the clearance rate; (iv) rabbits with combined passive humoral immunity and activated macrophages due to BCG, to see whether a synergistic enhanced clearance rate occurred; and (v) immune syphilitic rabbits, known to be resistant to challenge with *T. pallidum*, to compare their rate of clearance of treponemes with that of rabbits having only passive humoral immunity, and no cell-mediated immunity (Table 1).

Normal rabbits gradually cleared virulent treponemes from the blood (clearance time$_{50}$ = 1.5 h, where clearance time$_{50}$ is the time required for sufficient treponemes to be cleared from the rabbit circulation such that a blood sample taken at that time would establish infection in an indicator rabbit in only 50% of intradermal inoculation sites), but some treponemes were still present 8 h after inoculation, at the end of the sampling period. A similar clearance rate was observed with BCG-treated rabbits, indicating that activation of the fixed macrophages of the reticuloendothelial system did not result in enhanced phagocytosis of the treponemes. This treatment (BCG) was previously shown to enhance clearance of carbon particles (8) from the rabbit circulation due to increased phagocytosis by liver and spleen macrophages. The resistance of virulent *T. pallidum* to activated rabbit macrophages in vivo has been previously documented (1, 8, 18). Whether treponemes are phagocytosed in vivo to any significant degree is not known. Although electron micrographs have shown intracellular treponemes, the great majority are clearly extracellular in the early stages of the disease. *T. pallidum* will readily attach to host cells, but whereas antibody has been reported to enhance the attachment (12, 14, 16)

TABLE 1. *Clearance of virulent T. pallidum from the blood circulation of normal, BCG-activated, and immune syphilitic rabbits[a]*

Time of blood sampling of rabbits after i.v. inoculation with *T. pallidum*	Syphilitic lesion development in indicator rabbits after inoculation[b]				
	Normal rabbits[c]	BCG-treated rabbits[d]	Rabbits with passive humoral immunity to *T. pallidum*[e]	BCG-treated rabbits with passive humoral immunity to *T. pallidum*[f]	Rabbits immune to *T. pallidum*[g]
3 min	20/24	12/12	12/12	11/12	12/12
16 min	17/24	9/12	6/12	6/12	11/12
32 min	14/24	7/12	5/12	10/12	2/12
1 h	13/24	9/12	0/12	5/12	1/12
2 h	10/24	5/12	0/12	0/12	1/12
4 h	9/24	3/12	0/12	0/12	0/12
6 h	8/24	1/12	0/12	0/12	0/12
8 h	8/24	4/12	0/12	0/12	0/12
Clearance time$_{50}$[h]	1.5 h	1.5 h	16 min	54 min	20 min
Time for complete blood clearance of *T. pallidum*	>8 h	>8 h	32 min–1 h	1–2 h	2–4 h

[a] A total of six different clearance experiments were performed in which the number of *T. pallidum* inoculated intravenously ranged from 5.2×10^7 to 2.8×10^8, (mean, 1.3×10^8). *T. pallidum* were obtained from the testes of orchitic syphilitic rabbits by mincing tissue in an anaerobic maintenance medium (9).

[b] Inoculated intradermally with blood from rabbits with the indicated degree of immunity to *T. pallidum*. Indicator rabbits were housed at 18°C, and their backs were marked into a grid for intradermal inoculations and regularly shaved. They were examined daily, for 6 weeks after inoculation, to detect the development of dark-field-positive syphilitic lesions at the inoculation sites. Numerator indicates syphilis lesions. Denominator indicates inoculation sites.

[c] Six rabbits were used.

[d] Three rabbits were used. Intravenous infection with 1 mg of viable *Mycobacterium bovis*, strain BCG (Commonwealth Serum Laboratories, Melbourne, Australia) took place between 4 and 6 weeks before the *T. pallidum* clearance experiment. Immediately after the experiment they were Mantoux-tested (100 IU of tuberculin per 0.1 ml) and gave a positive result (20 mm diameter induration on day 3). This treatment was previously shown to enhance clearance of carbon particles from the rabbit circulation (8).

[e] A large pool of sterile immune syphilitic rabbit serum was prepared from rabbits infected 6 to 24 months previously with *T. pallidum* and which had been shown to be resistant to challenge with *T. pallidum*. In the clearance experiment, three donor rabbits were used, each receiving 7 lots of 10 ml of immune serum, intravenously, 6, 5, 4, 3, 2, and 1 day and 2 h before intravenous inoculation with *T. pallidum*. At the end of the clearance experiment the rabbits were bled for syphilis serology; rapid plasma reagin tests were all positive, and *T. pallidum* hemagglutination titers were 1/640; 1/2,560, and 1/2,560.

[f] Three rabbits were used. They were treated with BCG as in *d*, and with immune syphilitic rabbit serum as in *e*. Rabbits gave positive Mantoux tests as in *d*, and their syphilis serology was: rapid plasma reagin test negative and *T. pallidum* hemagglutination titers 1/1,280; 1/1,280, and 1/2,560. The negative rapid plasma reagin results were unexpected because the pool of immune serum used for passive immunization was strongly rapid plasma reagin positive.

[g] Three rabbits were used, all of which had been infected with *T. pallidum* 13 months previously. Syphilis serology; rapid plasma reagin test, positive; *T. pallidum* hemagglutination titers all 1/5,120. At the conclusion of the clearance experiment (8 h after intravenous inoculation), they were further challenged with 10^6 *T. pallidum* intradermally, but no lesions developed, confirming their immune state.

[h] See text.

others have reported that immune serum reduced the degree of attachment (5, 10). Antibodies to a treponemal mucopolysaccharidase have been postulated as responsible for the ability of immune serum to reduce the attachment of *T. pallidum* to host cells (4). Human blood monocytes did not phagocytose virulent *T. pallidum* in vitro, although attachment occurred (3). Blood phagocytes from rabbits infected with *T.* *pallidum* showed enhanced ability to reduce Nitro Blue Tetrazolium, suggesting that phagocytosis had taken place, although there was no correlation between the Nitro Blue Tetrazolium reduction and the symptoms of infection or the appearance of antibodies (19). In vitro phagocytosis of virulent and heat-killed *T. pallidum* with stimulant-induced rabbit peritoneal macrophages has been reported (13), and immune

serum significantly enhanced the phagocytosis. This process may also occur in vivo, but this assumption may be invalid since *T. pallidum* can grow in vivo but not in vitro. Hence, the structural integrity of the bacterium may not be maintained in vitro, leading to its easy phagocytosis. There is no good evidence as yet for any significant phagocytosis of virulent *T. pallidum* in vivo in rabbits or humans. Poor phagocytosis of *T. pallidum* may lead to slow antigenic processing of the bacterium and a delay in the establishment of immunity, as is observed in syphilis (11, 15, 17).

In rabbits protected by passive humoral immunity, clearance of treponemes was as rapid as in fully immune rabbits (clearance time$_{50}$ = 16 min; see Table 1). This suggests that humoral factors, probably antibodies, play an important role in the initial protection of an immune rabbit against *T. pallidum* infection, probably by a direct effect rather than via the involvement of the phagocytic cells of the reticuloendothelial system. Immune serum (transferred to normal rabbits) may be treponemicidal or (more likely) act by promoting the establishment of a protected intracellular location for the treponemes. Transfer of immune serum to normal rabbits has repeatedly been shown to ameliorate but not prevent the establishment of infection (when challenged with *T. pallidum*). The removal of virulent treponemes from the circulation by immune serum cannot be equated with complete treponemal destruction. Possibly the treponemes reside intracellularly, without growing, becoming extracellular at intervals, at which time they establish lesions should no antibody be present. This would explain the observation of delayed lesion development after transfer of passive humoral immunity to normal rabbits challenged with *T. pallidum*.

Alternatively, there may have been simple agglutination of the treponemes without decreasing real numbers of viable bacteria in the blood stream or a differential sequestering of the treponemes from the blood into other organs (e.g., lymph nodes) without an overall change in the number of virulent treponemes per rabbit.

Rabbits with passive humoral immunity to *T. pallidum* and having had prior treatment with BCG, did not show a synergistic enhanced clearance rate, it being similar to rabbits with passive humoral immunity (clearance time$_{50}$ = 54 min; see Table 1). This suggests that enhanced phagocytosis did not occur in the presence of immune serum, supporting previous observations (8).

The conclusion from this experiment is that circulating humoral factors, probably anti-*T. pallidum* antibodies, do play a role in immunity to reinfection in syphilis. The detection of trep-onemal neutralizing factors in immune serum supports this conclusion (2). The interesting question is why antibodies do not completely prevent the establishment of an infection. Presumably these antibodies are not bactericidal but only bacteriostatic in vivo. Further supporting this contention, virulent *T. pallidum* have been isolated intermittently from the blood stream of some immune syphilitic rabbits as much as 3 to 4 years after their initial infection had become latent (6, 7).

LITERATURE CITED

1. **Baughn, R., D. Musher, and J. Knox.** 1977. Effect of sensitization with *Propionibacterium acnes* on the growth of *Listeria monocytogenes* and *Treponema pallidum* in rabbits. J. Immunol. **118:**109–113.
2. **Bishop, N., and J. Miller.** 1976. Humoral immunity in experimental syphilis II. The relationship of neutralizing factors in immune serum to acquired resitance. J. Immunol. **117:**197–207.
3. **Brause, B., and R. Roberts.** 1978. Attachment of virulent *Treponema pallidum* to human mononuclear phagocytes. Br. J. Vener. Dis. **54:**218–224.
4. **Fitzgerald, T., and R. Johnson.** 1979. Mucopolysaccharidase of *Treponema pallidum*. Infect. Immun. **24:**261–268.
5. **Fitzgerald, T., R. Johnson, J. Miller, and J. Sykes.** 1977. Characterisation of the attachment of *Treponema pallidum* (Nichols strain) to cultured mammalian cells and the potential relationship of attachment to pathogenicity. Infect. Immun. **18:**467–478.
6. **Frazier, C., A. Bensel, and C. Keuper.** 1950. Phenomena of disease in rabbits fed cholesterol and inoculated with *Treponema pallidum*. II. Infectivity of blood. Am. J. Syph. Gonorrhea Vener. Dis. **34:**453–459.
7. **Frazier, C., A. Bensel, and C. Keuper.** 1952. Further observations on the duration of spirochetemia in rabbits with asymptomatic syphilis. Am. J. Syph. **36:**167–173.
8. **Graves, S., and R. Johnson.** 1975. Effect of pretreatment with *Mycobacterium bovis* (strain BCG) and immune syphilitic serum on rabbit resistance to *Treponema pallidum*. Infect. Immun. **12:**1029–1036.
9. **Graves, S., P. L. Sandok, H. M. Jenkin, and R. C. Johnson.** 1975. Retention of motility and virulence of *Treponema pallidum* (Nichols strain) in vitro. Infect. Immun. **12:**1116–1120.
10. **Hayes, N. S., K. E. Muse, A. M. Collier, and J. B. Baseman.** 1977. Parasitism by virulent *Treponema pallidum* of host cell surfaces. Infect. Immun. **17:**174–186.
11. **Kiraly, K.** 1976. Immunoallergologic aspects of syphilis, p. 71–105. *In* E. Rajka and S. Korossy (ed.), Immunological aspects of allergy and allergic diseases, vol. 8. Plenum Press, New York.
12. **Lamanna, C., and D. Hollander.** 1956. Demonstration of particulate adhesion of the Rickenberg type with the spirochete of syphilis. Science **123:**989–990.
13. **Lukehart, S., and J. Miller.** 1978. Demonstration of the

in vitro phagocytosis of *Treponema pallidum* by the rabbit peritoneal macrophages. J. Immunol. **121:**2014–2024.

14. **Metzger, M., and E. Michalska.** 1974. *Treponema pallidum* opsonophagocytic test. Arch. Immunol. **22:**745–758.

15. **Musher, D., R. Schell, and J. Knox.** 1976. The immunology of syphilis. Int. J. Dermatol. **15:**566–576.

16. **Nelson, R.** 1953. The immune-adherence phenomenon. An immunologically specific reaction between microorganisms and erythrocytes leading to enhanced phagocytosis. Science **118:**733–737.

17. **Pavia, C., J. Folds, and J. Baseman.** 1978. Cell-mediated immunity during syphilis. A review. Br. J. Vener. Dis. **54:**144–150.

18. **Schell, R., D. Musher, K. Jacobson, P. Schwethelm, and C. Simmons.** 1975. Effect of macrophage activation on infection with *Treponema pallidum*. Infect. Immun. **12:**505–511.

19. **Wicher, V., S. Blakowski, and K. Wicher.** 1977. Nitroblue tetrazolium test in experimental syphilis. Br. J. Vener. Dis. **53:**292–294.