Limited protection of rabbits against infection with *Treponema pallidum* by immune rabbit sera

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SUMMARY   After intradermal infection of rabbits with 3×10^6 *Treponema pallidum* (Melbourne 1 strain) samples of serum were taken at one, two, three, four, and six months after infection. Normal rabbits were passively immunised with these sera, challenged with intradermal doses (10^4, 10^3, 10^2, 10) of *T. pallidum,* and the latent periods of infection, lesion diameters, and the number of inoculation sites developing into lesions were observed. The sera taken at three, four, and six months reduced the number of intradermal inoculation sites that developed into syphilitic lesions after challenge with 10 *T. pallidum*. These same three sera also increased the latent period of infection after challenge with 10^4 *T. pallidum*. The transfer of 50 ml of immune serum per rabbit over a nine-day period before challenge had very little effect on the course of the challenge infection. Only a low level of immunity in rabbits to this strain of *T. pallidum* appears to be mediated by immune serum but this small degree of protection did increase with time after infection. Enhanced growth of *T. pallidum* in the serum-recipient rabbits did not occur, thus suggesting that none of the sera was immunosuppressive.

Introduction

It is generally recognised that immunity to *Treponema pallidum* in both humans and rabbits develops slowly (Turner and Hollander, 1957). Humans with untreated syphilis remain susceptible to reinfection until such time as their initial infection becomes latent (Magnuson *et al.,* 1956). Persons with primary and secondary lesions remain susceptible to exogenous *T. pallidum*. In rabbits the disease must progress for approximately three months before complete resistance to challenge is established (Turner and Hollander, 1957). The reasons for this slow onset of immunity are not known but may be due to the recently recognised immunosuppression that occurs early in syphilis (Pavia *et al.,* 1978). A circulating factor (Levene *et al.,* 1969) which decreases the ability of the host lymphocytes to be transformed appears to play a part. The immunosuppressive factor may be of bacterial origin,

resulting in 'antigenic overload' (Musher *et al.,* 1976), or of host origin—for example, an antibody (Medici, 1972). In either case, its concentration should theoretically rise soon after infection and then fall again as latency is established and the host becomes immune.

To investigate whether or not this occurs, samples of serum were taken at monthly intervals from infected rabbits until they became immune. These sera were used to immunise passively normal rabbits which were then challenged with *T. pallidum*; the course of infection was followed to see whether or not serum 'immunosuppressive' or serum 'protective' factors had been transferred.

The *T. pallidum* strain used in the study was isolated in 1977 from an anal chancre of a male homosexual in Melbourne, Australia. Since most recent work in the field of syphilis immunology has been carried out using the Nichols strain (Nichols, 1914), isolated many decades previously, we investigated the protective properties of immune rabbit serum raised against this current *T. pallidum* strain and compared it with information already available about immune serum against the Nichols strain.

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Materials and methods

ISOLATION OF TREPONEMA PALLIDUM (MELBOURNE 1 STRAIN)

This strain of *T. pallidum* was isolated in February 1977 from a small anal lesion (positive by darkfield microscopy) of a male homosexual by inoculation of the exudate into a rabbit testis. After 4½ months the rabbit developed antitreponemal antibodies and by six months treponemes were observed by darkfield microscopy of an aspirate from the orchitic testis. The rabbit was killed and an eluate from a minced testis transferred to another rabbit by intratesticular inoculation and the strain maintained in this manner.

HARVEST OF T. PALLIDUM

After intratesticular inoculation of approximately 5×10^7 *T. pallidum* per testis orchitis developed in about two weeks. Orchidectomy with an aseptic technique was performed on the rabbit immediately after it had been killed by intravenous injection of 4 ml sodium pentobarbitone (200 mg/ml). The testes were minced in sterile anaerobic *T. pallidum* maintenance medium (Graves *et al.*, 1975) and the eluted *T. pallidum* adjusted to the required concentration. Counts were made in a bacterial counting chamber under darkfield microscopy. The eluted *T. pallidum* usually had a concentration of about 10^7/ml. Dilutions were carried out under a stream of sterile, oxygen-free nitrogen.

EXPERIMENTAL INFECTION WITH T. PALLIDUM

Each of seven adult rabbits was inoculated intra-dermally into its shaved back with 3×10^6 *T. pallidum*. These rabbits were bled at monthly intervals to provide sera. Rabbits were housed at 18°C and fed antibiotic-free feed and water in unrestricted quantities.

SEROLOGICAL MONITORING

At monthly intervals antitreponemal antibody tests were performed on the individual sera and the pooled serum. Rapid plasma reagin (RPR) (Hynson, Wescott and Dunning, Baltimore) and *Treponema pallidum* haemagglutination (TPHA) tests (Fujizaki Pharmaceutical Co. Ltd., Shinjuku-ku, Tokyo) were performed according to the manufacturers' direction.

TRANSFER OF SERA

Sera taken each month from the infected rabbits were pooled and stored frozen until the final (six-month) samples were obtained. Each of the monthly pools were then sterilised by filtration through a 0.45 μm Millipore filter. Twenty-one adult male rabbits were selected as recipients of the serum and divided into seven groups of three rabbits: group 1 received no serum; group 2, normal serum; group 3, one-month serum; group 4, two-month serum; group 5 (one rabbit died during administration of the serum), three-month serum; group 6, four-month serum; and group 7, six-month serum. Each rabbit received a total of 50 ml serum, given in five intravenous doses of 10 ml at nine, seven, five, and two days and two hours before challenge.

T. PALLIDUM CHALLENGE OF RECIPIENT RABBITS

Each rabbit had its back shaved and marked into a 10-cm × 10-cm grid of 16 squares. The challenge doses of *T. pallidum* used were 10^4, 10^3, 10^2, and 10, all given intradermally (i.d.) in quadruplicate. Rabbits were kept shaved and were examined daily for the appearance of syphilitic lesions at the inoculation sites. The first sign of a definite induration at the site of inoculation was taken as the end of the latent period for that particular inoculum. The diameter of the zone of induration of each syphilitic lesion was measured 28 days after challenge. Rabbits were examined daily for 34 days after challenge and again on days 42 and 68. No new lesions appeared after day 32. During the observation period rabbits were housed at 18°C as before.

TEST OF IMMUNE STATUS OF DONOR RABBITS

A few days after the six-month serum collection from the seven infected rabbits, each of these rabbits was challenged intradermally with 10^6 *T. pallidum* at a shaved site on the back. None of these inoculation sites developed into syphilitic lesions, indicating that rabbits donating serum six months after infection were immune to rechallenge—that is, the serum taken from the six-month donor rabbits was in fact from immune rabbits.

Results

The donor rabbits, infected with 3×10^6 *T. pallidum* i.d., developed positive TPHA and RPR titres by one month after infection, with the exception of one rabbit which gave a negative result to the RPR test. By two months, the TPHA titres had reached a maximum (Table 1). However the only observed effect of the one-month and two-month sera on the recipient rabbits (after transfer of the serum and challenge with *T. pallidum*) was to produce apparently smaller lesions compared with normal rabbit serum and then only after a 10^4 challenge-dose (data not presented). Anti-*T. pallidum* antibodies present within two months of infection appeared to have very little host-protective ability.

Table 1 *Syphilis serology on pooled rabbit sera taken up to six months after infection with T. pallidum (3 × 10⁶ i.d., Melbourne 1 strain)*

Time after T. pallidum infection (months)	Test results	
	RPR	TPHA titre
0 (preinfection)	– *	<1/80
1	+ *	1/1280‡
2	+ *	1/5120§
3	+ †	1/5120§
4	+ †	1/5120§
6	+ †	1/5120§

+ Positive   – negative
*One of seven rabbits still negative
†All seven rabbits positive
‡Range of titres from individual rabbits, 1/320-1/5120
§Range of titres from individual rabbits, 1/1280-1/5120

Serum taken three months after infection retarded the development of lesions after challenge with 10^4 *T. pallidum* (Table 3) and reduced the number of inoculation sites that developed into syphilitic lesions after challenge with 10 *T. pallidum* (Table 2). It therefore had some protective effect.

Serum taken four months after infection reduced the size of the syphilitic lesions, retarded their development (increased latent period) after challenge with 10^4 *T. pallidum* (Table 3), and reduced the number of inoculation sites that developed into syphilitic lesions after challenge with 10 *T. pallidum* (Table 2). This serum also had some protective ability.

Serum taken six months after infection significantly reduced the number of inoculation sites that developed into syphilitic lesions after challenge with 10 *T. pallidum* (Table 2) and also retarded the development of lesions after challenge with 10^4 *T. pallidum* (Table 3). It was the most protective of the sera tested. However none of the sera had any significant effect on 10^3 and 10^2 *T. pallidum* challenge doses.

In general, none of the transferred sera was markedly successful in modifying the course of the *T. pallidum* infection. Depending on the criteria of protection used, different sera had varying (but minimal) protective ability. On the basis of the reduction in the number of inoculation sites developing into syphilitic lesions, the immune sera taken at three and six months were equally protective, while on the basis of the retardation of the development of syphilitic lesions, the immune sera taken at four and six months were equally protective.

Table 2 *Number of T. pallidum lesions (numerator) in serum-treated rabbits as a fraction of total intradermal inoculation sites (denominator) for four different T. pallidum (Melb. 1) inoculation doses after passive transfer of syphilitic rabbit sera*

Passive transfer to recipient rabbits (in months after T. pallidum infection)	T. pallidum lesions / Inoculation sites for four different T. pallidum inocula			
	10⁴	10³	10²	10
None	12/12	12/12	12/12	12/12
Normal rabbit serum	12/12	12/12	12/12	12/12
1	12/12	12/12	12/12	12/12
2	12/12	12/12	12/12	12/12
3	8/8	8/8	8/8	4/8
4	12/12	12/12	12/12	9/12
6	12/12	12/12	11/12	7/12

Table 3 *Latent period (days) for the development of syphilitic lesions in recipient rabbits after intradermal infection with graded doses of T. pallidum*

Passive transfer to recipient rabbits (in months after T. pallidum infection of donor rabbits)	Latent period (days) after four different Treponema pallidum challenge doses							
	10⁴		10³		10²		10	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
None	10·2	2·1	11·7	0·5	16·6	2·0	21·0	2·7
Normal rabbit serum	12·8	1·9	17·0	2·3	20·4	2·6	22·9	3·2
1	10·8	1·5	13·0	1·5	16·0	1·3	21·1	1·9
2	14·2	1·8	17·2	2·1	20·3	1·7	24·2	3·2
3	17·4	3·4*	18·5	2·4	22·2	3·5	24·5	1·7
4	16·2	1·2†	17·8	1·8	20·1	2·0	22·9	1·8
6	15·7	0·9†	17·7	1·6	19·9	2·7	24·7	3·6

*Significant difference (P<0·005) by Student's *t* test when compared to normal serum transfer
†Significant difference (P<0·001) by Student's *t* test when compared to normal serum transfer
SD = Standard deviation

Discussion

Using the Nichols strain of *T. pallidum*, a number of investigators have already shown that the transfer of immune rabbit serum to normal rabbits, and their subsequent challenge, does not completely protect the recipient rabbits (Perine *et al.,* 1973; Sepetjian *et al.,* 1973; Turner *et al.,* 1973; Graves and Johnson, 1975; Bishop and Miller, 1976a; Weiser *et al.,* 1976). Depending on the dose of immune serum given and its time of administration in relation to the challenge with *T. pallidum*, the development of syphilitic lesions may be retarded (that is, they have longer latent periods), with atypical morphology (smaller and less indurated), and regress more quickly, possibly without ulceration. With very low challenge doses of *T. pallidum*, lesions may even be completely prevented.

In our experiment we transferred five lots of 10-ml volumes of serum per rabbit over a nine-day period, the last dose being two hours before challenge with *T. pallidum*. The rationale for this procedure was to ensure a high level of transferred serum proteins throughout the tissues of the recipient rabbits at the time of first exposure to introduced *T. pallidum*. Sequential addition of immune serum to normal rabbits has produced rising antibody titres (Sepetjian *et al.,* 1973).

Where a protective effect was observed immune serum may either kill some of the introduced challenge *T. pallidum* or (more likely) be bacteriostatic. Thus, as the passively introduced antibodies decay the treponemes start to grow and establish a (modified) infection. Antibody and complement are apparently not bactericidal *in vivo* unlike *in vitro* (TPI test, Nelson and Mayer, 1949). This may be because *in vitro* the *T. pallidum* cannot grow and maintain its structural integrity. A similar phenomenon has been reported with *Haemophilus influenzae* type b, which is resistant to antibodies in a medium supporting growth but is lysed by antibodies in a medium that does not support growth (Newman *et al.,* 1973).

It is now well recognised that immunity to *T. pallidum* is slow to develop in both humans (Chesney, 1927) and rabbits (Eberson, 1921; Turner and Hollander, 1957). Recently Bishop and Miller (1976b) detected some immunity in rabbits as early as 11 days after infection, but not until three months were all their rabbits completely resistant to challenge.

In our experiments the first two serum samples taken after rabbit infection with *T. pallidum* (at one and two months) apparently had some protective ability as shown by a statistically significant reduction in the size of the syphilitic lesions produced

by a challenge with 10^4 *T. pallidum*. However no similar protection was found after challenge with 10^3, 10^2, or 10 *T. pallidum* making this protective effect of doubtful significance. The sera taken at three, four, and six months after infection not only retarded the appearance of the syphilitic lesions after challenge with 10^4 *T. pallidum* but also reduced the number of inoculation sites developing into lesions after challenge with 10 *T. pallidum*. In addition the serum taken at four months also reduced the size of the lesion after challenge with 10^4 *T. pallidum*. These observations indicate that protective factors were gradually being produced by the *T. pallidum*-infected rabbits. There was no evidence that any serum contained an immunosuppressive factor, although if it were present at low levels it may have been masked by the protective factors which were probably antibodies. In general, the longer the period of infection in the rabbit the greater the degree of passive immunity conferred by serum from that rabbit, but immunity was never complete.

Possibly, if serum had been taken less than one month after infection, the presence of immuno-suppressive factors in the serum could have been inferred should there have been more rapid growth of the challenge *T. pallidum* in the serum-recipient rabbits than in the control rabbits. Furthermore, the factor or factors responsible for early immuno-suppression in syphilis may possibly be cell-bound.

Normal rabbit serum gave some level of protection against a challenge with *T. pallidum* (Table 3), and consequently all comparisons were made between transfer with immune and normal serum rather than with no serum. The presence of rabbit protein on the surface of the introduced *T. pallidum* (Logan 1974) may have resulted in a cross-reaction to allotypic differences in these proteins owing to the immunisation of the recipient rabbit with the transferred immune rabbit serum proteins nine days before challenge.

In this study a new, recently isolated strain of *T. pallidum* (Melbourne 1) was used. All recent experimental work in syphilis immunology has involved the use of the Nichols strain, which may be atypical. Nichols (1914) himself thought this was so. Other workers have also suggested that there is serological heterogeneity among *T. pallidum* (Eberson, 1921; Chesney, 1927; Turner and Hollander, 1957). Our observations on the Melbourne 1 strain suggest that the basis of immunity is only partially humoral and in this respect it does not differ from the Nichols strain.

In hamsters the closely related bacterium, *Treponema pertenue,* can be prevented from producing cutaneous lesions by the passive transfer of immune serum (Schell *et al.,* 1978) but, as in

syphilis, immune serum does not completely protect the host from infection.

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