

Experimental infection of man with rabbit-virulent *Treponema paraluis-cuniculi*

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SUMMARY Virulent *Treponema paraluis-cuniculi* was inoculated intradermally into the arm of a human volunteer and into the shaved backs of 10 rabbits. An identical, but heat-killed, preparation was inoculated into the opposite arm of the volunteer as control. A superficial and transient infection developed in the volunteer, shown by a small zone of erythema that persisted for 24 days. The control preparation caused a smaller zone of erythema that disappeared after five days. A very poor immune response was detected by standard serological tests for syphilis. The inoculated rabbits developed lesions about six days after infection and seroconverted by 84 days.

The poor antitreponemal antibody response to *T paraluis-cuniculi* infection in the volunteer suggests that this naturally attenuated treponeme may not be suitable as a vaccine against infection with *T pallidum* in humans.

Introduction

Treponema paraluis-cuniculi, the causative agent of rabbit venereal spirochaetosis, is morphologically and antigenically related to *Treponema pallidum*.¹ Rabbits infected with *T paraluis-cuniculi*, both intratesticularly and venereally, show considerable protection against challenge with *T pallidum*,¹⁻³ although protection is not complete. Nevertheless, it has been suggested that this bacterium, as a naturally attenuated treponeme, might be suitable as a vaccine against human syphilis.^{2,4}

Only one early report⁴ of human inoculation with this bacterium has been published. Levaditi and Nicolau were inoculated, by scarification, with an unspecified number of *T paraluis-cuniculi* on the arm. No infection was established and the scarification scab became detached five days later. The Wassermann reaction remained negative. A rabbit inoculated with the same preparation became infected.

In an attempt to confirm the lack of virulence of *T paraluis-cuniculi* for man, one of us (SG) was

inoculated intradermally with 2×10^7 rabbit-virulent *T paraluis-cuniculi* on the left arm and with an identical heat-killed preparation on the right arm. Ten rabbits (as controls) were also inoculated intradermally with the viable preparation.

Materials and methods

TREPONEMAL SUSPENSION

T paraluis-cuniculi (strain 8816) obtained from the Center for Disease Control, Atlanta, Georgia, USA, (by courtesy of Dr A Balows) was inoculated into rabbit testes and the infection allowed to proceed for about one month, by which time mild orchitis has usually developed. The treponemes were harvested using a technique and anaerobic medium previously described.⁵ The treponeme suspension was clarified of crude particulate debris by centrifugation at $500 \times g$ for five minutes, and the supernatant contained 2×10^6 *T paraluis-cuniculi* per ml. It was concentrated 100-fold by centrifugation at $12\,000 \times g$ for 30 minutes and the sedimented treponemes were resuspended in one hundredth of the original volume of fresh medium. The final preparation contained 2×10^8 treponemes/ml, of which 99% were highly motile. A small sample of the preparation was killed by heating at 56°C for 30 minutes, resulting in the loss of treponemal motility.

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INOCULATION

The preparations (0.1 ml of each) were inoculated intradermally into the forearms of one of us (SG)—the viable preparation into the left arm and the heat-killed preparation into the right. The antigenic challenge was consequently 2×10^7 viable, plus 2×10^7 non-viable, *T paralis-cuniculi*. In addition, 10 normal male rabbits each received 0.1 ml of the viable preparation inoculated intradermally into their shaved backs.

CLINICAL MONITORING

Clinical monitoring was carried out by one of us (JD) and included full blood examination (biochemistry and cells) and examination for pyrexia, enlarged spleen, liver, and regional lymph nodes. Monitoring was carried out before inoculation and at various intervals thereafter for up to one year. The inoculation sites were examined daily and the zone of erythema measured (table I).

TABLE I *Macroscopic appearance of lesions after intradermal inoculation of a human volunteer with rabbit-virulent Treponema paralis-cuniculi*

Time after inoculation (days)	Left arm (test site)		Right arm (control site)	
	Response	Size (mm)*	Response	Size (mm)*
Preinfection	Normal		Normal	
2	E+O	4	E+O	3
3	E+O	4	E+O	2
4	E+O	4	E+O	2
5	E+O	4	Lesion almost invisible	
6	E	4	Normal	
7	E	5	"	
9	E	6	"	
13	E	5	"	
15	E	3	"	
19	Lesion almost invisible		"	
22	E	4	"	
24	E	3	"	
26	Normal		"	
30	Normal		"	

*Diameter of erythematous zone
E = erythema; O = oedema

SEROLOGICAL TESTS

Serological tests for syphilis were performed on the human sera at intervals after infection (table II). The rapid plasma reagin (RPR) test and *T pallidum* haemagglutination assay (TPHA) were performed in our laboratory; the Wassermann reaction (WR) in the bacteriology department of the Alfred Hospital; the Venereal Disease Research Laboratory (VDRL) and the fluorescent treponemal antibody-absorbed (FTA-ABS) tests in the serology department of the Fairfield Infectious Diseases Hospital; and the *T*

TABLE II *Response to serological tests for syphilis of human volunteer inoculated intradermally with rabbit-virulent Treponema paralis-cuniculi*

Time after inoculation (weeks)	Serological test results (titre)					
	RPR	WR	VDRL	TPHA	FTA-ABS	TPI
Preinfection	-	-	-	-	-	-
1	-	ND	ND	-	ND	-
2	-	ND	ND	-	ND	ND
3	-	ND	ND	-	ND	-
4	-	ND	ND	1/80	ND	ND
6	-	ND	ND	*	-	ND
8	-	ND	ND	*	ND	ND
12	-	1/8	ND	1/80	ND	-
16	-	-	-	1/80	-	-
22	-	-	-	1/80	-	-
52	-	-	-	-	-	-

ND = not done; - = negative

RPR = rapid plasma reagin test; WR = Wassermann reaction; VDRL = Venereal Disease Research Laboratory test; TPHA = *Treponema pallidum* haemagglutination test; FTA-ABS = fluorescent treponemal antibody-absorbed test; TPI = *Treponema pallidum* immobilisation test

*The titre was 1/160, but the unsensitized rbc controls gave a titre of 1/80, indicating the presence of a non-specific rbc-agglutinin in the serum.

pallidum immobilisation (TPI) test in the serology department of the Institute of Clinical Pathology and Medical Research.

Sera were coded so that the technicians (except in our laboratory) did not know that experimental sera were being examined.

MONITORING OF RABBITS

The inoculated rabbits were examined daily for the development of lesions, as shown by induration at the site of inoculation. They were tested serologically by the RPR test and TPHA 84 days after inoculation. Rabbits were maintained at 16-19°C and fed antibiotic-free food and water in unrestricted quantities.

Results

HUMAN INFECTION

Immediately after intradermal inoculation of the human volunteer with 0.1 ml of the viable and 0.1 ml of the heat-killed preparations both forearms became inflamed and oedematous over an area of about 40-50 cm². This reaction, which was probably due to foreign protein in the suspending medium (which contained 25% fetal calf serum), was similar on both arms. No systemic effects were noticed and no treatment with antihistamines was required. The reaction subsided after 48 hours, leaving a small erythematous zone around the inoculation site. On the control arm the erythematous zone gradually decreased in size and became invisible by day 6 (table I); on the infected arm it gradually increased to a

maximum size of 6 mm in diameter nine days after infection, decreased thereafter, and became invisible by day 26 (table I).

The different reaction at the two sites indicated that a very local transient infection had occurred on the infected arm. However, no systemic effects were observed. At no stage did blood examination or clinical examination show any abnormalities. Lymph nodes were not enlarged nor was the temperature raised. At 17 weeks after inoculation the volunteer developed an intercurrent, lower respiratory tract infection (confirmed by x-ray examination) that was successfully treated with amoxycillin (see Discussion).

RABBIT INFECTION

The detailed course of the infection in the rabbits has been reported.⁶ All 10 rabbits became infected, mostly by day 6, but two showed delayed lesions, which appeared 20 and 55 days after inoculation. The indurated rabbit lesions reached a maximum size of approximately 7 mm in diameter and persisted for a long time (minimum, 55 days; maximum, more than one year).

COMPARISON OF RESPONSE

The main difference between the infection in the rabbits and in the human volunteer was the lack of induration around the inoculation site in the latter. The early *T paraluis-cuniculi* lesions in the rabbits resembled early *T pallidum* lesions in rabbits. The lesions were erythematous and indurated. In the volunteer the *T paraluis-cuniculi* inoculation site showed erythema with only mild oedema, which gradually subsided (table I).

The volunteer produced a poor and limited humoral immune response when examined serologically (table II). The TPHA gave a marginally positive result (titre 1/80) between four and 22 weeks after infection (titres <1/80 were not considered positive in this test). The WR gave a transiently positive result (titre 1/8) 12 weeks after infection. All other serological tests, including the sensitive TPI and FTA-ABS tests, gave negative results during the one-year observation period.

The infected rabbits, in contrast, all gave positive results to the RPR test and TPHA when tested 12 weeks after infection. The TPHA titres ranged from 1/640 to 1/5120.⁶

Discussion

Although human inoculation with *T paraluis-cuniculi* has been reported once,⁴ we considered that the virulence of this bacterium for humans should be retested. Previously, the inoculum size was not

stated, the introduction was by scarification rather than by intradermal inoculation, and—since other serological tests for syphilis had not been developed by 1921—only the Wassermann reaction was used.

Our observation was not substantially different from that of Levaditi *et al.*,⁴ even though we did obtain a transient, superficial, and localised infection whereas they reported no infection. This discrepancy may have been due to differences in inoculum size. We detected a minimal serological response (the TPHA and WR gave transiently and weakly positive results) while previously⁴ the WR remained negative. This difference in the WR may also have been related to inoculum size. Unfortunately the TPHA was not available to the earlier investigators.

It appears that *T paraluis-cuniculi* may not be a suitable bacterium for stimulating a good immune response against *T pallidum* and will probably not be useful as a vaccinating agent against human syphilis. Nevertheless, the inoculation of larger numbers than 2×10^7 of viable *T paraluis-cuniculi* may produce a good antibody response. One cannot predict the effect of immune stimulation with 10^{10} or 10^{13} *T paraluis-cuniculi*.

In our experiment the concentration of syphilitic antibodies was too low to justify an ethical challenge infection with *T pallidum*. Although the relative contributions of humoral and cell-mediated immunity to overall immunity in syphilis is still a matter of dispute and experimentation, immune serum is partially protective,⁷⁻¹³ implying that antibodies do play some part in the immune state.

It may also be possible to induce a stronger antisyphilitic immune response by giving a second injection of *T paraluis-cuniculi*. Unfortunately the maintenance medium⁵ contained large amounts of fetal calf serum protein which, being strongly antigenic, may give rise to dangerous hypersensitivity reactions on second inoculation into the subject. The preparation also contained rabbit testicular protein, having been derived from rabbit testes. These problems could be solved by growing the bacterium in vitro in a protein-free medium, but unfortunately at present neither *T paraluis-cuniculi* nor *T pallidum* can be grown in vitro in any type of medium. A solution to this problem would greatly assist in finding a suitable vaccine for syphilis.

The human volunteer in our experiment developed a severe lower respiratory tract infection, which was confirmed by x-ray examination, 17 weeks after infection with *T paraluis-cuniculi*. Although his successful treatment with amoxycillin suggested that pneumococci were responsible, a high titre of antibodies to *Mycoplasma pneumoniae* (a bacterium which would not have responded to amoxycillin) was also present, indicating the possibility of a double

infection or else a previous infection with *M pneumoniae*. While this intercurrent infection may have been coincidental, it is also possible that the subject was in a transient state of immunosuppression, which made him more susceptible to other infections. Results from our laboratory have shown¹⁴ that rabbits infected with *T paraluiscuniculi* three months previously are more readily infected with *T pallidum* than are normal rabbits, indicating probable immunosuppression. On the other hand, rabbits infected five, seven, 12, and 30 months previously with *T paraluiscuniculi* showed some level of resistance to challenge with *T pallidum*, implying that the earlier immunosuppression was transient. Other workers have also claimed that immunosuppression occurs during rabbit and human syphilis.^{15 16}

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