Brit. J. vener. Dis. (1970) **46**, 451

Attempts at immunization against syphilis with avirulent *Treponema pallidum*

N. N. IZZAT, W. G. DACRES, J. M. KNOX, AND R. WENDE

From the Department of Dermatology and Syphilology, Baylor College of Medicine, Houston, Texas

Immunization against syphilis in experimental animals has been attempted by several investigators (Magnuson, Halbert, and Rosenau, 1947; Eagle and Fleischman, 1948; Waring and Fleming, 1951) and reviewed by others (Turner and Hollander, 1957; Cannefax, 1965; Wigfield, 1965). Most of these studies involved the administration of killed virulent *Treponema pallidum* harvested from testicular tissue with differing immunization periods and routes of immunization. The Reiter treponeme was also used by Gelperin (1951) to immunize rabbits against syphilis. Nearly all these attempts have met with little success. Recently, Miller (1969) and Metzger and Smogór (1969) have reported impressive results using the virulent strain of *T. pallidum*.

The present work describes attempts to render rabbits immune to syphilis with antigens prepared from freshly cultivated avirulent strains of *T. pallidum* (Nichols).

Material and Methods

New Zealand male rabbits, weighing 5 to 6 lb., were used throughout the investigation. All were housed in individual cages in a specially designed room that kept the environmental temperature at precisely 70°F. All animals had non-reactive serological tests for syphilis and no clinical symptoms suggestive of infection with *Treponema cuniculi*. The avirulent strain of *T. pallidum* used to challenge the immunized rabbits was obtained originally from Dr. G. R. Cannefax of the National Communicable Disease Center, Atlanta, Georgia. The virulent strain was maintained in the testes of normal rabbits and serially transferred every 11 days. The avirulent strain was cultivated in Spirolate broth medium following the method of Knox, Dacres, Short, and Glicksman (1967). The following preparations were used in attempts to immunize the rabbits.

(A) TREPONEMAL SEDIMENT

Sedimented organisms were accumulated by growing *T. pallidum* (avirulent strain) in Spirolate broth medium. Organisms were harvested using a Szent Georgi refrigerated centrifuge with a flow rate of 60–80 ml./min. The sedimented organisms were washed three times in 0·85 per cent. physiological saline and stored at −60°C. Before use, the culture cells were washed three times in phosphate buffered saline (pH 7·8) to remove all the Spirolate broth. The number of treponemes present in the sediment was determined microscopically on the basis of the number per high power field. Sufficient organisms were added to sterile 2 per cent. alum potassium sulphate (Baker), to give a final count of 2×10^6 per ml. Merthiolate at 1:10,000 was used as the preservative. The final preparation was injected subcutaneously at weekly intervals into three sero-negative rabbits for 10 weeks (0·5 ml. per injection). One additional rabbit was added to this group to serve as adjuvant control.

(B) TREPONEMAL SONICATE

Avirulent treponemes were harvested and washed as above. The cells were then suspended in physiological saline, 1:1 (v/v), and subjected to ultrasonication using a Biosonik apparatus. This was combined with *Escherichia coli* lipopolysaccharide, so as to contain 100 mg./ml. sonicated organisms and 2·4 mg./ml. *E. coli* lipopolysaccharide. The final mixture was administered to a total of sixteen rabbits, divided into two groups:

Group I

Six test rabbits and two control rabbits. All test rabbits received a total of 1,600 mg. sonicated avirulent treponemal antigen subcutaneously over a period of 16 weeks.

Group II

Six test rabbits. Each received a total of 1,600 mg. sonicated antigen over a 4-month period. Two additional rabbits were added to this group to serve as controls.

(C) LYSOZYME-TREATED TREPONEMES

10g. harvested and washed avirulent treponemes were suspended in 10 ml. phosphate buffered saline (pH 7·4) containing 40 μg./ml. lysozyme (Calbiochem). The suspension was kept in an incubator for one hour at 36°C. and checked frequently for loss of treponemal coiling. A suspension containing 90 per cent. uncoiled treponemes was centrifuged (37,000G) to remove the excess lysozyme and then re-suspended in an equal amount of saline and stored at −20°C. Before use, 1 ml. of this suspension was diluted to 4 ml. with physiological saline and 1 ml. of *E. coli* adjuvant was added. The final

Received for publication April 9, 1970

preparation was injected subcutaneously into seven rabbits; each received a total of 1,600 mg. of lysozyme treated antigen administered weekly over a 53-week period. Two control rabbits were used.

CHALLENGE WITH VIRULENT TREPONEMES

Unless otherwise stated, all rabbits were challenged intradermally at four sites on the lower back, with varying doses of virulent *T. pallidum* obtained from rabbit testes. Rabbits were observed daily for the development of chancres. All lesions that developed were subjected to darkfield examination. In addition serological tests (VDRL, TPI, and FTA-ABS) were performed before immunization, after immunization, and after challenge inoculation.

Results

In the first experiment, the spirochaete sediment-alum injections produced sero-conversion in two of the three animals after the fourth and eighth week of immunization. The spirochaete sediment-alum immunized rabbits were challenged after 10 weeks of immunization. The challenge doses were 4×10^6 virulent organisms injected into the testes, and 5×10^5 organisms inoculated intradermally at two sites on the back. 3 weeks after challenge, lesions developed at all sites in the test animals and control. Anticardiolipin titres increased further after the inoculation with challenge doses (Table I).

In the second experiment, the treponemal sonicate-adjuvant immunized rabbits and controls were challenged 2 weeks after the last immunization injection. Before challenge, all test and control rabbits in Groups I and II remained sero-negative. VDRL conversion occurred after challenge in all animals, including three controls (Table II). VDRL-antibody titres rose to higher levels in the test animals as compared to non-treated controls. All test and

TABLE I *Rabbits immunized with avirulent treponemal sediment in alum adjuvant*

Rabbit No.	*Initial VDRL*	*Serological test*	*Antibody titres*								
			Pre-challenge (wks)					*Post-challenge (wks)*			
			2	4	6	8	10	1	2	4	5
1002	NR	VDRL	NR	NR	NR	R:UND	R:UND	NR	WR	WR	WR
1004	NR	VDRL	NR	R:UND	R:UND	R:UND	R:UND		NR	R1:8	
1005	NR	VDRL	NR	NR	NR	NR	NR		WR	R1:16	R1:128
		FTA-ABS							R		R
		TPI									R
1006 Control	NR	VDRL						NR	NR	R:UND	R1:32

Abbreviations: NR Non-reactive; WR Weakly reactive; R:UND Reactive undiluted; R Reactive

TABLE II *Rabbits immunized with avirulent treponemal sonicate with* E. coli *adjuvant*

Group	*Rabbit no.*	*Total amount of sonicate per rabbit (mg.)*	*VDRL antibody titre*				
			Initial	*Pre-challenge (17 wks)*	*Post-challenge (wks)*		
					4	8	12
I	1183	1,600*	NR	NR	R 1:2	R 1:32	R 1:32
	1184		NR	NR	WR	WR	R:UND
	1185		NR	NR	NR	R 1:8	R 1:2
	1186		NR	NR	WR	WR	WR
	1187		NR	NR	WR	R 1:4	R:UND
	1188		NR	NR	WR	R 1:4	R 1:4
	Controls						
	1189		NR	NR	NR	R:UND	R 1:64
	1190		NR	NR	NR	NR	WR
II	1191	1,600†	NR	NR	NR	R 1:2	R:UND
	1192		NR	NR	R 1:2	R 1:2	R 1:2
	1193		NR	NR	R:UND	R 1:8	R 1:16
	1194		NR	NR	NR	R 1:8	R 1:4
	1195		NR	NR	NR	R 1:4	R 1:32
	1196		NR	NR	NR	R 1:4	R 1:2
	Controls						
	1197		NR	NR	NR	NR	NR
	1198		NR	NR	WR	R:UND	R 1:8

*Weekly immunization for 16 weeks
†Monthly immunization for 4 months

Abbreviations: NR Non-reactive; WR Weakly/reactive; R:UND Reactive undiluted; R Reactive

control animals in both groups developed darkfield positive chancres.

The lysozyme treated treponemes did not induce reactive VDRL tests in the seven treated rabbits (Table III); 6 weeks after challenge with fifty virulent organisms per site, all rabbits and both controls developed VDRL reactivity. These animals also developed darkfield positive lesions within 15 to 22 days after challenge.

TABLE III *Rabbits immunized with treponemal-lysozyme-treated cells*

Rabbit no.	*Antibody titres (VDRL)**		
	Initial VDRL	*Pre-challenge (53 wks)*	*Post-challenge (6 wks)*
1145	NR	NR	R 1:8
1147	NR	NR	R 1:4
1150	NR	NR	R 1:4
1152	NR	NR	R:UND
1153	NR	NR	R 1:4
1154	NR	NR	R 1:2
1155	NR	NR	R 1:4
Controls			
1156	NR		R 1:4
1157	NR		R 1:4

*Abbreviations: NR Non-reactive; WR Weakly reactive; R:UND Reactive undiluted; R Reactive

Discussion

The object of these experiments was to prepare antigens, which would cause the production of treponemal antibodies that would result in immunity against syphilis.

Repeated inoculation with treponemal sediment-alum mixture resulted in conversion in the serological reactivity in two of the three animals receiving the antigen. This is in contrast to rabbits immunized with treponemal-sonicate or lysozyme adjuvant mixtures, for these animals remained sero-negative until after the challenge was administered. The production of reactive sera in animals immunized with treponemal-sediment and the lack of such reactivity in animals receiving the sonicate or the lysozyme-treated preparations, suggests that the avirulent *T. pallidum* (Nichols) strain has antigens in common with those of pathogenic spirochaetes. These antigenic fractions were apparently lost during treatment with lysozyme and by sonication.

Despite the development of syphilitic antibodies after challenge, the serologically reactive rabbits in this experiment did not demonstrate resistance to infection. These results are not surprising, since Magnuson, Halbert, and Rosenau (1947), Eagle and Fleischman (1948), Waring and Fleming (1951), McLeod (1962), and Miller, Whang, and Fazzan (1963) reported similar results after their attempts at immunization using virulent *T. pallidum*. It may be that serological responses produced by such preparations do not relate to functional immunity or were not high enough to bestow protection. The contention of Magnuson, Thompson, and McLeod (1951) and Miller (1965) that antibodies measured by these serological tests play no role in prevention of infection is to be the subject of further studies in our laboratory.

Summary

Rabbits immunized with avirulent *T. pallidum* (Nichols) organisms, including spirochaetes collected from sediment, sonicated organisms, and lysozyme-treated organisms, were not protected from infection by challenge doses of virulent *T. pallidum*. Lysozyme-treated and sonicated antigenic preparations did not produce sero-reactivity, whereas immunization with avirulent treponeme sediment was capable of causing the conversion of VDRL tests from a non-reactive to a reactive state.

We wish to thank Misses C. R. Wills and S. E. McCotter for able technical assistance.

This investigation was supported by the John A. Hartford Foundation, Grant No. 2465.

References

CANNEFAX, G. R. (1965) *Brit. J. vener. Dis.*, **41**, 260

EAGLE, H., and FLEISCHMAN, R. (1948) *J. exp. Med.*, **87**, 369

GELPERIN, A. (1951) *Amer. J. Syph.*, **35**, 1

KNOX, J. M., DACRES, W. G., SHORT, D. H., and GLICKSMAN, J. M. (1967) *WHO/VDT/RES.*, **67**, 128

MAGNUSON, H. J., HALBERT, S. P., and ROSENAU, B. (1947) *J. vener. Dis. Inform.*, **28**, 267

——, THOMPSON, F. A., and MCLEOD, C. P. (1951) *J. Immunol.*, **67**, 41

MCLEOD, C. P. (1962) *Publ. Hlth Rep. (Wash.)*, **77**, 431

METZGER, M., and SMOGÓR, W. (1969) *Brit. J. vener. Dis.*, **45**, 308

MILLER, J. N. (1965) *J. Bact.*, **90**, 297

—— (1969) Abstract, A.M.A. Meeting, New York

——, WHANG, S. J., and FAZZAN, F. P. (1963) *Brit. J. vener. Dis.*, **39**, 195

TURNER, T. B., and HOLLANDER, D. H. (1957) "Biology of the Treponematoses", WHO Monogr. Ser. No. 35. WHO, Geneva

WARING, G. W., JR., and FLEMING, W. L. (1951) *Amer. J. Syph.*, **35**, 568

WIGFIELD, A. S. (1965) *Brit. J. vener. Dis.*, **41**, 275

Tentatives d'immunisation contre la syphilis avec *Treponema pallidum* avirulent

SOMMAIRE

Des lapins ayant reçu l'inoculation de *T. pallidum* (Nichols) avirulent, y compris de spirochètes recueillis de sédiments, d'organismes ultra-sonnés et d'organismes traités par lysozyme, ne furent pas protégés lors de l'inoculation ultérieure de *T. pallidum* virulent. Les préparations antigéniques traitées au lysozyme ou aux ultra-sons n'entraînèrent pas de séro-positivité alors que l'inoculation avec le sédiment de tréponèmes avirulents fut capable de faire passer les tests VDRL de la négativité à la positivité.