

RESISTANCE AND SEROLOGICAL CHANGES IN RABBITS IMMUNIZED WITH VIRULENT *TREPONEMA PALLIDUM* SONICATE

Nawfal N. Izzat, John M. Knox, William G. Dacres, and Edgar B. Smith

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

Abstract. Rabbits injected intramuscularly or intravenously or into the foot pad with virulent *T. pallidum* sonicate-adjuvant mixture developed VDRL and FTA-ABS antibodies. However, these sero reactive rabbits did not develop resistance against challenging doses of virulent *T. pallidum*.

Many attempts have been made to induce resistance against syphilis in rabbits and a number of good review articles have been published (1, 13, 15). Antigens from *Treponema pallidum* or related organisms have usually failed to produce significant resistance (4, 11). In all of these unsuccessful experiments the investigators used organisms which were killed by methods which could alter antigenic components. It is possible that the immunologically active antigens are substances which function only when introduced in the native state. Miller (9) and Metzger & Smogór (8) have reported successful immunization with the use of relatively unaltered treponemal antigens. These native antigens were prepared by irradiating virulent treponemes or by maintaining virulent treponemes at 4°C, with or without the addition of penicillin. We have used sonication in an attempt to free native antigens of *T. pallidum* without biochemically altering them.

MATERIALS AND METHODS

The virulent Nichols strain of *Treponema pallidum* was obtained from Dr G. R. Cannefax of the National Communicable Disease Center, Atlanta, Georgia.

Adult New Zealand male rabbits, weighing five to six pounds, with non-reactive VDRLs and no evidence of infection with *Treponema cuniculi*, were used throughout the study. They were housed in individual cages in a specially designed room that precisely kept the environmental temperature at 70°F.

Preparation of treponemal immunizing suspensions. Pathogenic *T. pallidum* (Nichols) was harvested in saline from infected rabbit testes. The steps in the isolation were as follows: (a) surgical removal of testes during the stage of firm orchitis; (b) thorough rinsing of testes with normal saline to remove excess blood; (c) mincing the testes and shaking in saline for 1 hour to diffuse spirochetes into the fluid phase; (d) decantation and centrifugation at 754 g for 15 min to remove cellular debris; and (e) centrifugation of supernatant at 3 015 g for 30 min to sediment all the spirochetes.

The organisms so harvested were suspended in saline at a concentration of 25 organisms per oil immersion field with $\times 10$ ocular and $\times 100$ objective. Samples (4 ml, containing approx. 5×10^8 organisms) were sonicated with Biosonik apparatus, mixed with 1 ml of *Escherichia coli* lipopolysaccharide and employed as an immunizing suspension.

Immunization procedure. Animals were injected with the above preparation in one of three ways. Three rabbits received weekly injections of 0.5 ml in the thigh muscle; three animals received the same dosage in the foot pad; and, three animals received 0.3 ml each week intravenously. All were injected for 16 weeks.

Infectivity tests. After the completion of the immunization schedule, all rabbits and three controls were challenged intradermally with 50 000 virulent *T. pallidum* suspended in 0.1 ml of 50% rabbit serum in saline. Each rabbit received four injections at four sites of the skin on the lower back. Rabbits were observed daily for the development of chancres. All lesions were subjected to dark-field examination.

Serological tests. Serological tests for syphilis (VDRL, FTA-ABS and TPI) were made 8 and 16 weeks before challenge and 4, 8, and 16 weeks after challenge. The Houston City Health Department Laboratory, Houston, Texas and the Venereal Disease Research Laboratory, National Communicable Disease Center, Atlanta, Georgia, performed these tests.

RESULTS

Administration of the treponemal sonicate-adjuvant mixture produced VDRL reactivity, with

Table I. Rabbit serological responses to virulent *T. pallidum sonicate*

Rabbit number	Immunization routes ^a	Initial VDRL	Serological tests ^b	Antibody titers				
				Pre-challenge (weeks)		Post challenge ^c (weeks)		
				8	16	4	8	16
1111	I.M.	NR	VDRL FTA-ABS TPI	R 1:32 R NR	R 1:16 R NR	R 1:8 R NR	R 1:4 R NR	R 1:2 R WR
1112	I.M.	NR	VDRL FTA-ABS TPI	R 1:16 R NR		Died		
1113	I.M.	NR	VDRL FTA-ABS TPI	R 1:8 R NR	R 1:8 R NR	R 1:8 R NR	R 1:4 R NR	R:UND R NR
1114	F.P.	NR	VDRL FTA-ABS TPI	R 1:2 R NR	R 1:16 R NR	R 1:8 R NR	R 1:4 R NR	R:UND R WR
1115	F.P.	NR	VDRL FTA-ABS TPI	R:UND R NR	R 1:2 R NR	R 1:2 R NR	R 1:4 R NR	R 1:4 R NR
1116	F.P.	NR	VDRL FTA-ABS TPI	R 1:16 R NR	R 1:8 R NR	R 1:4 R NR	R 1:2 R NR	R:UND R NR
1117	I.V.	NR	VDRL FTA-ABS TPI	R:UND R NR		Died		
1118	I.V.	NR	VDRL FTA-ABS TPI	R 1:8 R NR		Died		
1119	I.V.	NR	VDRL FTA-ABS TPI	R 1:2 R NR	R 1:8 R NR	R 1:4 R NR	R:UND R NR	R:UND R WR
1120	Control	NR	VDRL FTA-ABS TPI			R 1:8 R WR	R 1:16 R R	R 1:2 R R
1121	Control	NR	VDRL FTA-ABS TPI			R 1:8 R NR	R 1:2 R NR	WR R R
1122	Control	NR	VDRL FTA-ABS TPI			R 1:4 R NR	R 1:4 R NR	WR R NR

^a I.M. = intramuscular. F.P. = foot Pad. I.V. = intravenous.

^b NR = nonreactive. R:UND = reactive undiluted. R = reactive. WR = weakly reactive.

^c All rabbits had dark field positive lesions.

titers ranging from R:UND to 1:16. The anti-cardiolipin titers in the animals were not related to the routes of immunization (Table I). The FTA-ABS antibody appeared in all animals by 8 weeks prior to challenge, while the TPI remained non-reactive. Following challenge with virulent *T. pallidum*, dark-field positive lesions appeared at the

same time (15–20 days) in both test and control rabbits. The serological titers were maintained at the same level for 4 weeks after challenge and then there were gradual decreases. Only three of the test animals (1111, 1114, 1119) and two of the controls (1120, 1121) developed TPI reactivity.

DISCUSSION

The present study was an attempt to produce artificial immunity against syphilis through the use of *T. pallidum* (virulent Nichols strain) sonicate-adjuvant mixture.

Repeated intravenous, intramuscular, and foot pad inoculations in rabbits caused the production of antibodies that could be detected by VDRL and FTA-ABS tests. However, animals immunized over a 16 week period were not resistant to infection. These results are in agreement with the reports of Magnuson et al. (4); Eagle & Fleischman (2); Waring & Fleming (14); McLeod (6); and Miller et al. (11), following their attempts at immunization using virulent *T. pallidum*. Recently, Miller (10) and Metzger et al. (7) produced apparent resistance to syphilis in rabbits immunized with relatively unaltered virulent organisms. Both believe the protective antigens are situated in the peripheral layer of the treponemes. Miller (9) thinks that a heat-stable polysaccharide remaining in γ -irradiated treponemes was responsible for protection, while Metzger & Smogór (8) considered a heat-labile protein in their cold incubated organisms to be the protective antigen. Tani et al. (12), however, postulated that protective antigens are deep in the body of the treponemes, and must be "unmasked" prior to immunization. Using anti-formin-killed organisms, they too produced significant resistance to infection.

The sonicated material used in the present investigation is likely to contain both surface and deep antigens. This material was indeed antigenic, producing antibodies responsible for the conversion of VDRL and FTA-ABS tests from non-reactive to reactive. However, our antigen failed to produce protection against infection. Our findings and those of a previously reported study (3) support the belief of Magnuson et al. (5), that circulating antibodies may play little or no role in immunity against syphilis. One possible explanation of the sonicate's inability to produce protection, is that the total amount of antigen given to each animal was inadequate. Each rabbit received only 80 million sonicated organisms. Metzger & Smogór (8) produced resistance in animals immunized with 6 000 and 12 000 million organisms and found less protection in animals given 3 000 million organisms.

Further studies are in progress in our laboratory

to determine if higher doses of *T. pallidum* sonicate will succeed in producing immunity against syphilis.

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N. N. Izzat, Ph.D.
Department of Dermatology
Baylor College of Medicine
Texas Medical Center
Houston, Texas 77025
USA