Immunological studies on treponemal antigens

II. Serological changes and resistance to infection in rabbits immunized with culture supernatant of avirulent Treponema pallidum

N. N. IZZAT, E. B. SMITH, S. W. JACKSON AND J. M. KNOX

From the Department of Dermatology and Syphilology, Baylor College of Medicine, Houston, Texas 77025, and the Syphilology Research Laboratory, Veterans Administration Hospital, Houston, Texas 77031, U.S.A.

In a previous paper, Izzat, Smith, Jackson, and Knox (1971) reported the isolation of an antigenic substance from the supernatant of cultures of avirulent Treponema pallidum. The antigenic substance was shown to be nontoxic and glycoprotein in nature. Injections of this material into rabbits stimulated precipitin antibodies reactive with FTA-ABS desiccated antigen.

The object of the present investigation was to evaluate the efficiency of the isolated antigenic substance in producing resistance to challenge with virulent T. pallidum.

Material and Methods

The virulent Nichols strain of Treponema pallidum obtained from Dr. G. R. Canefax of the National Center of Disease Control, Atlanta, Georgia, was maintained in normal rabbit testes and was transferred successively every 2 weeks. This strain was used as the challenging agent throughout our study.

Adult New Zealand male rabbits, weighing 5 to 6 lb., which were seronegative to the VDRL test and showed no evidence of infection with Treponema caritculi, were used throughout this study. They were housed in individual cages in a specially designed room that maintained environmental temperature at precisely 70°F.

IMMUNIZATION MATERIAL

Using the extraction method cited in our previous paper (Izzat, Smith, and others, 1971), the ammonium sulphate precipitated antigen was concentrated and lyophilized. At the time of immunization this lyophilized material was suspended in physiological saline to a final concentration of 25 mg./ml. and mixed with complete Freund's adjuvant (v/v). For the control antigen, Spirolate medium subjected to the same extraction method and lyophilization was re-suspended in physiological saline to the same final concentration.

IMMUNIZATION PROCEDURE

Two groups of male rabbits were used. In the first group, four rabbits were immunized subcutaneously with the supernatant antigen mixed with complete Freund's adjuvant (v/v). They were given increasing doses (5 mg., 25 mg., 50 mg., 100 mg., and 100 mg.) at weekly intervals for 5 weeks. The total amount given to each rabbit was thus 280 mg. lyophilized supernatant or 168 mg. antigenic protein. Seven control rabbits were inoculated as follows: two with a total of 280 mg. lyophilized medium plus serum and adjuvant; one with 280 mg. medium plus adjuvant without the rabbit serum supplement; one with complete Freund's adjuvant; one with rabbit serum. Material given to the two latter rabbits was based on volume of injection rather than weight. The remaining two control rabbits served as controls for the challenging organisms and received saline only.

In the second group, eleven rabbits were immunized subcutaneously for 24 weeks, using the supernatant antigen re-suspended in the same manner as described above. This material was administered weekly with increasing increments (from 5 to 100 mg.) to a total dosage of 1,300 mg. supernatant antigen per animal. In the same group, five control rabbits were inoculated subcutaneously as follows: two with 1,300 mg. of the lyophilized medium plus serum and adjuvant; and the remaining three rabbits with saline only to serve as controls for the challenging organisms.

INFECTIVITY TESTS

After completion of the immunization schedule, two of the test animals in Group 1 were challenged intradermally with 25 × 10³ virulent Treponema pallidum cells suspended in 0.1 ml. of 50 per cent. rabbit serum in saline; each received four challenge injections on the skin of the lower back. The two remaining rabbits in this group, all the test rabbits of Group 2, and all the controls in both groups were challenged intradermally with 50 organisms per site. The challenging doses were determined according to the method of Izzat, Knox, Werth, and Dacres (1971). The animals were observed daily for the development of chancres. All lesions were subjected to darkfield examination.

 SEROLOGICAL TESTS

The VDRL, FTA-ABS, and TPI tests for syphilis were performed weekly on all rabbits throughout the immunization period and after challenge. The Houston City Health Department Laboratory and the Venereal Disease Research Laboratory, National Center for Disease Control, Atlanta, Georgia, performed these tests.
Results
Subcutaneous administration of 280 mg, supernatant antigen in complete Freund’s adjuvant resulted in VDRL seroconversion in three of the four test animals before challenge (Table IA). This seroreactivity increased quantitatively after challenge with virulent Treponema pallidum. All sera were reactive in the FTA-ABS tests by the end of the challenge period while the TPI tests remained non-reactive. All seven control animals (Table IB) became seropositive to the VDRL and FTA-ABS tests after challenge, but only one (Rabbit 1190) had shown seropositivity to the VDRL test before challenge. This rabbit had received 280 mg medium plus adjuvant.

All test and control animals (Table IA, B) developed darkfield positive lesions demonstrating lack of resistance to the challenge organisms.

Immunization with higher doses of the supernatant antigen (1,300 mg per rabbit) over a period of 24 weeks confirmed the above experimental data.

### TABLE IA Relationship between avirulent T. pallidum supernatant antigens and rabbit resistance in test animals

<table>
<thead>
<tr>
<th>Rabbit no.</th>
<th>Initial VDRL</th>
<th>Serological test</th>
<th>Pre-challenge (5 wks)</th>
<th>Challenge dose per site</th>
<th>Post-challenge (wks)</th>
<th>Resistance to challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>1186</td>
<td>NR VDRL</td>
<td>FTA-ABS TPI</td>
<td>R : UND</td>
<td>25 x 10³</td>
<td>R : 1 : 8</td>
<td>R : 1 : 2</td>
</tr>
<tr>
<td>1187</td>
<td>NR VDRL</td>
<td>FTA-ABS TPI</td>
<td>R : UND</td>
<td>25 x 10³</td>
<td>R : 1 : 8</td>
<td>R : 1 : 2</td>
</tr>
<tr>
<td>1188</td>
<td>NR VDRL</td>
<td>FTA-ABS TPI</td>
<td>R : UND</td>
<td>50</td>
<td>R : 1 : 2</td>
<td>R : 1 : 2</td>
</tr>
<tr>
<td>189</td>
<td>NR VDRL</td>
<td>FTA-ABS TPI</td>
<td>R : UND</td>
<td>50</td>
<td>R : 1 : 2</td>
<td>R : 1 : 2</td>
</tr>
</tbody>
</table>

NR—Nonreactive  WR—Weakly reactive  R—Reactive  R : UND—Reactive undiluted

### TABLE IB Relationship between avirulent T. pallidum supernatant antigens and rabbit resistance in control rabbits

<table>
<thead>
<tr>
<th>Rabbit no.</th>
<th>Initial VDRL</th>
<th>Serological test</th>
<th>Pre-challenge (5 weeks)</th>
<th>Post-challenge (wks)</th>
<th>Resistance to challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>1190</td>
<td>Lyophilized medium + serum with adjuvant</td>
<td>VDRL FTA-ABS TPI</td>
<td>R : UND</td>
<td>R : 1 : 2</td>
<td>R : 1 : 2</td>
</tr>
<tr>
<td>1191</td>
<td>Lyophilized medium + serum with adjuvant</td>
<td>VDRL FTA-ABS TPI</td>
<td>R : UND</td>
<td>R : 1 : 2</td>
<td>R : 1 : 2</td>
</tr>
<tr>
<td>1192</td>
<td>Lyophilized medium with adjuvant</td>
<td>VDRL FTA-ABS TPI</td>
<td>R : UND</td>
<td>R : 1 : 2</td>
<td>R : 1 : 2</td>
</tr>
<tr>
<td>1193</td>
<td>Rabbit serum only</td>
<td>VDRL FTA-ABS TPI</td>
<td>R : UND</td>
<td>R : 1 : 2</td>
<td>R : 1 : 2</td>
</tr>
<tr>
<td>1194</td>
<td>Adjuvant only</td>
<td>VDRL FTA-ABS TPI</td>
<td>R : UND</td>
<td>R : 1 : 2</td>
<td>R : 1 : 2</td>
</tr>
<tr>
<td>1195</td>
<td>None</td>
<td>VDRL FTA-ABS TPI</td>
<td>R : UND</td>
<td>R : 1 : 2</td>
<td>R : 1 : 2</td>
</tr>
<tr>
<td>1196</td>
<td>None</td>
<td>VDRL FTA-ABS TPI</td>
<td>R : UND</td>
<td>R : 1 : 2</td>
<td>R : 1 : 2</td>
</tr>
</tbody>
</table>

NR—Nonreactive  WR—Weakly reactive  R—Reactive  R : UND—Reactive undiluted
Nine of the eleven test animals demonstrated VDRL seroconversion. Simultaneously the two controls (Table IIA, B) that had received medium plus adjuvant developed reactivity to the VDRL test. Sera from all the test animals were reactive in the FTA-ABS test before challenge, but none of the sera from control animals showed such reactivity. After challenge with virulent *T. pallidum*, darkfield

TABLE II A  Resistance and serological changes of test rabbits immunized subcutaneously with lyophilized supernatant and challenged with virulent *Treponema pallidum*.

<table>
<thead>
<tr>
<th>Rabbit no.</th>
<th>Initial VDRL</th>
<th>Serological tests</th>
<th>Pre-challenge (wks)</th>
<th>Post-challenge (wks)</th>
<th>Resistance to (5 x 10 org./site)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1197</td>
<td>NR</td>
<td>VDRL FTA-ABS</td>
<td>4</td>
<td>2</td>
<td>NR</td>
</tr>
<tr>
<td>1198</td>
<td>NR</td>
<td>VDRL FTA-ABS</td>
<td>10</td>
<td>6</td>
<td>R</td>
</tr>
<tr>
<td>1199</td>
<td>NR</td>
<td>VDRL FTA-ABS</td>
<td>14</td>
<td>2</td>
<td>R:UND</td>
</tr>
<tr>
<td>1200</td>
<td>NR</td>
<td>VDRL FTA-ABS</td>
<td>20</td>
<td>10</td>
<td>R:1:2</td>
</tr>
<tr>
<td>1201</td>
<td>NR</td>
<td>VDRL FTA-ABS</td>
<td>24</td>
<td></td>
<td>R</td>
</tr>
</tbody>
</table>

TABLE II B  Resistance and serological changes of control rabbits immunized subcutaneously with lyophilized supernatant and challenged with virulent *Treponema pallidum*.

<table>
<thead>
<tr>
<th>Rabbit no.</th>
<th>Immunization material</th>
<th>Serological tests</th>
<th>Pre-challenge (wks)</th>
<th>Post-challenge (wks)</th>
<th>Resistance to challenge (5 x 10 org./site)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1208</td>
<td>Lyophilized medium + serum with adjuvant</td>
<td>VDRL FTA-ABS</td>
<td>4</td>
<td>2</td>
<td>All animals developed dark field positive lesions at all sites within 29 days post challenge</td>
</tr>
<tr>
<td>1209</td>
<td>Lyophilized medium + serum with adjuvant</td>
<td>VDRL FTA-ABS</td>
<td>10</td>
<td>10</td>
<td>R:1:2</td>
</tr>
<tr>
<td>1210</td>
<td>None</td>
<td>VDRL FTA-ABS</td>
<td>14</td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>1211</td>
<td>None</td>
<td>VDRL FTA-ABS</td>
<td>20</td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>1212</td>
<td>None</td>
<td>VDRL FTA-ABS</td>
<td>24</td>
<td></td>
<td>R</td>
</tr>
</tbody>
</table>

NR—Nonreactive  WR—Weakly reactive  R—Reactive  R : UND—Reactive undiluted
positive lesions appeared at the same time (24 to 29 days) in both test and control rabbits. The VDRL titres were maintained at essentially the same level for 2 weeks after challenge, and then gradually increased throughout the remainder of the challenge period (Table II A, B). The FTA-ABS tests were also reactive throughout this period.

Discussion

The antigenic complex isolated from the culture supernatant of avirulent *T. pallidum* is immunogenic. Animals inoculated subcutaneously with the supernatant antigen complex produced antibodies reactive in the VDRL and FTA-ABS tests. This finding is an agreement with that of Deacon and Hunter (1962), Király, Jobbágy, and Kováts (1967), Meyer and Hunter (1967), and Tringali and Cox (1970), who have reported the existence of common antigens between cultivatable and pathogenic treponemes. The fact that the VDRL antibodies were induced both in animals immunized with the supernatant and in animals immunized with the medium suggests the presence of a nonspecific antigen in the medium. This hypothesis is also supported by the related findings of Cannefax, Hanson and Skaggs (1968), Rathlev (1968), and Wilkinson and Ferguson (1968), who have demonstrated that several components of sorbents and uninoculated Reiter culture medium are able to remove nonspecific reactivity from nonsyphilitic human sera.

Results obtained by the FTA-ABS test on rabbits immunized with supernatant are of particular interest. All animals immunized with the supernatant material produced antibodies reactive in the specific FTA-ABS test. This reactivity indicates the presence of an antigen in the supernatant complex responsible for the induction of specific antibody in vivo. The reactivity also corresponds with the precipitin reaction between rabbit antisera and the FTA-ABS antigen observed in immunoelectrophoresis (Izzat, Smith, and others, 1971).

The results of serological tests on the rabbit sera after challenge failed to establish any relationship to resistance, since animals immunized with medium demonstrated higher VDRL titres than those immunized with supernatant antigen. Although the FTA-ABS test was reactive in both test and control animals after challenge, syphilitic chancre developed in both test animals and nonimmunized controls at approximately the same time. These findings as well as those of our previously reported study (Izzat, Dacres, Knox, and Wende, 1970) support the belief of Magnuson, Thompson, and McLeod (1951) and Miller, Whang, and Fazzan (1963) that circulating antibodies may play little or no role in immunity against syphilis.

Summary

Rabbit hyperimmune antisera experimentally produced against culture supernatant of avirulent *T. pallidum* were reactive in the VDRL and FTA-ABS tests. The VDRL reactivity was induced in both test and control animals, while the FTA-ABS reactivity was demonstrated only in the test animals. Hyperimmunization with the supernatant antigen did not protect animals against challenge doses of virulent *T. pallidum*.

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References


Rathlev, T. (1968) Ibid., 44, 295

Tringali, G. R., and Cox, P. M. (1970) Ibid., 46, 313


Études immunologiques sur les antigènes treponémiques

II. Modifications sérologiques et résistance à
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surnageat de cultures de *Treponema pallidum* non
virulent

SOMMAIRE

Des anti-sérum de lapins hyperimmunisés expérimентale-
ment avec le surnageat de cultures de *T. pallidum* non
virulent se montrèrent réactifs dans les épreuves de
VDRL et de FTA-ABS. La positivité au VDRL fut con-
statée à la fois chez les animaux considérés et chez
les témoins alors que la positivité pour le FTA-ABS ne
fut trouvée que pour les animaux hyperimmunisés.
L’hyperimmunisation avec l’antigel du surnageat ne pro-
tegea pas les animaux contre l’inoculation de *T.
pallidum*. 

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