Attempt to protect rabbits against experimental syphilis by passive immunization*

M. SEPETJIAN, D. SALUSSOLA, AND J. THIVOLET†
Laboratoire d’Hygiène, Domaine Rockefeller, Lyon, France

The role of humoural immunity, effected by circulating antibodies, in defence against a syphilitic infection is still uncertain. Turner (1939) showed that antibodies present in the serum of syphilitic patients neutralize virulent treponemes in vitro. This observation was the forerunner of the discovery by Nelson and Mayer (1949) of the antibodies that immobilize treponemes in vitro. Turner and Nelson (1950) reported that there was a direct relation between the titre of immobilizing antibodies and the resistance to re-infection. However, several authors (Magnuson, Thompson, and McLeod, 1951; Miller, 1967; Metzger, Michalska, Podwińska, and Smogor, 1969; Metzger and Smogor, 1969; Izzat, Smith, Jackson, and Knox, 1971) failed to confirm the relation between the antibody titre and resistance to infection.

However, it must be emphasized that in these experiments the production of the antibodies was induced actively, either by infecting the animal or by injecting attenuated treponemes or antigen fractions of treponemes. Under such conditions it can be presumed that the antibody production is not the only form of immune response in the animal. It is likely that cellular immunity will play a part in the defence reaction.

The present study has been made with the aim of establishing whether passive immunization with pre-formed antibody alone is able to ensure a partial protection against infection with T. pallidum in rabbits.

Methods

The clinical and serological changes in rabbits inoculated intradermally with pathogenic treponemes were observed in controls (C) and animals treated with injections of sera rich in antibodies (Protected = P).

ANIMALS

Five male fawn Burgundy rabbits weighing 6 to 7 lb, sero-negative by the fluorescent treponemal antibody (FTA) test and treponemal immobilization (TPI) test were used.

INOCULATION

A suspension of Treponema pallidum of the Nichols pathogenic strain (Tp) was prepared from an early orchitis. The suspension contained $8 \times 10^6$ Tp organisms/ml; each rabbit received on Day 0 (D0) six injections of 0.1 ml. intradermally on to the shaved skin of the back, that is $4.8 \times 10^6$ Tp per animal.

PROTECTION BY ANTIBODIES

Only two animals were given antibodies (P 10 and P 12), the remaining three rabbits were controls; the antibodies were in pooled sera from rabbits infected with syphilis. The serum had the following properties: protein content, 54.6 g./l; TPI titre, 1:140; FTA titre, 1:102,400 Kline titre, 1:8. Each protected animal was given eleven intravenous injections of 20 ml. The first injection was on D0, 1 hr before the inoculation of the Tp, the last on D11 (Fig. 1).

T.P. (4.8 X 10^6) at six sites

D0 D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12 D13 D14

* = 20 ml serum injected D0 - D14

FIG. 1 Method used for the protected rabbits

CLINICAL SURVEILLANCE

The animals were examined daily, the site of the inoculation inspected and the presence of Tp in the lesions checked by darkfield microscopy.
SEROLOGICAL SURVEILLANCE

Blood samples were taken daily or every other day and the FTA test carried out by the standard method. The anti-rabbit gamma globulin conjugate was a sheep anti-rabbit serum conjugated with fluorescein prepared by the Institut Pasteur, Paris; it was used at a dilution of 1 in 40.

Results

Clinical lesions (Table)
The control rabbits (C 1, 2, 3) all developed typical lesions of experimental syphilis at the site of the inoculation, starting on D₅ with erythema followed by rose-coloured indurated papules that ulcerated by D₂₈ and contained Tp as demonstrated by dark-field microscopy.

<table>
<thead>
<tr>
<th>Rabbits No.</th>
<th>Onset</th>
<th>Maximum</th>
<th>Disappearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day No.</td>
<td>Appearance</td>
<td>Day No.</td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
<td>5</td>
<td>16-30</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5</td>
<td>11-30</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>16-30</td>
</tr>
<tr>
<td>Protected</td>
<td>10</td>
<td>7</td>
<td>7-28</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>7</td>
<td>7-28</td>
</tr>
</tbody>
</table>

+a erythema  
++ ulcerated  
+++ extensive ulceration  
b number of sites showing various grades of lesions

The protected rabbits (P 10, 12) developed erythema at the injection site from D₁ to D₂₈ and a superficial papule at some of the inoculation sites. No ulceration occurred. The serum from incised and scraped indurated zones did not contain Tp.

Humoral responses (Fig. 2)
The control rabbits produced detectable amounts of antibody from D₁, with a FTA titre of 1:800; this increased rapidly up to a titre of 1:51,200 reached by D₂₈ by rabbit C2 and D₂₈ by C 3. Rabbit C 1 reached a maximum titre of 1:25,600 on D₁₉. After a slow fall the titres remained about 1:12,800 until D₁₉ when the animals were killed. The Kline test was positive from D₁ and the TPI positive from about D₂₈ in the three animals. These reactions remained positive throughout the evolution of the disease.

In the protected rabbits, from D₁ the FTA titre was 1:3200 (Fig. 2). The level of antibody continued to rise until D₁₈ reaching a maximum of 1:52,000. It then fell at a steady rate to a minimum of 1:200 by D₄₃. The titre remained unchanged until D₅₈; and then from D₄₅ it began to rise again in both animals. The maximum levels were 1:6400 for rabbit P 10 on D₁₅₁ and 1:12,800 for rabbit P 12 on D₁₁₅. These titres remained unchanged on D₁₄₅ when the experiment was ended.

Discussion

These experiments clearly demonstrate that injection of serum from syphilitic rabbits had a protective effect against primary infection. Clinically this was shown by the absence of ulceration and lack of Tp at the inoculation sites.

Quantitative measurements of the FTA titres showed that the immunological response evolved dissimilarly in the protected compared to the control animals. The controls produced a typical curve of antibody production in response to a primary infection, but that in the protected rabbits was very different. The antibody titres were raised from the outset and attained a level of 1:52,000 on D₁, 48 hrs after the last injection of serum. These antibodies acquired passively were eliminated progressively during the 34 days from D₁₈ to D₄₅, when the titre of 1:200 was very low compared to 1:6400 and 1:25,600 in the controls. The elevation
of antibody titre in the protected rabbits after D₅₀ was probably due to active immunity from an asymptomatic infection, but the titres attained were lower than those in the controls.

Hence passive immunization had a protective effect which could suppress the primary clinical and serological manifestations of syphilis. However, the late elevation of the FTA titre suggests that the Tp were not completely eliminated.

The experimental design was chosen after earlier studies, not reported here, had shown that protection of a rabbit by immune serum requires a high titre of circulating antibody to be maintained in the animal. This involves the use of serum from a pool of sera of very high titre, and repeated large intravenous injections. The antibody must be given at the outset of the infection, hence the injections should precede the inoculation of the Tp.

The number of Tp inoculated (4.8 × 10⁶ per rabbit) was very high. We used this number of organisms as the demonstration of protection against such a massive infection would be spectacular and unequivocal. However, this might be the explanation why the protection failed to prevent the development of an asymptomatic infection.

Summary
Passive immunization with massive amounts of serum from syphilitic rabbits rich in antibody gave a marked protection against inoculation with 4.8 × 10⁶ T. pallidum. This was demonstrated clinically and serologically.

References
—— and SMOGOR, W. (1969b) Ibid., 45, 308
TURNER, T. B. (1939) Ibid., 69, 867

Essai de protection des lapins vis-à-vis de la syphilis expérimentale par immunisation passive

SOMMAIRE
L’immunisation passive avec d’importantes quantités de sérum provenant de lapins syphilitiques riches en anticorps a donné une protection nette vis-à-vis de l’inoculation de 4.8 × 10⁶ T. pallidum, ainsi que ceci fut démontré cliniquement et sérologiquement.