Susceptibility of rabbits venereally infected with Treponema paraluis-cuniculi to superinfections with Treponema pallidum

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SUMMARY Three female rabbits, venereally infected with Treponema paraluis-cuniculi between five and eight months previously, had treponeme-containing genital lesions and positive results to serological tests for syphilis. These rabbits and four normal female rabbits were challenged with Treponema pallidum intradermally on the shaved back with triplicate doses of $10^4$, $10^3$, $10^2$, and 10 treponemes (all at different sites). Significantly fewer syphilitic lesions developed in the rabbits previously infected with Treponema paraluis-cuniculi (4/36) compared with the control rabbits (37/48), and the mean size of the lesions (diameter of induration) was significantly less (4 mm compared with 10 mm). It appears that previous venereal infection of female rabbits with Treponema paraluis-cuniculi induced a level of cross-immunity against infection with Treponema pallidum. Protection was however not complete.

Introduction

Treponema paraluis-cuniculi and Treponema pallidum, which cause rabbit venereal spirochaetosis and human syphilis respectively, are closely related morphologically and antigenically. Previous studies have shown an appreciable level of immunity to Treponema pallidum in rabbits inoculated intratesticularly with Treponema paraluis-cuniculi between six and eight months previously.

In the experiment now reported venereally infected female rabbits (the natural route of infection with Treponema paraluis-cuniculi) were challenged with low serial 10-fold doses of Treponema pallidum ($10^4$, $10^3$, $10^2$, and 10) intradermally on the shaved back, and the degree of syphilitic infection was measured by the percentage of inoculation sites at which lesions developed and their size. A comparison was made with normal female rabbits challenged with Treponema pallidum to ascertain the degree of protection against Treponema pallidum offered by the previous venereal infection with Treponema paraluis-cuniculi.

Materials and methods

INFECTION WITH Treponema paraluis-cuniculi

A male rabbit (rabbit No 82673) was experimentally infected with Treponema paraluis-cuniculi and placed in a pen with four normal female rabbits, as described. The four female rabbits became venereally infected, as shown by the development of treponeme-containing lesions and seroconversion about five months after cohabitation with the infected male rabbit. Six months afterwards, when lesions were still present in three of the four female rabbits, they were challenged with Treponema pallidum.

CHALLENGE WITH Treponema pallidum (Nichols strain)

Treponema pallidum was obtained from the testes of a rabbit inoculated intratesticularly with $5 \times 10^7$ Treponema pallidum about 10 days previously. Details of procedure and elution medium used were as published. The same anaerobic medium was used to dilute the Treponema pallidum to $10^7$/ml and thence in serial 10-fold dilutions to $10^2$/ml (under anaerobic conditions).

The four control and four Treponema paraluis-cuniculi-infected rabbits had their backs shaved and marked into a $3 \times 4$ grid, each of the 12 squares being approximately 15 mm in length. Into each square 0.1 ml of Treponema pallidum suspension was inoculated.
intraderrmally, so that each rabbit received triplicate inoculations of $10^4$, $10^3$, $10^2$, and $10^1$ *Treponema pallidum*.

The rabbits were examined twice a week for five weeks and the development of indurated syphilitic lesions on the backs was recorded. The rabbits' backs were kept shaved during this period.

**SEROLOGICAL MONITORING**

The rapid plasma reagin (RPR) test was carried out according to the manufacturer's instructions (Commonwealth Serum Laboratories, Melbourne, Australia). Results were recorded as strongly positive, weakly positive, or negative. The *Treponema pallidum* haemagglutination assay (TPHA) was performed using the kit produced by Fujizoki Pharmaceutical Company Ltd. The manufacturer's instructions were followed. Only titres greater than 1/80 were considered positive and therefore indicative of past or current treponemal infection. Known positive and negative control sera were included in each test series. Previous studies have shown that results to these two tests become positive in venereal rabbit spirochaetosis.

**Results**

One of the *T. paraluis-cuniculi*-infected rabbits was excluded from the experimental analysis because it had been badly attacked by the male rabbit and had numerous mating bites and infected claw injuries on its back, making detection of classical indurated *T. pallidum* lesions at the site of inoculation very difficult.

As shown in the table, the rabbits which had previously been infected venereally with *T. paraluis-cuniculi* had developed some degree of protection against challenge with *T. pallidum*, as shown by the smaller number of lesions which developed at the inoculation sites and their smaller size, even though the protection was not complete.

After venereal infection of the three female rabbits with *T. paraluis-cuniculi* they developed positive results to the RPR test and TPHA. The uninfected control rabbits had negative results. After challenge with *T. pallidum* the test rabbits continued to have positive results and the results for the control rabbits became positive in both serological tests.

**Discussion**

Turner and McLeod and Turner et al carried out studies on the susceptibility of rabbits experimentally infected intratesticularly with *T. paraluis-cuniculi* to challenge with *T. pallidum* (Nichols strain and strain CJ). They found considerable, but not complete, protection. As they comment, their experiments were a severe test of the immune system of the *T. paraluis-cuniculi*-infected rabbits, as their intradermal challenge inocula were approximately $2 \times 10^6$ *T. pallidum*. Nevertheless some protection was evident by the absence, or slow development, or smaller size of the lesions after challenge with *T. pallidum*. Their results were sufficiently impressive for them to write, "While no (syphilis) vaccine is in sight, the problem (human syphilis) is sufficiently important to warrant the exploration of all avenues of approach, including the potentialities of *T. cuniculi* as an immunising agent."

Subsequent work, reported by Turner and Hollander involved inoculating rabbits intratesticularly with *T. paraluis-cuniculi* (strain A) and eight months thereafter challenging with a low dose (500 treponemes) of *T. pallidum* (Nichols) intradermally on the shaved back. As in the earlier experiments partial protection was obtained.

The purpose of the experiment reported in this paper was to determine whether or not rabbits naturally infected by the venereal route (as distinct from experimental intratesticular inoculation) with *T. paraluis-cuniculi* were resistant to low challenge doses of *T. pallidum* (Nichols) given intradermally. From the results it appears that significant protection did occur. Low challenges (10 and $10^2$) of *T. pallidum* failed to cause any observable lesions in the

**TABLE Development of syphilitic lesions in normal rabbits and in rabbits previously infected with Treponema paraluis-cuniculi on intradermal (id) challenge with four different doses of Treponema pallidum (Nichols strain)**

<table>
<thead>
<tr>
<th><em>Treponema pallidum</em> challenge doses (id)</th>
<th>Normal female rabbits*</th>
<th><em>Treponema paraluis-cuniculi</em>-infected female rabbits†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No of lesions/sites inoculated</td>
<td>Diameter of lesions (mm) (mean ± SD)</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>$10^4$</td>
<td>10/12</td>
<td>11·0 ± 6·9</td>
</tr>
<tr>
<td>$10^3$</td>
<td>10/12</td>
<td>11·1 ± 3·5</td>
</tr>
<tr>
<td>$10^2$</td>
<td>11/12</td>
<td>7·8 ± 5·7</td>
</tr>
<tr>
<td>10</td>
<td>6/12</td>
<td>8·3 ± 3·6</td>
</tr>
<tr>
<td>Total</td>
<td>37/48</td>
<td></td>
</tr>
</tbody>
</table>

*Four rabbits
†Three rabbits
NL = no lesion
immunised rabbits and higher challenges (10³ and 10⁴) caused significantly fewer and significantly smaller lesions in the immunised rabbits (table).

It appears that *T paraluis-cuniculi* does offer some protection to rabbits against low challenge doses of *T pallidum*. Would it do the same for humans, and could it be a potential vaccine against syphilis? Further studies, in both rabbits and humans, are required to clarify this important question.

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