Treponema-specific and antilipoidal 19S(IgM) antibodies in penicillin-treated and untreated rabbits after infection with Treponema pallidum

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SUMMARY The titres of treponema-specific and antilipoidal 19S(IgM) antibodies were determined in rabbits infected intratesticularly with Treponema pallidum. One group of rabbits was treated with penicillin the other served as control. Using different serological tests it was shown that 19S(IgM) antibodies were still detectable eight months after infection at about the same titres in both groups. In contrast, 19S(IgM) antibody titres in patients with syphilis became undetectable within three to six months after penicillin treatment. It is suggested therefore that the rabbit is not a reliable model for studying the effect of penicillin in human T pallidum infections.

Introduction

Since the experiments of Haensell and Parodi the rabbit has been the animal of choice in research into syphilis. Thus, experimentally infected rabbits have been widely used to test the various antisyphtilic drugs, including penicillin.

The presence of Treponema pallidum in the tissues of infected rabbits after penicillin treatment is difficult to prove. The results of staining methods are uncertain because differentiation between T pallidum and Treponema cuniculi is often impossible.

In previous investigations several authors have suggested that the demonstration of antigen-specific 19S(IgM) antibodies is a reliable indicator of the persistence of antigen in the host. This suggestion was observed in viral infections and in syphilis by Atwood and Miller, O'Neill and Nicol, Müller and Loa, and Schmidt. It should be possible therefore to study the effect of penicillin treatment on the elimination of T pallidum in infected rabbits using the decrease in treponema-specific 19S(IgM) antibodies as an indicator. Furthermore, the titres of 19S(IgM) antibodies after penicillin treatment should indicate whether or not the immunological reaction of the infected rabbit is comparable to that of man.

Material and methods

INFECTION OF RABBITS
Eighteen male albino rabbits weighing between 2 and 2.5 kg were used. Their sera were tested for antilipoidal as well as for treponema-specific antibodies by the cardiolipin complement-fixation (CF) test, the T pallidum haemagglutination assay (TPHA), and the fluorescent treponemal antibody (FTA) test and gave negative results. One uninfected animal served as control throughout the experiment.

Seventeen animals were infected with T pallidum (Nichols strain). Under ether anaesthesia 2 ml of a suspension containing about $5 \times 10^6$ treponemes/ml were injected into the testicles (a total dose of about $1 \times 10^7$ treponemes. The rabbits developed a typical orchitis between the seventh and tenth day after infection.

TREATMENT
Eight of the animals were treated from day 21 to day 36 after infection with hydracillin, containing 0.75 megaunits procaine penicillin G and 0.25 megaunits penicillin G sodium per 2 ml. The rabbits received 0.3 ml hydracillin intramuscularly (that is, 0.15 megaunits of the penicillin preparation). In T pallidum immobilisation (TPI) tests with heated guinea pig serum, and using controls with defined penicillin concentrations, it could be shown that the serum penicillin concentration was more than 0.03 IU/ml in the rabbits’ sera for 20 days.

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Under pentobarbital narcosis, blood samples were collected by cardiac puncture at one, two, three, and eight months after infection. The sera were inactivated for 30 minutes at 56°C in a waterbath and tested on the day of collection or the following day after storage at 4°C. The room temperature for the animals was 22°C throughout the experiment.

**SEPARATION OF GLOBULIN FRACTIONS (GEL FILTRATION)**

The separation of the 19S(IgM) fraction of the rabbits' sera was performed either by Sephadex G 200 or by Ultrogel AcA 34 using samples of 0.7 ml serum and columns 40 cm long and 1.5 cm in diameter. Phosphate-buffered saline (pH 7.3; 15 mS conductivity at 22°C) was used as eluant.

**ESTIMATION OF ANTIBODY TITRES**

Titres of the 19S(IgM) antibodies in the different tests were calculated from three peak fractions (each 1.3 ml) of the first elution maximum of the column. The fractions were not concentrated. Using immunofluorescence and 7S(IgG)-specific anti-rabbit serum (from goats) no 7S(IgG) antibodies were found in the 19S(IgM) fractions.

**INDIRECT HAEMAGGLUTINATION**

The TPHA was performed quantitatively on whole sera as well as on the fractions after gel filtration following the manufacturer’s instructions (Fujizoki Pharmaceuticals Co Ltd, Tokyo).

**INDIRECT IMMUNOFLUORESCENCE**

The standard method for the quantitative FTA test was used. Twofold dilutions were tested, starting with a dilution of 1/5 in sera and 1/2 in the fractions after gel filtration. No absorption by ultrasonicate of *Treponema phagedenis* was carried out because the animals had no treponema-specific antibodies at the time the experiments were started. Treponemal infections other than with *T pallidum* (Nichols strain) could, furthermore, be excluded by parallel investigations of the uninfected control animal.

For demonstration of treponema-specific antibodies an FITC-labelled polyvalent anti-rabbit globulin serum (from goats) was used (Deutsche Bio-Mérieux GmbH, Nürtingen, lot No 01106) at a dilution of 1/30. An anti-rabbit IgM serum with μ-chain specificity was prepared in guinea pigs in our laboratory after the method of Fink et al and was conjugated with FITC by the method of Wagner and Heinrich. Unfixed FITC was removed from the serum by filtration through Sephadex G 25. Finally, the anti-rabbit IgM serum was absorbed with rabbit IgG. This preparation was used for the 19S-IgM-FTA-ABS test in the fractions after gel filtration at a dilution of 1/20. Working dilutions of conjugates were established by titration.

**ANTI-LIPOIDAL CF TEST**

For the CF test, with cardiolipin as antigen, a microtitre Kolmer technique was used.

### Results

**TPHA TEST**

The antibody titres in the different tests of sera from eight treated and nine untreated rabbits after intratesticular infection with *T pallidum* were expressed as mean values at the different times of cardiac puncture (figs 1-4). All infected animals developed
Treponema-specific and antilipoidal 19S(IgM) antibodies in penicillin-tested and untreated rabbits

treponema-specific antibodies (reactive in the TPHA test) with high titres (between 1/2560 and 1/81920) (fig 1). The mean was estimated to be more than 1/20 000. In the following eight months the TPHA test titre remained high in the untreated group and fell to a mean of about 1/4000 in the treated group.

The 19S(IgM) TPHA antibody titres in the peak fractions showed a nearly analogous pattern in treated and untreated rabbits. Antibodies of this type, with titres between 1/8 and 1/16, persisted in both groups of animals for as long as eight months after infection.

IMMUNOFLUORESCENCE TESTS
A comparable response of antibody development was demonstrated in the treated and untreated rabbits by the FTA and 19S-IgM-FTA tests (fig 2). A high increase in 19S(IgM) antibodies after infection was followed by a rapid decrease in the following three months. Five months later 19S(IgM) antibodies were still demonstrable by the 19S-IgM-FTA test at titres of about 1/5 in treated as well as in untreated animals.

FIG 2 FTA test titres in whole serum and in the 19S(IgM) fraction after gel filtration of penicillin-treated or untreated rabbits infected with T pallidum (Nichols strain). (For symbols of the curves see fig 1.)

CF TEST
Similarly, complement-fixing antilipoidal antibodies rose to mean titres of 1/3000 or 1/1000 in the first month after infection and decreased to between 1/16 and 1/32 at the eighth month after infection (fig 3). 19S(IgM) antilipoidal antibodies still persisted in both groups at a titre of about 1/6 at the end of the experiment.

FIG 3 Antilipoidal CF antibody titres in whole serum and in the 19S(IgM) fractions after gel filtration of penicillin-treated or untreated rabbits infected with T pallidum (Nichols strain). (For symbols of the curves see fig 1.)

CONTROL
The uninfected control rabbit developed neither treponema-specific nor antilipoidal antibodies during the observation period of eight months.

HUMAN SPHILIS
For comparison the antibody reaction of patients with treated or untreated primary syphilis (demonstrated by the 19S(IgM) or 7S(IgG) FTA-ABS test) is shown in fig 4. Treatment with penicillin (1 megaunit Clemizol penicillin intramuscularly for 12 consecutive days) resulted in a decrease in 19S(IgM) antibodies to an undetectable level within three to six
months; this type of antibody as well as the 7S(IgG) type might persist in untreated patients for more than eight months. Details of our investigations of the effect of treatment with penicillin or other antibiotics in human syphilis will be published later.

Discussion

The immune response to intratesticular infection of rabbits with *T. pallidum* is not affected by therapeutically effective treatment with penicillin (about 50 000 IU/kg/body weight daily). Eight months after infection and about seven months after treatment there were no differences in the titres of 19S(IgM) antibodies in treated and untreated rabbits by the different tests.

Using the TPHA and the FTA and antilipoidal CF tests it was shown that in treated as well as in untreated rabbits a high increase of 19S(IgM) antibodies was followed by a decrease to a low level at eight months after infection.

From the immunological point of view it might be suggested that the identical immune response of treated and untreated rabbits shows an equivalent stage of chronic or healed infection. Investigations in patients with syphilis led to the suspicion that the demonstration of treponema-specific 19S(IgM) antibodies by the immunofluorescence technique is a sign that pathogenic treponemes persist in the host.6,9 This has not been adequately proved in experimental syphilis in rabbits.

Our results in no way contradict those of Paris-Hamelin et al.15 These authors estimated 19S(IgM) antibodies using the FTA test and TPHA at dilutions of 1/100 and 1/40 respectively and so were not able to detect the low titres of 19S(IgM) antibodies.

The observations made on 19S(IgM) antibodies in typical cases of primary syphilis in man after penicillin treatment differ from those in experimentally infected rabbits. In cured patients treponema-specific 19S(IgM) antibodies disappeared between three and six months after treatment had started. In contrast, this type of antibody can persist in the serum of untreated patients for eight months or longer.

The reasons for the different immunological reactions to penicillin treatment for syphilis in humans and rabbits are not known. Nevertheless, from the results of our experiments we suggest that the rabbit is an unsuitable model for studies of the effect of penicillin in human syphilis.

References

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