Protective Immunization against *Treponema pallidum* Using Specific Immune Complexes—an Attempt

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Rabbits were immunized using isolated and dissolved specific immune complexes obtained from patients suffering from early syphilis. After immunization the rabbits were infected intradermally with living *Treponema pallidum*. Some of them did not develop lesions at the infection sites. In comparison to controls there was a delayed and reduced production of antibodies. The effect of immunization may be induced by a treponemal protein antigen as a constituent of the specific immune complexes. *Key-words: Syphilis; Antigen; Antibody.*
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Circulating immune complexes (IC) in early syphilis (S) were first assayed by Sølling et al. (1). We were able to demonstrate that IC in S persist up to one year following adequate tretment in some cases (2, 3). Furthermore, it has been shown that IC contain specific antibodies (2) and specific antigen (4). To detect the specific antibodies isolated and dissolved IC were examined in the specific S-tests (TPHA-, FTA-ABS- and TPI-test). The specific antigen was verified by immunization of rabbits using isolated and dissolved IC obtained from patients suffering from early S. The results concerning the specificity of the IC were confirmed by Wozniczko-Orlowska & Milgrom (5) in human S and by Baughn et al. (6) in experimental S using a different technique. The present experimental study was designed to examine the protective effect of immunization using specific IC obtained from patients with secondary S.

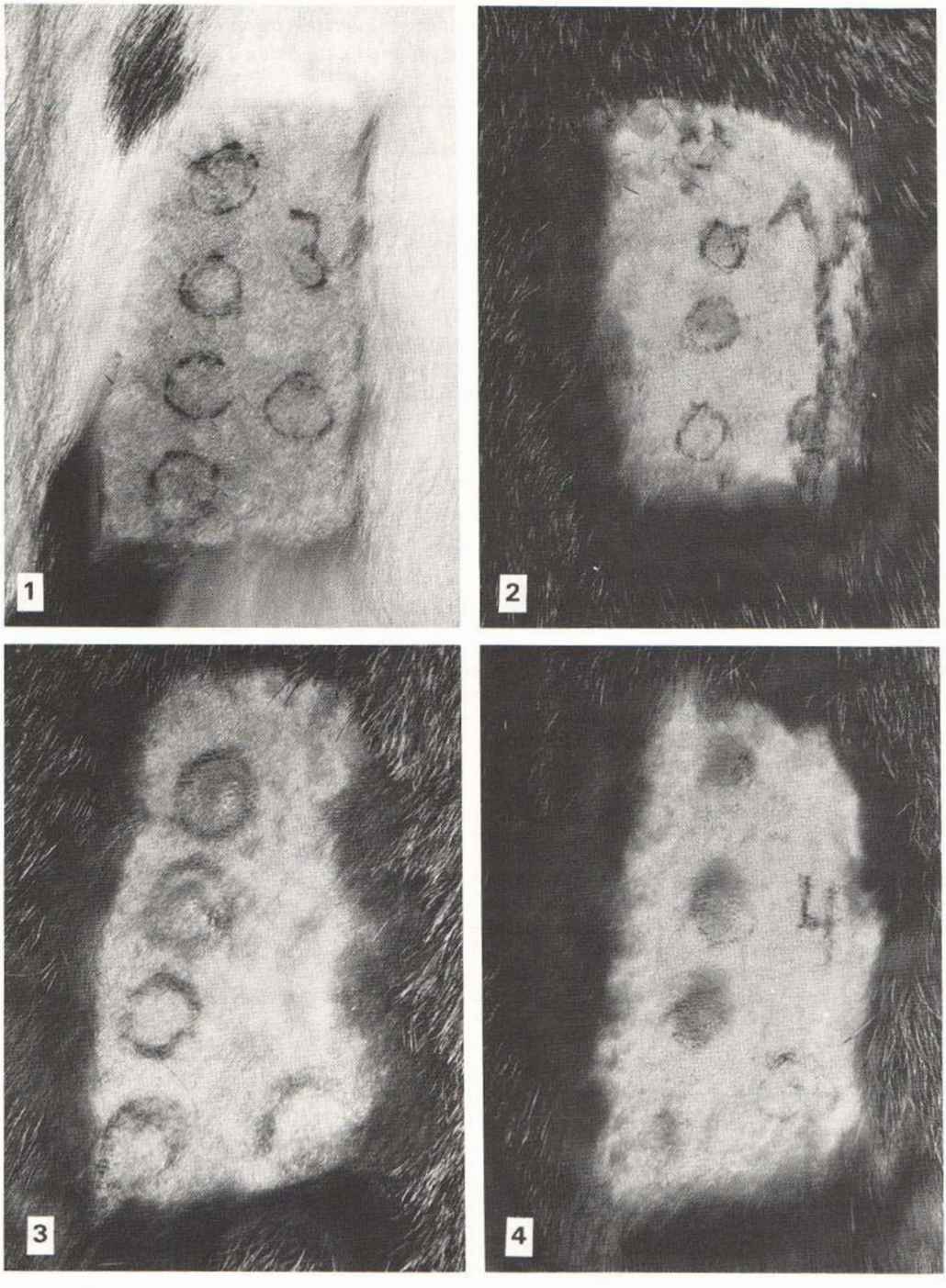
MATERIAL AND METHODS

Immune complexes

IC for the immunization of rabbits were obtained from the serum of a 24-year-old male patient suffering from early syphilis, three days after treatment was completed (reactivity of serum: TPHA- and FTA-ABS-test positive, TPI-test 88% specific immobilization) and from two other patients (15 and 22 years old, respectively) before treatment (reactivity of serum: TPHA- and FTA-ABS-test positive, TPI-test 24 and 36% specific immobilization respectively). IC were assayed by polyethylene glycol (PEG) precipitation method according to Diezel et al. (7). IC levels were extremely elevated with 6.95 mg protein/ml serum before and 6.0 mg/ml after treatment in the first patient and there were medium levels with 4.0 and 4.3 mg/ml, respectively in the other patients (mean value as determined in 100 healthy controls: 2.6±0.9 mg/ml). For isolation IC were washed three times with barbital-HCl buffer containing 3.5% PEG to remove solved IgG or IgM and dissolved in 0.15 mol/l NaCl solution. The solution was adjusted to 2 mg protein/ml and mixed with equal volumes of complete Freund's adjuvans for primary immunization. For the following injections the IC were dissolved in saline alone.

Animals

Female outbred rabbits (12 for the experiment and 6 controls) were housed individually at 18–20°C and were given antibiotic-free food and water. They were selected for experiments by testing serum in



Figs. 1–4. Intensity and type of lesions on shaven back of rabbits immunized with specific immune complexes (1–3) and challenged with living *Treponema pallidum* (Nichol's strain) in comparison to controls (4): no reaction (1); transitory and unsignificant reaction (2); delayed but typical reaction at the infection sites where the highest Treponeme doses had been given (3); full reaction (4).

Table 1. *Final titers (mean ± SD) in TPHA-test of serum samples from 12 rabbits immunized with IC from patients with early S and then infected with living T. pallidum intradermally in comparison to 6 controls*

	Final titers (mean ± SD)		
	Immunized		
Days after infection	With success (8 animals)	Without success (4 animals)	Controls (6 animals)
19	1: 30±24	1: 24±11	1: 37±30
28	1: 104±114	1: 64±0	1: 69±47
37	1: 104±48	1: 128±0	1: 608±790
49	1: 80±55	1: 320±271	1: 800±969
64	1: 176±96	1: 768±362	1: 1 565±1 968
79	1: 288±161	1: 1 276±1 080	1: 4 000±4 039
93	1: 256±181	1: 1 280±1 080	1: 2 901±2 069
120	1: 132±98	1: 1 536±724	1: 1 792±2 031
145	1: 136±91	1: 3 072±1 448	1: 2 250±1 848

routine antitreponemal tests (TPHA-, FTA-ABS- and TPI-test) to exclude especially an inapparent infection with Treponema paraluis-cuniculi.

Immunization

0.25 ml IC solution mixed with complete Freund's adjuvans were injected into each foot of the rabbits. Six weeks later they received 1.0 ml solution intramuscularly on the first day and intravenously on the second and third days. The same procedure was repeated one week later. The first blood sample was taken one week after the last injection.

Infection

T. pallidum, Nichols strain, was maintained by rabbit testicular passages, Treponemes were extracted from the infected testicles in saline. Tissue debris was removed by centrifugation at low speed. Treponemal suspension was mixed with an equal volume of heat-inactivated normal rabbit serum. The Treponemes were counted by darkfield microscopy using a "Siedentopf" condenser. The concentration was adjusted to 8×10^6 motile *T. pallidum* per ml serum saline.

The immunized as well as the control animals were infected intradermally at 4 sites on the shaven back with varying numbers of Treponemes (2.4×10^4, 4.8×10^4, 9.6×10^4 and 19.2×10^4) one week after the last injection of IC for immunization. The rabbits were examined daily for lesion development and photographed on 9th, 14th and 21st day after infection. Blood samples were obtained from all the rabbits about every two weeks after infection.

Serologic testing

Rabbit blood samples were tested by FTA-ABS-, TPHA- and TPI-test.

RESULTS

The immunized rabbits developed antibodies reactive only in TPHA-test in a final titer of 1:16, but not reactive in FTA-ABS- and TPI-test. After intradermal infection with living *T. pallidum* the immunized rabbits demonstrated varying reactions at the infection sites: on third (4 animals) did not develop any lesions, one third (4 animals) showed transitory and insignificant lesions and one third (4 animals) developed delayed and diminished but typical lesions at sites where the highest Treponeme doses had been given (Figs. 1, 2, 3). The control animals developed typical lesions at all infection sites (Fig. 4). As expected the serum samples of control animals became reactive in TPHA-test until the 19th day after

Table II. *Specific immobilization in per cent (mean ± SD) in TPI-test of serum samples from 12 rabbits immunized with IC from patients with early S and then infected with living T. pallidum intradermally in comparison to 6 controls*

Days after infection	Specific immobilization in per cent (mean ± SD)		
	Immunized		
	With success (8 animals)	Without success (4 animals)	Controls (6 animals)
19	7±9	4±6	3±5
28	28±34	20±10	21±11
37	27±30	12±11	25±27
49	33±26	34±2	36±14
64	34±26	22±2	66±10
79	35±26	30±8	66±10
93	35±22	42±8	75±11
120	30±9	52±10	95±9
145	25±9	72±9	87±5

infection. The serum samples from immunized rabbits showed a slowly increasing reactivity in TPHA-test to a low titer and also a low reactivity in TPI-test when there were no or only transitory lesions at the infection sites. These rabbits were considered to be immunized with success (Table I and II).

When the rabbits developed delayed but typical lesions at the infection sites the reactivity of their serum samples increased slowly up to the same titers as those of the controls until the 145th day in TPHA-test, consequently the decrease in reactivity was delayed in comparison with the controls. The TPI-test became reactive at the same time. These animals were considered to be immunized without success (Tables I and II). There were not significant differences between immunized animals and controls in FTA-ABS-test.

DISCUSSION

Despite recent progress in the identification of treponemal protein antigens (8, 9, 10, 11), the nature of all treponemal antigens is not clear yet, no specific protective antigen has been identified and it seems doubtful if a treponemicide antibody does exist (12). Some authors (13, 14) suppose that a "protective antigen" is present inside the *T. pallidum* organism which has to be liberated to become immunogenic.

Metzger and co-workers (15, 16) believe in a heat-labile antigen, a protein component of *T. pallidum* as carrier of immunogenicity, being responsible for success in their immunizing experiments.

Miller (17, 18) has tried to explain his successfull immunizing experiments using γ-irradiated Treponemes by humoral mechanisms in connection with weak antigenicity of the outer envelope of *T. pallidum*

In our recent experiments the antibody response after immunization with IC was very weak. There was a varying humoral immune response in a number of immunization experiments using ICs obtained from several patients. The highest antibody level was achieved in a previous experiment with a final titer of 1 : 128 in TPHA-test (4).

In spite of the weak immune response a protective effect was induced. Immunization

with ICs altered the local reaction at the infection sites as well as the humoral immune response of an experimental intradermal infection with living Tp.

It is assumed that the effect of protective immunization dependes on the presence of treponemal protein antigen constituent(s) in the ICs. This protein antigen may be able to initiate the production of treponemicide antibodies and to stimulate effector T-cells. We suppose that an unlimited growth and multiplication of *Treponema pallidum* was prevented in rabbits considered to be immunized with success. Consequently, the amount of treponemal antigen was small and cleared rapidly by the immune system of the host. Because of the small amount of antigen the humoral immune response was only weak and the antibody level was low compared with the controls and the animals considered to be immunized without success.

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