Immunity to Syphilis

I. Passive Transfer in Rabbits with Hyperimmune Serum

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Passive transfer of humoral immunity to experimental syphilis in rabbits was accomplished with large doses of hyperimmune serum. When the serum was administered before inoculation of *Treponema pallidum*, the onset of chancres was delayed; when it was given early in the course of infection, a transient period of arrest and healing of the chancres was imposed.

Current knowledge and concepts of immunity to syphilis have been well summarized by Turner (12).

Although man possesses little or no innate immunity to syphilis, he develops a substantial degree of acquired immunity to the disease. Immunity is acquired slowly, possibly because the protective immunogen is weak, and wanes after elimination of the organism either naturally or by antibiotic therapy. Despite many years of extensive investigation, nothing definitive is known about the mechanisms involved in acquired immunity or the identity of the protective immunogen(s). There are no compelling reasons to rule out the potential contribution of either humoral or cellular immune forces to total immunity. The demonstration of "protective antibodies" in the serum of infected rabbits by Eberson (1) and of treponema immobilizing (TPI) antibody by Nelson and Mayer (7) initially supported the concept that immunity to syphilis is due solely to humoral antibodies. However, the later finding that there is no certain correlation between the serum titers of TPI antibody and immunity (4) and the evidence that strong delayed hypersensitivity to treponeme antigens develops in the course of disease have led a number of investigators to suggest that immunity in syphilis is primarily cellular in nature. Although phagocytosis of live treponemes is not known to occur, it is possible that cellular immune forces could operate in other ways than by destruction within phagocytes, such as by the direct effects of lymphokines (8) or through the agency of cytophilic antibodies on "immune cells."

The experimental approach of determining the infectivity of virulent organisms mixed with immune serum, initially employed by Metch-nikoff and Roux (5), in 1905, has been used by most subsequent investigators. This is unfortunate because the in vitro susceptibility of *Treponema pallidum* to TPI antibody appears to be an in vitro aging artifact resulting from progressive loss of the surface mucoid layer (uncoating) which is presumed to afford the treponeme in vivo protection against antibody (6, 12). The most obvious approach for determining the mechanisms of acquired immunity to syphilis would be to attempt to transfer resistance passively with large doses of immune serum or cells or combinations thereof. Since a perusal of the literature did not reveal that trials of this sort had been made, the present experiments with immune serum were performed.

MATERIALS AND METHODS

New Zealand male rabbits weighing from 1.5 to 3.0 kg were used. They were subjected to serological screening for *T. cuniculi* infection by the VDRL and FTA tests and were caged individually in a room in which the ambient temperature did not exceed 70 F (21 C). They were maintained on an antibiotic-free diet. The inoculum was prepared from a uniform suspension of *T. pallidum* (Nichols strain) stored in liquid nitrogen, and consisted of motile organisms that were resistant to the immediate immobilizing effect of TPI antibody and complement and, hence, must have possessed intact mucoid coats. The suspending medium was TPI medium containing 10% dimethylsulfoxide and 10% normal rabbit serum. Chancres were produced by inoculating 10^6 *T. pallidum* intracutaneously (i.c.) at each of six sites on the shaved back. The number of organisms in the suspension used for inoculation was determined by spreading 5 μliters of the material under a cover slip (22 by 22 mm) rimed with vaseline. The number of organisms was calculated by counting 50 high-power darkfields and multiplying the total by 10^5.

Since there is a tendency for the individual chancres to vary considerably in size, every effort was made to achieve uniformity of inoculation with respect to depth of injection into the skin and numbers of organisms deposited at each injection site. Attention was paid to keeping organisms well suspended, charging syringes but once, randomizing sites injected on the same and different animals in experimental and control groups, etc. After inoculation, the animals were observed daily for the appearance of chancres; the mean diameter of induration and character of the lesions were recorded, and darkfield tests were done periodically on a representative chancre. At most sites, beginning chancres were evident by day 3.

Normal pooled serum was prepared from blood specimens obtained by intracardiac aspiration. A pool of hyperimmune serum (immune serum) was prepared, in a similar manner, from rabbits that had been inoculated i.c. with the standard dose of *T. pallidum* every 3 months until they were strongly resistant to skin reinfection (usually 3 to 6 months). The immune sera were collected 5 days after the last challenge dose of *T. pallidum*; they were titrated for VDRL, FTA and TPI antibodies and were pooled; the titers were as follows: VDRL, 1:64; TPI, 1:256; and FTA, 1:2048. All pooled sera were subjected to membrane filtration (0.45 μm; Millipore Corp.) prior to storage without preservative at -20 C.

RESULTS

Varying doses of pooled immune rabbit serum were first tested for their capacity to alter the course of early chancres. Control rabbits consisted of untreated animals and animals given normal pooled serum. Skin lesions were established in 16 animals with the standard inoculum of 10^6 *T. pallidum*. Twenty-four hours after chancres first appeared on each animal (i.e., on day 4 or 5), a dose of 80 ml of hyperimmune serum per kg body weight was administered intraperitoneally (i.p.) to each of eight of the animals (Fig. 1). Controls consisted of four animals given normal serum and four animals not given serum. The chancres in both the immune serum-treated and control animals
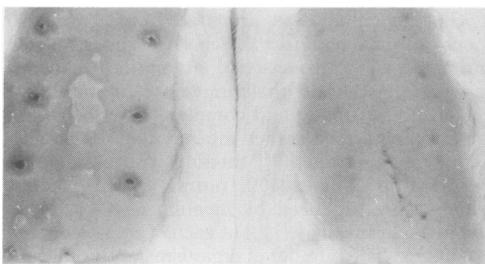
increased progressively in size from small papules at day 3 after treponeme inoculation, to large, intensely indurated, elevated, darkfield-positive lesions between 10 and 15 mm in diameter by day 10. However, after day 10, the chancres in the animals given immune serum receded, and most of them were inconspicuous between weeks 3 and 4. Because of the small numbers of organisms persisting in the lesions, prolonged search was often necessary to demonstrate treponemes by darkfield examination of the exudates obtained by scraping the injection sites. The inconspicuous lesions in these animals were in sharp contrast to the large chancres in control animals, which continued to progress for some time after day 8 to become necrotic and teeming with organisms. The difference in the size and character of the chancres of immune serum-treated and control rabbits was most apparent between weeks 3 and 4 of the experiment (Fig. 2). Between weeks 4 and 5, the lesions in six of the eight immune serum-treated rabbits began to enlarge and to develop induration, whereas the chancres of control rabbits started to resolve and heal. The subsequent course and character of these resurgent chancres in the immune serum-treated animals were similar to the course of the chancres in the control animals earlier in the experiment.

Initial attempts to prevent or to delay the onset of chancres in rabbits by giving a high i.p. dose of the same pool of immune serum (80 ml/kg) 24 h prior to inoculation of the standard dose of 10^6 *T. pallidum* were not successful. Although in these experiments the onset times of chancres did not differ in immune serum-treated and control animals, the chancres in treated animals did not attain sizes as large as the chancres in control animals and were not as
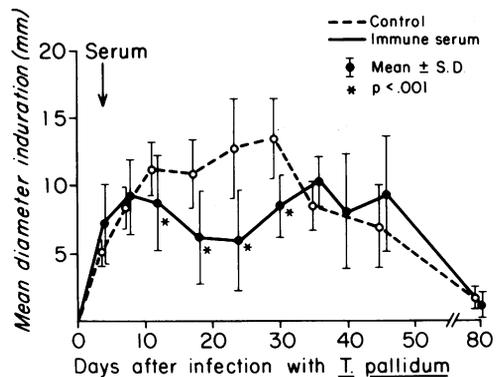
FIG. 1. *Influence of hyperimmune serum on developing skin chancres in rabbits. Control rabbit (left) bearing progressing ulcerated chancres; hyperimmune serum-treated rabbit (right) bearing arrested chancres.*

FIG. 2. *Influence of hyperimmune serum on the development of cutaneous syphilitic chancres in rabbits.*

indurated. However, when the experiment was repeated with a lower challenge dose of *T. pallidum* (10^3), the onset time of chancres in the immune serum-treated animals was significantly delayed (Table 1), and the chancres healed earlier.

An attempt was next made to delay the onset of chancres for a prolonged period of time by serial i.p. doses of immune serum. Two rabbits were repeatedly injected with 80 ml of immune serum per kg, administered at intervals of 3 to 5 days. One received five doses, and the other received seven doses of immune serum. Chancres did not appear during immune serum treatment but developed 4 to 12 days after the treatment was discontinued.

DISCUSSION

As indicated in the introduction, no attempts have been made by others to passively transfer immunity to syphilis by use of large doses of immune serum, and trials with small doses have been uniformly unsuccessful. Finger and Landsteiner (2) failed to prevent the development of skin lesions of secondary syphilis in two patients by the local subcutaneous injection of immune monkey serum. Kemp and Fitzgerald (3) postulated that the offspring of immune female rabbits might be resistant to syphilitic infection due to passive transfer of maternal antibody via the placenta or colostrum. That they were unable to show any resistance in such offspring was not surprising in that the rabbits were not challenged with virulent *T. pallidum* until 12 to 14 weeks of age, when little, if any, passively transferred maternal antibody would still have been present. Sheldon et al. (10) produced focal manifestations of what they regarded to be the Jarisch-Herxheimer reaction in rabbits with early syphilitic skin lesions by administering a single intravenous dose of 70 ml of pooled serum from rabbits with late, untreated syphilis. Unfortunately no mention was made of the subsequent course of the skin lesions. It is possible that in these experiments humoral immunity

was transferred but not documented. Finally, Tani and Aikawa (11) demonstrated that acquired immunity to syphilis can be passively transferred by parabiosing a rabbit with syphilitic infection of long duration to one with early lesions. Of the 35 pairs of parabionts surviving 9 days or longer, all but 7 parabionts with early chancres showed definite healing of their lesions, as manifested by a decrease in induration and the disappearance of spirochetes from the lesions. Since both antibodies and immunocompetent cells are exchanged in parabiosis, the relative contributions of cellular and/or humoral forces to the immunity achieved cannot be assessed.

The present observations provide strong evidence that immunity to syphilis can be passively transferred with serum, presumably as the result of specific antibodies. To ensure results which would be meaningful with respect to immune forces operating in the living animal, initial experiments were conducted in which immune serum was administered to animals bearing established lesions, on the assumption that the organisms present in such lesions would have fully reconstituted their protective capsular coats following inoculation. A transient arrest in the development of the lesions was observed. When immune serum was administered prior to inoculation with *T. pallidum*, a delay in the onset of chancres was observed. Although the manner by which antibodies may act is unknown, it is possible that they serve to immobilize the organisms and/or to destroy them when combined with complement. Reynolds (9) observed that treponemes inoculated into the subcutaneous tissues of immune rabbits did not disseminate from the site of inoculation, whereas in nonimmune rabbits the organisms entered the regional lymphatics within minutes and were rapidly disseminated via the draining lymph nodes and blood stream. The promptness of this localizing effect in the immune animal suggests that the immune forces involved were humoral rather than cellular. Our

TABLE 1. *Capacity of hyperimmune serum to delay the onset of skin chancres in rabbits produced with 10^3 T. pallidum*

Treatment	No. of rabbits	Dose of serum[a]	Onset of chancres (days)	Significance[b]
Hyperimmune serum	4	80 ml/kg	10.3 ± 1.2[c]	$P < .05$
Normal serum	4	80 ml/kg	7.5 ± 0.5	NS
None	5	None	7.0 ± 0.7	

[a] The sera were injected i.p. 24 h before inoculation.
[b] Unpaired rank sum test. NS, Not significant.
[c] Mean ± standard deviation.

observation that early chancres, initially suppressed by immune serum, later ran the normal cycle of development and healing seen in control animals, implies that the effect of immune serum was primarily that of preventing an increase in numbers of organisms; at least its effect was not one of killing all or perhaps even the majority of organisms present when the serum was given. This suggests that multiplying organisms are the principal targets of humoral antibodies in vivo.

ACKNOWLEDGMENTS

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