Acquired Resistance and Expression of a Protective Humoral Immune Response in Guinea Pigs Infected with *Treponema pallidum* Nichols

CHARLES S. PAVIA* AND CAROL J. NIEDERBUHL

Trudeau Institute, Saranac Lake, New York 12983

Resistance to cutaneous syphilitic reinfection in strain 2 and strain 13 guinea pigs developed gradually 3 to 7 months after primary infection and reached maximum levels at 6 to 7 months after the induction of primary cutaneous disease. Associated with this acquired resistance was the occurrence of Arthus reactions and anamnestic-type antibody responses. Passive transfer of immune serum containing high-titered treponemal antibody into normal strain 2 guinea pigs significantly delayed the appearance and markedly diminished the severity and duration of skin lesions that developed after these recipients were challenged with treponemes but did not prevent the dissemination of organisms to the draining lymph nodes. These findings provide direct evidence that syphilitic infection elicits the formation of serum factors that are, at least, partially protective against symptomatic disease.

Syphilitic infection evokes a complex antibody response in the diseased host, resulting in the formation of two varieties of antibody (25). One of these is the nonspecific Wasserman or anticardiolipin antibody, and the other type of antibody specifically reacts with several components of the organism (8, 9). Currently available data support the notion that humoral immunity plays a limited protective role against treponemal infections, since individuals who contract syphilis can progress through three well-defined stages of the disease despite the formation of abundant quantities of both varieties of antibodies (21, 25). Also, in experimental disease, untreated animals have been shown to harbor virulent treponemes in certain tissues as a form of latency and are subject to intermittent spirochetemia, even in the presence of high titers of circulating antibody (6, 25). Furthermore, vaccines consisting of dead or purified antigen preparations of *Treponema pallidum* that induce the production of antibodies (10, 11, 25) generally have been unsuccessful in providing protection against treponemal infection, although considerable success was achieved in rabbits immunized with large numbers of motile organisms attenuated by gamma-irradiation (14). It has been shown, however, based on the results of passive immunization experiments that immune serum or immunoglobulins from syphilitic rabbits (3, 23, 24, 27) and hamsters (1) can confer partial or complete protection against syphilitic infection to normal challenged recipients. Related in vitro studies (4, 5) have shown that immune syphilitic serum and immunoglobulins in the presence of complement can neutralize the infectivity of virulent *T. pallidum* as well as modify host immune responses in vitro (2, 15, 16).

In terms of evaluating immune effector mechanisms involved in protection against syphilis, experimental *T. pallidum* infection in the guinea pig has yet to be widely used as a model of the human venereal disease. Recently, however, it has been shown (18, 19, 28) that outbred and certain inbred strains of guinea pigs are capable of developing, on a regular basis, symptomatic disease after exposure to large numbers of *T. pallidum* organisms and that various components of both humoral and cell-mediated immunity become activated in these animals during experimental infection. In the present study we reexamined the development of acquired resistance and the potential protective effects of immune serum with an inbred guinea pig model of human syphilis (18). Our results demonstrate that peak resistance to cutaneous disease occurred 6 to 7 months after primary treponemal infection and that serum from guinea pigs immune to challenge infection with *T. pallidum* Nichols has the capacity to confer a moderate degree of protection against cutaneous disease in passively immunized, challenged recipients.

MATERIALS AND METHODS

Animals. Strain 2 guinea pigs were bred under barrier-sustained conditions at the Animal Breeding Facility of the Trudeau Institute from animals originally obtained from the Division of Research Services, National Institutes of Health, Bethesda, Md. Strain 13 guinea pigs were purchased from ARI Breeding Laboratories, East Bridgewater, Mass. Strain 2 (GPLA-B.1, S, Ia. 2,4,5,6) and strain 13 (GPLA-B.1, S, Ia. 1,3,5,6,7) guinea pigs have been inbred since 1906. Reciprocal skin grafts between these two strains are usually rejected within 17 days (26). Both strains share the serologically detectable histocompatibility antigens determined by the GPLA loci (equivalent to murine H-2K/D gene products) but differ by several other antigens (Ia) determined by complex linked genes in three subregions of the I region. Adult (age, 3 to 10 months) animals weighing 400 to 1,100 g each and having negative reactions in either the fluorescent treponemal antibody assay (18) or the Sera-Tek treponemal antibody (MHA-TP) test (Ames Div., Miles Laboratories, Inc., Elkhart, Ind.) were used in these experiments. Outbred New Zealand White male rabbits with negative MHA-TP reactions were supplied by the Animal Breeding Facility of the Trudeau Institute or purchased from ARI Breeding Laboratories. All animals were housed in an air-filtered environment maintained at 20 ± 2°C.

Bacteria and infections. The virulent Nichols strain of *T. pallidum* was generously provided by James Folds, University of North Carolina, Chapel Hill. A sample of the original inoculum was found to be free of known rodent viral

* Corresponding author.

pathogens according to results of routine serological screening performed by the Animal Diagnostic Testing Service of Microbiological Associates, Walkersville, Md. Before the guinea pigs were infected, their hair was removed from the areas chosen for the injection sites with electric clippers, and after inoculation, they were kept hair free by periodic clipping. Guinea pigs were inoculated intradermally (i.d.) with various numbers of treponemes (see Results) in a volume of 0.1 ml at multiple sites in the hind-leg region. At selected monthly intervals after primary treponemal infection, guinea pigs received a second (challenge) injection of 10^7 or 10^6 T. pallidum organisms. Pathogenic organisms for these injections were grown in the testes of rabbits without the use of cortisone and were obtained by extraction of infected rabbit testicular tissue as detailed elsewhere (20, 22). The extraction procedure was carried out at room temperature under an atmosphere of 1.5% O_2–5% CO_2–93.5% N_2. Motile organisms were enumerated by the method described by Fieldsteel et al. (7) after staining them with acridine orange and viewing them under UV illumination (18). The size of developing lesions was monitored against time by measuring changes in the diameter of the infected skin sites with dial calipers. Only treponeme-containing chancres exhibiting erythema and induration followed by ulceration were considered to be typical syphilitic lesions. Flat erythematous skin reactions lacking treponemes were considered to be negative or atypical lesions. Challenged guinea pigs also were followed for the development of Arthus sensitivity and anamnestic-type antibody responses. In some experiments, guinea pigs received an i.d. injection of heat-killed (56°C for 1 h) T. pallidum or an inoculum of testes extract obtained after subjecting a suspension of freshly harvested treponemes to high-speed centrifugation (12,800 × g for 20 min). This supernatant was frozen and thawed several times and, based on microscopic examination, contained no visible treponemes or only small numbers (less than 10^4/ml) of nonmotile organisms. In additional experiments, guinea pigs were given seven daily injections of the broad-spectrum antibiotic Claforan (cefotaxime sodium; Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.), at a dosage of 50 mg/kg to terminate syphilitic infection. This antibiotic was chosen over other more commonly used antimicrobial agents such as penicillin or its derivatives since there is evidence that these latter drugs are toxic to guinea pigs (26), presumably as a result of overgrowth of gram-negative organisms in the gut.

Detection of antibodies. Whole blood was collected from animals before infection and at various intervals up to 8 months postinoculation. Plasma or serum was assayed for the presence of treponemal antibodies by the MHA-TP test as previously described (18) and in accordance with the instructions supplied by the manufacturer. It should be noted that while serum or plasma was not heat inactivated before testing, all samples with equivalent titers (irrespective of their origin) gave uniform hemagglutination-reactivity patterns.

Preparation of immune and normal sera for passive transfer experiments. Fifteen strain 2 guinea pigs were initially infected i.d. with a total of 1.5×10^7 to 2×10^7 T. pallidum. After inoculation, each guinea pig developed typical treponeme-containing, chancrelike skin lesions (18). Four to seven months later, these T. pallidum-infected guinea pigs were challenged with an i.d. injection of 10^7 treponemes. After another 1 to 2 months, guinea pigs were bled by intracardiac puncture or by venous aspiration to obtain serum. A group of 10 normal syngeneic guinea pigs with

negative MHA-TP titers were similarly bled to obtain normal serum. The pooled immune sera and the pooled normal sera were filter (pore size, 0.45 μm) sterilized and stored at −70°C until use.

Passive transfer experiments. Recipients were injected intravenously with 4 to 5 ml of immune or normal serum per kg of body weight. Two hours later, serum recipients were challenged i.d. at duplicate sites with 10^7 or 10^6 freshly harvested T. pallidum. At 2, 4, 6, 8, and 10 days postchallenge infection, each guinea pig was injected intramuscularly in the thigh with 1.0 ml of either immune or normal serum. Animals were observed daily for the development of skin lesions.

Detection of T. pallidum organisms in infected tissues. At 1 month and between 4 and 6 months after the initiation of serum transfer experiments, 6 to 10 guinea pigs were bled and sacrificed. Popliteal and inguinal lymph nodes were removed aseptically for infectivity tests in serologically negative, normal recipient rabbits. Excised tissues were minced finely with scissors and forceps and suspended in a small volume of basal reduced medium (18). After centrifugation at 100 × g for 10 min to remove gross material and most of the lymphoid cells, samples of the remaining supernatant were reacted with an equal volume of acridine orange (100 μg/ml) in phosphate-buffered saline (pH 7.2). These preparations were then examined immediately for the presence of motile treponemes, which stain bright green when viewed under UV illumination. Viability and virulence of organisms recovered from these suspect infected tissue preparations, as well as from prepared samples of lymph nodes in which treponemes were not observed microscopically, were determined by injecting concentrated extracts of minced tissue into the shaven back of normal recipient rabbits. These test rabbits then were monitored for the development of typical syphilitic skin lesions and also examined periodically for treponemes in fluid samples removed from scrapings of obvious skin lesions as previously described for guinea pigs infected i.d. (18). The pattern of lesion development in these rabbits challenged with guinea pig lymph node extracts was compared with the rate of lesion formation in rabbits simultaneously infected i.d. with known numbers (i.e., 10^2, 10^3, 10^4, 10^5, 10^6) of treponemes freshly harvested from orchitic rabbit tissue. This latter procedure enabled us to construct a standard lesion-growth curve from which a reasonably accurate estimate of the number of virulent organisms present in these lymph node preparations can be made. This method of extrapolation is a slight modification of the technique previously described by Turner and Hollander (25) for estimating the number of virulent treponemes found in a given sample of infected host tissue.

RESULTS

Resistance to challenge infection with T. pallidum. Ten groups of guinea pigs (6 to 10 animals per group) were challenged i.d. with 10^7 or 10^6 organisms at 3, 4, 5, 6, or 7 months after a primary infection with 10^7 treponemes. During this period there was progressive development of resistance to challenge infection (Fig. 1). This finding was based on the lack of formation of typical chancrelike, treponeme-containing lesions occurring at the challenge sites. Typical ulcerative lesions similar to those previously described (18, 19) (and as depicted elsewhere [see Fig. 4B]) occurred in a parallel group of normal control guinea pigs undergoing primary syphilitic infection. At 3 months post-primary infection only slightly more than 50% of the animals were immune

to reinfection, while from 7 months onward no cutaneous lesions developed in any animals during an observation period extending beyond 20 weeks postchallenge. It should be noted, however, that viable treponemes (between 10^3 and 10^4) could be recovered from the draining popliteal and inguinal lymph nodes taken from lesion-resistant guinea pigs several weeks after challenge infection (Fig. 1). At 6 to 7 months after primary infection, eight separate groups of three unchallenged guinea pigs were given antibiotic therapy with Claforan. At 2, 4, 8, and 16 weeks later, these treated guinea pigs were challenged i.d. with 10^7 T. pallidum. None of these challenged animals given prior drug therapy developed lesions at test injection sites, nor were viable organisms recovered from their lymph nodes (Fig. 1). In parallel experiments, it was determined that treating normal guinea pigs with equivalent doses of Claforan 2 to 16 weeks before challenge afforded no protection against symptomatic disease, nor did this prevent treponemal invasion of the draining lymph nodes (data not shown). The patterns of resistance to cutaneous challenge infection described above were similar in both strain 2 and strain 13 male and female guinea pigs.

Intense Arthus-type reactions were produced after i.d.

FIG. 2. Example of a typical Arthus reaction (arrow) occurring 3 to 7 months after a primary infection in a strain 2 guinea pig challenged i.d. with 10^7 T. pallidum 6 to 10 h previously. At 3, 6, and 12 h postchallenge, the size of erythematous and indurated skin reactions in a group of six previously infected guinea pigs averaged 8.1, 8.8, and 8.5 mm in diameter, respectively. Injections of extracts of normal rabbit testes elicited only faint skin reactions measuring <3 mm.
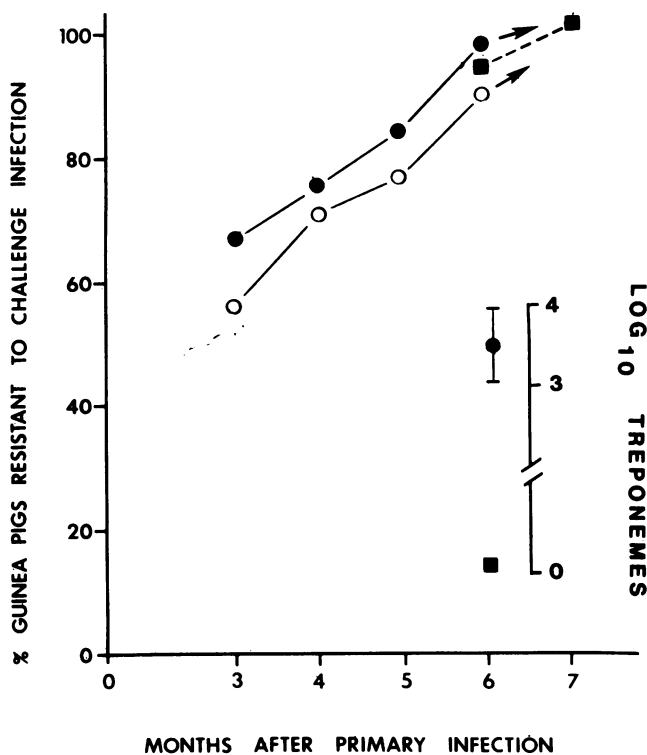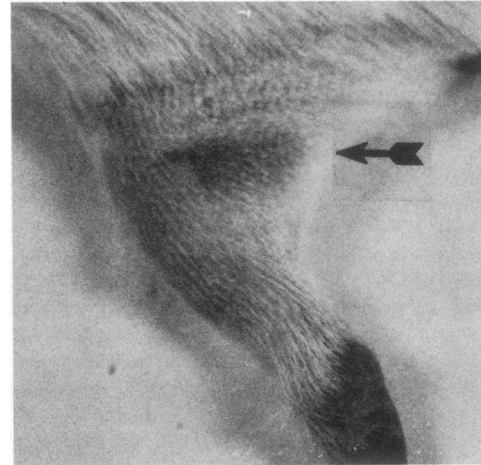

FIG. 1. Temporal development of resistance to reinfection. At 3 to 7 months after primary syphilitic infection, separate groups of strain 2 and strain 13 guinea pigs were challenged i.d. with 10^6 (●) or 10^7 (○) treponemes and then monitored for lesion development. At 6 to 7 months after primary infection, eight additional groups of guinea pigs (■) were cured of syphilitic infection after receiving seven daily injections of the antibiotic Claforan (50 mg/kg) and then challenged with 10^7 T. pallidum 2 to 16 weeks later. There were 6 to 10 guinea pigs per time point in the groups not receiving Claforan. Individual data points on the lower right refer to the mean number of viable T. pallidum ± standard error of the mean recovered from the lymph nodes of the untreated (●) or antibiotic-treated (■) groups of infected guinea pigs.

challenge with live or dead treponemes, beginning at 3 months after the primary immunizing T. pallidum infection. Test injection sites exhibited local swelling and erythema 3 to 6 h postchallenge (Fig. 2). These reactions increased to a maximum size 8 to 20 h later with the development of extensive swelling and reddening of the entire thigh region of the rear leg. The circumscribed areas of erythema present at 20 h at the injection sites persisted for another 12 to 24 h, and a central zone of necrosis usually occurred at many of the test sites, resulting in some residual skin thickening. In control experiments, previously infected guinea pigs were challenged i.d. with an extract of infected rabbit testes lacking motile treponemes. Testing with this noninfectious preparation caused only slight local erythematous reactions that led to some skin thickening within 12 to 24 h, which then disappeared.

Associated with this evidence of substantial resistance to challenge infection and of skin sensitivity to T. pallidum was the occurrence of a marked rise in the MHA-TP antibody titer. Within 1 month after challenge, the serum of resistant guinea pigs attained titers of antitreponemal antibody averaging 1,920, whereas maximal prechallenge titers averaged only 480. This represents a fourfold increase in the level of circulating antibody in resistant guinea pigs. It should be noted that serum taken from guinea pigs injected with noninfectious material with or without dead treponemes or from guinea pigs injected with viable treponemes and given subsequent antibiotic therapy was either nonreactive or attained barely detectable levels of antibody (data not shown).

Serum-mediated transfer of antitreponemal immunity. In the next series of experiments, we tested the ability of serum or plasma taken from immune guinea pigs to transfer protection against syphilitic infection to normal syngeneic recipients. Immune guinea pig serum was obtained from 15 donor guinea pigs that had been infected intradermally 4 to 7 months previously and had been challenged i.d. with 10^7 or 10^6 T. pallidum organisms (Fig. 1). Cutaneous, treponeme-containing lesions developed in 50% of guinea pigs infused
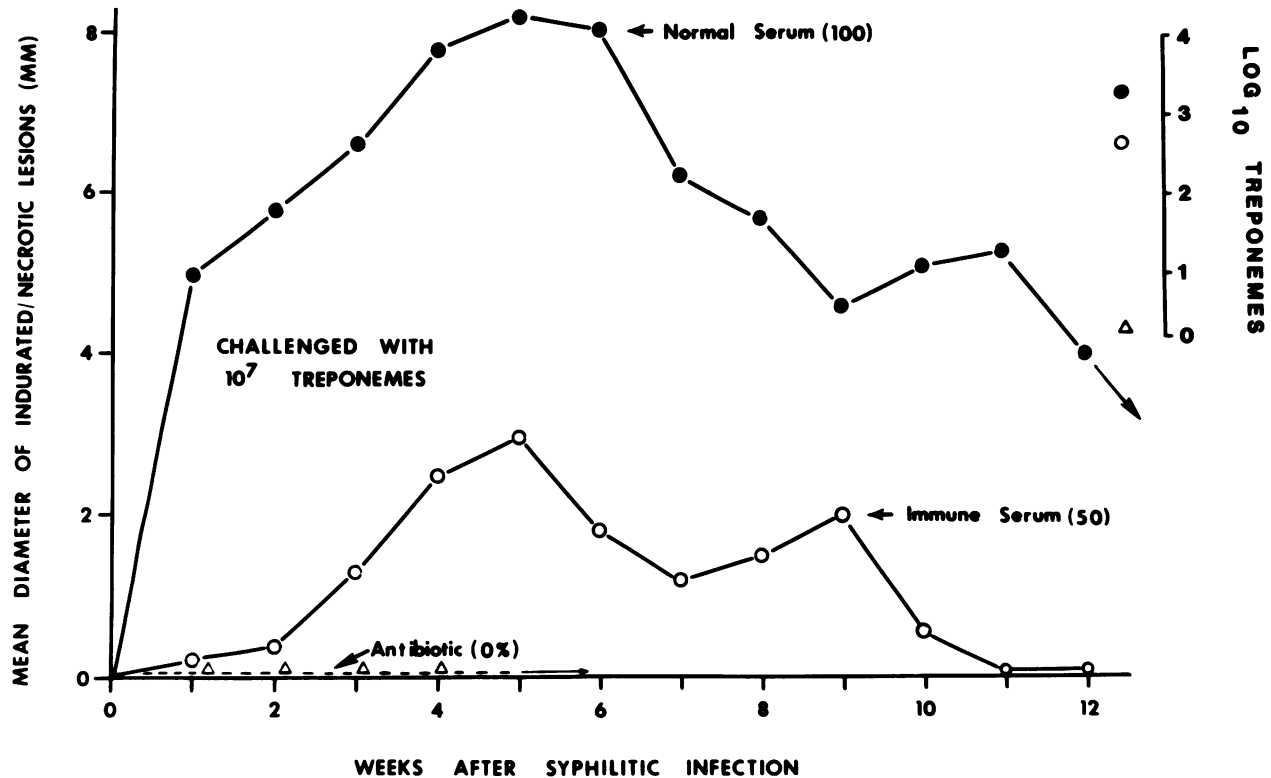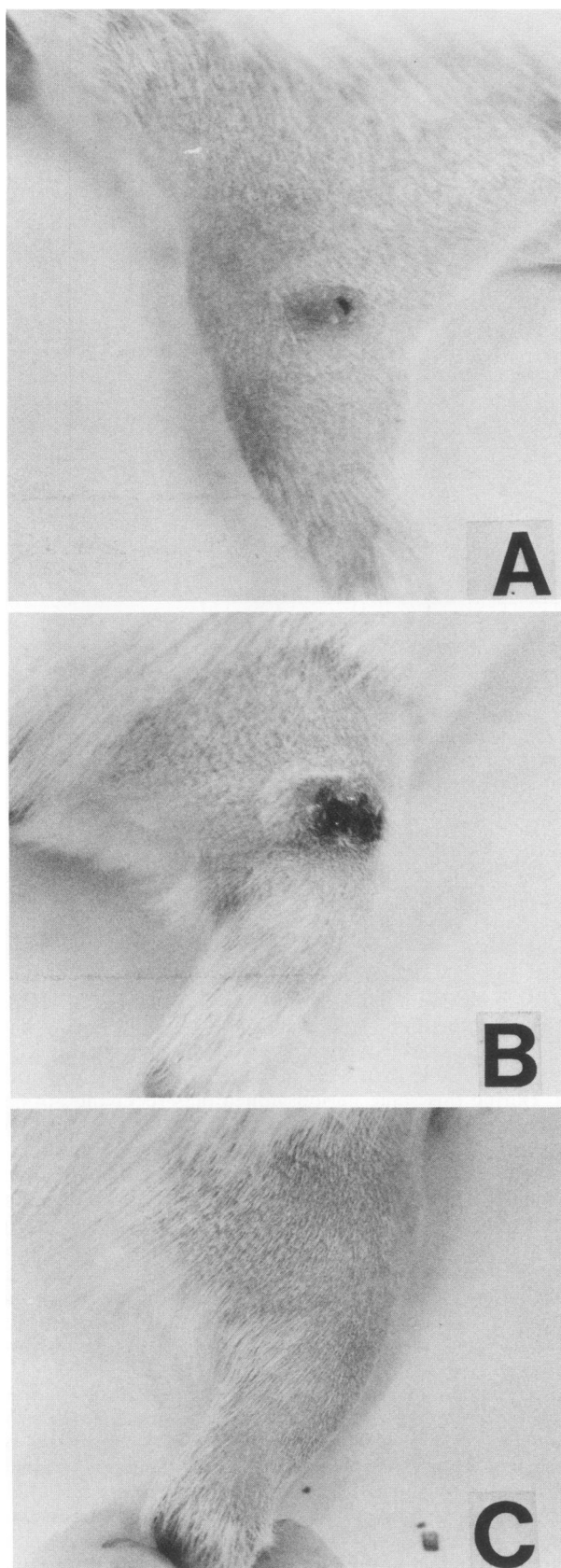
WEEKS AFTER SYPHILITIC INFECTION

FIG. 3. Capacity of passively transferred immune guinea pig serum to protect against an i.d. challenge with 10^7 treponemes (O). Appropriate controls included a group of challenged guinea pigs receiving normal serum (●) or normal guinea pigs given no serum but receiving antibiotic treatment with Claforan (△) for the first 7 days postchallenge. Numbers within parentheses indicate the percentage of guinea pigs developing treponeme-containing cutaneous lesions. Each experimental group consisted of six age- and sex-matched strain 2 guinea pigs. Individual data points on the upper right refer to the mean number of viable *T. pallidum* recovered from the lymph nodes of each of the three different groups of challenged guinea pigs.

with hyperimmune serum after challenge with 10^7 treponemes (Fig. 3). Skin lesions which did develop in these animals were characterized by (i) a prolonged incubation period and shorter duration, (ii) a smaller size (measuring 3 mm or less in average diameter) with only slight erythema or induration, and (iii) only slight ulceration and necrosis at their peak stage (Fig. 4A) when compared with the disease pattern of the indurated and severely necrotic lesions occurring in the group of challenged guinea pigs receiving normal serum (Fig. 4B). The remaining challenged guinea pigs (50%) from the immune serum recipient group did not develop cutaneous syphilitic lesions in a manner analogous to a parallel control group of infected guinea pigs receiving simultaneous antibiotic treatment (Fig. 3). A greater degree of protection occurred in animals given immune serum and challenged with the lower dose of 10^6 treponemes (Fig. 4C). Only 17% of these guinea pigs developed typical syphilitic skin lesions which exhibited erythema and induration for a transient period (at 4 and 5 weeks postchallenge) at the injection site (data not shown). In marked contrast, however, unprotected guinea pigs of the entire control group developed typical ulcerative lesions measuring from 3 to 7 mm in average size and lasting for at least a 12-week observation period. Necrotic skin lesions also occurred in an additional control group of challenged guinea pigs infused with serum from donors previously inoculated with a noninfectious extract of rabbit testes (Fig. 4B).

Levels of antibody in recipient guinea pigs. To determine whether passive immunization resulted in a substantial rise

in circulating antibody titer, recipient guinea pigs were monitored for MHA-TP levels at periodic postchallenge intervals (Fig. 5). At the time of transfer into recipients, pooled donor immune serum had a mean titer of 1,920, while pooled donor nonimmune serum was negative in the MHA-TP test. For the first 4 weeks of the post-serum transfer and challenge period, relatively high levels of antibody (range of mean titers, 120 to 320) were maintained in recipient guinea pigs. During this same period guinea pigs receiving normal serum remained negative or were only weakly reactive (range of group mean titers, 20 to 40) for MHA-TP antibody. Between 4 and 11 weeks postchallenge there was a gradual decline in antibody titers in the immune serum recipients, while during this same period the control challenge group exhibited a progressive increase in antibody titers until maximum levels were reached, usually from 8 weeks onward. After 11 weeks, circulating antibody levels of the immune serum recipient guinea pigs began to rise substantially until peak titers were attained from 16 weeks onward (Fig. 5).

Dissemination of treponemal infection. Because of their exquisite susceptibility to very low numbers (≤200) of treponemes (13), rabbits were chosen as test recipients of extracts of suspected infected tissue of challenged guinea pigs. Similar to guinea pigs belonging to the control challenged groups, immune serum recipients showed evidence of disseminated infection (Fig. 3) based on the formation of treponeme-containing skin lesions after transfer of tissue extracts into test recipient rabbits. These results are consis-

tent with the ability of immune serum recipient guinea pigs to eventually produce antitreponemal antibodies (presumably as a consequence of in vivo proliferation of *T. pallidum*) at a level greater than that which previously was attained by injections of immune serum or than that in a group of unchallenged guinea pigs receiving immune serum alone (Fig. 5).

DISCUSSION

Maximum resistance to challenge syphilitic infection develops relatively slowly in both humans (25) and experimentally infected animals (4, 25). During the early stage of syphilis some immunity is generated, which increases gradually as the disease progresses through its various phases until maximum levels are attained during the later stages. Usually a period ranging from several months to several years elapses before this process becomes completed. Our results involving the homologous challenge of guinea pigs at various intervals after primary syphilitic infection are consistent with these earlier findings. By 3 months after initial infection, at a time when all primary lesions have completely healed (18), a major proportion of previously infected guinea pigs were resistant to challenge. At succeeding monthly intervals, resistance increased gradually and became firmly established at 6 to 7 months post-initial infection. An additional important finding was the ability of infected guinea pigs given antibiotic therapy at 6 to 7 months to maintain complete resistance to challenge infection. Without antibiotic treatment, however, viable treponemes were recoverable from the draining lymph nodes of previously infected and challenged (lesion-resistant) guinea pigs, probably as a consequence of the original immunizing infection. Anamnestic-type antibody responses and strong Arthus reactions also occurred in these animals, suggesting a possible relationship between resistance to syphilitic infection and these expressions of heightened humoral immune responses. In this regard, there is a growing body of evidence based on passive immunization experiments that humoral factors generated in response to syphilitic infection confer various levels of resistance (1, 3, 23, 24, 27) to *T. pallidum* infection in experimental animals. The data presented here are in close agreement with these earlier findings by showing that serum from guinea pigs resistant to challenge syphilitic infection confers a limited degree of protection against symptomatic disease in immune serum recipients challenged with the virulent Nichols strain of *T. pallidum*. The inability of immune serum to provide complete protection was evident not only in the development of temporary, treponeme-containing lesions in a small percentage of challenged guinea pigs receiving serum but also by the degree of dissemination of organisms from the primary challenge site to the regional lymph nodes of the host. Based on infectivity tests with normal rabbit recipients (Fig. 3), low but significant numbers (ranging from 100 to 1,000) of virulent treponemes were recoverable from these sites, beyond the original focus of

FIG. 4. Representative examples of cutaneous lesions of reduced severity occurring in unprotected guinea pigs (50%) infused with immune serum and challenged with 10^7 treponemes (A), typical necrotic lesions exhibited by challenged guinea pigs receiving normal serum or by guinea pigs infused with serum from donors injected with a noninfectious extract of orchitic rabbit testes (B), and no lesions in protected challenged recipients of immune serum (C).
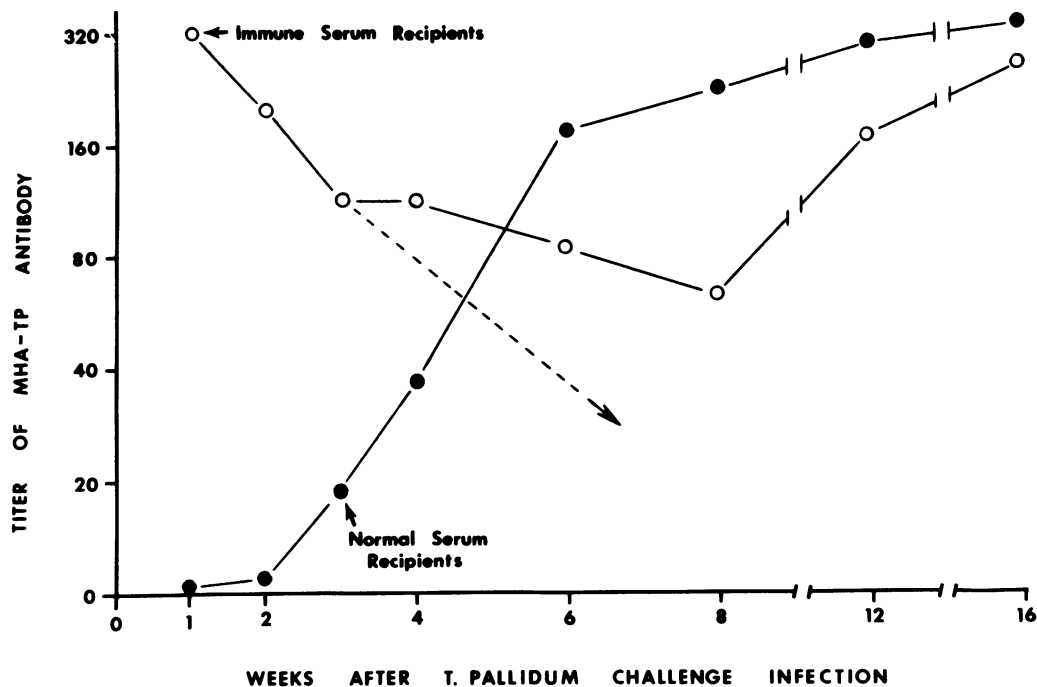
FIG. 5. Levels of MHA-TP antibody in challenged guinea pigs receiving immune (○) or normal (●) serum. Each point represents the mean of the reciprocal of the highest dilution exhibiting at least 1+ reactivity. The dashed lines indicate declining MHA-TP antibody titers in a matched control group of uninfected (unchallenged) guinea pigs receiving immune syphilitic serum alone. Challenged guinea pigs are the same as those described in the legend of Fig. 3.

challenge organisms introduced i.d. Immune serum therefore is unable to prevent this migration of treponemes, so that the degree of protection observed was at best partial, resulting in only a delay or reduction in the overall number of disseminated organisms. This latter finding correlates well with previous studies in the rabbit (3, 4), whereby immune serum has been shown to inhibit cutaneous disease but is ineffective in preventing dissemination of organisms to distal sites in the challenged host.

To our knowledge, only two published reports (5, 23) have presented evidence that immunoglobulins isolated from immune serum are active in vivo and in vitro in the expression of antisyphilis immunity. In support of this finding we recently observed (C. Pavia, C. J. Niederbuhl, and J. Saunders, Immunology, in press) that fractions of immune serum enriched for guinea pig immunoglobulins are partially protective against syphilitic infection. Further analysis of guinea pig immunoglobulin G from immune serum by Western blotting techniques (Pavia et al., in press) revealed immune reactivity with several treponemal proteins similar to what was previously reported for human (9) and rabbit (8) syphilitic sera. It is possible that guinea pigs and rabbits could not be completely protected due to the infusion of insufficient amounts of the essential protective humoral factors contained in immune serum such as antibody. During the first few weeks of challenge infection, recipient guinea pigs contained fairly high levels of circulating antitreponemal antibody which was predominantly of donor origin since several weeks elapsed before matched control guinea pigs started synthesizing significant levels of antibody. After this period and for the next several weeks thereafter, MHA-TP antibody titers declined gradually in the sera of these recipient animals, at a rate which was consistent with the reported in vivo half-life (6 to 7 days) for guinea pig immunoglobulins (22).

In view of the relatively small amount of hyperimmune sera used in our passive immunization experiments, our data could be interpreted as supporting an important role for humoral factors in antisyphilis immunity. Previously, in the work of Bishop and Miller (3), excellent protection against syphilitic infection in rabbits was achieved by the daily infusion of immune serum for a period lasting 37 consecutive days postchallenge. Taking into account the average body weight of the rabbits used in their study, most of their protected animals received just under 400 ml of serum each. This substantial amount of transferred serum effectively neutralized the vast majority of treponemes present in the infectious inoculum, which numbered only 4,400 organisms. Lesions did develop later on, however, after the discontinuance of immune serum injections. Similar, but somewhat less impressive, results involving the use of enormous quantities of serum have been reported by others (24, 27). It is important to point out that because these earlier studies involved multiple injections of homologous antisera into allogeneic rabbit recipients, the evidence for the protective effects of antibodies in syphilitic serum must be reassessed based on our current understanding of allotypic markers on rabbit immunoglobulins (12) and regulation of the immune response by idiotypes (20). It is possible that the emergence of antiallotypic or antiidiotypic responses, or both, as previously suggested by Bishop and Miller (3), could have occurred in these passively immunized rabbits resulting either in an amplification of the actual level of protection attributable to transferred immune serum alone or in some neutralization of the humoral antitreponemal factors. By comparison, in the experiments described here, we observed a limited degree of protection in guinea pigs receiving approximately 4 ml of hyperimmune serum per kg of body weight on the day of challenge, followed by five more injections of 1.0 ml of serum on alternate days postchallenge.

For the entire experimental period no animal received more than 10 ml of hyperimmune serum which, based on the resulting delay in lesion development or lack of lesion formation, or both, had the capacity to neutralize large numbers (between 10^6 and 10^7) of the challenge population of treponemes. It is noteworthy that by using inbred guinea pigs in this study the possible interference by antiallotypic responses has been avoided, although we cannot totally exclude the formation of antiidiotypic antibodies directed against immunoglobulins present in the transferred serum.

In conclusion, based on the results obtained so far by the technique of passive immunization, it is clear that immune serum and antitreponemal immunoglobulins from syphilitic hamsters (1), rabbits (3, 23, 24, 27), and guinea pigs (Fig. 3) can confer either complete or partial protection against cutaneous or disseminated *T. pallidum* infection. As a consequence, it may become necessary to reassess the overall importance of the antisyphilis humoral response, at least to the extent of its ability to effectively control the number of treponemes present at the primary focus of infection, which slows, but does not always prevent, dissemination of challenge organisms. This effector mechanism would, therefore, provide valuable time to the infected host in allowing for the development of a cellular immune response (17; C. Pavia and C. Niederbuhl, J. Immunol., in press) before being overwhelmed by vast numbers of replicating treponemes.

LITERATURE CITED

1. **Azadegan, A. A., R. F. Schell, and J. L. LeFrock.** 1983. Immune serum confers protection against syphilitic infection on hamsters. Infect. Immun. **42:**42–47.
2. **Baughn R. E., and D. M. Musher.** 1982. Reappraisal of lymphocyte responsiveness to concanavalin A during experimental syphilis: evidence that glycosaminoglycans in the sera and tissues interfere with active binding sites on the lectin and not with lymphocytes. Infect. Immun. **35:**552–559.
3. **Bishop, N. H., and J. N. Miller.** 1976. Humoral immunity in experimental syphilis. I. The demonstration of resistance conferred by passive immunization. J. Immunol. **117:**191–196.
4. **Bishop, N. H., and J. N. Miller.** 1976. Humoral immunity in experimental syphilis. II. The relationship of neutralizing factors in immune serum to acquired resistance. J. Immunol. **117:**197–207.
5. **Blanco, D. R., J. N. Miller, and P. A. Hanff.** 1984. Humoral immunity in experimental syphilis: the demonstration of IgG as a treponemicidal factor in immune rabbit serum. J. Immunol. **133:**2693–2697.
6. **Collart, P., P. Franceshini, and P. Durel.** 1971. Experimental rabbit syphilis. Br. J. Vener. Dis. **47:**389–400.
7. **Fieldsteel, A. H., F. A. Becker, and J. G. Stout.** 1977. Prolonged survival of *Treponema pallidum* (Nichols strain) in cell-free and tissue culture systems. Infect. Immun. **18:**173–182.
8. **Hanff, P. A., N. H. Bishop, J. N. Miller, and M. A. Lovett.** 1983. Humoral immune response in experimental syphilis to polypeptides of *Treponema pallidum.* J. Immunol. **131:** 1973–1977.
9. **Hanff, P. A., T. E. Fehniger, J. N. Miller, and M. A. Lovett.** 1982. Humoral immune response in human syphilis to polypeptides of *Treponema pallidum.* J. Immunol. **129:** 1287–1291.
10. **Izzat, N. N., J. M. Knox, W. G. Dacres, and E. B. Smith.** 1971. Resistance and serological changes in rabbits immunized with virulent *Treponema pallidum* sonicate. Acta Dermato-venereol. **51:**157–160.
11. **Izzat, N. N., and E. Yazdi.** 1973. Immunological studies on treponemal cellular antigens. I. Serological changes and resistance to infection in rabbits immunized with treponemal nucleic acids. Br. J. Vener. Dis. **49:**338–341.
12. **Kindt, T. J.** 1975. Rabbit immunoglobulin allotypes: structure, immunology and genetics. Adv. Immunol. **21:**35–86.
13. **Magnuson, H. J., H. Eagle, and R. Fleischman.** 1948. The minimal infectious inoculum of *Spirochaeta pallida* (Nichols strain), and a consideration of its rate of multiplication in vivo. Am. J. Syph. **32:**1–18.
14. **Miller, J. N.** 1973. Immunity in experimental syphilis. VI. Successful vaccination of rabbits with *Treponema pallidum,* Nichols strain, attenuated by gamma irradiation. J. Immunol. **110:**1206–1215.
15. **Pavia, C. S., J. D. Folds, and J. B. Baseman.** 1976. Depression of lymphocyte response to concanavalin A in rabbits infected with *Treponema pallidum* (Nichols strain). Infect. Immun. **14:**320–322.
16. **Pavia, C. S., J. D. Folds, and J. B. Baseman.** 1977. Selective in vitro response of thymus-derived lymphocytes from *Treponema pallidum*-infected rabbits. Infect. Immun. **18:**603–611.
17. **Pavia, C. S., J. D. Folds, and J. B. Baseman.** 1978. Cell-mediated immunity during syphilis. A review. Br. J. Vener. Dis. **54:**144–150.
18. **Pavia, C. S., and C. J. Niederbuhl.** 1985. Experimental *Treponema pallidum* infection in inbred guinea pigs: development of lesions and formation of antibodies. Genitourin. Med. **61:**75–81.
19. **Pierce, C. S., K. Wicher, and S. Nakeeb.** 1983. Experimental syphilis: guinea pig model. Br. J. Vener. Dis. **59:**157–168.
20. **Rajewsky, K., and T. Takemari.** 1983. Genetics, expression and function of idiotypes. Annu. Rev. Immunol. **21:**35–86.
21. **Schell, R. F., and D. M. Musher (ed.).** 1983. Pathogenesis and immunology of treponemal infection, p. 101–120. Marcel Dekker, Inc., New York.
22. **Sell, S.** 1964. Evidence for species' differences in the effect of serum gamma-globulin concentration on gamma-globulin catabolism. J. Exp. Med. **120:**967–986.
23. **Titus, R. G., and R. S. Weiser.** 1979. Experimental syphilis in the rabbit: passive transfer of immunity with immunoglobulin G from immune serum. J. Infect. Dis. **140:**904–913.
24. **Turner, T. B., P. H. Hardy, Jr., B. Newman, and E. E. Nell.** 1973. Effects of passive immunization on experimental syphilis in the rabbit. Johns Hopkins Med. J. **133:**241–251.
25. **Turner, T. B., and D. H. Hollander.** 1957. Biology of the treponematoses. WHO Monogr. Ser. **35:**1–277.
26. **Wagner, J. E., and P. J. Manning (ed.).** 1976. Biology of the guinea pig, p. 106, p. 273. Academic Press, Inc., New York.
27. **Weiser, R. S., D. Erickson, P. L. Perine, and N. N. Pearsall.** 1976. Immunity to syphilis: passive transfer in rabbits using serial doses of immune serum. Infect. Immun. **13:**1402–1407.
28. **Wicher, K., V. Wicher, and R. F. Gruhn.** 1985. Differences in susceptibility to infection with *Treponema pallidum* (Nichols) between five strains of guinea pig. Genitourin. Med. **61:**21–26.