INFECTION AND IMMUNITY, Aug. 1986, p. 305–311
0019-9567/86/080305-07$02.00/0

Vol. 53, No. 2

Enhanced Primary Resistance to *Treponema pallidum* Infection and Increased Susceptibility to Toxoplasmosis in T-Cell-Depleted Guinea Pigs

CHARLES S. PAVIA
Trudeau Institute, Saranac Lake, New York 12983

Received 18 February 1986/Accepted 1 May 1986

Strain 2 guinea pigs made T-cell deficient by thymectomy and irradiation and protected with syngeneic bone-marrow cells (TXB guinea pigs) have a surprisingly high level of resistance to cutaneous syphilis and to the dissemination of treponemes to the draining lymph node. Compared with normal euthymic controls infected with *Treponema pallidum* Nichols, syphilitic TXB guinea pigs developed fewer and less severe skin lesions and their lymph nodes contained lower numbers of treponemes. Associated with this evidence for enhanced innate resistance was the ability of the TXB host to produce, during each test interval of a primary infection, more antitreponemal antibodies than that of their euthymic counterparts. Similar levels of partial protection against cutaneous and disseminated syphilitic infection and elevated antibody levels occurred in challenged normal guinea pigs passively immunized with lymphocytes from *T. pallidum*-infected TXB donors. In contrast, the capacity of the TXB host to be protected against a lethal infection with the unrelated intracellular protozoan parasite *Toxoplasma gondii* was greatly impaired unless it received an intravenous infusion of normal syngeneic thymocytes. These seemingly paradoxical results are explained primarily in terms of a residual T-helper-cell population in the TXB guinea pig which is large and competent enough to generate antisyphilis, but not anti-*Toxoplasma*, immunity.

Recent studies (1, 20–22, 25, 26) have shown that acquired resistance to infection with the pathogenic spirochete *Treponema pallidum* involves both humoral and cellular components of the defense system of the host for its expression. In this regard, while there is convincing evidence for the T-cell-mediated nature of antitreponemal immunity in endemic syphilis (25), there is equally convincing evidence (21) that resistance to the venereal Nichols strain of *T. pallidum* can be induced by both T and B cells. Also, for both of these disease-causing species of treponemes, specific antibody appears to play a prominent role in preventing lesions from developing fully (3, 21, 29) and in restricting the number of treponemes that disseminate to the lymph nodes during an active infection (1, 22). However, the manner in which T cells interact with each other as well as with other cells of the immune system in providing protection or in limiting the full expression of effective immunity throughout the various stages of syphilis has yet to be determined.

Relevant information on the nature of the host-treponeme relationship may result from studying the course of syphilis in the immunocompromised host. It is well known, for example, that the administration of the anti-inflammatory drug cortisone into rabbits increases yields of treponemes in infected testes, causes a significant delay in the usual healing process, and delays the generation of humoral and cellular antitreponemal responses (8, 11, 30). Other immunosuppressive drugs given to syphilitic rabbits reduce the number of lymphocytes and macrophages found in lesions and increase the number of animals developing secondary-like disseminated lesions (16). Unlike experimentally infected rabbits, however, syphilitic guinea pigs treated with cortisone do not experience a more aggravated clinical course of disease than do normal controls (32). In attempting to further delineate the immune mechanisms responsible for protection against syphilis, we used thymectomized, irradiated, bone-marrow-reconstituted (TXB) guinea pigs as the experimental hosts of treponemal infection in the experiments described here. Such immunocompromised animals, while lacking the full complement of functionally mature T cells, possessed a preexisting state of enhanced antitreponemal immunity, yet could not survive a usually nonlethal infection with the intracellular protozoan parasite *Toxoplasma gondii*. These results indicate a differential susceptibility of TXB guinea pigs to infections caused by an intracellular pathogen versus those caused by a predominantly extracellular bacterium that may involve unique parasitic strategies or host-parasite interactions.

MATERIALS AND METHODS

Animals. Young (2- to 4-week-old) and adult (3- to 20-month-old) strain 2 guinea pigs having negative reactions in the Sera-Tek treponemal antibody (MHA-TP) test (Ames Div., Miles Laboratories, Inc., Elkhart, Ind.) or in the indirect *Toxoplasma* hemagglutination assay (TPM-Test; Wampole Laboratories, Div. Carter-Wallace, Inc., Cranbury, N.J.) were obtained from the Animal Breeding Facility of the Trudeau Institute. Outbred New Zealand white male rabbits having negative MHA-TP reactions were supplied by the Animal Breeding Facility or were purchased from F & M Rabbitry, Malone, N.Y. Male and female 12- to 20-week-old AB6 and B6D2 mice to be used for the routine maintenance of *Toxoplasma gondii* parasites by serial peritoneal passage were provided by the Animal Breeding Facility. All animals were housed in an air-filtered environment maintained at 20 ± 2°C.

T-cell-depleted (TXB) guinea pigs. Among the most commonly used laboratory rodents, only guinea pigs possess a cervical thymus (31), consisting of two symmetrical lobes in the precardium mediastinum and neck region, where it surrounds the trachea ventrally and laterally. TXB guinea pigs were produced from 2- to 4-week-old strain 2 guinea pigs that had been anesthetized with 40 mg of ketamine

hydrochloride (Ketaset; Bristol Laboratories, Syracuse, N.Y.) per kg. by making a midline incision (20 to 25 mm in length) at the level of the mandibular angles and then surgically removing the thymus glands. The incision wound was closed with metal autoclips and covered with antiseptic powder. At 2 to 4 weeks later, animals were exposed to 900 rads whole-body (lethal) irradiation from a cesium-137 source with a midphantom dose rate of 29.5 rad/min and were immediately infused intravenously with 10^8 syngeneic bone marrow cells taken from the long bones of sex- and age-matched donors. After another 6 to 10 weeks, the surviving TXB guinea pigs (>90%) were used in the infectivity experiments. In additional experiments, TXB guinea pigs were reconstituted with an intravenous infusion of 10^9 thymocytes from normal syngeneic donors 2 to 7 days prior to sensitization with complete Freund adjuvant (CFA) or before infection with *Toxoplasma gondii* (described below).

Microorganisms and infections. The Nichols strain of *T. pallidum* was maintained by serial passage in rabbit testes without the use of cortisone (19, 20), and treponemes were enumerated as previously described (19). The virulent RH strain of *Toxoplasma gondii* was obtained from Jack Remington (Palo Alto Medical Research Foundation, Palo Alto, Calif.). A sample of the original inoculum was found to be free of known rodent viral 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, the hair was removed with electric clippers from the areas chosen for the injection sites, and after inoculation they were kept hairless by periodic clipping. Pathogenic parasites for these injections were grown intraperitoneally in normal mice by twice-weekly serial passage of *Toxoplasma gondii* tachyzoites. Peritoneal exudates from mice infected with 10^6 *Toxoplasma gondii* organisms 3 to 4 days earlier were collected by washing the peritoneal cavity with 4 to 5 ml of RPMI 1640 medium. This yielded approximately 2×10^8 parasites per mouse. After enumeration of parasites in a hemocytometer, cell numbers were adjusted to the desired concentration, and the suspensions were used to infect guinea pigs. Normal euthymic guinea pigs and TXB guinea pigs were inoculated intradermally (i.d.) with various numbers (see Results) of treponemes or *Toxoplasma gondii* tachyzoites in 0.1 ml at duplicate sites in the hind-leg region. Within groups of identically treated animals, lesions developed at a relatively uniform rate and with a similar degree of severity (see Results). The size of developing lesions over time was monitored by measuring changes in the diameter of the infected skin sites with dial calipers.

Detection of antibodies. Whole blood was collected from animals before infection and at various intervals up to 16 weeks 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.

Detection of *T. pallidum* in infected tissues. At periodic intervals after challenge infection of guinea pigs, infected skin sites or obvious lesions and the draining lymph nodes were biopsied or removed entirely. Numbers of motile, virulent treponemes present in these excised tissue specimens were determined either by direct microscopic examination (19, 22) or by injecting concentrated extracts of minced tissues into the shaven back of normal recipient rabbits, as detailed elsewhere (20, 21). The pattern of lesion development in the rabbits receiving extract material was compared with the rate of lesion formation in a separate matched group of rabbits infected i.d. with various numbers (ranging from 10^2 to 10^6, at semilog intervals) of treponemes freshly harvested from orchitic rabbit tissue. Data are reported as the logarithm of the mean number of virulent organisms.

Lymphocyte responses to mitogens. Spleen and lymph node cells from normal and TXB guinea pigs were prepared and cultured in microtiter plates (Costar, Cambridge, Mass.) with optimal concentrations of the T-cell mitogens concanavalin A (2 μg per culture) and phytohemagglutinin (5 μg per culture) and of the B-cell mitogen lipopolysaccharide (10 μg per culture) following procedures detailed previously (21). Each culture contained 5×10^5 cells in culture medium supplemented with 5×10^{-5} M mercaptoethanol. After 70 h at 37°C in an atmosphere of 5% CO_2–95% air, cultures were pulse-labeled with 0.5 μCi of [^{3}H]thymidine (specific activity, 5.0 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) for an additional 8 h before samples were collected onto glass fiber filter paper with the aid of a semiautomated microharvester (Bellco Glass, Inc., Vineland, N.J.). Samples were counted for radioactivity in a liquid scintillation spectrometer after each fiber disk had been placed into a plastic scintillation vial containing a toluene-based scintillation fluid. Data are expressed as mean counts per minute of [^{3}H]thymidine incorporation of triplicate samples.

Immunizations. Guinea pigs were sensitized to mycobacterial antigens by being injected in each footpad with 0.1 ml of complete Freund adjuvant (CFA) containing dead *Mycobacterium tuberculosis* $H_{37}Rv$ (Trudeau Mycobacterial Collection, TMC no. 102) at a concentration of 1 mg/ml. Bacteria for the CFA were grown in Proskauer-Beck medium for 10 days after the pellicle had been removed, heated at 100°C for 30 min, and lyophilized. After 3 to 4 weeks, guinea pigs were injected i.d. in the rear leg with 10 μg of purified protein derivative (PPD) in buffered diluent (Tubersol; Squibb/Connaught, Inc., Princeton, N.J.), and their delayed-type hypersensitivity skin reactions were measured at 12-, 24-, and 48-h intervals.

In separate experiments, TXB donor guinea pigs were immunized against syphilis as previously described for thymus-intact guinea pigs (21) by (i.d.) infection with 2×10^7 virulent *T. pallidum* cells. At 6 to 8 months later, the infected guinea pigs were treated with the broad-spectrum antibiotic Claforan (cefotaxime sodium; Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.) at a daily dose of 50 mg/kg for seven consecutive days to terminate the primary syphilitic infection (20). After another 2 weeks, spleen and lymph node cells were harvested from these guinea pigs and used in passive immunization experiments; this time point was chosen because it corresponded to peak resistance to challenge infection on the basis of lack of formation of any cutaneous syphilitic lesions and the failure of organisms to disseminate to the draining lymph nodes (20). Control lymphoid cells were obtained from uninfected TXB guinea pigs treated with Claforan 2 weeks earlier. After preparation of single-cell suspensions (21) and just prior to cell transfer it was determined that 80 to 85% of the viable nucleated lymph node cells reacted with a fluorescein-conjugated goat anti-guinea pig immunoglobulin G reagent (Miles Laboratories, Elkhart, Ind.) and the remaining cells (i.e., 15 to 20%) reacted with a monoclonal anti-guinea pig T-cell antibody (7, 21).

Statistical analysis. For experiments dealing with PPD skin

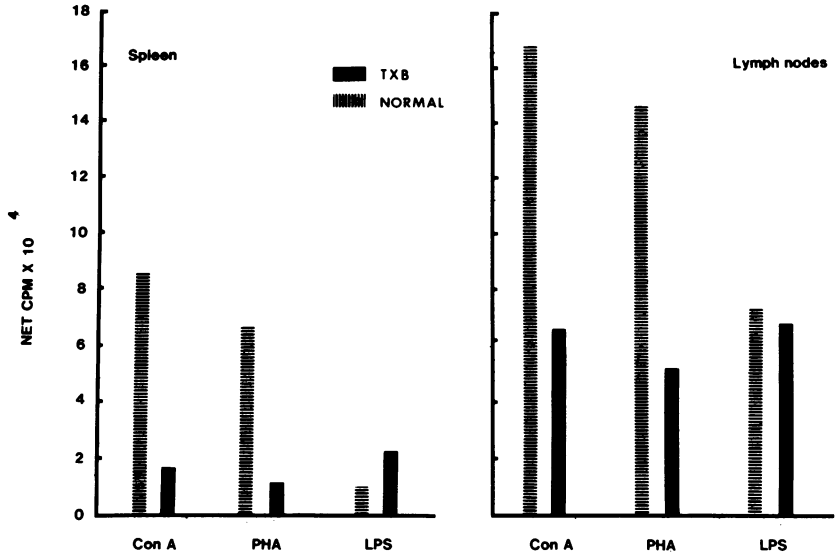

FIG. 1. Mean counts per minute of [^{3}H]thymidine incorporation of triplicate (mitogen-stimulated) cultures after subtraction of counts per minute (<3,000) of unstimulated background cultures. Each culture contained 5×10^5 spleen or lymph node cells from TXB (solid bars) or euthymic (hatched bars) guinea pigs. The data are taken from 12 separate experiments.

tests, enumeration of treponemes in the lymph nodes, and measurement of antibody levels, differences in these data among the separate test groups of guinea pigs were analyzed by Student's *t* test. Except for the experiments depicted in Fig. 1, each experiment, which involved one to three guinea pigs per test group, was repeated three to five times.

RESULTS

Immunological features of strain 2 guinea pigs depleted of T cells. Prior to infection of TXB guinea pigs with *T. pallidum* or *Toxoplasma gondii*, initial experiments were conducted to determine the degree of T-cell deficiency in these animals. The in vitro proliferative responses of the spleen and lymph node cells to the T-cell mitogens concanavalin A and phytohemagglutinin A were reduced by approximately 80 and 60%, respectively, in the TXB group (12 animals) compared with the lymphoblastic responses of the spleen and lymph node cells taken from 12 normal euthymic guinea pigs (Fig. 1). Lymphocytes from TXB guinea pigs exhibited normal or slightly elevated reactivity to the B-cell mitogen lipopolysaccharide.

Three different groups of guinea pigs (eight animals per group) were skin tested with 10 μg of PPD 3 to 4 weeks following injection of CFA. When examined 24 to 48 h after injection of skin test antigen, TXB guinea pigs showed evidence of weak or negative delayed-type hypersensitivity skin reactions, exhibiting small zones of erythema measuring 3.5 to 4.0 mm in diameter and a trivial degree of local skin thickening (Fig. 2). In contrast, normal euthymic guinea pigs and TXB guinea pigs infused with normal syngeneic thymocytes prior to CFA sensitization displayed strong tuberculin sensitivity when given a cutaneous challenge of 10 μg of PPD. Typical skin reactions for both groups of sensitized guinea pigs were highly indurated and intensely erythematous, measured between 11.0 and 12.5 mm in diameter 24 to 48 h after challenge (Fig. 2), and were significantly different in size ($P < 0.001$) from those of the TXB group.

Clinical course of *T. pallidum* infection and humoral responses of normal versus TXB guinea pigs. Two groups of normal guinea pigs and two groups of TXB guinea pigs (12 to 15 guinea pigs per group) were challenged at duplicate sites with 10^7 or 10^6 live *T. pallidum* cells. Although 92% of normal guinea pigs developed typical treponeme-containing ulcerative skin lesions after challenge with 10^6 and 10^7 treponemes, cutaneous lesions developed in only 33 and 47%, respectively, of similarly challenged TXB guinea pigs (Fig. 3). For the latter group, treponemes were detected during the early postchallenge period in some of the challenge sites, and lesions which did develop were delayed in their appearance, smaller, and less severe and healed sooner than was the case for the disease pattern for euthymic guinea pigs. TXB guinea pigs also exhibited enhanced levels of protection by virtue of significantly ($P < 0.001$) fewer numbers of virulent treponemes disseminating from the inoculation site to the draining lymph nodes relative to the sizable numbers of organisms recovered from the lymph nodes of infected normal hosts (Fig. 4).

Anti-treponemal antibody (MHA-TP) levels were monitored at periodic postchallenge intervals. For both groups of guinea pigs, a low but measurable amount of antibody became detectable at 2 weeks of infection; this was followed by a gradual rise in antibody titers until maximum levels were reached at the end of a 16-week observation period (Fig. 4). The MHA-TP procedure also showed that, during each test interval, TXB guinea pigs produced significantly (about two- to fourfold) ($P < 0.01$) higher levels of antibodies than did their euthymic counterparts.

Passive transfer of anti-treponemal immunity with cells from TXB guinea pigs. Because TXB guinea pigs demonstrated relatively high levels of resistance to syphilis, passive transfer experiments were designed to determine whether a comparable degree of protection could be conferred to normal recipient guinea pigs infused with spleen and lymph node cells from syphilitic TXB (B-cell-enriched) guinea pigs. In this latter group, only 14 and 42% of recipient guinea pigs developed cutaneous lesions after challenge with 10^6 and 10^7 treponemes, respectively (Table 1). In contrast, typical treponeme-containing chancres occurred in the entire group

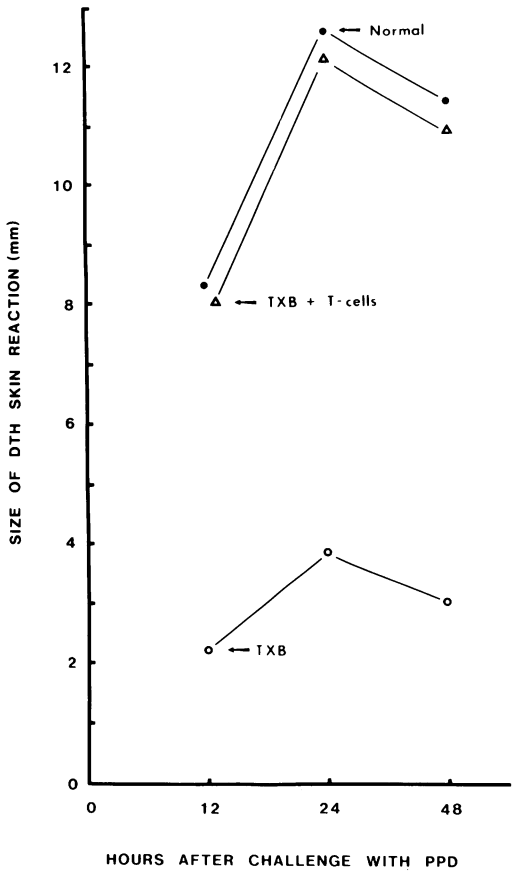

FIG. 2. Graphic representation of the results of skin tests of normal guinea pigs (●), TXB guinea pigs (○), and TXB guinea pigs infused with 10^9 normal thymocytes (△). All three groups of guinea pigs were challenged i.d. with 10 μg of PPD after sensitization with CFA. There were eight guinea pigs per group.

of 14 guinea pigs receiving lymphocytes from uninfected TXB guinea pigs, and significantly higher numbers of virulent treponemes could be recovered from the draining lymph nodes of these unprotected recipients.

Levels of antibody in challenged recipient guinea pigs. To determine whether passive immunization resulted in a substantial rise in circulating antibody titers, recipient guinea pigs were monitored for MHA-TP antibody levels during the early postchallenge period. Within 4 weeks after cell transfer and treponemal challenge, relatively high levels of antibody (range of group mean titers, 640 to 1,280) were produced in guinea pigs infused with lymphocytes from immune TXB hosts (Table 1). During the same period, guinea pigs receiving lymphoid cells from unimmunized TXB donors had much lower MHA-TP antibody levels (range of mean titers, 40 to 80).

Clinical course of toxoplasmosis in normal and TXB guinea pigs. To further evaluate whether the phenomenon of reduced susceptibility of TXB guinea pigs was unique for treponemal (bacterial) infection, an experiment was performed to compare the clinical course of the parasitic disease toxoplasmosis in TXB and fully immunocompetent guinea pigs. Two groups of guinea pigs (12 euthymic and 10 TXB animals) were infected i.d. with 10^6 *Toxoplasma gondii* tachyzoites at duplicate sites. In contrast to the mild and self-limiting infection in normal, thymus-intact guinea pigs, toxoplasmosis introduced by the i.d. route resulted in an acutely lethal form of the disease in the TXB host (Fig. 5). During the first 7 to 10 days of infection, TXB guinea pigs showed signs of serious illness, such as fever, loss of appetite, moderate to extreme weight loss, labored breathing, and mild diarrhea, so that by day 16 of infection 80% of these animals had died, whereas all euthymic guinea pigs survived for an observation period lasting 8 weeks postchallenge.

An attempt was made to reverse the increased susceptibility of TXB guinea pigs to *Toxoplasma* infection by infusing them with thymocytes from syngeneic donors. As shown above (Fig. 2), an intravenous injection of 10^9 thymocytes into TXB hosts 2 to 7 days before sensitizing them to CFA caused a marked restoration of their capacity to generate anti-*Mycobacteria* immunity based on the elicitation of near-normal delayed-type hypersensitivity reactions in response to PPD. Similarly, such thymocyte-mediated reconstitution also enabled all challenged TXB guinea pigs to survive what usually develops into a rapidly fatal *Toxoplasma*-induced infection (Fig. 5).

DISCUSSION

Sufficient evidence exists (21, 25) in support of the concept that T cells are important for the development of immunity to syphilis, either by triggering B cells to differentiate and produce antibody (21) or by activating macrophages (18) for enhanced phagocytosis (12) of treponemes, or by both mechanisms (27), depending upon the experimental situation. It was therefore at first surprising to find in the studies presented here that guinea pigs lacking T cells have an enhanced innate resistance to cutaneous and disseminated *T. pallidum* infection relative to that of their euthymic counterparts. Indeed, this unexpected finding appears to contradict our previously published results (21), as well as those reported by others (25), showing that T cells can passively transfer protection against syphilis in normal recipients and that T cells predominantly infiltrate lesions in situ at around the time healing commences (10). Thymus-derived lymphocytes are, however, important for the development of anti-

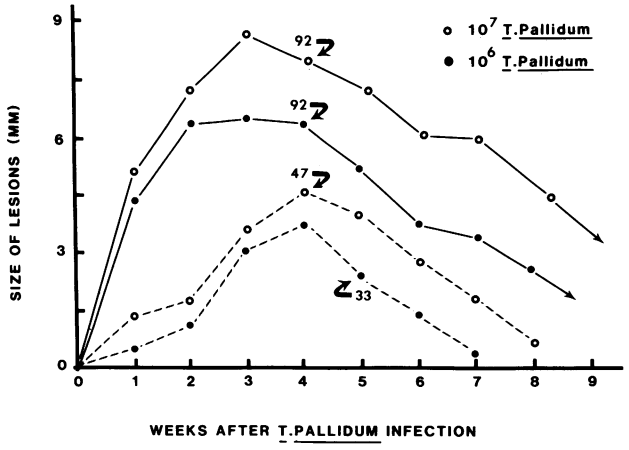

FIG. 3. Evidence that TXB guinea pigs (dashed lines) are more resistant to cutaneous disease than are normal euthymic guinea pigs (solid lines) after i.d. challenge, at duplicate sites, with 10^7 (○) or 10^6 (●) treponemes. Numbers adjacent to arrows refer to the percentage of guinea pigs developing typical chancrelike, treponeme-containing skin lesions for each challenge group. The arrows pointing downward from the lesion-growth curves represent the gradual-to-total regression of lesion size occurring after 9 weeks for the two indicated test groups. There were 12 to 15 guinea pigs in each group.

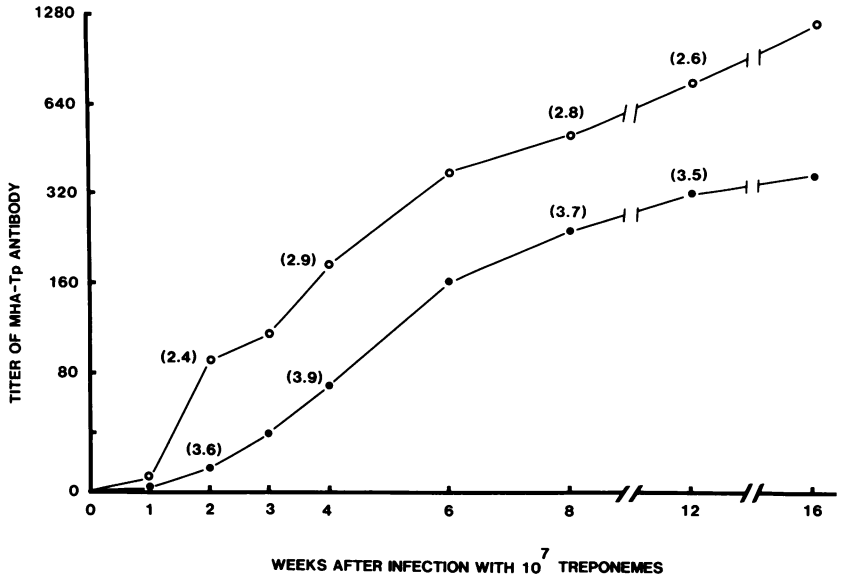

FIG. 4. Levels of MHA-TP antibody in normal (●) or TXB (○) guinea pigs challenged i.d. with 2×10^7 treponemes. Each point represents the mean of the reciprocal of the highest dilution exhibiting at least 1+ reactivity. Individual numerical values in parentheses refer to the mean $\log_{10}$ number of viable treponemes recovered, at the indicated time points, from the lymph nodes of each of the two different groups of challenged guinea pigs. There were three guinea pigs in each experimental group per time point.

Toxoplasma immunity in the guinea pig in a manner analogous to the disease pattern in athymic nude mice infected with virulent *Toxoplasma gondii* parasites (9). This was indicated by the generally lethal course of toxoplasmosis in the TXB host and was even more firmly established by the restoration of protective immunity by an infusion of syngeneic thymocytes (Fig. 5).

What could account for these apparent discrepancies in the response of the TXB guinea pigs to infection with *T. pallidum* and *Toxoplasma gondii*? Although the TXB animals used in this study exhibited diminished cell-mediated immune responses consistent with those reported by other investigators (4, 28) for guinea pigs lacking T cells, it is possible that our TXB guinea pigs, similarly to mice made T-cell deficient by thymectomy and irradiation (14), may still retain a residual T-cell population that is large enough to generate anti-syphilis immunity but not anti-*Toxoplasma* immunity. Such a possibility seems likely in view of recently obtained serological data (manuscript submitted for publication) showing that there is incomplete elimination of all peripheral T cells in the TXB guinea pig. This finding was based on the ability of a small but significant proportion of lymphocytes from TXB guinea pigs to react in vitro with a highly specific monoclonal anti-guinea pig T-cell antibody (7, 21). It should also be realized that the mode and rate of multiplication for the two microorganisms used in this study are markedly different. It has been estimated that pathogenic *T. pallidum* replicates almost exclusively in an extracellular

TABLE 1. Ability of lymphocytes from *T. pallidum*-immune TXB guinea pigs to confer resistance to treponemal challenge

Expt group (7 guinea pigs/group)	No. developing lesions (lesions/sites)	Log_{10} virulent treponemes in lymph nodes ± SEM[a]	MHA-TP titer[b]
Euthymic guinea pigs given cells from noninfected TXB donors and challenged with 10^7 treponemes[c]	7 (14/14)	3.58 ± 0.15	≤80
Euthymic guinea pigs given cells from immune TXB donors and challenged with 10^7 treponemes	3 (6/14)	2.74 ± 0.12[d]	640–1,280
Euthymic guinea pigs given cells from noninfected TXB donors and challenged with 10^6 treponemes	7 (13/14)	3.41 ± 0.13	≤80
Euthymic guinea pigs given cells from immune TXB donors and challenged with 10^6 treponemes	1 (2/14)	2.35 ± 0.11[d]	640–980

[a] At 2 to 3 months postchallenge, extracts of guinea pig lymph nodes were injected i.d. into normal rabbits, and the number of viable *T. pallidum* cells was estimated as detailed in Materials and Methods.

[b] Range of MHA-TP antibody titer in euthymic recipients of lymphocytes from nonimmune or *T. pallidum*-immune TXB donors at 2 to 4 weeks after cell transfer and treponemal challenge.

[c] Age- and sex-matched syngeneic recipient guinea pigs were infused intravenously with 1×10^8 to 2×10^8 lymphocytes in 2.0 ml of RPMI 1640 medium; cells were transferred 24 to 48 h before treponemal challenge.

[d] $P < 0.001$ when compared with corresponding control groups.

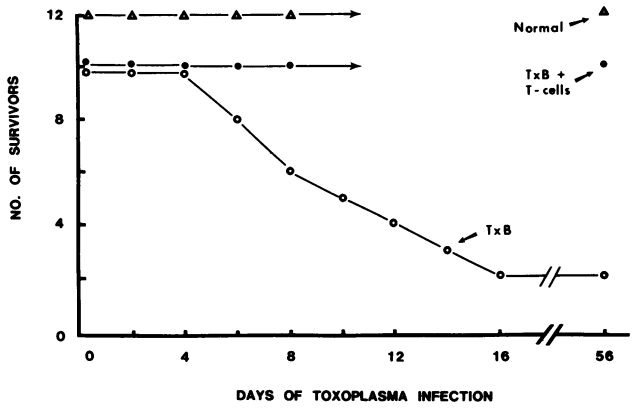

FIG. 5. Evidence that an i.d. challenge with 2×10^6 *Toxoplasma gondii* organisms causes a lethal infection in TXB guinea pigs (○) but not in euthymic guinea pigs (△), nor in TXB guinea pigs (●) reconstituted with syngeneic thymocytes. Arrows leading from the symbols △ and ● indicate that all animals from these two groups survived up to 56 days after challenge infection.

environment every 30 to 33 h (30), whereas *Toxoplasma gondii* is an obligate intracellular parasite with a division time of 4 to 6 h (23). The increased dependence of the host on a fully intact T-cell population in its defense against a virulent intracellular parasite relative to an extracellular one may well reflect either such different growth properties in vivo or other unique parasitic strategies and virulence determinants (23, 27). In this context, it is especially noteworthy that during certain bacterial infections a similar inverse relationship occurs in mice deprived of T cells, based on their impairment in generating an anti-tuberculous immune response but not in their capacity to develop anti-*Listeria* immunity (14).

An alternative explanation of the observed enhancement of resistance to primary syphilitic infection in TXB guinea pigs is their capacity to generate a more vigorous antitreponemal antibody response than their euthymic litter mates, partly as a direct consequence of the removal of a T-suppressor cell subset. Also, in this regard, lymphocytes from *T. pallidum*-infected TXB guinea pigs have the ability to passively transfer significant levels of resistance to syphilis (Table 1), resulting in the almost immediate production of substantially high levels of anti-treponemal antibody in the protected recipients. This result correlates well with our earlier findings (21) of partial protection conferred upon the recipients of syphilis-immune B cells challenged with virulent treponemes. Consistent with the T-suppressor cell hypothesis are the recent findings in TXB mice in a model of T-cell-mediated regression of established tumors (15), showing that the TXB tumor bearer, unlike normal intact mice, is incapable of generating enough T cells to suppress an anti-tumor immune response. Therefore, adoptive immunotherapy against a tumor implant can be achieved in a TXB recipient, but not in normal recipients that consistently produce T-suppressor cells as a result of tumor challenge, thereby blocking the anti-tumor function of donor T cells. On the basis of this finding, it is therefore possible that syphilis in TXB guinea pigs, and perhaps also in newborn guinea pigs (17), is less severe than in fully competent adults because of the diminished capacity of the TXB and newborn host to produce suppressor cells in response to treponemal infection, which could otherwise interfere with certain emerging host immune responses. A somewhat similar suppressor-type mechanism mediated primarily by humoral factors has already been postulated as a contribution to the delay in an effective host response during human syphilis and in the rabbit model of this disease (2, 18, 33).

An additional consideration is that TXB guinea pigs, like congenitally athymic mice (6, 13) and TXB mice (5), may be able to protect themselves from certain infections through the production of activated, fixed tissue macrophages, having enhanced bactericidal properties. This situation could arise following leakage of the normal gut flora through the intestinal epithelium after damage either by irradiation or by bacterial penetration in the absence of T-dependent immunoglobulin (6). Such a process may lead to nonspecific activation of the reticuloendothelial system, induced by bacterial lipopolysaccharide released into the circulation (6), or may cause B cells to release macrophage-activating lymphokines. Related to this possibility are reports (24, 34) demonstrating that B lymphocytes can produce migration inhibition factor following stimulation with nonspecific mitogens or antigens. In this regard, however, while others (6, 13) have demonstrated activated macrophages in mice depleted of T cells, preliminary experiments from this laboratory (unpublished data) showed that peritoneal macrophages from TXB guinea pigs do not have increased treponemicidal or toxoplasmacidal activity. The absence of activated macrophages in the TXB guinea pig peritoneum does not, however, preclude the presence of activated tissue macrophages near the challenge site such as in the skin, where the treponemal organisms are typically introduced for inducing a primary infection and lesion and which serves as the focal point of bacterial dissemination.

In conclusion, if antisyphilis immunity is heavily dependent upon a purely cell-mediated response, as it appears to be with toxoplasmosis (23), then the T-cell-depleted guinea pig would be expected, compared with euthymic guinea pigs infected with *T. pallidum*, to be more susceptible to syphilis by undergoing an exacerbated clinical course of the disease, or there would be unrestricted growth of treponemes in vivo, perhaps leading to the death of the affected host. Surprisingly, however, none of these conditions occurred in TXB guinea pigs following treponemal challenge. In fact, they displayed a level of resistance to syphilis superior to that of normal guinea pigs. Such results, together with those reported previously (5, 6, 13, 14) in other, related, experimental systems, stress the need for care in interpreting studies of antimicrobial immunity which involve the use of hosts made immunodeficient by genetic mutations, chemotherapy, surgical procedures, or irradiation.

While it is still unclear how T cells, B cells, macrophages, antibody, and other host factors interact with each other and with the treponeme during an ongoing syphilitic infection, it can be concluded from the results presented here and from those reported earlier (21, 22) that the B lymphocyte, in cooperation with a small residual subset of T-helper cells, plays an important role in the defense of the host against syphilis through the production of specific protective antibodies. These data strongly suggest, moreover, that there is a minimal but necessary T-cell requirement in the expression of antitreponemal immunity in the guinea pig model of human syphilis.

ACKNOWLEDGMENTS

I greatly appreciate the excellent technical assistance of Carol J. Niederbuhl and Kathie E. Curnick.

This work was supported by Public Health Service grant AI-19824 from the National Institute of Allergy and Infectious Diseases and by a grant from the J.M. Foundation.

LITERATURE CITED

1. **Azadegan, A. A., F. 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.** 1983. Immunoregulatory effects in experimental syphilis, p. 271–296. *In* R. E. Schell and D. M. Musher (ed.), Pathogenesis and immunology of treponemal infection. Marcel Dekker, Inc., New York.
3. **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.
4. **Campos-Neto, A., H. Levine, and S. F. Schlossman.** 1978. T cell regulation of specific B cell responses. J. Immunol. **121**: 2235–2240.
5. **Chan, C., P. A. L. Kongshavn, and E. Skamene.** 1977. Enhanced primary resistance to *Listeria monocytogenes* in T cell-deprived mice. Immunology **32**:528–537.
6. **Cheers, C., and R. Waller.** 1975. Activated macrophages in congenitally athymic "nude" mice and in lethally irradiated mice. J. Immunol. **115**:844–847.
7. **Chiba, J., T. M. Chused, W. M. Leiserson, S. E. Zweig, and E. M. Shevach.** 1983. Production and characterization of monoclonal antibodies to guinea pig lymphoid differentiation antigens. J. Immunol. Methods **63**:247–261.
8. **DeLamater, E. D., V. R. Saurino, and F. Urbach.** 1952. Studies on the immunology of spirochetes. I. Effect of cortisone on experimental spirochetosis. Am. J. Syph. **36**:127–139.
9. **Lindberg, R. E., and J. K. Frenkel.** 1977. Toxoplasmosis in nude mice. J. Parasitol. **63**:219–221.
10. **Lukehart, S. A., S. A. Baker-Zander, R. M. C. Lloyd, and S. Sell.** 1980. Characterization of lymphocyte responsiveness in early experimental syphilis. II. Nature of cellular infiltration and *Treponema pallidum* distribution in testicular lesions. J. Immunol. **124**:461–467.
11. **Lukehart, S. A., S. A. Baker-Zander, R. M. C. Lloyd, and S. Sell.** 1981. Effect of cortisone administration on host-parasite relationships in early experimental syphilis. J. Immunol. **127**:1361–1368.
12. **Lukehart, S. A., and J. N. Miller.** 1978. Demonstration of the *in vitro* phagocytosis of *Treponema pallidum* by rabbit peritoneal macrophages. J. Immunol. **121**:2014–2023.
13. **Nickol, A. D., and P. F. Bonventre.** 1977. Anomalous high native resistance of athymic mice to bacterial pathogens. Infect. Immun. **18**:636–645.
14. **North, R. J.** 1973. Importance of thymus-derived lymphocytes in cell-mediated immunity to infection. Cell. Immunol. **7**:166–176.
15. **North, R. J.** 1984. Models of adoptive T cell-mediated regression of established tumors. Contemp. Top. Immunobiol. **13**:243–257.
16. **Pacha, J. M., M. Metzger, W. Smogor, E. Michalska, J. Podwinska, and J. Ruczkowska.** 1979. Effect of immunosuppressive agents on the course of experimental syphilis in rabbits. Arch. Immunol. Ther. Exp. **27**:45–51.
17. **Pavia, C. S.** 1986. Transfer of resistance to syphilitic infection from maternal to newborn guinea pigs. Infect. Immun. **51**:365–368.
18. **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.
19. **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.
20. **Pavia, C. S., and C. J. Niederbuhl.** 1985. Acquired resistance and expression of a protective humoral response in guinea pigs infected with *Treponema pallidum* Nichols. Infect. Immun. **50**:66–72.
21. **Pavia, C. S., and C. J. Niederbuhl.** 1985. Adoptive transfer of anti-syphilis immunity with lymphocytes from *Treponema pallidum* infected guinea pigs. J. Immunol. **135**:2829–2834.
22. **Pavia, C. S., C. J. Niederbuhl, and J. Saunders.** 1985. Antibody-mediated protection of guinea pigs against infection with *Treponema pallidum*. Immunology **56**:280–287.
23. **Remington, J. S., and J. L. Krahenbuhl.** 1982. Immunology of *Toxoplasma gondii*, p. 327–371. *In* A. J. Nahmias and R. J. Reilly (ed.), Immunology of human infection, part II. Viruses and parasites. Plenum Publishing Co., New York.
24. **Rocklin, R. E., R. P. MacDermott, L. Chess, S. F. Schlossman, and J. R. David.** 1974. Studies on mediator production by highly purified human T and B lymphocytes. J. Exp. Med. **140**:1303–1316.
25. **Schell, R. F., J. K. Chan, J. L. LeFrock, and O. Bagasra.** 1980. Endemic syphilis: transfer of resistance to *Treponema pallidum* strain Bosnia A in hamsters with a cell suspension enriched for thymus-derived cells. J. Infect. Dis. **141**:752–758.
26. **Schell, R. F., J. L. LeFrock, and J. K. Chan.** 1982. Transfer of resistance with syphilitic immune cells: lack of correlation with mitogenic activity. Infect. Immun. **35**:187–192.
27. **Schell, R. F., and D. M. Musher (ed.).** 1983. Pathogenesis and immunology of treponemal infection. Marcel Dekker, Inc., New York.
28. **Shu, S., L. S. Fonseca, J. T. Hunter, and H. J. Rapp.** 1983. Mechanisms of immunological eradication of a syngeneic guinea pig tumor. II. Effect of methotrexate treatment and T cell depletion of the recipient on adoptive immunity. Transplantation **35**:56–61.
29. **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.
30. **Turner, T. B., and D. H. Hollander.** 1957. Biology of the treponematoses. W.H.O. Monogr. Ser. **35**:1–277.
31. **Wagner, J. E., and P. J. Manning (ed.).** 1976. Biology of the guinea pig, p. 57. Academic Press, Inc., New York.
32. **Wicher, K., and A. Jakubowski.** 1964. Effect of cortisone on the course of experimental syphilis in the guinea pig. Br. J. Vener. Dis. **40**:213–216.
33. **Wright, D. J. M., and A. S. Grimble.** 1974. Why is the infectious stage of syphilis prolonged? Br. J. Vener. Dis. **50**:45–49.
34. **Yoshida, T., H. Sonoyaki, and S. J. Cohen.** 1973. The production of migration inhibition factor by B and T cells of the guinea pig. J. Immunol. **138**:784–797.