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Immune T Cells Sorted by Flow Cytometry Confer Protection against Infection with *Treponema pallidum* subsp. *pertenue* in Hamsters

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The role of cell-mediated immunity against infection with *Treponema pallidum* subsp. *pertenue* in humans or experimental animals is unclear. Hamsters injected subcutaneously in the hind paws with 4×10^6 unfractionated lymph node cells or enriched lymph node T cells (immunoglobulin negative, Ia negative) from *T. pallidum* subsp. *pertenue*-immune hamsters were resistant to challenge with *T. pallidum* subsp. *pertenue*. The popliteal lymph nodes of hamsters that received immune cells weighed less and had significantly fewer treponemes than did lymph nodes from hamsters infused with cells from nonimmune donors. Furthermore, recipients of immune T cells failed to develop antitreponemal antibodies 21 days after challenge. Enriched T cells were obtained by flow cytometric separation by using monoclonal anti-Ia antibody 14-4-4s, which identified hamster B cells. Flow cytometric analysis by two-color immunofluorescent staining with anti-hamster-immunoglobulin and monoclonal anti-Ia antibody 14-4-4s confirmed that monoclonal anti-Ia antibody 14-4-4s recognized B cells. In addition, lymph node cells obtained after treatment with anti-Ia monoclonal antibody 14-4-4s and complement were 97% T cells, as determined by monoclonal antibody 20, a hamster T-cell marker. These results demonstrated that highly enriched T cells (immunoglobulin negative, Ia negative) from *T. pallidum* subsp. *pertenue*-immune hamsters conferred partial protection on hamsters against infection with *T. pallidum* subsp. *pertenue*.

The mechanism by which humans and experimental animals acquire resistance to infection with *Treponema pallidum* subsp. *pertenue*, the etiological agent of yaws (or frambesia), is complex. We showed previously (4) that passive transfer of *T. pallidum* subsp. *pertenue* immune serum conferred complete protection on hamsters against challenge with *T. pallidum* subsp. *pertenue*. The donors of the immune serum, however, exhibited cutaneous lesions, and their lymph nodes teemed with treponemes at the time of serum collection. Furthermore, administration of immune serum to hamsters infected for more than 1 week failed to impair or influence the progression of infection. These results suggested that hamsters can develop the humoral response needed to protect them against *T. pallidum* subsp. *pertenue* challenge; however, these components are insufficient to destroy the treponemes present in the donor hamsters. These observations suggest that other mechanisms, particularly cell-mediated immunity, may be involved (15).

Chan et al. (7) demonstrated that enriched T cells confer protection against challenge with *T. pallidum* subsp. *pertenue* on recipient hamsters. Anti-Thy-1 antibody and rabbit anti-hamster thymocyte serum were used to confirm isolation of enriched T cells. Witte and Streilein (27), however, demonstrated that Thy-1 antigen is present on hamster T cells and the majority of resting B lymphocytes. This observation is not unique, since the pattern of expression of Thy-1 on lymphocytes from other species is diverse (27). The report of Witte and Streilein (27) indicated that in the experiments of Chan et al. (7), the transferred T cells may have been contaminated with B cells.

In the present study, enriched T cells were obtained by flow cytometry with a monoclonal antibody specific for hamster B cells. The purity of the T-cell preparations was then verified by monoclonal antibody specific for hamster T cells and by responses in functional assays. Resistance to challenge with *T. pallidum* subsp. *pertenue* was transferred with enriched T cells. The protected hamsters did not develop detectable antibody to treponemal antigens.

MATERIALS AND METHODS

Animals. Male and female inbred LSH/Ss Lak hamsters 6 to 9 weeks old were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, Mass.). Hamsters weighing 80 to 100 g were housed three or four per cage at an ambient temperature of 21°C.

Organism. *Treponema pallidum* subsp. *pertenue* (Haiti B) was originally isolated from a lesion on the lower abdomen of an 11-year-old patient with typical generalized frambesiform yaws (24). The strain has been maintained by passage in hamsters (22). In our laboratory, the inguinal lymph nodes of the hamsters were removed aseptically 5 to 6 weeks after intradermal injection with 10^6 viable treponemes in the inguinal region. The lymph nodes were teased apart in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) containing 5% fetal bovine serum (Hyclone Laboratories, Inc., Logan, Utah) and forced through sterile 60-mesh stainless-steel wire mesh filters. After centrifugation at $270 \times g$ for 3 min to remove cellular debris, the number of treponemes in the supernatant was determined by dark-field microscopy (13). The supernatant fluid was dispensed into vials at a concentration of 5×10^6 treponemes per ml and stored at −70°C until used.

Antibody reagents. The following monoclonal antibodies were used in this study. Hybridoma cell line 14-4-4s, producing monoclonal murine anti-Ia antibody (MAb 14-4-4s), was kindly provided by Colleen Hayes (University of Wis-

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consin, Madison). This monoclonal antibody, which has a specificity for Ia.7 in the Ia chart and I-E^k for the mouse (14), was grown in RPMI 1640 medium containing 10% fetal bovine serum. Five days after cultivation the supernatant was centrifuged at 270 × *g*, collected, dispensed in 5-ml aliquots and stored at −20°C. The aliquots were then thawed on the day of experimentation and used at a final dilution of 1:10.

MAb 20 (hamster pan T-cell marker) ascites fluid was provided by Joan Stein-Streilein (University of Miami, Miami, Fla.) (26). The ascites fluid was maintained at −70°C, thawed, and used at a 1:50 dilution. Fluorescein isothiocyanate (FITC)-conjugated and unconjugated goat anti-hamster immunoglobulin and FITC-conjugated goat anti-rabbit immunoglobulin were purchased from Organon Teknika–Cappel Corp. (West Chester, Pa.). They were used at a final dilution of 1:20.

MAb 14-4-4s and MAb 20 were labeled with biotin (9, 23). Briefly, 2 mg of antibody solution was incubated with 0.25 mg of biotin-HO-succinimide (Sigma Chemical Co., St. Louis, Mo.) in 100 μl of dimethyl sulfoxide (Sigma) and stirred for 2 h. The solution was then dialyzed against Dulbecco phosphate-buffered saline (DPBS; Sigma) overnight with several changes of buffer. The resulting solution was sterilized by filtration (pore size, 0.45 μm; Corning Glass Works, Corning, N.Y.) and stored at −70°C until use. Avidin-FITC and streptavidin-phycoerythrin conjugates were purchased from Becton Dickinson Immunocytometry Systems (Mountain View, Calif.).

Preparation of lymph node cells. Single-cell suspensions of lymph node cells were prepared by teasing the nodes apart with forceps and gently pressing them through a stainless-steel 60-mesh screen into RPMI 1640 medium containing 5% fetal bovine serum. The cells were then washed twice with RPMI 1640 medium and suspended to 2 × 10^7 cells per ml in DPBS.

Flow cytometric analysis and isolation of T cells. In one-color analysis, 2 × 10^6 lymph node cells were incubated with FITC-conjugated goat anti-hamster immunoglobulin antiserum (1:10, heavy and light chain specific) for 30 min, washed twice with DPBS, fixed with 2% formaldehyde, and stored at 4°C in the dark until analyzed. In two-color analysis, 2 × 10^6 cells were incubated with FITC-conjugated goat antibody to hamster immunoglobulin and washed twice with DPBS; biotinylated MAb 14-4-4s was then added. RPC 5, an immunoglobulin G2a (IgG2a) antibody at 1.2 mg/ml of protein, was used as a control antibody (Bionetics Laboratory Products Division, Charleston, S.C.). After 30 min of incubation at 4°C, the cells were washed twice with DPBS and incubated with phycoerythrin-conjugated streptavidin (Becton Dickinson) for 30 min at 4°C. Surface immunofluorescence of 10,000 cells was determined with a cell sorter (Epics C; Coulter Electronics, Inc., Hialeah, Fla.). For sorting, cells were stained and suspended in RPMI 1640 medium containing 5% fetal bovine serum and 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) buffer solution. The cells were sorted at 2,500 to 3,000 cells per s.

Cytometrically enriched T cells were obtained by treating lymph node cells with labeled MAb 14-4-4s. Briefly, 2 × 10^8 pooled immune or normal cells in 5 ml of RPMI 1640 medium containing 5% fetal bovine serum were incubated with 0.5 ml of biotinylated MAb 14-4-4s for 45 min. The suspension of cells was washed twice by centrifugation at 270 × *g* for 10 min and then incubated with avidin-FITC for 45 min. After two more washes, the cells were suspended to 4 × 10^6 cells per ml in RPMI 1640 medium containing 5% fetal bovine serum and processed by flow cytometry. The nonlabeled T cells were collected, centrifuged, and suspended to 4 × 10^7 cells per ml.

Passive transfer of resistance. Thirty hamsters were infected intradermally in the inguinal regions with 5 × 10^5 viable *T. pallidum* subsp. *pertenue*. Ten to sixteen weeks after infection, the hamsters were treated with penicillin (4,000 U) to terminate infection. Pooled immune lymph node cells were obtained from 25 of these hamsters 10 days after treatment with penicillin; no treponemes were observed in the suspensions of immune cells. The other five animals did not become infected when rechallenged with *T. pallidum* subsp. *pertenue*. No spirochetes were detected in their lymph nodes 28 days after challenge. Cells from noninfected hamsters were also obtained 10 days after treatment with penicillin. Immune or normal lymph node cells (1 × 10^6 or 4 × 10^6 cells per hamster) were injected subcutaneously into the right hind paw. The hamsters were then challenged intradermally by injecting 5 × 10^5 viable *T. pallidum* subsp. *pertenue* in the popliteal region and were sacrificed 3 weeks after infection. Although inbred hamsters were used in this investigation, all recipients of cells were irradiated as an additional precaution to guarantee the survival of the transferred cells. Gamma irradiation (500 rads) was delivered with a Cobalt 60 irradiator (Packer Co., Cleveland, Ohio). Hamsters survived this level of radiation without reconstitution with normal hamster bone marrow cells.

Assessment of protection. The lymph node weight and the number of treponemes in the nodes were used as parameters for evaluating the responses of passively immunized hamsters to treponemal infection. The weights of the popliteal lymph nodes were determined with a Mettler H32 balance. The number of treponemes per lymph node was determined by using a modification of the procedure described by Miller (13). Duplicate slides of each homogenized lymph node were prepared, and 100 fields per slide were examined for treponemes by dark-field microscopy. Data are presented as the mean number of treponemes per lymph node.

Mitogen studies. Single-cell suspensions of lymph node cells from normal or infected hamsters were prepared and diluted to 2 × 10^6 cells per ml in RPMI 1640 medium with 10% heat-inactivated fetal bovine serum containing 100 U of penicillin and 100 μg of streptomycin (Hazelton, Denver, Pa.) per ml. Suspensions of 0.1 ml of medium containing 2 × 10^5 unfractionated or fractionated lymph node cells were cultured in flat-bottomed 96-well microdilution plates (Costar, Cambridge, Mass.) at a final volume of 0.2 ml per well. Two mitogens, concanavalin A (ConA) (Sigma) and lipopolysaccharide (LPS) from *Escherichia coli* 55:B5 (Sigma), were prepared according to the instructions of the manufacturer and diluted in RPMI 1640 medium containing fetal bovine serum. They were added to cell cultures in concentrations of 2 μg of ConA per ml and 50 μg of LPS per ml. Cultures were incubated in triplicate at 37°C for 48 h in a humid atmosphere of 5% CO_2 and 95% air. Eight hours before harvesting, each culture was pulsed with 50 μCi of [^{3}H]thymidine (specificity, 6.7 μCi/mole; Dupont, NEN Research Products, Boston, Mass.) per ml. Cultures were harvested with a multiple-cell harvester (Whittaker, M.A. Bioproducts, Inc., Walkersville, Md.) onto fiberglass filter paper. The filter paper disks were placed in Biosafe II liquid scintillation fluid (Research Products International Corp., Mount Prospect, Ill.), and cells were counted in a scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.). Results were expressed as counts per minute ± standard error.

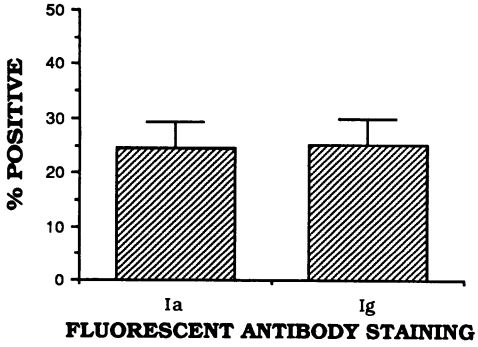

FIG. 1. Fluorescent-antibody staining of lymph node cells from hamsters infected with *T. pallidum* subsp. *pertenue* for more than 10 weeks. The lymph node cells were stained with either biotinylated MAb 14-4-4s and avidin-FITC (Ia) or goat anti-hamster immunoglobulin-FITC (Ig).

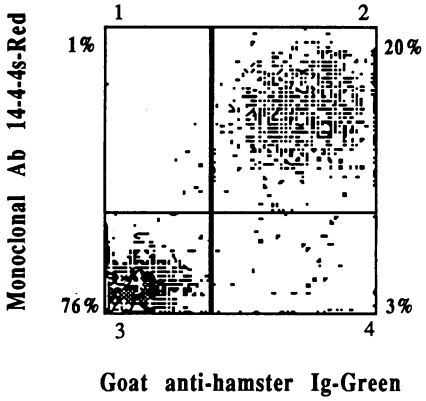

FIG. 2. Coexpression of immunoglobulin and Ia on hamster lymph node B cells determined by two-color immunofluorescence analysis. Hamster lymph node cells were stained with FITC-conjugated goat anti-hamster immunoglobulin and then with biotinylated MAb 14-4-4s and streptavidin-phycoerythrin.

Complement-mediated lysis. MAb 14-4-4s was added to suspensions of 2×10^6 lymph node cells per ml at a final dilution of 1:10 and maintained at 4°C for 60 min. Cells were washed twice by centrifuging at $270 \times g$ for 10 min with DPBS and suspended to 2×10^6 cells per ml, and a 1:1 mixture of baby rabbit complement and guinea pig complement was added at a final concentration of 1:10. The solution was incubated at 37°C for 60 min, washed, and resuspended. Viability was determined by eosin orange exclusion.

Test for treponemal antibody. A treponemal antibody test (Sera Tek; Fujizoki Pharmaceutical Co., Ltd., Tokyo, Japan) was obtained from Ames Co. (Elkhart, Ind.). The test was performed as described by the manufacturer, except that sera from infected hamsters were serially diluted with absorbing diluent to obtain quantitative titers.

Statistical analysis. Data were analyzed by the analysis of variance procedure. When a significant F ratio indicated reliable mean differences, the Student *t* test was used to examine pairs of means. The alpha level was set at 0.05 before the experiments were started.

RESULTS

Identification of hamster B cells. Goat anti-hamster immunoglobulin and MAb 14-4-4s, which recognizes Ia antigen, stained the same proportion (25%) of lymph node cells obtained from hamsters infected with *T. pallidum* subsp. *pertenue* for more than 10 weeks (Fig. 1). To further prove that MAb 14-4-4s identified B cells, we used two-color flow cytometry to confirm that the Ia epitope resided mainly on immunoglobulin-positive cells. After dual staining with MAb 14-4-4s-phycoerythrin (PE)- and FITC-conjugated goat antiserum to hamster immunoglobulin, 20% of the Ia-positive cells were also positive for surface immunoglobulin (Fig. 2, quadrant 2). Only 1% of the total lymphocyte population was immunoglobulin negative, Ia positive (Fig. 2, quadrant 1) while 3% of the cells were immunoglobulin positive, Ia negative (Fig. 2, quadrant 4). The majority (76%) of the cells (Fig. 2, quadrant 3) were immunoglobulin negative, Ia negative.

When lymph node cells were depleted of Ia-positive cells (from 30.1 to 4.5%) with MAb 14-4-4s and complement, they were significantly enriched for T cells (97.6%) as determined by use of MAb 20, a pan T-cell marker (Table 1). There was a simultaneous depletion in immunoglobulin-positive cells, from 30 to 6.8%. As a further test of cell depletion, mitogenicity of different cell populations was tested by using ConA as a T-cell mitogen and LPS as a B-cell mitogen. Lymph node cells depleted of Ia-positive cells showed a significant ($P < 0.05$) increase in response to ConA and a decrease ($P < 0.05$) in response to LPS (Fig. 3).

Transfer of protection with immune T cells. Lymph node cells from immune and normal hamsters were enriched for T cells (Ia negative) by treatment with MAb 14-4-4s and complement. Hamsters which received 4×10^6 nonfractionated immune lymphocytes or lymphocytes enriched for T cells were protected against challenge with *T. pallidum* subsp. *pertenue* (Table 2). This protection was indicated by a significant decrease ($P < 0.05$) in both lymph node weight and number of treponemes compared with recipients of normal T cells. Recipients of 4×10^6 immune T cells also did not develop a detectable antitreponemal antibody titer. By contrast, recipients of 4×10^6 unfractionated immune lymphocytes developed a substantial antitreponemal antibody titer (1:5,120). Animals that received only 10^6 immune unfractionated lymphocytes or enriched T cells were not protected from treponemal challenge.

Immune and normal T cells were then obtained by flow cytometry with fluorescein-labeled MAb 14-4-4s (Table 3). A significant ($P < 0.05$) decrease was observed in the lymph node weights and numbers of treponemes in recipients of 4×10^6 immune T lymphocytes (immunoglobulin negative, Ia negative) compared with the weights and numbers for recipients of normal T lymphocytes and for the control group.

TABLE 1. Comparison of fluorescent-antibody staining of lymphocytes obtained from immune hamster lymph nodes before and after depletion of B cells with MAb 14-4-4s and complement[a]

Fluorescent antibody	% of antibody-staining cells	
	Before depletion	After depletion
Goat anti-hamster immunoglobulin	30.1	6.8
Anti-Ia (14-4-4s)	30.1	4.5
MAb 20[b]	64.9	97.6

[a] Lymph node cells were obtained from hamsters infected with *T. pallidum* subsp. *pertenue* for more than 10 weeks.
[b] MAb 20 recognizes hamster T lymphocytes.

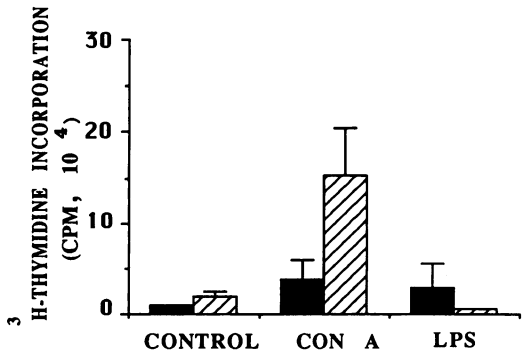

FIG. 3. Responses of hamster lymph node cells to ConA and LPS before (■) and after (▨) treatment with MAb 14-4-4s and complement.

Antitreponemal antibody was not detected in recipients of immune or normal T lymphocytes 21 days after challenge with *T. pallidum* subsp. *pertenue*.

DISCUSSION

The results of this investigation showed that cytometrically enriched T cells (immunoglobulin negative, Ia negative) from hamsters immune to *T. pallidum* subsp. *pertenue* infection could confer protection on recipient hamsters against challenge with *T. pallidum* subsp. *pertenue*. Purified T cells, depleted of B cells, were obtained by using MAb 14-4-4s. The T-cell protection was not associated with the development of antitreponemal antibodies.

In the late 1970s, Chan et al. (7) presented evidence that immune T cells (Thy-1-positive cells) confer protection against challenge with *T. pallidum* subsp. *pertenue*. However, Witte and Streilein (27) showed that hamster T and B cells express the Thy-1 antigen. Chan et al. (7) also detected a substantial amount of antibody (1:640) to treponemal antigens in recipients of immune T cells. Although this concentration of antibody was fourfold lower than that in recipients of immune T and B cells, it is now known that hamsters challenged with *T. pallidum* subsp. *pertenue* can produce treponemicidal antibodies as early as 1 week after infection (4). In fact, serum obtained from hamsters infected for 1 week conferred partial protection on recipients against treponemal challenge (3, 4). Similarly, Pavia and Niederbuhl (16) were able to transfer antisyphilis immunity with T cells obtained from *T. pallidum*-infected guinea pigs. These investigators also detected high levels of antitreponemal antibodies 1 week after challenge with *T. pallidum* in recipients of T cells. Wicher et al. (25) conferred protection on guinea pigs by using purified T cells. Although the humoral response to treponemal antigen was lower in recipients of T cells than in controls, antibody was produced. Collectively, these observations suggested that Chan et al. (7), Pavia and Niederbuhl (16), and Wicher et al. (25) transferred both T and B cells.

TABLE 2. Effects of cell transfer on lymph nodes and on conferral of resistance to infection with *T. pallidum* subsp. *pertenue*

Type and no. of cells transferred[a]	Mean ± SE for lymph nodes		Reciprocal antibody titer
	Wt (mg)	No. of treponemes (10^3)	
Normal T cells (4×10^6)	6.7 ± 2.6	250 ± 64	Negative
Immune T cells (Ia^-)			
1×10^6	5.6 ± 1.4	160 ± 44	Negative
4×10^6	2.5 ± 1.2[b]	37 ± 27[b]	Negative
Immune whole cells			
1×10^6	6.0 ± 2.3	320 ± 129	Negative
4×10^6	3.6 ± 1.6[b]	32 ± 21[b]	5,120

[a] Hamsters (three per group) were sacrificed 21 days after infection.
[b] $P < 0.05$.

TABLE 3. Effects of cell transfer on lymph nodes and against infection with *T. pallidum* subsp. *pertenue*

Cells transferred[a]	Mean ± SE for lymph nodes	
	Wt (mg)	No. of treponemes (10^3)
Normal		
Whole cells	8.0 ± 1.6	703 ± 146
T cells	9.0 ± 1.8	1,100 ± 95
Immune		
Whole cells	4.8 ± 0.5[b]	36 ± 12[b]
T cells	4.3 ± 1.0	111 ± 45[b]
None	8.7 ± 1.0	1,082 ± 185

[a] Hamsters were sacrificed 21 days after infection. There were four animals per group except the control group, which contained eight.
[b] $P < 0.05$.

In the present investigation, murine anti-Ia MAb 14-4-4s was used to obtain enriched T cells. It has been reported that anti-mouse Ia antibody identifies an Ia homolog on hamster B cells (28). Witte and Streilein (28) demonstrated that hamster B cells, but not T cells, express Ia antigen. While we cannot exclude the possibility that activated hamster T cells may express Ia antigen for a short period of time, our data indicate that immune T cells obtained from hamsters infected with *T. pallidum* subsp. *pertenue* more than 10 weeks earlier had little or no Ia antigen expression. Flow cytometric analysis of Ia and immunoglobulin expression showed that Ia antigen was not expressed on hamster T cells at the time immune T cells were collected. The Ia-negative T cells, presumably including memory cells, were capable of conferring resistance to *T. pallidum* subsp. *pertenue* infection. These same cells did not induce antitreponemal antibody production.

The following evidence was provided to confirm the specificity of MAb 14-4-4s for hamster B cells. Goat anti-hamster serum and MAb 14-4-4s recognized the same percentage of cells in the lymph nodes of infected hamsters. Complement-mediated lysis by MAb 14-4-4s resulted in a lymphocyte population of less than 7% immunoglobulin-positive and 5% Ia-positive cells. The remaining cells were 97% T cells, identified by MAb 20, a hamster pan T-cell marker. In addition, two-color cytometric analysis showed that both immunoglobulin and Ia markers were present on the same population of lymphocytes. Therefore, we concluded that the enriched T cells were depleted of B cells.

Although T cells can confer protection on recipient hamsters challenged with *T. pallidum* subsp. *pertenue*, treponemes were detected in the lymph nodes. The inability of recipients of immune T cells to eliminate *T. pallidum* subsp. *pertenue* completely is an important observation.

Direct T-cell-mediated cytotoxicity has been shown in some bacterial infections (12, 18).

Immune T cells, when activated by exposure to antigen (presumably in the presence of antigen-presenting cells), may secrete lymphokines and/or cytokines that can kill treponemes directly (18). This direct killing does not require the presence of antibody or B cells.

Examination of the histological changes in rabbit testes showed that *T. pallidum* was gradually cleared after infiltration of T cells and the accumulation of macrophages (11). Recently we showed that phagocytosis of *T. pallidum* subsp. *pertenue* by macrophages proceeded slowly (1). Resident and activated macrophages reduced the number of treponemes by only 50 to 65% after incubation for 24 h, even in the presence of immune serum. This suggests that the T-cell–macrophage interaction in the popliteal lymph node may be able to bring about bacteriostasis; however, it may be ineffective in inducing a bactericidal event against treponemes. A sufficient number of macrophages or activated macrophages may not be available to induce killing of treponemes. The macrophage population of the lymph node is approximately 2 to 5% and does not increase after infection (5).

The immune response to *T. pallidum* subsp. *pertenue* is complex. Infection of hamsters with *T. pallidum* subsp. *pertenue* produces chronic skin lesions that persist for several months (22). Hamsters infected for 10 weeks or longer quickly resolve their lesions after treatment with penicillin and are thereafter resistant to reinfection (21). The inability of hamsters to eliminate *T. pallidum* subsp. *pertenue* in the absence of antimicrobial treatment is a paradox, as their serum can kill treponemes in vitro and confer complete protection on recipients (4). In addition, we showed that T cells can confer protection.

The nature of *T. pallidum* subsp. *pertenue* infection, which is protracted despite the development of effective humoral and cell-mediated immunity, implies that treponemes are protected from destruction. When recipients of T cells were infected with *T. pallidum* subsp. *pertenue*, acquisition of host protein by the treponemes may have prevented their destruction by macrophages. Alderete and Baseman (2) showed that host proteins are both loosely and avidly associated with the outer envelope of treponemes. Fitzgerald et al. (8) also demonstrated that a coating of glycosaminoglycans inhibited complement-dependent antibody killing of treponemes. These molecules may be host derived. Therefore, treponemes may evade direct cell killing, antibody-dependent phagocytosis, or complement-mediated antibody lysis by coating themselves with protective "self" molecules that prevent immune recognition. Processes such as aging or fixation, which remove the outer envelope, increase antibody-treponeme interaction (10, 17). Recently, Radolf et al. (19, 20) demonstrated that treponemes possess few surface protein antigens. This observation also may account for the inability of macrophages to phagocytize treponemes even in the presence of antibody and immune T cells.

In conclusion, we demonstrated that T cells can confer protection against *T. pallidum* subsp. *pertenue* in the absence of antitreponemal antibody. Additional studies are needed to determine the mechanism by which T cells confer protection and to determine whether the response can be augmented to prevent infection with *T. pallidum* subsp. *pertenue*.

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