Borrelia burgdorferi-Specific T Lymphocytes Induce Severe Destructive Lyme Arthritis†

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This is the first documentation that *Borrelia burgdorferi*-specific T lymphocytes are involved in the pathogenesis of Lyme arthritis. We present direct evidence that T lymphocytes obtained from inbred LSH hamsters vaccinated with a whole-cell preparation of Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in adjuvant conferred on naive recipient hamsters the ability to develop severe destructive arthritis when challenged with *B. burgdorferi* sensu stricto isolates C-1-11 and 297. By contrast, recipients infused with normal T lymphocytes and challenged with *B. burgdorferi* sensu stricto isolates C-1-11 and 297 failed to develop severe destructive arthritis. The T lymphocytes transferred were obtained from the lymph nodes of vaccinated and nonvaccinated hamsters by depleting B lymphocytes by using monoclonal antibody 14-4-4s (<1% B lymphocytes by flow cytometric analysis). The enriched T lymphocytes showed enhanced proliferation to stimulation with concanavalin A and failed to respond to lipopolysaccharide. Moreover, only the enriched T lymphocytes from vaccinated hamsters proliferated on exposure to a whole-cell preparation of *B. burgdorferi* sensu stricto isolate C-1-11 in the presence of mitomycin-treated syngeneic antigen-presenting cells. These results demonstrate that *B. burgdorferi*-specific T lymphocytes primed by vaccination with a whole-cell preparation of inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in adjuvant are involved in the development of severe destructive arthritis. Additional experiments are needed to define the precise mechanism(s) responsible for the development of Lyme arthritis.

We showed previously (26) that severe destructive arthritis developed in vaccinated hamsters after challenge with isolates of *Borrelia burgdorferi* sensu lato. Specifically, severe destructive arthritis was readily evoked in inbred LSH hamsters vaccinated with a whole-cell preparation of Formalin-inactivated *B. burgdorferi* sensu lato in adjuvant when challenged with the homologous (vaccine) isolate before high levels of protective borreliacidal antibody developed. Once high levels of protective borreliacidal antibody developed, vaccinated hamsters were protected from homologous challenge and the development of arthritis. Vaccinated hamsters, however, still developed severe destructive arthritis when challenged with other isolates of the three genomic groups of *B. burgdorferi* sensu lato (*B. burgdorferi* sensu stricto, *Borrelia garinii*, and *Borrelia afzelii*) (29, 30), despite high levels of isolate (vaccine)-specific borreliacidal antibody.

These findings are important and demonstrate that adverse effects may develop in vaccinated hamsters if protective antibody is not induced before infection with the Lyme borreliosis spirochete. The mechanism(s) responsible for the development of severe destructive arthritis is unknown and needs to be elucidated. We, therefore, sought direct evidence for T-lymphocyte involvement in the development of Lyme arthritis. We present the first direct evidence that T lymphocytes obtained from hamsters vaccinated with a whole-cell preparation of Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in adjuvant can confer on naive recipient hamsters the ability

to develop severe destructive arthritis when challenged with isolates of *B. burgdorferi* sensu stricto.

MATERIALS AND METHODS

Hamsters. Six- to 8-week old inbred LSH/Ss hamsters were obtained from our breeding colony located at the Wisconsin State Laboratory of Hygiene. Hamsters weighing 60 to 120 g were housed three per cage at an ambient temperature of 21°C. Food and water were available ad libitum.

Organisms. Low-passage (<5) virulent *B. burgdorferi* sensu stricto isolates C-1-11 and 297, representing two distinct seroprotective groups among isolates of *B. burgdorferi* sensu stricto (29, 30), were cultured in modified Barbour-Stoenner-Kelly (BSK) medium (4) at 32°C to a concentration of 5×10^7 spirochetes per ml. Samples (500 μ l each) were dispensed into 1.5-ml screw-cap tubes (Sarstedt, Newton, N.C.) containing 500 μ l of BSK supplemented with 30% glycerol (Sigma, St. Louis, Mo.), sealed, and stored in liquid nitrogen. When needed, a frozen suspension of spirochetes was thawed, and an aliquot was used to inoculate fresh BSK. The culture was incubated at 32°C for 72 h and diluted with fresh BSK to yield 10^7 spirochetes per ml. Spirochetes were enumerated by dark-field microscopy and with a Petroff-Hauser counting chamber.

Preparation of vaccine. A whole-cell *B. burgdorferi* sensu stricto isolate C-1-11 vaccine was prepared as described previously (26). Briefly, *B. burgdorferi* sensu stricto isolate C-1-11 was grown in 4 liters of BSK to 5×10^8 spirochetes per ml. Spirochetes were harvested by centrifugation ($10,000 \times g$, 30 min) after three washes with phosphate-buffered saline (PBS [pH 7.4]). The pellet was suspended in 1% Formalin and incubated at 32°C for 30 min. The Formalin-inactivated spirochetes were then washed three times with PBS by centrifugation and suspended in PBS. Samples (500 μ l each) containing 5×10^9 spirochetes were dispensed into 1.5-ml screw-cap tubes (Sarstedt) and stored at -70°C . Subsequently, frozen samples of Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 were thawed and suspended in 10 ml of an aluminum hydroxide gel adjuvant (HPA-3; Reheis, Inc., Berkeley Heights, N.J.) before use.

Vaccination of hamsters. Hamsters were vaccinated as described previously (26). Briefly, hamsters were mildly anesthetized with ether contained in a nose-and-mouth cup and vaccinated intramuscularly in each hind leg with a single dose of 0.2 ml of 10^8 cells of Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in adjuvant. The protein concentration was 50 to 100 μ g per inoculum, determined by using the Bio-Rad (Hercules, Calif.) protein assay kit. Controls consisted of nonvaccinated hamsters inoculated with 0.2 ml of PBS.

Preparation of hamster sera and lymph node cells. Immune sera and lymph

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node cells were obtained from 50 hamsters after 5 weeks of vaccination with a whole-cell preparation of Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in adjuvant. Concomitantly, normal sera and lymph node cells were obtained from 50 nonvaccinated noninfected hamsters. Hamsters were anesthetized with ether and bled by intracardiac puncture. The blood was allowed to clot, and sera were separated by centrifugation at $500 \times g$, pooled, dispensed into 1.2-ml aliquots, and frozen at -20°C until needed. Popliteal and inguinal lymph nodes were removed aseptically from vaccinated and nonvaccinated hamsters; teased apart in Dulbecco's modified Eagle's medium (DMEM; Sigma) containing 10% heat-inactivated (56°C for 30 min) fetal-calf serum (HyClone Labs., Inc., Logan, Utah), 2-mercaptoethanol (5×10^{-5} M; Sigma), penicillin (100 U/ml; Sigma), and streptomycin (100 $\mu\text{g}/\text{ml}$; Sigma); and pressed through a sterile stainless steel 60-mesh screen into DMEM. The cells were washed twice with PBS by centrifugation and suspended in DMEM to a concentration of 10^7 cells per ml.

Antibody reagents. Hybridoma cell line 14-4-4s (ATCC HB-32) secreting murine monoclonal antibody (MAb) 14-4-4s has specificity for I-E^k of murine H-2 haplotypes and Ia.7 in the Ia chart (32). It also recognizes a surface cell marker on hamster B lymphocytes (27, 28, 52, 53). Hybridoma 14-4-4s was grown in DMEM at 37°C in a humidified atmosphere of 7.5% CO_2 . After 10 days, culture supernatants were collected by centrifugation at $500 \times g$, sterilized by filtration with a 0.22- μm pore-size filter apparatus (Nalgene Labware Div., Rochester, N.Y.), dispensed into 12-ml aliquots, and frozen at -20°C until used. Unconjugated goat anti-mouse immunoglobulin, heavy and light chain specific (Organon Teknica Corp., Durham, N.C.), was used to coat tissue culture dishes for panning and enrichment of T lymphocytes. For flow cytometric analysis of lymphocyte populations, fluorescein isothiocyanate (FITC)-conjugated goat anti-hamster immunoglobulin, heavy and light chain specific (Boehringer Mannheim Biochemicals, Indianapolis, Ind.), and FITC-conjugated goat immunoglobulin (whole immunoglobulin; Sigma) were used.

Isolation of T lymphocytes. Methods for purification of hamster T lymphocytes with MAb 14-4-4-s were previously described (27, 28, 52, 53). Briefly, T lymphocytes were obtained from single-cell suspensions of lymph node cells from vaccinated and nonvaccinated hamsters by removing adherent B lymphocytes in immunoglobulin-coated tissue culture dishes. In some experiments, vaccinated hamsters were challenged with 10^6 *B. burgdorferi* cells 72 h before T lymphocytes were obtained. The immunoglobulin-coated dishes (100 by 20 mm; Corning Glass Works, Corning, N.Y.) were prepared by coating the surfaces of dishes with 100 μg of unconjugated goat anti-mouse immunoglobulin in coupling buffer (15 mM Na_2CO_3 , 35 mM NaHCO_3 [pH 9.6]) overnight at 4°C and washing the dishes three times with PBS before use. Single-cell suspensions of 10^7 immune and normal lymph node cells per ml were incubated with MAb 14-4-4s in DMEM for 30 min at 4°C . The suspensions of cells were washed twice with PBS by centrifugation, suspended to 10^7 cells per ml, poured over the immunoglobulin-coated tissue culture dishes, and incubated for 60 min at 4°C . Nonadherent cells were then collected, poured over another set of immunoglobulin-coated dishes, and incubated for 60 min at 4°C . This process was repeated three more times. After the last panning cycle, the nonadherent cells containing T lymphocytes were washed twice with PBS by centrifugation and suspended to 5×10^7 cells per ml in DMEM. Cell viability determined by trypan blue (Sigma) exclusion was greater than 99%. Subsequently, the enriched T lymphocytes were analyzed by flow cytometry; examined for proliferation to stimulation with concanavalin A (Con A), lipopolysaccharide (LPS), and a whole-cell preparation of *B. burgdorferi* sensu stricto isolate C-1-11; and used in adoptive transfer studies. We also showed previously (27, 28) that enriched T lymphocytes ($>95\%$ by flow cytometric analysis) obtained by using MAb 14-4-4s displayed enhanced proliferation to stimulation with Con A and failed to respond to LPS.

Analysis of lymphocytes by flow cytometry. One-hundred-microliter samples containing 5×10^5 lymph node cells obtained before and after panning were stained with FITC-conjugated goat anti-hamster immunoglobulin (1:100) for 15 min at 4°C . Samples were then washed twice with PBS by centrifugation, fixed with 2% paraformaldehyde (Sigma), and kept in the dark until analyzed by flow cytometry. Controls included samples stained with FITC-conjugated goat immunoglobulin (1:100). All cell samples were analyzed by using a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, Mountain View, Calif.) with FACScan LYSIS II software for data acquisition. Initially, lymphocytes were detected and differentiated from other cell types by forward scatter, side scatter, and FITC fluorescence. Data for 5,000 to 10,000 cells were acquired. Cell samples were then analyzed by histogram profiles of FITC fluorescence by using FACScan LYSIS II software. Gates were established for cell samples stained with FITC-conjugated goat immunoglobulin. The percentage of B lymphocytes was determined by a percent shift in FITC fluorescence of FITC-conjugated goat anti-hamster immunoglobulin-stained lymphocytes.

T-lymphocyte proliferation assays. Lymph node cells obtained from vaccinated and nonvaccinated hamsters before and after panning were incubated with Con A and LPS. One-hundred-microliter samples containing 5×10^5 lymph node cells in DMEM were added to flat-bottom wells of a 96-well microtiter plate (Becton Dickinson Labware, Lincoln Park, N.J.). Con A (2 $\mu\text{g}/\text{ml}$; Sigma) and LPS from *Escherichia coli* 55:BS (50 $\mu\text{g}/\text{ml}$; Sigma) (27, 28) were added to yield a final volume of 200 μl per well. Controls included lymph node cells cultured in DMEM without mitogens. All assays were performed in triplicate and incubated at 37°C for 48 h in a humidified atmosphere of 7.5% CO_2 . Cell

proliferation was determined by using a colorimetric assay employing 2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxyanilide inner salt (XTT; Sigma) and phenazine methosulfate (PMS; Sigma) (17, 35, 47). Twenty microliters of XTT (1 mg/ml)–1% PMS in DMEM was added to each well and incubated at 37°C for 12 to 16 h in a humidified atmosphere of 7.5% CO_2 . The colorimetric reaction was then measured spectrophotometrically at 450 nm.

In other experiments, enriched T lymphocytes obtained after panning from vaccinated and nonvaccinated hamsters were incubated with mitomycin-treated syngeneic antigen-presenting cells (APCs) and a whole-cell preparation of *B. burgdorferi* spirochetes. Fifty-microliter samples containing 5×10^5 T-lymphocytes in DMEM were added to flat-bottom wells of a 96-well microtiter plate. Syngeneic APCs were obtained from spleens of normal noninfected hamsters. Spleens were surgically removed, aseptically teased apart, and pressed through a sterile stainless steel 60-mesh screen into DMEM. Splenocytes were separated by Ficol-Hypaque (density, 1.077; Sigma) gradient centrifugation, washed twice with PBS, and suspended to 10^7 cells per ml in DMEM. Mitomycin (50 $\mu\text{g}/\text{ml}$; Sigma) was added, and the cell suspension was incubated at 37°C for 30 min in a humidified atmosphere of 7.5% CO_2 . Cell suspensions were washed three times with PBS by centrifugation and suspended to 10^7 cells per ml in DMEM. Fifty microliters of mitomycin-treated APCs containing 10^6 splenocytes in DMEM was added to wells containing T lymphocytes. A whole-cell preparation of *B. burgdorferi* sensu stricto isolate C-1-11 was obtained from a 96-h culture, centrifuged at $10,000 \times g$ for 5 min, washed five times with PBS by centrifugation, and suspended to 10^9 spirochetes per ml in DMEM. One hundred microliters containing 10^6 spirochetes and their 10-fold dilutions were added to each well containing T lymphocytes and APCs. Controls included T lymphocytes and APCs in DMEM without spirochetes. All assays were performed in triplicate and incubated at 37°C for 4 to 6 days in a humidified atmosphere of 7.5% CO_2 . T-lymphocyte proliferation was determined by the colorimetric assay employing XTT and PMS (17, 35, 47) as described above.

Adoptive transfer of T lymphocytes and sera. Adoptive transfer of T lymphocytes and serum was carried out in separate experiments. Immune T lymphocytes and sera were obtained from 50 hamsters vaccinated for 5 weeks with a whole-cell preparation of Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in adjuvant. Concomitantly, normal T-lymphocytes and sera were obtained from 50 nonvaccinated noninfected hamsters. Cells and sera were processed as described above.

Two groups of six hamsters each were mildly anesthetized with ether contained in a nose-and-mouth cup and injected subcutaneously in each hind paw with 0.1 ml of PBS containing 5×10^6 immune or normal T lymphocytes. Eighteen to 24 h later, three hamsters from each group were challenged subcutaneously in the hind paws with 0.1 ml of 10^6 viable *B. burgdorferi* sensu stricto isolate C-1-11 cells or injected with 0.1 ml of BSK. In other experiments, recipients of immune or normal T lymphocytes were challenged with 10^6 viable *B. burgdorferi* sensu stricto isolate 297 cells.

The same experimental approach was used to transfer immune and normal sera and to challenge recipient hamsters with viable spirochetes. However, 1.0 ml of immune or normal serum was injected intravenously in the sublingual vein at daily intervals for 7 days. Three days after the first injection (day 0), all recipients were challenged with viable spirochetes. Controls for the adoptive transfer experiments included vaccinated and nonvaccinated hamsters challenged with viable spirochetes or injected with BSK.

Assessment of arthritis. Swelling of the hind paws of hamsters challenged with isolates of *B. burgdorferi* sensu stricto C-1-11 and 297 was used to evaluate the inflammatory response. The hind paws were measured every other day for 21 days with a plethysmograph (Buxco Electronics, Sharon, Conn.). Measurements were obtained by mildly anesthetizing hamsters with ether contained in a nose-and-mouth cup and carefully dipping a hind paw into a column of mercury to the ankle and measuring the amount (in milliliters) of mercury displaced. The mean plethysmograph value was obtained from three hamsters (six hind paws) per group and was used as an index of severity of swelling from arthritis. Mercury displacement was standardized with a volume calibrator.

Recovery of *B. burgdorferi* from tissues. Twenty-one days after challenge, hamsters were euthanized by CO_2 inhalation. The urinary bladder, spleen, left kidney, and heart of each hamster were removed aseptically, homogenized through a 5-ml syringe, and inoculated into 5 ml of BSK supplemented with rifampin (100 $\mu\text{g}/\text{ml}$; Sigma) and phosphomycin (100 $\mu\text{g}/\text{ml}$; Sigma). Cultures were incubated at 32°C and examined weekly for 2 weeks by dark-field microscopy for motile spirochetes. If spirochetes were not detected, 0.5 ml of the culture was inoculated into 4 ml of fresh BSK supplemented with antibiotics and examined weekly for 3 weeks. If spirochetes were not detected, the culture was considered negative.

Preparation of tissues for histology. The hind legs of hamsters were amputated 21 days after challenge at the midfemur, fixed in 10% neutral buffered Formalin, placed in decalcifying solution (Lerner Laboratories, Pittsburgh, Pa.) for 18 h, and stored in 10% Formalin prior to processing. The knees and hind paws were bisected longitudinally, embedded in paraffin, cut into 6- μm sections, placed on glass slides, and stained with hematoxylin and eosin. Hind legs within each hamster group were randomly selected for histopathological examination.

Statistics. All values obtained were tested by the analysis of variance. The Fisher least-significant-difference test (46) was used to examine pairs of means

FIG. 1. Proliferative responses of lymph node cells from vaccinated (■) and nonvaccinated (▨) hamsters before and after depletion (panning) of B lymphocytes to stimulation with Con A and LPS. Proliferative responses were determined by using a colorimetric assay employing XTT and PMS and measuring the optical density spectrophotometrically at 450 nm (O.D. 450 nm). Optical density readings are expressed as the means $\pm$ standard errors of the means (error bars) for triplicate samples.

when a significant F ratio indicated reliable mean differences. The alpha level was set at 0.05 before the experiments were started.

RESULTS

Isolation of enriched T lymphocytes. Enriched T lymphocytes were obtained from lymph node cells of 50 vaccinated and nonvaccinated hamsters by depleting B lymphocytes with MAb 14-4-4s, which recognizes a surface cell marker on hamster B lymphocytes (27, 28, 52, 53). Flow cytometric analysis showed that the percentages of B lymphocytes present in the samples (24.7 and 12.4% for vaccinated and nonvaccinated hamsters, respectively) were significantly reduced after panning with MAb 14-4-4s ($P < 0.01$). Enriched T lymphocytes from vaccinated and nonvaccinated hamsters contained only 0.9 and 0.5% B lymphocytes, respectively. When enriched T lymphocytes were incubated with *B. burgdorferi* organisms in vitro, anti-*B. burgdorferi* antibodies were also not detected in the supernatants. In addition, when the enriched T lymphocytes were stimulated in vitro with Con A, a T-cell mitogen, enhanced proliferative responses were detected (Fig. 1). By contrast, the proliferative responses after stimulation with LPS, a B-cell mitogen, were significantly reduced ($P < 0.01$).

T-lymphocyte specificity for *B. burgdorferi*. The in vitro proliferative responses of enriched T lymphocytes from vaccinated hamsters exposed to whole-cell preparations containing 10^8 , 10^7 , 10^6 , 10^5 , and 10^4 *B. burgdorferi* sensu stricto isolate C-1-11 spirochetes per ml were significantly enhanced compared with the proliferative responses of enriched T lymphocytes from nonvaccinated hamsters ($P < 0.01$) (Fig. 2). Maximum proliferative responses were obtained with 10^6 to 10^8 spirochetes per ml. Only slight differences were detected among the proliferative responses of enriched T lymphocytes obtained from nonvaccinated hamsters with and without exposure to spirochetes and enriched T-lymphocytes responses of vaccinated hamsters not exposed to *B. burgdorferi* spirochetes.

Severe destructive arthritis adoptively conferred by T lymphocytes. We next examined the ability of T lymphocytes obtained from vaccinated hamsters to confer adoptively severe destructive arthritis on naive recipient hamsters. T lymphocytes from 50 vaccinated and nonvaccinated hamsters, obtained by depleting B lymphocytes by using MAb 14-4-4s, were injected into naive recipient hamsters which were then challenged with *B. burgdorferi* sensu stricto isolates C-1-11 and 297. Figure 3 shows that recipients of immune T lymphocytes from hamsters vaccinated for 5 weeks developed severe swelling of the hind paws. Swelling was detected 6 days after challenge and increased rapidly, with peak swelling occurring 14 to 16 days after challenge before gradually decreasing. By contrast, recipients of normal T lymphocytes from nonvaccinated hamsters developed slight swelling. The swelling developed slowly (day 10) and was significantly less ($P < 0.01$) and of shorter duration than the swelling detected in recipients of immune T lymphocytes. Nonvaccinated hamsters challenged with *B. burgdorferi* sensu stricto isolates C-1-11 and 297 also developed slight swelling. The swelling was significantly less at days 14 and 16 after challenge ($P < 0.01$) than the swelling detected in recipients of normal T lymphocytes challenged with isolates C-1-11 and 297. In addition, nonvaccinated hamsters injected with BSK failed to develop swelling of the hind paws. Similarly, recipients of T lymphocytes from vaccinated and nonvaccinated hamsters injected with BSK failed to develop any clinical and pathological manifestations of Lyme borreliosis. When these experiments were repeated, similar results were obtained. Tissues of recipients and donors of T lymphocytes challenged with isolates C-1-11 and 297 grew spirochetes when cultured in BSK (Table 1).

In other experiments, naive recipient hamsters were infused with T lymphocytes from hamsters vaccinated for 1 and 3 weeks. Again, immune T lymphocytes adoptively conferred severe destructive arthritis on recipients challenged with *B. burgdorferi* sensu stricto isolates C-1-11 and 297. We also observed that development of severe destructive arthritis re-

FIG. 2. Proliferative responses of enriched T lymphocytes from vaccinated (■) and nonvaccinated, noninfected (▨) hamsters with and without exposure to different concentrations of a whole-cell preparation of *B. burgdorferi* sensu stricto isolate C-1-11. Proliferative responses were determined by using a colorimetric assay employing XTT and PMS and measuring the optical density spectrophotometrically at 450 nm (O.D. 450 nm). Optical density readings are expressed as the means $\pm$ standard errors of the means (error bars) for triplicate samples.

quired a challenge with viable spirochetes. Arthritis was not evoked in recipients after challenge with an inoculum of 10^6 or multiple injections (daily for 7 days) of 10^6 dead *B. burgdorferi* sensu stricto isolate C-1-11 or 297 cells.

Histopathology of hind paws of recipients of *B. burgdorferi*-specific T lymphocytes. Erosive and destructive arthropathy was detected in the joints of the hind paws of recipients of T lymphocytes obtained from vaccinated hamsters 21 days after challenge with *B. burgdorferi* sensu stricto isolate C-1-11 (Fig. 4C). The histopathology and severity of the arthropathy were similar to those detected in the joints of the hind paws of vaccinated hamsters challenged with *B. burgdorferi* sensu stricto isolate C-1-11 (Fig. 4E). The synovia of the tibiotarsal and intertarsal joints displayed chronic hypertrophy and hyperplasia characterized by bridging of villi mixed with fibrin-forming pannus tissue, erosion of the articular cartilage, and focal destruction of underlying bone (Fig. 4C and E). A cellular infiltrate of neutrophils, macrophages, mast cells, lymphocytes, and plasma cells was also present in the subsynovial and peri-articular tissues. Chronic arthritis was pronounced and characterized by fibromyxoid changes, with residual granulation tissue and occasional cartilaginous metaplasia. By contrast, the histopathology of the hind paws of nonvaccinated hamsters (Fig. 4D) and recipients of normal T lymphocytes (Fig. 4B) challenged with *B. burgdorferi* sensu stricto isolate C-1-11 displayed only synovitis (Fig. 4B and D). A cellular inflammatory infiltrate was present, but the joint spaces were free of significant alterations including the lack of pannus tissue formation and absence of erosive and destructive arthropathy. Nonvaccinated hamsters injected with BSK failed to develop any significant histopathological changes (Fig. 4A). Likewise, recipients of immune and normal T lymphocytes and vaccinated hamsters injected with BSK failed to develop any significant histopathological changes. In other experiments, recipients of

immune T lymphocytes challenged with *B. burgdorferi* sensu stricto isolate 297 also displayed erosive and destructive arthropathy similar to the histopathological changes shown in Fig. 4C and E.

Failure of *B. burgdorferi*-specific immune serum to induce arthritis. The purpose of this experiment was to determine whether treatment of naive recipient hamsters with immune sera obtained from hamsters vaccinated for 5 weeks with a whole-cell preparation of Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in adjuvant could induce arthritis after challenge with the homologous isolate and *B. burgdorferi* sensu stricto isolate 297, representing a different seroprotective group (29, 30). Immune and normal sera were injected into naive recipient hamsters at daily intervals for 7 days. Three days after the first injection, all hamsters were challenged with *B. burgdorferi* sensu stricto isolates C-1-11 and 297. No significant differences were detected in hind paw swelling among the recipients of immune and normal sera and nonvaccinated hamsters challenged with *B. burgdorferi* sensu stricto isolates C-1-11 and 297 (Fig. 5). Spirochetes were recovered in BSK from all tissues of recipients of normal serum challenged with *B. burgdorferi* sensu stricto isolates C-1-11 and 297 (Table 2). No spirochetes were recovered from the tissues of recipients of C-1-11-specific immune serum challenged with the homologous spirochete. Spirochetes, however, were recovered from the tissues of recipients of C-1-11-specific immune serum challenged with *B. burgdorferi* sensu stricto isolate 297 (Table 2). The joints of the hind legs of nonvaccinated hamsters and recipients of immune or normal sera challenged with *B. burgdorferi* sensu stricto isolates C-1-11 and 297 were also examined for erosive and destructive arthropathy. Severe destructive arthritis was not detected, and no significant differences in histopathology were observed.

FIG. 3. Development of hind paw swelling in naive hamsters adoptively immunized with immune (triangles) and normal (inverted triangles) T lymphocytes and challenged with *B. burgdorferi* sensu stricto isolates C-1-11 (open symbols) and 297 (closed symbols). Controls included nonvaccinated hamsters challenged with *B. burgdorferi* sensu stricto isolates C-1-11 (◇) and 297 (●) or injected with BSK (●).

DISCUSSION

There is a scarcity of information on the role of cell-mediated immunity, specifically the role of T lymphocytes, in the pathogenesis of Lyme borreliosis. T lymphocytes from patients with chronic Lyme arthritis and experimentally infected animals respond vigorously to stimulation with *B. burgdorferi* antigens in vitro (22, 23, 31, 37, 45, 54). *B. burgdorferi*-specific

human T-lymphocyte clones that recognize the outer surface proteins A and B, flagellum, and heat-shock proteins 60 and 70 have also been established (23, 24, 43, 44, 54–56). In addition, delayed-type hypersensitivity is present in humans (2) and has been elicited in several inbred strains of mice presensitized with *B. burgdorferi* antigens (36, 38). Other studies, however, have failed to demonstrate delayed-type hypersensitivity reactions in experimental animals or have argued against a direct role for T-lymphocyte responses in the pathogenesis of Lyme borreliosis (7, 8).

Recently, we suggested that cell-mediated immunity was responsible for the severe destructive arthritis that developed in vaccinated hamsters after challenge with isolates of *B. burgdorferi* sensu lato (26). Briefly, severe destructive arthritis was evoked in hamsters vaccinated with a whole-cell preparation of Formalin-inactivated *B. burgdorferi* sensu stricto in adjuvant when hamsters were challenged with the homologous isolate before specific borreliacidal antibody developed. Once borreliacidal antibody developed, vaccinated hamsters were protected from challenge and the development of arthritis. Vaccinated hamsters, however, still developed severe destructive arthritis when challenged with isolates of the three genomic groups of *B. burgdorferi* sensu lato (*B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii*), belonging to different seroprotective groups (29, 30). In this report, we present the first direct evidence that *B. burgdorferi*-specific T lymphocytes are responsible for the development of severe destructive arthritis. When

TABLE 1. Recovery of *B. burgdorferi* sensu stricto isolates C-1-11 and 297 from the tissues of recipients of immune or normal T lymphocytes

T-lymphocyte group ^a	No. of animals with indicated isolate in:							
	C-1-11				297			
	Bladder	Spleen	Kidney	Heart	Bladder	Spleen	Kidney	Heart
Recipients								
Normal	3	2	3	3	3	2	1	2
Immune	2	3	2	3	3	3	2	1
Donors								
Normal	3	3	3	3	3	2	1	3
Immune	2	3	1	2	3	3	3	3

^a Normal or immune T lymphocytes were injected into naive recipient hamsters which were then challenged with *B. burgdorferi* sensu stricto isolates C-1-11 and 297. Hamsters were sacrificed 21 days after challenge. There were three hamsters in each group.

FIG. 4. Histopathology of hind paws of naive hamster adoptively immunized with immune (C) and normal (B) T lymphocytes and challenged with *B. burgdorferi* sensu stricto isolate C-1-11. Controls included vaccinated (E) and nonvaccinated (D) hamsters challenged with *B. burgdorferi* sensu stricto isolate C-1-11 and nonvaccinated hamsters injected with BSK (A). Arrows indicate areas of infiltration of inflammatory cells into the synovium.

naive recipient hamsters were infused with T lymphocytes obtained from hamsters vaccinated with a whole-cell preparation of Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in adjuvant, they developed severe destructive arthritis after challenge with *B. burgdorferi* sensu stricto isolates C-1-11 and 297.

The successful demonstration that *B. burgdorferi*-specific T lymphocytes were responsible for the adoptive transfer of severe destructive arthritis was confirmed by immunological en-

richment and characterization of the transferred cells. The transferred cells were enriched for T lymphocytes by depleting B lymphocytes with MAb 14-4-4s, which is specific for hamster B lymphocytes (27, 28, 52, 53). The enriched T lymphocytes contained <1% B lymphocytes by flow cytometric analysis. In addition, the enriched T lymphocytes had enhanced proliferative responses to stimulation with Con A but failed to respond to stimulation with LPS. Furthermore, only the enriched T lymphocytes from vaccinated hamsters proliferated after expo-

FIG. 5. Development of hind paw swelling in naive hamsters adoptively immunized with immune (triangles) and normal (inverted triangles) sera and challenged with *B. burgdorferi* sensu stricto isolates C-1-11 (open symbols) and 297 (closed symbols). Controls included nonvaccinated hamsters challenged with *B. burgdorferi* sensu stricto isolates C-1-11 (◇) and 297 (◆) or injected with BSK (●).

sure to *B. burgdorferi* antigens in the presence of mitomycin-treated syngeneic APCs.

Development of the severe destructive arthritis in naive recipient hamsters was not caused by immune serum or its components. When naive hamsters were administered immune sera from hamsters vaccinated for 5 weeks with a whole-cell preparation of Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in adjuvant and challenged with the homologous isolate, no swelling of the hind paws was detected. By contrast, only slight swelling of the hind paws occurred when recipients were challenged with *B. burgdorferi* sensu stricto isolate 297. This isolate belongs to a different seroprotective group (29, 30). The degree of swelling was similar to that of recipients of normal serum and nonvaccinated hamsters challenged with *B. burgdorferi* sensu stricto isolates C-1-11 and 297. Our results confirm previous studies (15, 16, 39, 40, 42) that immune serum

cannot induce arthritis. In addition, the histopathologies of the hind paws of recipients of immune and normal sera and nonvaccinated hamsters challenged with these spirochetes were similar. No severe destructive arthritis was detected.

Our results demonstrate that *B. burgdorferi*-specific T lymphocytes are responsible for the development of severe destructive arthritis. There is considerable evidence that T lymphocytes also play a major role in the progression of chronic and erosive arthropathies in rodents activated by immunization with mycobacterial antigens in adjuvant (5, 13, 14, 18, 19, 50), type-II collagen (3, 11, 12, 21, 33), and streptococcus cell wall antigens (1, 6, 34, 51). However, the precise mechanism(s) by which *B. burgdorferi*-specific T lymphocytes cause the induction and/or progression of severe destructive Lyme arthritis is unknown. When immunocompetent nonvaccinated hamsters (10, 26, 41) or recipient hamsters infused with normal T lympho-

TABLE 2. Recovery of *B. burgdorferi* sensu stricto isolates C-1-11 and 297 from the tissues of recipients of immune or normal serum

Group ^a	No. of animals with indicated isolate in:							
	C-1-11				297			
	Bladder	Spleen	Kidney	Heart	Bladder	Spleen	Kidney	Heart
Normal	3	3	3	3	3	3	2	3
Immune	0	0	0	0	3	1	3	2

^a Normal or immune serum was administered to naive recipient hamsters at daily intervals for 7 days. Three days after the first serum injection, all hamsters were challenged with *B. burgdorferi* sensu stricto isolates C-1-11 and 297. Hamsters were sacrificed 21 days after challenge. There were three hamsters in each group.

cytes are challenged with *B. burgdorferi* sensu stricto isolates C-1-11 and 297, they develop synovitis that wanes gradually. Development of severe destructive arthritis is prevented. By contrast, vaccinated hamsters (26) or hamsters infused with *B. burgdorferi*-specific T lymphocytes develop severe destructive arthritis when challenged with the same isolates of *B. burgdorferi* sensu lato. It is postulated that accumulation of activated T lymphocytes within the tissues of the hind paws of hamsters, differences in frequencies of the subpopulations of activated T lymphocytes, or modifications of the cytokine environment in the presence or absence of APCs may be responsible for the development of severe destructive arthritis. Additional experiments are needed to define the mechanisms responsible for the onset, control, and progression of Lyme arthritis in non-vaccinated and vaccinated hamsters. The ability of vaccinated hamsters and recipients infused with *B. burgdorferi*-specific T lymphocytes to develop severe destructive arthritis provides a unique opportunity to elucidate these mechanisms.

Severe destructive arthritis was induced by interaction of *B. burgdorferi*-specific T lymphocytes with viable spirochetes. When recipients of *B. burgdorferi*-specific T lymphocytes were challenged with dead spirochetes or daily injections of dead spirochetes for 7 days, swelling of the hind paws was not detected. These results confirm our previous finding that killed low-passage *B. burgdorferi* organisms, even after multiple injection in the hind paws of irradiated hamsters, could not induce arthritis (20). Our results suggest that antigens, immune stress proteins, or factors inherent to live *B. burgdorferi* organisms and not found in or released by dead spirochetes are responsible for the induction of arthritis. Determination and elimination of these arthritis-producing factors are critical for developing a safe vaccine. Exposure to infected ticks could evoke adverse effects if the vaccine contained the arthritis-inducing components.

It is interesting that the onset, peak, and resolution of severe destructive arthritis were similar in vaccinated hamsters (26) and recipients of *B. burgdorferi*-specific T lymphocytes challenged with *B. burgdorferi* sensu stricto isolates C-1-11 and 297. Swelling of the hind paws of vaccinated and adoptively immunized hamsters was detected early after challenge and increased rapidly, with peak swelling occurring on days 14 to 16 after challenge before gradually decreasing. Furthermore, the histopathologies of the hind paws of the challenged vaccinated hamsters and recipients of *B. burgdorferi*-specific T lymphocytes were indistinguishable. These results suggest that accumulation of other cell types, besides *B. burgdorferi*-specific T lymphocytes, is necessary for the initial development and resolution of arthritis. Macrophages have been implicated in playing a critical role in the development of Lyme arthritis because they produce modulatory cytokines in the presence of spirochetes and activated T lymphocytes (9, 20, 45). *B. burgdorferi*-specific T lymphocytes in the presence (induction of arthritis) or absence (resolution phase) of spirochetes could produce products with both macrophage-activating and macrophage-deactivating properties. The role that immune T lymphocytes and macrophages plays in the development of arthritis is central to our understanding the pathogenesis of Lyme arthritis.

Our results showed that *B. burgdorferi*-specific T lymphocytes could not confer protection on adoptively immunized hamsters against infection with isolates of *B. burgdorferi* sensu stricto. When recipients of *B. burgdorferi*-specific T lymphocytes were challenged with *B. burgdorferi* sensu stricto isolates C-1-11 and 297, spirochetes were readily recovered from their tissues after cultivation in BSK. These results confirmed the findings of de Souza et al. (8). These investigators showed that adoptive transfer of enriched T lymphocytes from chronically

infected mice failed to protect recipients against infection. Likewise, Schaible et al. (36) failed to detect resistance against infection with *B. burgdorferi* sensu stricto isolate B31 in mice demonstrating delayed-type hypersensitivity. Collectively, these results (8, 36) show that T lymphocytes are not involved in the development of cell-mediated resistance. T lymphocytes, however, are involved in the development of severe destructive arthritis.

The finding that vaccinated hamsters (26) or hamsters adoptively immunized with *B. burgdorferi*-specific T lymphocytes develop severe destructive arthritis when challenged with the Lyme borreliosis spirochete is important. We have found an animal model of Lyme arthritis that may closely resemble a subset of Lyme patients that develop chronic Lyme arthritis. Steere and colleagues (49) have shown that approximately 60% of patients from North America infected with *B. burgdorferi* develop intermittent episodes of arthritis. Within 2 to 3 years after infection, 10% of these patients develop continuous joint inflammation. These patients have an increased frequency of the class II major histocompatibility complex molecule HLA-DR4 and demonstrate treatment-resistant arthritis (48). Lengle-Janben et al. (25) also showed that T-cell responses to *B. burgdorferi*, specifically those of OspA-reactive T-cell lines, were preferentially recognized in these patients compared with patients with treatment-responsive arthritis. Additional studies with the hamster model of severe destructive Lyme arthritis may define the subset of T lymphocytes involved in the induction of arthritis and the component(s) of the spirochete responsible for the priming of these cells.

In summary, we have demonstrated that *B. burgdorferi*-specific T lymphocytes can adoptively transfer the ability to induce arthritis in naive recipient hamsters challenged with isolates of *B. burgdorferi* sensu stricto. Additional experiments are needed to define the specific mechanism(s) involved. These studies are important for the development of a safe vaccine and determining means to modulate the immune response to prevent the development of the signs and symptoms of Lyme arthritis.

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