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Involvement of CD4$^+$ T Lymphocytes in Induction of Severe Destructive Lyme Arthritis in Inbred LSH Hamsters

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We determined that *Borrelia burgdorferi*-specific CD4$^+$ T lymphocytes are responsible for the development of severe destructive Lyme arthritis and affect the production of borreliacidal antibody. Severe destructive Lyme arthritis was readily evoked in immunocompetent inbred LSH hamsters vaccinated with a whole-cell preparation of Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in adjuvant when challenged with *B. burgdorferi* sensu stricto isolate 297. When vaccinated hamsters were depleted of CD4$^+$ T lymphocytes by the administration of monoclonal antibody GK1.5 and challenged, they failed to develop severe destructive arthritis. Similarly, nonvaccinated hamsters with or without the depletion of CD4$^+$ T lymphocytes failed to develop severe destructive arthritis. In addition, depleting CD4$^+$ T lymphocytes impaired the development of borreliacidal antibody in vaccinated and nonvaccinated hamsters challenged with the Lyme borreliosis spirochete. These findings show that CD4$^+$ T lymphocytes are important for the recognition of arthritogenic and protective antigens of *B. burgdorferi* sensu lato isolates. Additional studies are needed to define the mechanisms responsible for the development of severe destructive Lyme arthritis and the production of borreliacidal antibody.

Recently, we showed that severe destructive Lyme arthritis developed in vaccinated hamsters after a challenge with isolates of *Borrelia burgdorferi* sensu lato and suggested a role for cell-mediated immunity (17). Specifically, severe destructive arthritis was readily evoked in immunocompetent inbred LSH hamsters vaccinated with a whole-cell preparation of Formalin-inactivated *B. burgdorferi* organisms 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 a 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, *B. garinii*, and *B. afzelii*), despite high levels of isolate (vaccine)-specific borreliacidal antibody (17).

Subsequently, we demonstrated that *B. burgdorferi*-specific T lymphocytes were responsible for the development of severe destructive Lyme arthritis. *B. burgdorferi*-specific T lymphocytes obtained from immunocompetent 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 (18). These findings demonstrate that T lymphocytes play a critical role in the development of Lyme arthritis.

In this study we provide further evidence that *B. burgdorferi*-specific CD4$^+$ T lymphocytes are directly involved in the development of severe destructive Lyme arthritis.

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MATERIALS AND METHODS

Hamsters. Six- to eight-week-old inbred LSH/Ss WSLH 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 (21, 22), were cultured in modified BSK medium (3) at 32°C to a concentration of 5×10^7 spirochetes per ml. Five-hundred-microliter samples were dispensed into 1.5-ml screw-cap tubes (Sarstedt, Newton, N.C.) containing 500 μl 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 with a Petroff-Hausser counting chamber.

Vaccine preparation. A whole-cell *B. burgdorferi* sensu stricto isolate C-1-11 vaccine was prepared as described previously (17). 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 × *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 by centrifugation and suspended in PBS. Five-hundred-microliter samples 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 (17). 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 Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in adjuvant. The protein concentration was 50 to 100 μg per inoculum, determined with the Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, Calif.). Controls consisted of nonvaccinated hamsters inoculated with 0.2 ml of PBS.

Antibody reagents. Hybridoma cell line GK1.5 (ATCC TIB-207) secreting rat monoclonal antibody (MAb) GK1.5 (isotype IgG2b) has specificity for the CD4 (L3T4a) molecule on murine helper T lymphocytes (6, 45, 49). It also recognizes

the CD4 molecule on hamster helper T lymphocytes (20) but does not recognize monocytes or macrophages. The GK1.5 hybridoma cell line was cultured in Dulbecco's modified Eagle medium (Sigma) containing 10% heat-inactivated (at 56°C for 30 min) fetal calf serum (HyClone Laboratories, Inc., Logan, Utah), 2-mercaptoethanol (5×10^{-5} M; Sigma), penicillin (100 U/ml; Sigma), and streptomycin (100 μg/ml; Sigma) at 37°C in a humidified atmosphere of 7.5% CO_2. After 10 days, GK1.5 culture supernatants were collected by centrifugation at 500 × *g*, sterilized by filtration with a 0.22-μm-pore-size filter apparatus (Nalgene Labware Division, Rochester, N.Y.), dispensed into 45-ml aliquots, and frozen at −20°C until needed. Ascites containing MAb GK1.5 were also generated in 30 BALB/c mice (Jackson Lab, Bar Harbor, Maine). Mice were primed with a single intraperitoneal injection of 0.5 ml of incomplete Freund's adjuvant (Sigma) (7, 27) for 7 days, exposed to 500 rads of a sublethal dose of whole-body gamma radiation with a Cobalt 60 irradiator (Picker Corp., Cleveland, Ohio), and injected intraperitoneally with a 4-day culture of 5×10^6 GK1.5 hybridomas. After 14 days, the peritoneal cavities of mice were tapped for ascites with a 20-ml syringe attached to a 23-gauge needle. Mice were euthanized by CO_2 inhalation. MAb GK1.5 was purified and concentrated from ascites and culture supernatants by membrane affinity chromatography by a recombinant protein G system (Amicon, Beverly, Md.). Fractions containing MAb GK1.5 were pooled, and protein concentrations were determined with the Bio-Rad protein assay kit. Pooled fractions were then frozen at −20°C until used for in vivo depletion studies. Nonspecific rat immunoglobulin (Ig) (Organon Teknika Corp., Durham, N.C.) was used as a control for the in vivo depletion studies. For flow cytometric staining of hamster CD4+ T lymphocytes, phycoerythrin (PE)-conjugated rat anti-mouse CD4 Ig (isotype IgG2a; Boehringer Mannheim Biochemicals, Indianapolis, Ind.) was used. Control antibodies included PE-conjugated rat IgG2a Ig (PharMingen, San Diego, Calif.).

In vivo depletion of CD4+ T lymphocytes. Eighteen hamsters vaccinated for 2 weeks and 18 nonvaccinated hamsters were mildly anesthetized with ether and injected intraperitoneally with 0.5 ml of PBS containing 0.5 mg of MAb GK1.5 for three consecutive days (−3, −2, and −1 before the challenge). Hamsters were then challenged (day 0) and also injected with 0.5 ml of PBS containing 0.25 mg of MAb GK1.5 every other day for 2 weeks. Controls included 18 vaccinated and 18 nonvaccinated hamsters injected intraperitoneally on the same schedule with similar concentrations of nonspecific rat Ig. In other experiments, MAb GK1.5 was administered for only three consecutive days before vaccinated and nonvaccinated hamsters were challenged.

Infection of hamsters with *B. burgdorferi*. On the day of the challenge (day 0), vaccinated and nonvaccinated hamsters with and without treatment with MAb GK1.5 or nonspecific rat Ig were mildly anesthetized with ether and challenged subcutaneously in each hind paw with 0.2 ml of BSK containing 10^6 viable *B. burgdorferi* sensu stricto isolate 297. Controls included vaccinated and nonvaccinated hamsters injected with 0.2 ml of BSK.

Preparation of hamster sera and lymphocytes. Sera and lymphocytes were obtained from vaccinated and nonvaccinated hamsters treated with MAb GK1.5 or nonspecific rat Ig 8 h before infection and at 7 and 14 days after the challenge with *B. burgdorferi* sensu stricto isolate 297. At each interval, groups containing three hamsters were anesthetized with ether and bled by intracardiac puncture. The blood was allowed to clot, and sera were separated by centrifugation at 500 × *g*, pooled, dispensed into 1.2-ml aliquots, and frozen at −20°C until used. The popliteal and inguinal lymph nodes and spleens were surgically removed, aseptically teased apart, and pressed through a sterilized stainless steel 60-mesh screen into Dulbecco's modified Eagle medium. Lymphocytes from the popliteal and inguinal lymph nodes were pooled, washed twice by centrifugation with PBS, and suspended to 10^7 cells per ml in Dulbecco's modified Eagle medium. Splenic lymphocytes were obtained similarly, except they were separated from erythrocytes and other cells by Ficoll-Hypaque (density 1.077; Sigma) gradient centrifugation.

Flow-cytometric analysis of lymphocytes from CD4-depleted hamsters. One-hundred-microliter samples containing 10^6 lymphocytes from spleens and lymph nodes of each group of hamsters were stained with PE-conjugated rat anti-mouse CD4 Ig (1:100) for 15 min at 4°C. Samples were washed twice by centrifugation with PBS, fixed with 2% paraformaldehyde (Sigma) and kept in the dark until analyzed. Control samples were stained with PE-conjugated rat IgG2a Ig (1:100). All samples were analyzed with a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, Mountain View, Calif.) with FACScan LYSYS II software for data acquisition and single-color analysis. Initially, lymphocyte populations were detected and differentiated from other cell types by forward scatter, side scatter, and PE fluorescence. Data for 5,000 to 10,000 cells were acquired. Samples were then analyzed by histogram profiles of PE fluorescence with FACScan LYSYS II software. Gates were established with sample controls for CD4+ T lymphocytes stained with PE-conjugated rat IgG2a Ig. The percentages of CD4+ T lymphocytes in each sample were determined by a percent shift in the PE fluorescence of PE-conjugated rat anti-mouse CD4 Ig-stained lymphocytes.

Assessment of arthritis. The amount of swelling of the hind paws of hamsters challenged with *B. burgdorferi* sensu stricto isolate 297 was used to evaluate the inflammatory response. The hind paws were measured every other day for 14 days with a plethysmograph (Buxco Electronics, Sharon, Conn.). Measurements were obtained by mildly anesthetizing hamsters with ether contained in a nose-and-mouth cup, carefully dipping a hind paw to the ankle into a column of mercury, and measuring the amount (in ml) 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.

TABLE 1. Percentages of CD4+ T lymphocytes in lymph nodes and spleens of vaccinated and nonvaccinated hamsters[a]

Hamster group	% CD4+ T lymphocytes in:					
	Lymph nodes at:			Spleens at:		
	8 h	Day 7	Day 14	8 h	Day 7	Day 14
CD4+ depleted						
Nonvaccinated	0.4	0.1	4.4	0.2	0.4	2.8
Vaccinated	0.5	0.2	4.1	0.1	0.2	1.9
Control						
Nonvaccinated	34.7	41.0	32.3	14.4	21.6	14.5
Vaccinated	53.0	41.2	38.5	42.7	39.3	20.6

[a] Hamsters were treated and not treated with MAb GK1.5 8 h before being challenged and at 7 and 14 days after being challenged with *B. burgdorferi* sensu stricto isolate 297. CD4+ T lymphocytes were stained with PE-conjugated rat anti-mouse CD4 Ig. Percentages were determined by flow cytometric analysis.

Preparation of tissues for histology. The hind legs of deceased hamsters were amputated 14 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.

Measuring borreliacidal antibody by flow cytometry. Sera from vaccinated and nonvaccinated hamsters with and without treatment with MAb GK1.5 or nonspecific rat Ig were heat inactivated at 56°C for 30 min, diluted 1:10 with fresh BSK, and filter sterilized through a 0.22-μm-pore-size filter apparatus (Acrodisc; Gelman Sciences, Ann Arbor, Mich.). A frozen suspension of *B. burgdorferi* sensu stricto isolate 297 was thawed, inoculated into fresh BSK, and incubated at 32°C for 72 h. Spirochetes were enumerated by dark-field microscopy and a Petroff-Hausser counting chamber and adjusted to contain 5×10^5 spirochetes per ml with BSK. One-hundred-microliter samples of the spirochetal suspension were added to round-bottom wells of a 96-well microtiter plate (GIBCO Laboratories, Grand Island, N.Y.). Subsequently, 100 μl of sera or their twofold dilutions from vaccinated and nonvaccinated hamsters with and without treatment with MAb GK1.5 or nonspecific rat Ig and 20 μl of sterile guinea pig complement (hemolytic titer, 200 50% hemolytic complement units per ml; Sigma) were added to each well of the microtiter plate. The plate was shaken gently and incubated at 32°C for 16 h. All assays were performed in duplicate. After the incubation of assay samples, 100 μl was removed and diluted 1:5 with PBS (pH 7.4) and 50 μl of acridine orange (AO, 5.4 nM; Sigma) was added (19). Controls included samples containing normal serum with viable or heat-killed (56°C for 30 min) spirochetes in BSK-containing complement. The samples were then analyzed with a FACScan flow cytometer with FACScan LYSYS II software for data acquisition. Initially, viable and heat-killed spirochetes were detected and differentiated from BSK, serum, and complement particles by forward scatter, side scatter, and AO fluorescence. Live gating was performed only on profiles of spirochetes during data acquisition to exclude all BSK, serum, and complement particles. Data were acquired for 1 min. Assay samples were then analyzed by histogram profiles of AO fluorescence with FACScan LYSYS II software. Gates were established for viable and heat-killed spirochetes on the basis of their incorporation of AO. Three parameters were evaluated: events per minute (the number of labeled spirochetes), percent shift in AO fluorescence (the number of dead spirochetes), and mean channel fluorescence (the intensity of fluorescence-labeled spirochetes). Borreliacidal activity was determined by a decrease in events per minute and increases in percent shift in AO fluorescence and mean channel fluorescence compared with values obtained with normal serum. The borreliacidal antibody titer was determined as the last dilution of immune serum that killed spirochetes compared with normal serum (19).

Statistics. All measurements and values obtained were compared and tested by the analysis of variance. The Fisher least-significant-difference test (42) was used to examine pairs of means when a significant *F* ratio indicated reliable mean differences. The alpha level was set at 0.05 before the experiments were started.

RESULTS

In vivo depletion of CD4+ T lymphocytes with MAb GK1.5. When vaccinated and nonvaccinated hamsters were treated with MAb GK1.5, significant decreases ($P < 0.001$) in levels of CD4+ T lymphocytes were detected at all intervals in the

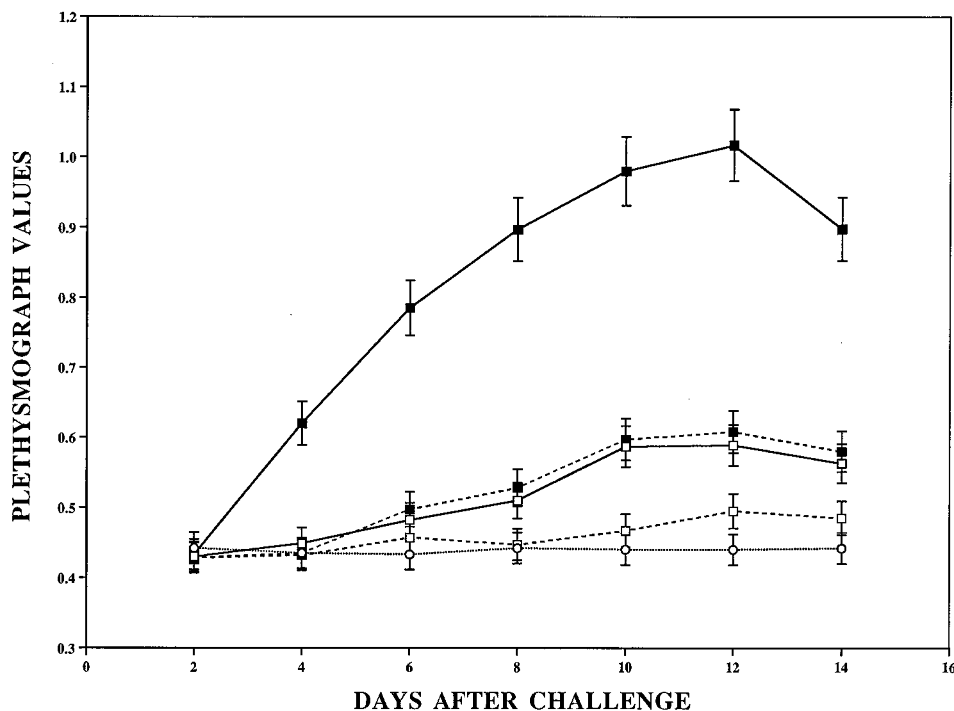

FIG. 1. Development of hind paw swelling in vaccinated (filled squares) and nonvaccinated hamsters (open squares) with (– – – –) and without (▬▬) depletion of CD4$^+$ T lymphocytes. Hamsters were challenged with *B. burgdorferi* sensu stricto isolate 297 14 days after vaccination. Controls included nonvaccinated hamsters injected with BSK (○). The mean plethysmograph value for three hamsters per group (six hind paws) at each interval is expressed as the mean ± the standard error.

lymph nodes and spleens compared with vaccinated and nonvaccinated hamsters treated with nonspecific rat Ig (Table 1). When these experiments were repeated, similar results were obtained.

Depletion of CD4$^+$ T lymphocytes abrogates the development of severe destructive Lyme arthritis. When hamsters vaccinated for 14 days were challenged with *B. burgdorferi* sensu stricto isolate 297, severe swelling of the hind paws developed (Fig. 1 and 2C). Swelling was detected 4 days after the challenge and peaked at day 12 (at a plethysmograph value of 1.02 ± 0.02) before decreasing (Fig. 1). By contrast, vaccinated hamsters depleted of CD4$^+$ T lymphocytes and challenged with *B. burgdorferi* sensu stricto isolate 297 developed only mild swelling of the hind paws (Fig. 1 and 2B). The severity and duration of swelling paralleled the swelling detected in the hind paws of nonvaccinated hamsters challenged with *B. burgdorferi* sensu stricto isolate 297. In addition, nonvaccinated hamsters depleted of CD4$^+$ T lymphocytes developed less swelling of the hind paws after being challenged with *B. burgdorferi* sensu stricto isolate 297 than nonvaccinated hamsters with intact CD4$^+$ T lymphocytes (Fig. 1). Nonvaccinated hamsters injected with BSK failed to develop any swelling of the hind paws (Fig. 1 and 2A). As additional controls, vaccinated and nonvaccinated hamsters with or without depletion of CD4$^+$ T lymphocytes were injected with BSK. They also failed to develop swelling of the hind paws.

In other experiments, vaccinated hamsters were treated with MAb GK1.5 only before the challenge (days −3, −2, and −1) with *B. burgdorferi* sensu stricto isolate 297. Flow cytometric analysis showed that CD4$^+$ T lymphocytes were not detected in lymphoid organs (≤3%). However, the percentage of CD4$^+$ T lymphocytes increased fourfold by day 10 after the challenge. These hamsters developed severe destructive arthritis by day 35 after the challenge.

Histopathology of the hind paws. Fourteen days after the challenge with *B. burgdorferi* sensu stricto isolate 297, an erosive and destructive arthritis was detected in the hind paws of vaccinated hamsters (Fig. 3C). These results confirmed our previous findings (17). The histopathology and severity of the arthropathy showed that the synovia of the tibiotarsal and intertarsal joints displayed chronic hypertrophy and hyperplasia characterized by bridging of villi mixed with fibrin (pannus tissue formation), erosion of the articular cartilage, and focal destruction of underlying bone. A cellular infiltrate of neutrophils, macrophages, mast cells, lymphocytes, and plasma cells was also present in the subsynovial and periarticular 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 vaccinated hamsters depleted of CD4$^+$ T lymphocytes and infected with *B. burgdorferi* sensu stricto isolate 297 was less severe (Fig. 3E). A cellular inflammatory infiltrate was present, but the joint spaces were free of significant alterations, showing a lack of pannus tissue formation and an absence of erosive and destructive arthropathy. Similar histopathological findings of synovitis without erosive and destructive arthropathy were demonstrated in nonvaccinated hamsters with or without the depletion of CD4$^+$ T lymphocytes and challenged with *B. burgdorferi* sensu stricto isolate 297 (Fig. 3B and 3D). Nonvaccinated hamsters injected with BSK failed to develop any significant histopathological changes (Fig. 3A). Similarly, vaccinated and nonvaccinated hamsters depleted of CD4$^+$ T lymphocytes failed to develop any significant histopathological changes when injected with BSK.

Effects of depletion of CD4$^+$ T lymphocytes on the development of borreliacidal antibody. When nonvaccinated immunocompetent hamsters were challenged with *B. burgdorferi* sensu stricto isolate 297, borreliacidal antibody developed (Table 2).

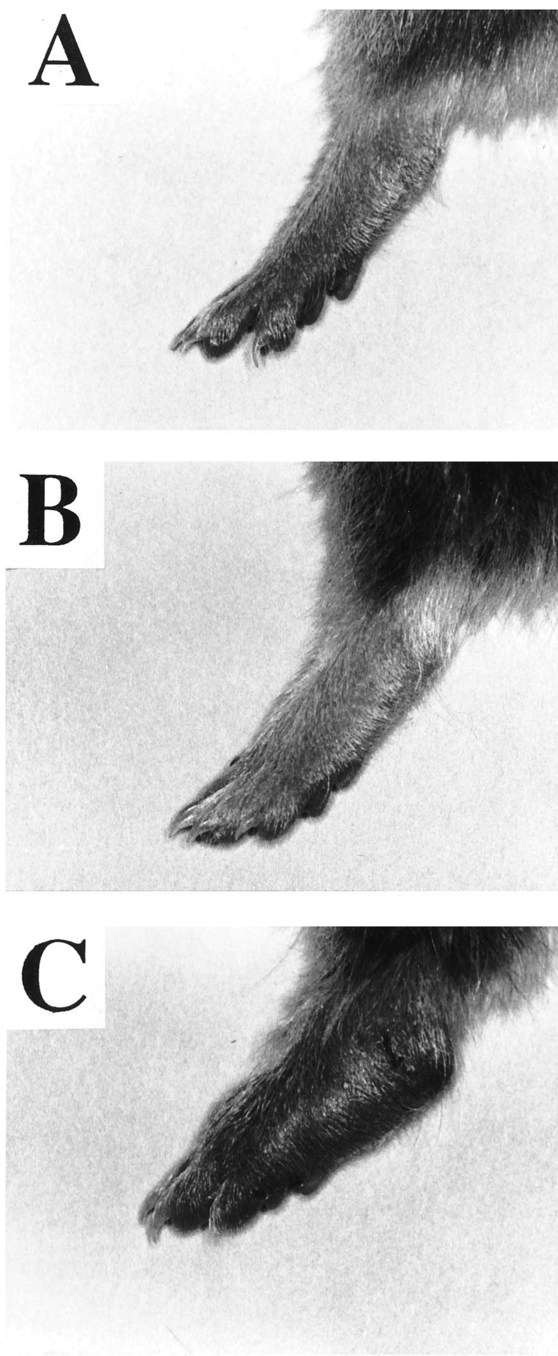

FIG. 2. Appearance of the left hind paws of vaccinated hamsters with (B) and without (C) depletion of CD4$^+$ T lymphocytes 12 days after the challenge with *B. burgdorferi* sensu stricto isolate 297. Nonvaccinated hamsters with and without the depletion of CD4$^+$ T lymphocytes and challenged with *B. burgdorferi* sensu stricto isolate 297 showed swelling similar to that of vaccinated hamsters depleted of CD4$^+$ T lymphocytes (B). Nonvaccinated noninfected hamsters injected with BSK failed to develop any swelling (A).

Borreliacidal antibody increased 16-fold by day 14 of the challenge. By contrast, borreliacidal antibody failed to develop in nonvaccinated hamsters depleted of CD4$^+$ T lymphocytes and challenged with *B. burgdorferi* sensu stricto isolate 297. Although borreliacidal antibody developed in vaccinated hamsters depleted of CD4$^+$ T lymphocytes, the borreliacidal antibody titers were two- to fourfold lower at 7 and 14 days after the challenge than levels of borreliacidal antibody detected in vaccinated hamsters not depleted of CD4$^+$ T lymphocytes.

DISCUSSION

There is mounting evidence that T lymphocytes participate in the development of Lyme arthritis. CD4$^+$ T lymphocytes are the dominant T lymphocytes isolated from the synovium of Lyme arthritis patients (43). T lymphocytes also respond vigorously to stimulation with *B. burgdorferi* antigens in vitro (14, 28, 33, 52). In addition, specific human CD4$^+$ T-lymphocyte clones established from patients with chronic Lyme arthritis have been shown to recognize *B. burgdorferi* outer surface proteins (Osp) A and B, the flagellum, and heat shock proteins 60 and 70 (15, 40, 41, 53, 54). Others have suggested that the antibody response to OspA is a marker for a critical T-lymphocyte response that prolongs Lyme arthritis (10, 11). Collectively, these investigations suggest that T lymphocytes play an important role in the induction, development, and maintenance of Lyme arthritis.

These approaches, however, have fallen short of proving that T lymphocytes are involved in the pathogenesis of Lyme arthritis. The results of this investigation, however, have provided direct evidence that CD4$^+$ T lymphocytes are involved in the development of severe destructive Lyme arthritis. When hamsters vaccinated with a whole-cell preparation of Formalin-inactivated *B. burgdorferi* organisms in adjuvant were depleted of CD4$^+$ T lymphocytes and challenged, they failed to develop severe destructive arthritis. This was supported by histopathologic examination. These results also confirmed our previous finding that nonfractionated T lymphocytes obtained from vaccinated hamsters can confer on naive recipient hamsters the ability to develop severe destructive arthritis when challenged with isolates of *B. burgdorferi* sensu lato (18). This study extends these findings by showing that a specific subpopulation of T lymphocytes, particularly CD4$^+$ T lymphocytes, is involved in the development of severe destructive Lyme arthritis. MAb GK1.5 (anti-L3T4) used to deplete hamsters of CD4$^+$ T lymphocytes has been shown previously to recognize hamster helper T lymphocytes (20).

A direct role for CD4$^+$ T lymphocytes in the development of severe destructive Lyme arthritis is not surprising, since CD4$^+$ T lymphocytes have been shown to be involved in the development of rheumatoid arthritis in humans (9, 30, 46, 47) and the induction of type II collagen arthritis in DBA mice (29). The treatment of humans with anti-CD4 antibody and the depletion of CD4$^+$ T lymphocytes in DBA mice have also been shown to inhibit arthritis. What is surprising is the extent of involvement of CD4$^+$ T lymphocytes in the development of severe destructive Lyme arthritis. The treatment of vaccinated hamsters with MAb GK1.5 completely abrogated the ability of vaccinated hamsters to develop clinical and histopathological manifestations of severe destructive Lyme arthritis when challenged with *B. burgdorferi* organisms; however, hamsters still developed synovitis. If the repopulation of vaccinated hamsters with CD4$^+$ T lymphocytes was allowed by decreasing the number of anti-CD4 treatments, severe destructive arthritis was readily detected.

The precise mechanism(s) by which *B. burgdorferi*-specific CD4$^+$ T lymphocytes are involved in the development of severe destructive Lyme arthritis is unknown. We showed that nonvaccinated hamsters (Fig. 2B and 3B) or nonvaccinated hamsters infused with normal T lymphocytes (18) challenged with the Lyme borreliosis spirochete develop only synovitis. By contrast, vaccinated hamsters (Fig. 2C and 3C) or naive ham-

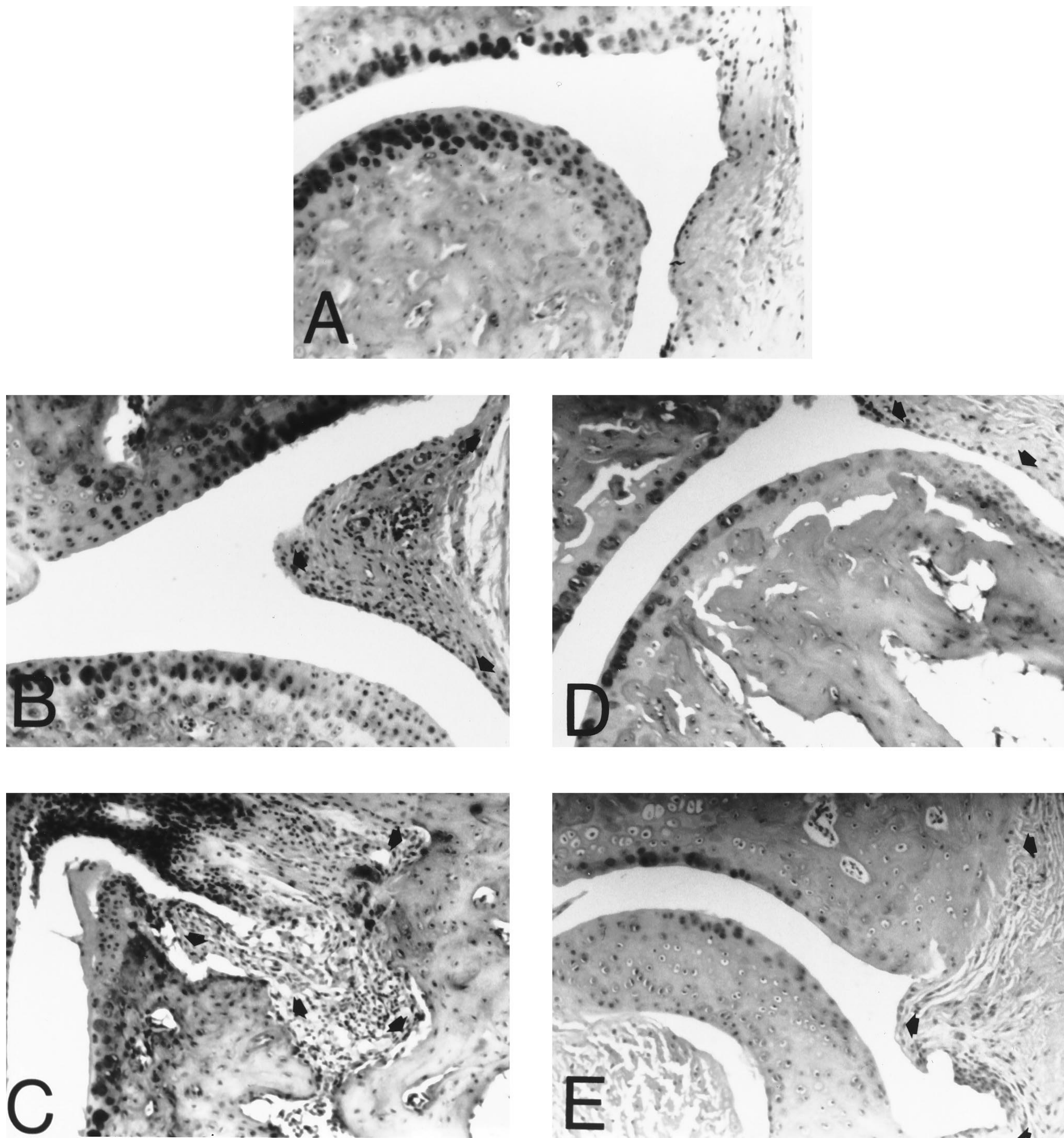

FIG. 3. Histopathology of the hind paws of vaccinated (E) and nonvaccinated (D) hamsters depleted of CD4$^+$ T lymphocytes 14 days after the challenge with *B. burgdorferi* sensu stricto isolate 297. Controls included vaccinated (C) and nonvaccinated (B) hamsters infected with *B. burgdorferi* sensu stricto isolate 297 and nonvaccinated noninfected hamsters (A) injected with BSK. Arrowheads demonstrate areas of infiltration of inflammatory cells into the synovium.

sters infused with *B. burgdorferi*-specific T lymphocytes obtained from vaccinated hamsters and challenged with isolates of *B. burgdorferi* sensu lato (18) develop severe destructive arthritis. This demonstrates that T lymphocytes primed by vaccination with inactivated *B. burgdorferi* organisms in alum, especially CD4$^+$ T lymphocytes, are necessary for the development of severe destructive arthritis. Our results suggest that the accumulation of primed CD4$^+$ T lymphocytes within the tissues of the hind paws of hamsters and the interaction with viable *B. burgdorferi* organisms results in the development of severe destructive arthritis. If primed CD4$^+$ T lymphocytes are not present and hamsters are challenged with viable *B. burgdorferi* organisms (17, 18), severe destructive arthritis does not develop and only synovitis is detected. Preliminary histopathological studies suggest the development of synovitis is monocyte/macrophage mediated and the development of severe de-

TABLE 2. Determination of borreliacidal antibody titers in vaccinated and nonvaccinated hamsters with or without the depletion of CD4^{+} T lymphocytes

Hamster group	Borreliacidal antibody titer at:[a]		
	8 h	Day 7	Day 14
CD4^{+} depleted			
Nonvaccinated	<20	<20	<20
Vaccinated	80[b]	320	320
Control			
Nonvaccinated	<20	160	320
Vaccinated	20	640	1,280

[a] Borreliacidal antibody titers were determined with sera obtained 8 h before infection and 7 and 14 days after the challenge with *B. burgdorferi* sensu stricto isolate 297.

[b] The borreliacidal antibody titer was 1:20 prior to the administration of MAb GK1.5.

structive arthritis requires both monocyte/macrophage and primed CD4^{+} T-lymphocyte populations. Additional experiments are needed to define the arthritogenic components of viable *B. burgdorferi* sensu lato, the contribution of CD4^{+} T-lymphocyte subpopulations, the involvement of cytokines, and the role of antigen-presenting cells. These experiments are needed to define the mechanisms that control and cause the continuous exacerbation of severe destructive Lyme arthritis in hamsters.

Recently, Keane-Myers and Nickell (12) showed that depletion of subsets of T lymphocytes altered the pathogenesis of *B. burgdorferi* infection in C3H/HeN and BALB/c mice. When CD4^{+} T lymphocytes were depleted in these mice, the severity of arthritis was enhanced, suggesting that CD8^{+} T lymphocytes may be responsible for the exacerbation of the arthritis. By contrast, the depletion of CD8^{+} T lymphocytes enhanced the resistance of mice to infection and prevented arthritis, suggesting that CD4^{+} T lymphocytes control the development of arthritis. These results conflict with those presented in this study. We showed that the depletion of CD4^{+} T lymphocytes in vaccinated hamsters challenged with *B. burgdorferi* sensu stricto isolate 297 prevented the development of severe destructive arthritis. An explanation may be that CD4^{+} T lymphocytes are the major T-lymphocyte population activated after vaccination with inactivated whole spirochetes in adjuvant in hamsters. The elimination of this cell population prevented the induction of severe destructive arthritis. However, when nonvaccinated hamsters were depleted of CD4^{+} T lymphocytes and infected with *B. burgdorferi* sensu stricto isolate 297, they developed only synovitis. This suggests that other cells, perhaps CD8^{+} T lymphocytes, are responsible for the development of synovitis in nonvaccinated hamsters. Collectively, these findings suggest that CD4^{+} T lymphocytes play a major role in controlling or preventing the induction of events leading to the development of synovitis in nonvaccinated hamsters and severe destructive arthritis in vaccinated hamsters.

Depleting CD4^{+} T lymphocytes also impaired the development of borreliacidal antibody. When nonvaccinated hamsters were depleted of CD4^{+} T lymphocytes, they failed to develop borreliacidal antibody after the challenge. By contrast, nonvaccinated hamsters with intact CD4^{+} T lymphocytes developed a 16-fold increase in levels of borreliacidal antibody. These results suggest that the development of borreliacidal antibody is CD4^{+} T lymphocyte dependent. Other investigators have also suggested that T lymphocytes are involved in the generation of protective antibody responses against *B. burgdorferi* sensu lato (32). Since borreliacidal antibody is necessary for the development of protection against infection with *B. burgdorferi* sensu lato (21, 22, 34–36), the roles played by CD4^{+} T lymphocytes or their subpopulations in promoting the recognition of protective antigens and maintaining sustained levels of protective antibody need to be defined for the development of an effective vaccine.

We also found that the production of borreliacidal antibody occurred independently of CD4^{+} T-lymphocyte support. When vaccinated hamsters were depleted of CD4^{+} T lymphocytes, two unexpected findings were made. A fourfold higher borreliacidal antibody titer was detected (day 0) in vaccinated hamsters following treatment with MAb GK1.5 than was found in nontreated vaccinated hamsters. In addition, the borreliacidal antibody titers increased after the challenge, although they were two- to fourfold lower than those detected in vaccinated hamsters with intact CD4^{+} T lymphocytes. The initial elevated borreliacidal antibody response detected in vaccinated hamsters depleted of CD4^{+} T lymphocytes may be due to polyclonal activation of *B. burgdorferi*-specific B lymphocytes. The lysis of CD4^{+} T lymphocytes by MAb GK1.5 and complement may have released T-lymphocyte factors that caused polyclonal proliferation or the activation of B lymphocytes with the production of borreliacidal antibody. In support of this explanation, supernatants and cytokines from CD4^{+} T lymphocytes have been shown to induce polyclonal proliferation of B lymphocytes with the production of antibody (2, 8, 15, 16). Once vaccinated, hamsters depleted of CD4^{+} T lymphocytes were challenged and *B. burgdorferi* antigens could also act as mitogens (5, 23, 37, 48, 51) and cause *B. burgdorferi*-specific B lymphocytes to produce borreliacidal antibody independently of CD4^{+} T lymphocytes.

Our results demonstrate that *B. burgdorferi*-specific CD4^{+} T lymphocytes primed by vaccination with a whole-cell preparation of Formalin-inactivated *B. burgdorferi* spirochetes in adjuvant mediate the development of severe destructive Lyme arthritis and influence the production of borreliacidal antibody. In mice and humans, CD4^{+} T lymphocytes can be divided into two distinct subsets, Th1 and Th2, by different effector functions and cytokine profiles (1, 25, 26, 31, 38, 39, 44, 50). Th1 lymphocytes participate in delayed-type hypersensitivity, macrophage activation, and antibody-dependent cell cytotoxicity and characteristically produce interleukin-2 and gamma interferon. Th2 lymphocytes participate in aiding B lymphocytes in antibody production and characteristically produce interleukin-4, -5, and -10. Although cytokine profiles of *B. burgdorferi*-specific CD4^{+} T-lymphocyte subpopulations were not performed, we suspect that the development of severe destructive Lyme arthritis is caused by Th1-like lymphocytes. In support, *B. burgdorferi*-specific human CD4^{+} T-lymphocyte clones from chronic Lyme arthritis patients (54) and *B. burgdorferi*-stimulated lymphocytes from disease-susceptible C3H/HeJ mice (13, 24) displayed Th1 cytokine profiles. We also suspect that CD4^{+} T lymphocytes involved in the production of borreliacidal antibody may be of the Th2-like lymphocyte lineage. Experiments are in progress to define the subpopulations of CD4^{+} T lymphocytes responsible for the development of severe destructive Lyme arthritis and borreliacidal antibody.

In conclusion, we demonstrated that *B. burgdorferi*-specific CD4^{+} T lymphocytes mediate the development of severe destructive Lyme arthritis and influence the production of borreliacidal antibody. Understanding the role of T lymphocytes in the pathogenesis of Lyme arthritis will promote the development of an efficacious and safe vaccine. The vaccine must exclude *B. burgdorferi* antigens containing T-lymphocyte epitopes that are involved in the development of severe destruc-

tive Lyme arthritis and include *B. burgdorferi* antigens that augment the production of protective borreliacidal antibody.

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