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Macrophages and Enriched Populations of T Lymphocytes Interact Synergistically for the Induction of Severe, Destructive Lyme Arthritis

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Hamsters receiving both macrophages exposed to Formalin-inactivated *Borrelia burgdorferi* (Mϕ-FBb) and enriched populations of either immune or naive T lymphocytes developed severe swelling of the hind paws when infected with *B. burgdorferi*. Swelling was detected 6 days after infection, peaked on day 10, and gradually decreased. Swelling was also observed in the hind paws of hamsters infused with only Mϕ-FBb or only enriched populations of either immune or naive T lymphocytes after infection with *B. burgdorferi*. However, the swelling detected in these hamsters was less severe and of shorter duration. In addition, hamsters receiving both macrophages not exposed to Formalin-inactivated *B. burgdorferi* (Mϕ-NFBb) and enriched populations of either immune or naive T lymphocytes failed to develop severe swelling after infection with *B. burgdorferi*. No swelling was also observed in hamsters infused with both Mϕ-FBb and enriched populations of immune T lymphocytes and then inoculated with spirochetal growth medium. We further showed that macrophages and enriched populations of T lymphocytes did not interact synergistically for controlling *B. burgdorferi* infection, as spirochetes were readily recovered from the tissues of all cell transfer recipients infected with *B. burgdorferi*. These findings demonstrate that hamsters infused with both Mϕ-FBb and enriched populations of either immune or naive T lymphocytes develop a more fulminate arthritis after infection with *B. burgdorferi* than recipients infused with either cell type alone. These findings suggest that macrophages and T lymphocytes interact synergistically for the induction of severe, destructive Lyme arthritis.

Lyme borreliosis is the most frequently reported tick-borne illness in the United States (42), with over 13,000 cases reported in 1996 (7). Classically, Lyme borreliosis is characterized by an expanding skin lesion (erythema migrans) accompanied by constitutional symptoms that include fatigue, headache, mild stiff neck, bone and muscle aches, and fever (43, 46). Other clinical manifestations include mild heart conduction system blockage, neurologic abnormalities, and polyarthropathies (26, 31, 34). Arthritis is frequently the first recognized complication of Lyme borreliosis in the United States (46), with intermittent episodes developing in approximately 60% of afflicted individuals (46). In severe cases, chronic inflammatory Lyme arthritis can lead to cartilage and bone erosion (45, 46).

We showed that severe, destructive Lyme arthritis (SDLA) could be induced in vaccinated hamsters after infection with isolates of *Borrelia burgdorferi* sensu lato (19). An erosive and destructive arthropathy was readily detected in the joints of the hind paws of vaccinated hamsters after infection with *B. burgdorferi*. Recently, we showed that macrophages play a major effector role in the pathogenesis of SDLA (11). SDLA was induced in recipients of macrophages obtained from *B. burgdorferi*-vaccinated or nonvaccinated hamsters exposed in vitro to Formalin-inactivated *B. burgdorferi* and then infected with viable *B. burgdorferi*. Clinical and histopathologic manifestations of SDLA occurred 8 days after infection and persisted for months. In addition, Lim et al. (20) showed that *B. burgdorferi*-specific T lymphocytes could induce SDLA. Approximately 8 days were required before manifestations of SDLA developed in recipients of *B. burgdorferi*-specific T lymphocytes. Collectively, these findings suggest that transferred macrophages may promote the recruitment and accumulation of endogenous T lymphocytes into the tissues of the hind paws of recipient hamsters. Cooperation between macrophages and T lymphocytes may be required before SDLA develops.

In this study, we demonstrated that macrophages and enriched T-lymphocyte preparations interacted synergistically for the induction of SDLA. Hamsters infused with both macrophages exposed to Formalin-inactivated *B. burgdorferi* (Mϕ-FBb) and enriched preparations of either immune or naive T lymphocytes developed SDLA after infection with *B. burgdorferi* more rapidly than did recipients infused with either cell type alone.

MATERIALS AND METHODS

Hamsters. Six- to 13-week-old inbred LSH hamsters were obtained from our breeding colony located at the Wisconsin State Laboratory of Hygiene. Hamsters weighing 100 to 140 g were housed three per cage at an ambient temperature of 21°C. Food and water were provided ad libitum.

Organisms. Low-passage (<5) virulent *B. burgdorferi* sensu stricto isolates 297 and C-1-11 were cultured once in modified Barbour-Stoenner-Kelly (BSK) medium (5) at 32°C to a concentration of 5×10^7 spirochetes per ml. Five-hundred-microliter samples were then dispensed into 1.5-ml screw-cap tubes (Sarstedt, Newton, N.C.) containing 500 μl of BSK supplemented with 20% glycerol (Sigma, St. Louis, Mo.), and the tubes were sealed and stored at −70°C. When needed, a frozen suspension of spirochetes was thawed and an aliquot was used to inoculate fresh BSK. Spirochetes were enumerated by dark-field microscopy with a Petroff-Hausser counting chamber.

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Vaccine preparation. *B. burgdorferi* sensu stricto isolate C-1-11 was grown in 3 liters of BSK to 5×10^7 spirochetes per ml. Spirochetes were pelleted by centrifugation (15,000 × *g*, 20°C, 15 min) and washed three times with phosphate-buffered saline (PBS; pH 7.4). The washed pellet of spirochetes was resuspended in 1% Formalin and incubated at 32°C for 30 min with periodic mixing. The Formalin-inactivated spirochetes were then washed three times by centrifugation (18,000 × *g*, 10°C, 20 min) and resuspended in PBS. Five-hundred-microliter samples containing 5×10^9 spirochetes were dispensed into 1.5-ml screw-cap tubes and stored at −70°C.

Vaccination of hamsters. Frozen samples of Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 were thawed and suspended in 10 ml of a 1% suspension of aluminum hydroxide (Imject alum; Pierce, Rockford, Ill.). Sixty hamsters were mildly anesthetized with ether contained in a nose-and-mouth cup and were vaccinated intramuscularly in each hind leg with 0.2 ml of a solution containing 75 to 100 μg of borrelial protein in alum.

Recovery of macrophages. Hamsters were mildly anesthetized with ether contained in a nose-and-mouth cup and injected intraperitoneally with 5 ml of 3% aged thioglycolate (Sigma) in PBS. Four days after injection, hamsters were euthanized by inhalation of CO_2, and 20 ml of cold Hanks balanced salt solution (Sigma) was injected intraperitoneally. The peritoneal cavity was massaged for 1 min, and the exudate cells were recovered by aspiration with a syringe. The suspension of peritoneal exudate cells was centrifuged at 500 × *g* for 10 min at 4°C. The supernatants were then decanted, and the cells were resuspended in a solution containing Dulbecco's modified Eagle's medium (Sigma) supplemented with 10% heat-inactivated (56°C for 30 min) fetal calf serum (HyClone Laboratories, Inc., Logan, Utah), 5×10^{-5} M 2-mercaptoethanol (Sigma) without antimicrobial agents or with 100 U of penicillin (Sigma) per ml, and 100 μg of streptomycin (Sigma) per ml. Subsequently, the cell suspensions were poured over polystyrene tissue culture dishes (100 by 20 mm; Corning Glass Works, Corning, N.Y.) and incubated at 37°C in an atmosphere of 7.5% CO_2 for 4 h. After incubation, the tissue culture dishes were rinsed twice with 12-ml portions of warm Hanks balanced salt solution to remove nonadherent cells. Giemsa-stained smears of isolated cells showed a homogeneous population of macrophages, with no other types of leukocytes visible. Furthermore, we showed previously by flow-cytometric analysis that macrophage preparations obtained by panning were free of lymphocyte contamination (11).

Incubation of macrophages with *B. burgdorferi*. Macrophages were exposed to either viable or nonviable *B. burgdorferi* sensu stricto isolate 297. A frozen suspension of Formalin-inactivated or heat-killed *B. burgdorferi* was thawed and mixed with PBS to a concentration of 10^8 spirochetes per ml. In addition, a suspension containing viable *B. burgdorferi* sensu stricto isolate 297 (10^8 per ml) was prepared from a 6- to 9-day-old culture. The culture was centrifuged at 18,000 × *g* for 7 min, and the pellet was resuspended in Dulbecco's modified Eagle's medium without antimicrobial agents. Subsequently, the spirochete suspensions (1 ml) were added to 9 ml of Dulbecco's modified Eagle's medium and poured onto tissue culture dishes containing 10^6 macrophages. The cultures were then incubated for 4 h at 37°C in an atmosphere of 7.5% CO_2. After incubation, tissue culture dishes were washed twice with 12-ml portions of warm Hanks balanced salt solution to remove remaining nonadherent cells and spirochetes. Five milliliters of cold, nonenzymatic cell lifter (Sigma) was added to each tissue culture dish, and the cultures were incubated at 4°C for 30 min. Macrophages exposed to *B. burgdorferi* were removed by vigorously tapping and gently scraping the inside of the tissue culture dishes with a sterile rubber policeman. Macrophage suspensions from several tissue culture dishes were aspirated, pooled, and centrifuged at 500 × *g* for 10 min at 4°C. After centrifugation, the supernatant was decanted and the pellet was resuspended in cold PBS. Macrophage viability was determined by trypan blue (Sigma) exclusion. Controls included macrophages not exposed to *B. burgdorferi.*

Antibody reagents. Hybridoma cell line 14-4-4s (ATCC HB-32) secreting murine monoclonal antibodies (MAb) recognizes a surface cell marker on hamster B lymphocytes (21, 22, 51, 52). Hybridoma 14-4-4s was grown in Dulbecco's modified Eagle's medium at 37°C in a humidified atmosphere of 7.5% CO_2. After 7 days, the culture supernatant was collected after centrifugation at 500 × *g* for 10 min at 4°C, dispensed into 12-ml aliquots, and frozen at −20°C until used. Unconjugated goat anti-mouse immunoglobulin, heavy and light chain specific (Organon Teknika Corp., Durham, N.C.), was used to coat tissue culture dishes for panning and isolation of T lymphocytes. In the flow-cytometric analysis of T-lymphocyte preparations, a phycoerythrin-conjugated goat anti-hamster immunoglobulin, specific for both heavy and light chains (Boehringer Mannheim Biochemicals, Indianapolis, Ind.), was used for the detection of B lymphocytes. $CD4^+$ T lymphocytes were detected with a phycoerythrin-conjugated rat anti-mouse CD4 (L3T4) antibody (Boehringer Mannheim Biochemicals). This antibody has specificity for the CD4 (L3T4) molecule on murine and hamster T lymphocytes. Phycoerythrin-conjugated goat and rat immunoglobulins were used as isotype controls.

Isolation of enriched populations of T lymphocytes. The isolation of enriched populations of hamster T lymphocytes by using MAb 14-4-4s has been described previously by Liu et al. (21, 22) and others (51, 52). Briefly, enriched populations of T lymphocytes were isolated from the inguinal lymph nodes of hamsters 14 days after vaccination with Formalin-inactivated *B. burgdorferi* and from those of nonvaccinated hamsters. B lymphocytes were removed from the lymph node suspensions with immunoglobulin-coated tissue culture dishes. The dishes (100 by 20 mm; Corning Glass Works) were prepared by coating the surfaces 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 four times with PBS before use. Single-cell suspensions of 10^7 immune and nonimmune lymph node cells per ml were incubated with MAb 14-4-4s in Dulbecco's modified Eagle's medium for 30 min at 4°C. During this 30-min incubation, the cell suspension was periodically mixed. The suspensions of cells were washed twice with PBS by centrifugation (500 × *g* for 10 min at 4°C), resuspended 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 by gently rinsing the tissue culture dishes with cold Dulbecco's modified Eagle's medium. Enriched T-lymphocyte suspensions obtained from several tissue culture dishes were aspirated, pooled, and centrifuged at 500 × *g* for 10 min at 4°C. After centrifugation, the supernatant was decanted and the pellet was resuspended in Dulbecco's modified Eagle's medium. The suspension was poured over another set of immunoglobulin-coated dishes and incubated for 60 min at 4°C. This process was repeated three times. After the last panning cycle, the cells were washed twice with PBS by centrifugation (500 × *g* for 10 min at 4°C) and resuspended in PBS. Cell viability was determined by trypan blue (Sigma) exclusion. Enriched T-lymphocyte preparations obtained by this method were shown to contain <5% B-lymphocyte contamination by flow-cytometric analysis (20). Giemsa-stained smears of B-lymphocyte-depleted lymph node cells obtained by this method showed a homogeneous population of lymphocytes with no other types of leukocytes visible.

Analysis of enriched T-lymphocyte preparations by flow cytometry. One-hundred-microliter samples containing 10^5 lymph node cells obtained before and after panning with MAb 14-4-4s were stained for the presence of B lymphocytes or $CD4^+$ T lymphocytes. B lymphocytes were stained with a phycoerythrin-conjugated goat anti-hamster immunoglobulin, specific for both heavy and light chains (Boehringer Mannheim Biochemicals; 1:100), for 15 min at 4°C. $CD4^+$ T lymphocytes were stained with a phycoerythrin-conjugated rat, anti-mouse CD4 (L3T4) antibody (Boehringer Mannheim Biochemicals; 1:100) for 15 min at 4°C. This antibody has specificity for the CD4 (L3T4) molecule on murine and hamster T lymphocytes. Samples were then washed twice with PBS by centrifugation, fixed with 1% paraformaldehyde (Sigma), and kept in the dark until analyzed by flow cytometry. Phycoerythrin-conjugated goat and rat antibodies were used as isotype controls. Other controls included unstained suspensions of lymph node cells. All samples were analyzed by using a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, Mountain View, Calif.) with FACScan LYSYS II software for data acquisition. Cells were detected by forward scatter, side scatter, and phycoerythrin fluorescence. Data from 5,000 cells were acquired. Cell samples were then analyzed by means of histogram profiles of phycoerythrin fluorescence evaluated with FACScan LYSYS II software. Gates were established by using unstained samples and samples stained with the isotype control antibodies. The percentages of B lymphocytes and $CD4^+$ T lymphocytes present in the cellular suspensions were determined by the percent shifts in the phycoerythrin fluorescences of the stained cells.

Cell transfer and infection of hamsters. Three hamsters per group were mildly anesthetized and injected subcutaneously in each hind paw with 0.4 ml of PBS containing 10^6 viable macrophages and/or T lymphocytes. Within 13 to 14 h after the cell transfer, recipient hamsters were mildly anesthetized and infected subcutaneously in each hind paw with 0.2 ml of BSK medium containing 10^5 viable *B. burgdorferi* sensu stricto isolate 297 spirochetes. This concentration of spirochetes can readily be detected in hamsters when their tissues are cultivated in BSK medium. The viability of the spirochetes was determined by motility and dark-field microscopy.

Assessment of arthritis. Swelling of the hind paws of hamsters was used to evaluate the inflammatory response. Prior to experimentation, hamsters were randomly chosen and their hind paws were measured before they were assigned to cages. After infusion of cells, the hind paws of recipient and control hamsters were measured periodically for 21 days with a dial type vernier caliper (Fisher Scientific, Pittsburgh, Pa.) graduated in 0.1-mm increments. Measurements were obtained by mildly anesthetizing each hamster and carefully measuring the width and thickness of each hind paw. The caliper values obtained from three hamsters (six hind paws) per group were summed and divided by six to determine the mean caliper value of the severity of swelling.

Preparation of tissues for histology. Twenty-one days after infection, hamsters were euthanized, and the hind legs were amputated at the midfemur, fixed in 10% neutral buffered zinc Formalin, placed in decalcifying solution (Lerner Laboratories, Pittsburgh, Pa.) for 30 h, and stored in 10% zinc formalin prior to processing. The knees and hind paws were bisected longitudinally, placed in embedding cassettes (Fisher Scientific), embedded in paraffin and cut into 6-μm sections, and the sections were placed on glass slides and stained with hematoxylin and eosin. The hind legs were randomly selected for histopathological examination and scored by a blinded observer.

Statistics. The mean caliper values among groups were tested by analysis of variance, with Minitab statistical analysis software. The Fisher least-significant-difference test (48) 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. The standard error of the mean was also calculated for each mean caliper value.

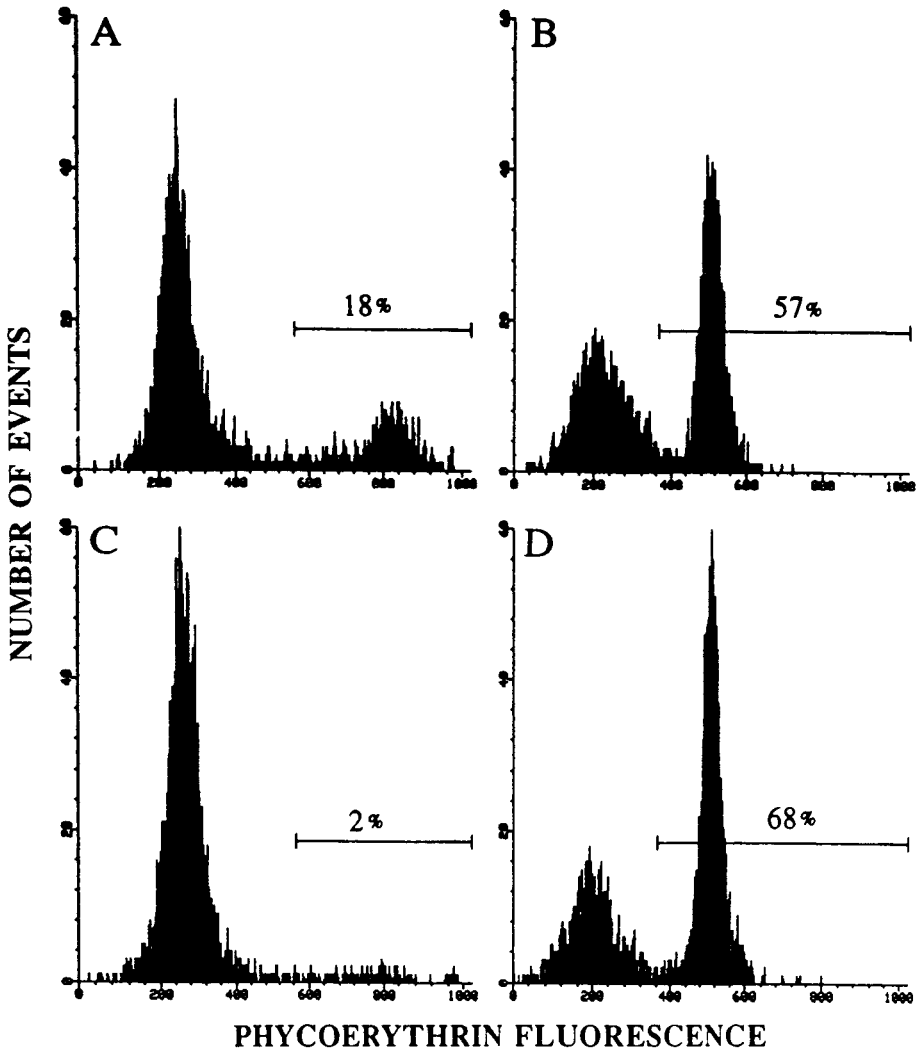

FIG. 1. Flow cytometric analysis of enriched T-lymphocyte preparations obtained from the inguinal lymph nodes of *B. burgdorferi*-vaccinated hamsters before (A and B) and after (C and D) panning with MAb 14-4-4s. Cell preparations were stained with a phycoerythrin-conjugated goat anti-hamster immunoglobulin (A and C) or a phycoerythrin-conjugated rat anti-mouse CD4 (L3T4) antibody (B and D) which has specificity for the CD4 (L3T4) molecule on murine and hamster T lymphocytes. The percentages of B lymphocytes (A and C) and $CD4^+$ T lymphocytes (B and D) present in the cell preparations are reported as the percent shifts in the phycoerythrin fluorescences of stained cells.

RESULTS

Flow-cytometric examination of enriched T-lymphocyte preparations. This study was conducted to extend our previous findings that SDLA could be induced in naive recipient hamsters infused with Mϕ-FBb and infected with *B. burgdorferi* (11). To evaluate the cellular interactions responsible for the potentiation of SDLA, we performed adoptive cell transfers with both Mϕ-FBb and enriched populations of either naive or immune T lymphocytes. Immune T lymphocytes were obtained from the inguinal lymph nodes of *B. burgdorferi*-vaccinated hamsters. Flow-cytometric analysis of enriched populations of immune T lymphocytes included in the suspensions of macrophages showed that the populations were relatively free of contamination with B lymphocytes (2%) but were enriched (68%) for $CD4^+$ T lymphocytes (Fig. 1). Similar results were obtained when T lymphocytes were isolated from the inguinal lymph nodes of naive hamsters.

Macrophages and enriched populations of T lymphocytes interact synergistically for the induction of SDLA. We determined the ability of Mϕ-FBb and macrophages not exposed to Formalin-inactivated *B. burgdorferi* (Mϕ-NFBb) obtained from vaccinated hamsters to induce SDLA synergistically when transferred to naive recipient hamsters in the presence or absence of enriched populations of either naive or immune T lymphocytes after infection with *B. burgdorferi*. Swelling of the hind paws was inordinately severe in recipients infused with both Mϕ-FBb and immune T lymphocytes (Fig. 2 and 3A). Swelling was detected 6 days after infection, peaked on day 10, and gradually decreased (Fig. 2). When recipients were infused with both Mϕ-FBb and naive T lymphocytes (Fig. 2), the onset of swelling was delayed and the severity was less than the swelling detected in recipients of both Mϕ-FBb and immune T lymphocytes ($P < 0.001$). Recipients of only Mϕ-FBb also developed severe swelling of the hind paws (Fig. 2). However, the onset of swelling was delayed and the swelling was less severe than that detected in recipients of both Mϕ-FBb and either immune or naive T lymphocytes ($P < 0.001$). The latter results affirm our previous finding (11) that Mϕ-FBb can in-

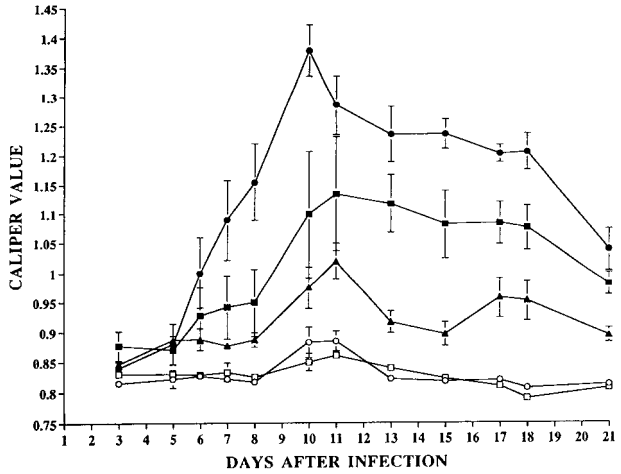

FIG. 2. Development of swelling of the hind paws of naive recipient hamsters infused with both Mϕ-FBb and enriched populations of either immune (●) or naive (■) T lymphocytes after infection with *B. burgdorferi*. Controls included recipient hamsters infused with only Mϕ-FBb (▲) and those infused with only enriched populations of either immune (○) or naive (□) T lymphocytes; all controls were then infected with viable *B. burgdorferi*. There were three hamsters per group. Data are means ± standard errors of the means.

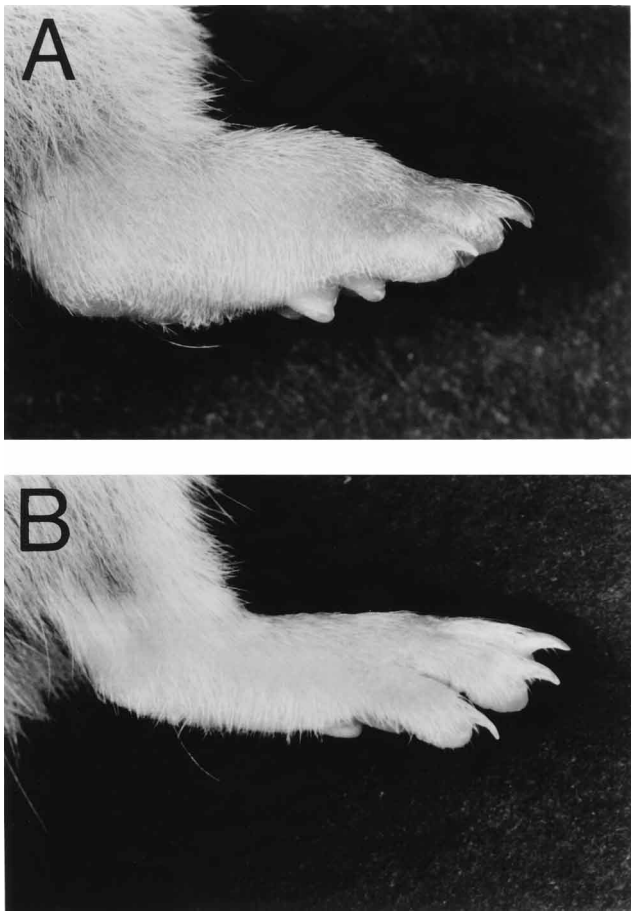

FIG. 3. Appearance of the hind paws of hamsters infused with both Mϕ-FBb and enriched populations of immune T lymphocytes. Hamsters were then infected with viable *B. burgdorferi* (A) or injected with spirochetal growth medium (B). Photographs were taken of randomly selected hamster paws 10 days after the cell transfer.

duce SDLA in recipients after infection with *B. burgdorferi*. By contrast, only slight swelling (days 10 and 11) was detected in the hind paws of recipients infused with only immune or naive T lymphocytes (Fig. 2) or in recipients infused with Mϕ-NFBb with or without immune or naive T lymphocytes (Fig. 4). Furthermore, no swelling was detected in recipients infused with both Mϕ-FBb and immune (Fig. 3B) or naive T lymphocytes (data not shown) after inoculation with spirochetal growth medium. When these studies were repeated (five times) similar results were obtained.

Macrophages exposed to other preparations of *B. burgdorferi* and enriched populations of immune T lymphocytes induced SDLA. We determined the abilities of macrophages exposed in vitro to viable, Formalin-inactivated, or heat-killed *B. burgdorferi* and immune T lymphocytes to induce SDLA in recipient hamsters after infection with *B. burgdorferi*. Severe swelling of the hind paws was detected in recipients infused with macrophages exposed to the various preparations of *B. burgdorferi* and immune T lymphocytes (Fig. 5). Swelling was detected 5 to 6 days after infection, peaked between 8 and 10 days, and gradually decreased. By contrast, hamsters infused with both Mϕ-NFBb and immune T lymphocytes developed only slight swelling (days 10 and 11) of the hind paws after infection with *B. burgdorferi* (Fig. 5).

Macrophages and enriched populations of T lymphocytes were unable to clear infection with *B. burgdorferi*. *B. burgdorferi* organisms were recovered from the bladders and spleens of recipient hamsters infused with Mϕ-FBb and Mϕ-NFBb in the presence or absence of either immune or naive T lymphocytes after infection with *B. burgdorferi* (Table 1). *B. burgdorferi* organisms were also recovered from recipient hamsters infused with only immune or naive T lymphocytes after infection with *B. burgdorferi* (Table 1).

Histopathology of hind paw swelling. Histopathologic alterations present in tissue sections of the hind paws of hamsters were graded from four to zero. Grade four described tissue sections with the most destructive and fulminate histopathologic changes. Conversely, grade zero described tissue sections with no observable histopathologic alterations. The hind paws of hamsters infused with both Mϕ-FBb and immune T lymphocytes demonstrated grade four histopathology (Fig. 6A). The papillary and reticular layers of the dermis showed a florid acute and chronic inflammation. A prominent edema was present in the soft tissues, and atrophic changes were observed in the skeletal muscle. The synovial linings of the tibiotarsal and intertarsal joints were hypertrophic, with focal vilous formation. Subsynovial edema and chronic inflammation were also present. An examination of the small bones showed them to be surrounded and focally destroyed by the inflammatory process. The hind paws of hamsters infused with both Mϕ-FBb and naive T lymphocytes demonstrated grade three histopathology. The pathologic alterations observed in these hamsters were similar to those observed in the tissue sections taken from hamsters infused with both Mϕ-FBb and immune T lymphocytes but were slightly less severe. The hind paws of hamsters receiving only Mϕ-FBb demonstrated grade two histopathology (Fig. 6C). An extensive cellular infiltrate was present in the periarticular soft tissues. The synovial linings of the tibiotarsal and intertarsal joints were hypertrophic, with subsynovial edema present. Focal bone erosion was also observed in the small bones of these hamsters but was not as severe as in hamsters infused with both Mϕ-FBb and either immune or naive T lymphocytes. These findings were similar to those previously reported (11). In contrast, hamsters infused with both Mϕ-NFBb and either immune or naive T lymphocytes

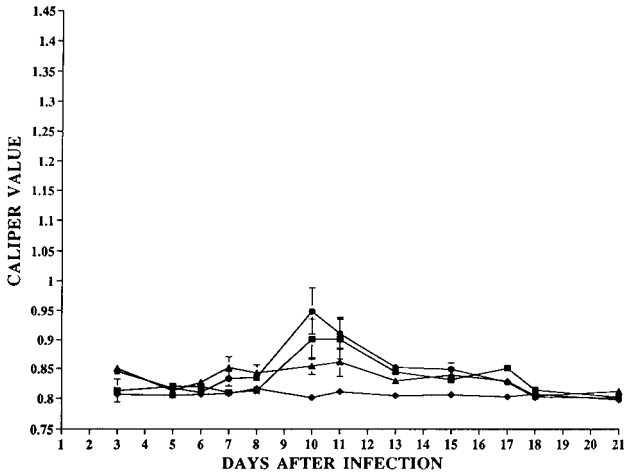

FIG. 4. Development of swelling of the hind paws of naive recipient hamsters infused with only Mϕ-NFBb (▲) or with enriched populations of either immune (●) or naive (■) T lymphocytes after infection with *B. burgdorferi*. Other hamsters were infused with both Mϕ-FBb and enriched populations of immune T lymphocytes and then injected with spirochetal growth medium (◆). There were three hamsters per group. Data are means ± standard errors of the means.

demonstrated grade one histopathology. Hamsters infused with only Mϕ-NFBb or only immune or naive T lymphocytes also demonstrated grade one histopathology (Fig. 6D). Mild focal synovial and subsynovial inflammations were present in the tibiotarsal and intertarsal joints of these animals. However, the joint spaces were free of any significant histopathologic changes. Hamsters infused with both Mϕ-FBb and immune T lymphocytes and inoculated with spirochetal growth medium demonstrated no observable histopathologic alterations in either the tibiotarsal or intertarsal joints (Fig. 6B).

DISCUSSION

Considerable effort has been focused on the delineation of the immune cells responsible for the pathogenesis of Lyme arthritis. Early studies suggested that T lymphocytes were involved. The responses of *B. burgdorferi*-specific T lymphocytes isolated from the synovial fluid or peripheral blood were elevated in individuals with Lyme arthritis (17, 30, 39, 49, 56). Further studies demonstrated that T lymphocytes from individuals with stage three Lyme arthritis responded more vigorously to stimulation with *B. burgdorferi* antigens than T lymphocytes from individuals with less progressive forms of Lyme arthritis (18, 53). Other studies (1, 9, 28, 44) have suggested that macrophages are also involved in the induction of Lyme arthritis. Elevated levels of macrophage-associated molecules such as interleukin 1 (IL-1), tumor necrosis factor, prostaglandin E_2, and collagenase have been detected in the synovial fluid extracts and serum of individuals with Lyme arthritis. IL-1 and tumor necrosis factor are known to activate osteoclastic cartilage and bone reabsorption and may be responsible for the arthritic manifestations associated with Lyme arthritis (4, 55). In addition, macrophages can be stimulated by *B. burgdorferi* to produce nitric oxide (24). Nitric oxide has been associated with the induction of other arthritic diseases and may play a role in the induction of Lyme arthritis (27, 50).

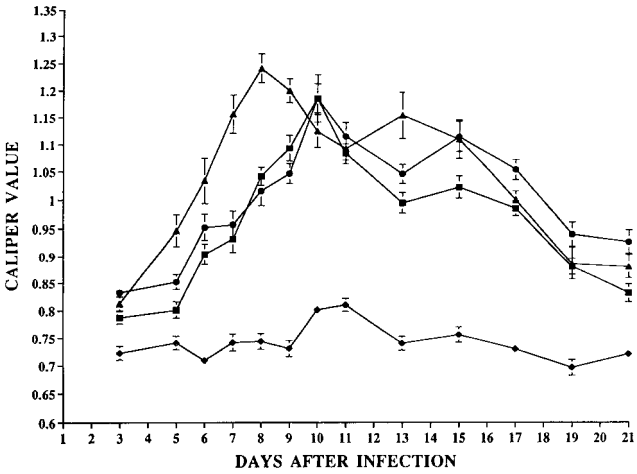

FIG. 5. Development of swelling of the hind paws of naive recipient hamsters infused with macrophages exposed in vitro to Formalin-inactivated (●), heat-killed (■), or viable (▲) *B. burgdorferi* and enriched populations of immune T lymphocytes. Other hamsters were infused with both Mϕ-NFBb and enriched populations of immune T lymphocytes (◆). Hamsters were then infected with viable *B. burgdorferi*. There were three hamsters per group. Data are means ± standard errors of the means.

TABLE 1. Isolation of *B. burgdorferi* sensu stricto isolate 297 from the tissues of recipient hamsters infused with Mϕ-FBb or Mϕ-NFBb in the presence or absence of enriched populations of either immune or naive T lymphocytes

Tissue source	No. of cultures positive[a] with indicated treatment							
	Mϕ-FBb			Mϕ-NFBb			No macrophages	
	Immune T cells	Naive T cells	No T cells	Immune T cells	Naive T cells	No T cells	Immune T cells	Naive T cells
Bladder	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Spleen	3/3	3/3	2/3	3/3	2/3	3/3	3/3	2/3

[a] Recipients were infected with the Lyme disease spirochete and sacrificed 21 days after infection. Results are presented as the number of positive cultures per the total number of hamsters for which cultures were performed. There were three hamsters per group.

These studies, however, have provided only indirect evidence for the putative cells responsible for the pathogenesis of Lyme arthritis. We showed previously that T lymphocytes (20) played a major role in the induction of SDLA. T lymphocytes obtained from hamsters vaccinated with a whole-cell preparation of inactivated *B. burgdorferi* in alum induced SDLA when naive recipient hamsters were infected with *B. burgdorferi* (20). In addition, we demonstrated (11) that macrophages obtained from either vaccinated or nonvaccinated hamsters and exposed in vitro to Formalin-inactivated *B. burgdorferi* induced SDLA in the hind paws of naive recipient hamsters when they were infected with *B. burgdorferi.*

In this study, we confirmed and extended our previous finding (11) that Mϕ-FBb without T lymphocytes could induce SDLA in naive recipients infected with *B. burgdorferi*. Swelling of the hind paws of recipient hamsters was detected 6 days after infection and peaked on days 10 to 11. However, when hamsters were infused with both Mϕ-FBb and enriched populations of naive T lymphocytes, the severity and duration of the swelling of the hind paws was further enhanced. Our results suggest that Mϕ-FBb can activate naive T lymphocytes and that this activation results in an augmentation of SDLA. In support, blood-derived dendritic cells exposed in vitro to *B. burgdorferi* have been shown to activate naive autologous T lymphocytes (13). Collectively, these results suggest that T

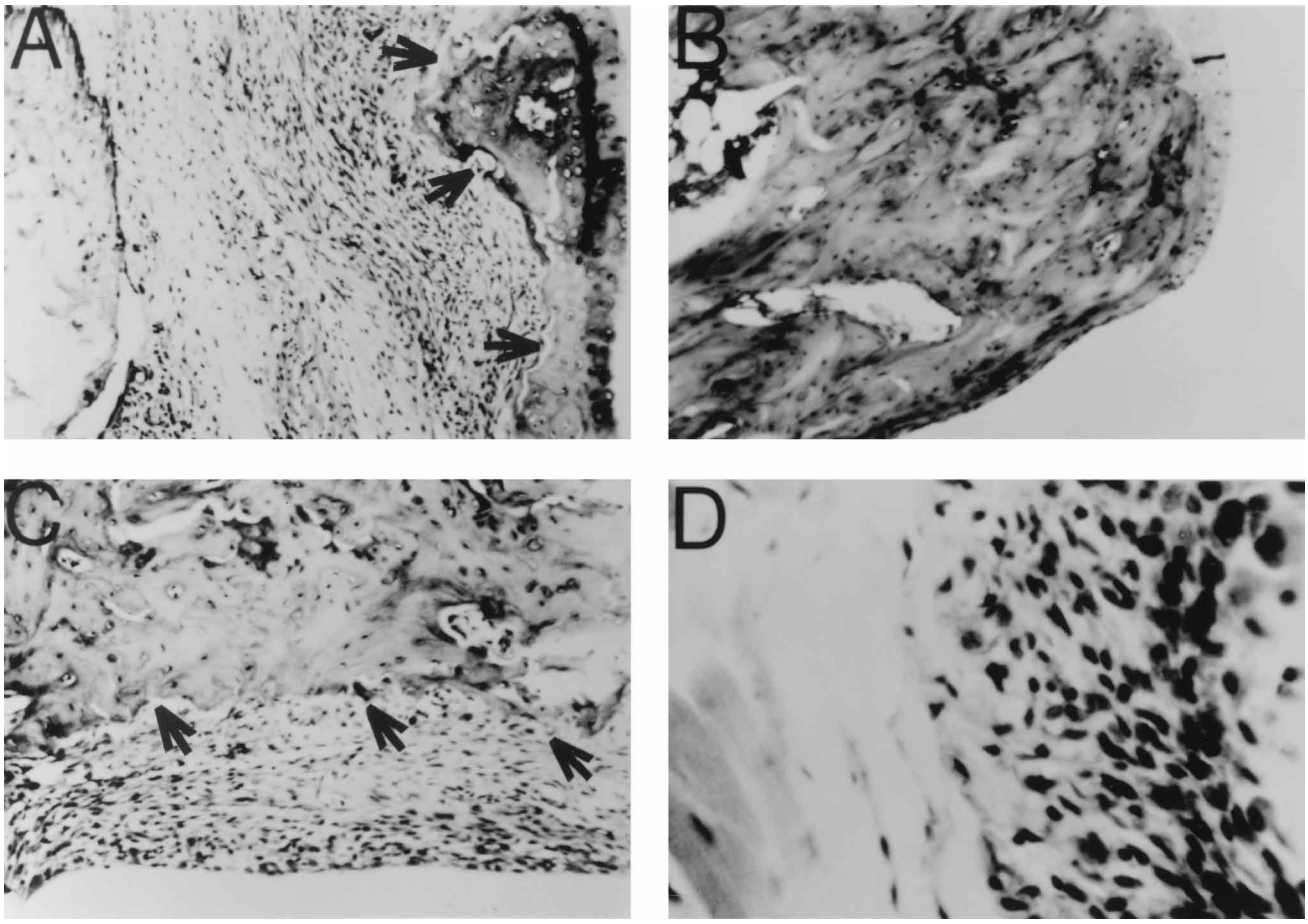

FIG. 6. Histopathology of the hind paws of naive cell transfer recipients. SDLA was detected in recipient hamsters infused with both Mϕ-FBb and enriched populations of T lymphocytes (A) or only Mϕ-FBb (C) after infection with *B. burgdorferi*. Arrows indicate areas where the inflammatory process has eroded bone and/or the synovial lining of the joint (A and C). No SDLA was detected in recipients infused with only enriched populations of T lymphocytes (D). The only remarkable histopathologic changes observed in these recipients were mild synovial and subsynovial inflammations (D). No histopathologic alterations were detected in recipients infused with both Mϕ-FBb and enriched populations of immune T lymphocytes after inoculation with spirochetal growth medium (B). The joint spaces of these recipients were clear and devoid of any inflammation. Hamsters were randomly selected for histopathologic examination and scored by a blinded observer.

lymphocytes enhance the induction of arthritis when combined with macrophages that have or are processing *B. burgdorferi.*

In this study, we also showed that macrophages and enriched populations of T lymphocytes, especially $CD4^+$ T lymphocytes, interacted synergistically for the induction of SDLA. Recipients of both Mϕ-FBb and immune T lymphocytes developed extremely severe swelling in the hind paws after infection with *B. burgdorferi*. The inordinate swelling of the hind paws was detected rapidly after infection (day 6) and gradually decreased. Swelling of the hind paws was also detected in recipients infused with both Mϕ-FBb and naive T lymphocytes or with only Mϕ-FBb after infection with *B. burgdorferi*. However, the swelling detected in these animals was less severe and significantly delayed (2 to 4 days) compared with the swelling detected in hamsters infused with both Mϕ-FBb and immune T lymphocytes. Histopathologic examination confirmed that macrophages and T lymphocytes interacted synergistically for the induction of SDLA. By contrast, no swelling was detected in hamsters receiving both Mϕ-FBb and immune T lymphocytes after inoculation with spirochetal growth medium.

It is interesting that hamsters infected with *B. burgdorferi* develop only a mild arthritis (19, 29) while hamsters infused with both Mϕ-FBb and enriched populations of T lymphocytes develop SDLA after infection with *B. burgdorferi*. Macrophages incubated in vitro are exposed to inordinate amounts of borrelial antigen for processing and presentation to T lymphocytes. This suggests that processing of borrelial antigens by macrophages and presentation to T lymphocytes may be more efficient in vitro than in vivo. Another explanation may be that delivery of macrophages and T lymphocytes directly into the hind paws of naive recipient hamsters augments the normal recruitment and accumulation of endogenous immune cells that would ordinarily induce only mild arthritis after infection with *B. burgdorferi*. In support of this explanation, we showed that recipients infused with both Mϕ-FBb and enriched populations of T lymphocytes developed severe swelling in the hind paws more rapidly after infection with *B. burgdorferi* than recipients infused with either cell type alone.

We hypothesize that the presentation of borrelial antigens by macrophages elicits the release of lymphokines by *B. burgdorferi*-specific T lymphocytes. The release of lymphokines may trigger an indirect mechanism that results in the induction of SDLA or may further activate macrophages to release compounds which directly or indirectly result in the induction of SDLA. In support of this hypothesis, hamsters receiving both Mϕ-NFBb and either immune or naive T lymphocytes failed to develop SDLA after infection with *B. burgdorferi*. These macrophages had no opportunity to process and present borrelial

antigens to T lymphocytes. The identification of modulatory cytokines elicited by hamster macrophages and T lymphocytes has been complicated by the unavailability of species-specific antibody reagents. However, other studies (16, 54, 55) support this hypothesis. It has been shown previously that individuals with progressive forms of Lyme arthritis frequently have T lymphocytes that respond vigorously to *B. burgdorferi* antigens and liberate lymphokines characteristic of the Th1 phenotype. The existence of highly polarized Th1 lymphokine patterns has also been demonstrated for mice susceptible to Lyme arthritis (15, 25). It is well established that T lymphocytes of the Th1 lineage are involved in the activation of macrophages (32). Macrophage activation may be the limiting factor for the induction of SDLA.

Several investigations have shown that macrophages phagocytize *B. burgdorferi* (2, 3, 14, 33, 37). Subsequently, it was demonstrated that viable *B. burgdorferi* organisms were preferentially internalized via coiling phagocytosis (36, 38). Studies using other microbes showed that motility was the trigger mechanism for coiling phagocytosis (8, 47). These studies suggest that macrophages may process viable and nonviable *B. burgdorferi* differently. So we exposed macrophages in vitro to viable and nonviable (Formalin-inactivated or heat-killed) *B. burgdorferi*. Hamsters receiving macrophages exposed to either viable or nonviable *B. burgdorferi* and enriched populations of immune T lymphocytes developed severe swelling in the hind paws when infected with *B. burgdorferi*. Our results suggest that macrophages process viable and nonviable (Formalin-inactivated or heat-killed) *B. burgdorferi* similarly. Furthermore, our results suggest that Formalin treatment of borrelial antigens does not constrain antigen presentation to T lymphocytes, as has been described previously (10). These findings are consistent with those obtained by Rittig et al. (36, 37). They demonstrated that viable and antibiotic-inactivated *B. burgdorferi* were similarly internalized by macrophages.

Although macrophages and enriched populations of immune T lymphocytes interacted synergistically for the induction of SDLA, they did not play a significant role in controlling *B. burgdorferi* infection. Spirochetes were readily recovered from the tissues of recipients infected with *B. burgdorferi*. We also showed previously that spirochetes could be readily recovered from the tissues of hamsters infused with only Mϕ-FBb (11) or only *B. burgdorferi*-specific T lymphocytes (20). It has been demonstrated that protection against *B. burgdorferi* infection is conferred primarily by B lymphocytes (40) and that protective antibodies against *B. burgdorferi* are generated independently of T lymphocytes (12). In support of this, we showed that borreliacidal antibodies eliminated *B. burgdorferi* from the host (41) and could kill the Lyme spirochete in vitro (23). We also showed that human serum containing borreliacidal antibodies could protect hamsters from infection with *B. burgdorferi* (6). By contrast, Rao and Frey (35) showed that protective resistance to *B. burgdorferi* could be conferred on mice with a $CD4^+$ T-lymphocyte clone. This clone was shown to produce IL-4 and hence may elicit rapid production of borreliacidal antibodies that eliminate *B. burgdorferi* from recipient mice. However, the mechanism of protection conferred upon recipients of this T-lymphocyte clone remains unelucidated. Our findings (6, 11, 20, 23, 41) and those of others (12, 40) suggest that the generation of humoral immunity, however, is more important for controlling *B. burgdorferi*. Macrophages and T lymphocytes play a major role in the development of Lyme arthritis but are not directly involved in the clearance of spirochetes from an infected host.

In conclusion, we have demonstrated that macrophages and enriched populations of T lymphocytes interacted synergistically for the induction of SDLA. Hamsters infused with both Mϕ-FBb and enriched populations of either immune or naive T lymphocytes developed SDLA after infection with *B. burgdorferi*. The arthritis detected in hamsters infused with both Mϕ-FBb and T lymphocytes was more fulminate than the arthritis detected in hamsters receiving either cell type alone. We further showed that hamsters receiving macrophages exposed to either viable or nonviable *B. burgdorferi* and enriched populations of immune T lymphocytes also developed SDLA after infection with *B. burgdorferi*. Further studies are needed to delineate which T lymphocyte subset(s) is responsible for activating macrophages to induce SDLA. These studies are important for the characterization of the cell types and immune mechanisms responsible for the induction of SDLA. These studies may facilitate the development of new therapeutic options to circumvent the development of Lyme arthritis in individuals infected with *B. burgdorferi.*

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