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Macrophages Exposed to *Borrelia burgdorferi* Induce Lyme Arthritis in Hamsters

BRIAN K. DU CHATEAU,[1,2] DOUGLAS M. ENGLAND,[3,4] STEVEN M. CALLISTER,[5] LONY C. L. LIM,[1,2,6] STEVEN D. LOVRICH,[5] AND RONALD F. SCHELL[1,2,6]*

Wisconsin State Laboratory of Hygiene[1] and Departments of Bacteriology,[2] Pathology and Laboratory Medicine,[3] and Medical Microbiology and Immunology,[6] University of Wisconsin, Madison, Wisconsin 53706; Department of Pathology, Meriter Hospital, Madison, Wisconsin 53715[4]; and Microbiology Research Laboratory, Gundersen Medical Foundation, La Crosse, Wisconsin 54601[5]

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The mechanism(s) by which Lyme arthritis is induced has not been elucidated. In this study, we showed that macrophages have a direct, effector role in the pathogenesis of Lyme arthritis. Severe destructive arthritis was induced in recipients of macrophages obtained from *Borrelia burgdorferi*-vaccinated and nonvaccinated hamsters exposed to Formalin-inactivated *B. burgdorferi* in vitro and then challenged with the Lyme spirochete. Swelling of the hind paws was detected within 8 h of infection, increased rapidly, and peaked at 21 h. This initial swelling decreased, and by day 4 only slight swelling was detected. Severe swelling of the hind paws was detected 8 days after infection and increased rapidly, with peak swelling occurring on day 11. Histopathologic examination affirmed that macrophages exposed to Formalin-inactivated spirochetes induced a severe destructive Lyme arthritis. The onset and severity of the severe destructive arthritis were dependent on the number of macrophages transferred. By contrast, macrophages not exposed to Formalin-inactivated *B. burgdorferi* failed to induce severe destructive arthritis in recipients after challenge with *B. burgdorferi*. Similarly, severe destructive arthritis was not detected in recipients of macrophages injected with spirochetal growth medium. Our results also showed that transferred macrophages could not protect hamsters from infection with *B. burgdorferi*, as spirochetes were readily recovered from their tissues when cultured. These findings demonstrate that macrophages exposed to *B. burgdorferi* are directly involved in the induction of Lyme arthritis.

Arthritis is a frequent complication of Lyme borreliosis (44). Intermittent episodes of arthritis develop in approximately 60% of individuals, especially in North America, infected with *Borrelia burgdorferi* (44). In severe cases, chronic inflammatory Lyme arthritis exhibits cartilage and bone erosion which leads to permanent joint dysfunction (23, 43, 44). When dogs (1), hamsters (18, 39), mice (4, 36), monkeys (3), and rats (5) are infected with *B. burgdorferi*, they develop moderate to severe arthritis. The severity of the arthritis in these models is greatly influenced by the maturity of the immune system. Suppressing or restricting components of the immune response greatly enhances the severity of Lyme arthritis (6, 35, 39). Collectively, these approaches have shown the importance of the immune response in the induction of Lyme arthritis.

The mechanism(s) by which Lyme arthritis is induced is only now being deciphered. Recently, we demonstrated that severe destructive Lyme arthritis could be readily induced in mature immunocompetent inbred LSH hamsters (23). When hamsters were vaccinated with nonviable *B. burgdorferi* contained in alum, a severe destructive arthritis was induced after infection with *B. burgdorferi*. Lim et al. (24) showed that induction of severe destructive arthritis was dependent on *B. burgdorferi*-specific T lymphocytes. Continued studies (25) showed that vaccinated hamsters treated with anti-$CD4^+$ antibody failed to develop severe destructive Lyme arthritis when infected with *B. burgdorferi*. Other investigators (19, 20, 30) have also shown that T cells and their subsets can exert antagonistic influences on the induction of Lyme arthritis.

In this study, we demonstrate that macrophages also play a major role in the induction of severe destructive Lyme arthritis. Macrophages obtained from either vaccinated or nonvaccinated hamsters and exposed to Formalin-inactivated *B. burgdorferi* in vitro induced a severe destructive arthritis in the hind paws of naive recipient hamsters when challenged with viable *B. burgdorferi.*

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.

Organisms. Low-passage-number (<10) virulent *B. burgdorferi* sensu stricto isolate 297 was cultured once in modified Barbour-Stoenner-Kelly (BSK) medium (11) 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 Chemical Co., St. Louis, Mo.), sealed, and stored at −70°C. When needed, a frozen suspension of spirochetes was thawed, and a sample was used to inoculate fresh BSK. Spirochetes were enumerated in a Petroff-Hausser counting chamber.

Preparation of vaccine. *B. burgdorferi* organisms were grown in 2 liters of BSK to 5×10^7 spirochetes per ml. Spirochetes were pelleted by centrifugation (10,000 × *g*, 15°C, 10 min) and washed three times with phosphate-buffered saline (PBS; pH 7.4). The washed pellet 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 (12,000 × *g*, 10°C, 15 min) and resuspended 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.

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

Recovery of macrophages. Forty-five vaccinated and 30 nonvaccinated hamsters were mildly anesthetized with ether contained in a nose-and-mouth cup and

* Corresponding author. Mailing address: Wisconsin State Laboratory of Hygiene, 465 Henry Mall, Madison, WI 53706. Phone: (608) 262-3634. Fax: (608) 265-3451.

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 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), 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 of warm Hanks balanced salt solution to remove nonadherent cells. Giemsa-stained smears of the isolated cells showed a homogeneous population of macrophages with no other types of leukocytes visible.

Analysis of macrophage preparations by flow cytometry. Macrophages were obtained from vaccinated hamsters. T lymphocytes were purified from the inguinal lymph nodes of hamsters by using methods previously described (26, 27, 47, 48). One-milliliter samples containing 5×10^6 macrophages or T lymphocytes were incubated with normal hamster serum for 1 h, washed twice with PBS by centrifugation, and stained with phycoerythrin-conjugated rat anti-[L3T4, mouse (CD4)] antibody (1:100; Boehringer Mannheim) 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 2% paraformaldehyde (Sigma), and kept in the dark until analyzed by flow cytometry. A phycoerythrin-conjugated rat immunoglobulin G2a antibody was used as an isotype control. Other controls included unstained cellular suspensions of macrophages and T lymphocytes. 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 for 5,000 cells were acquired. Cell samples were then analyzed by histogram profiles of phycoerythrin fluorescence, using FACScan LYSYS II software. Gates were established by using unstained samples and samples stained with the isotype control antibody. The percentage of $CD4^+$ T lymphocytes present in the cellular suspensions was determined by a percent shift in the phycoerythrin fluorescence of the phycoerythrin-conjugated rat anti-[L3T4, mouse (CD4)]-stained cells. No $CD4^+$ T lymphocytes or B lymphocytes were detected in the macrophage suspensions.

Incubation of macrophages with *B. burgdorferi*. A frozen suspension of Formalin-inactivated spirochetes was thawed and mixed with PBS to contain a concentration of 5.5×10^8 spirochetes per ml. Subsequently, the spirochete suspension (1 ml) was added to 11 ml of Dulbecco's modified Eagle's medium and poured onto a tissue culture dish containing 10^7 macrophages. The culture was incubated for 4 h at 37°C in an atmosphere of 7.5% CO_2. After incubation, the tissue culture dish was washed twice with 12 ml of warm Hanks balanced salt solution to remove nonadherent cells and Formalin-inactivated spirochetes. Five milliliters of cold, nonenzymatic cell lifter (Sigma) was then added to the tissue culture dish and 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 dish with a sterile rubber policeman. Macrophage suspensions from several tissue culture dishes were aspirated, pooled, and centrifuged at 1,500 × *g* for 10 min at 4°C. After centrifugation, the supernatant was decanted and the macrophages were resuspended in cold PBS. Macrophage viability was determined by trypan blue exclusion. Controls included macrophages not exposed to *B. burgdorferi*.

Cell transfer and infection of hamsters. Three hamsters per group were mildly anesthetized and injected subcutaneously in each hind paw with 0.2 ml of PBS containing various concentrations of viable macrophages ranging from 3×10^3 to 9×10^6 with or without exposure to Formalin-inactivated *B. burgdorferi*. Within hours after transfer of macrophages, recipient hamsters were mildly anesthetized and infected subcutaneously in each hind paw with 0.2 ml of BSK containing 10^5 to 10^6 viable *B. burgdorferi* sensu stricto isolate 297. In other experiments, recipients were infected 12 to 17 h after transfer of macrophages. This concentration of spirochetes can easily be detected in hamsters when their tissues are cultivated in BSK. The viability of spirochetes was determined by motility.

Assessment of arthritis. Swelling of the hind paws of hamsters was used to evaluate the inflammatory response. Hind paws were periodically measured for 21 days with a plethysmograph (Buxco Electronics, Sharon, Conn.). Measurements were obtained by mildly anesthetizing hamsters, carefully submerging a hind paw into a column of mercury to the ankle, and measuring the amount (in milliliters) of mercury displaced. The mean plethysmograph value obtained from three hamsters (six hind paws) per group was used as an index of severity of swelling. Mercury displacement was standardized with a volume calibrator.

Recovery of spirochetes. Twenty-one days after infection, hamsters were euthanized by CO_2 inhalation. The urinary bladder and spleen were aseptically removed, homogenized, and placed in 5 ml of fresh BSK. Cultures were incubated at 32°C and examined weekly for 2 weeks by dark-field microscopy for motile spirochetes. If spirochetes were not detected, 1 ml of the culture was inoculated into 4 ml of fresh BSK, incubated at 32°C, and examined weekly for 3 weeks.

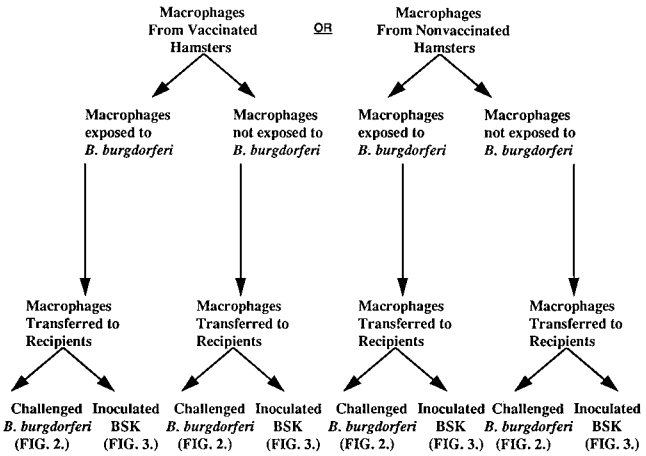

FIG. 1. Preparations of macrophages used for the induction of severe destructive Lyme arthritis.

Preparation of tissues for histology. After euthanization, the hind legs of hamsters were amputated at the mid-femur, fixed in 10% neutral buffered zinc Formalin, placed in decalcifying solution (Lerner Laboratories, Pittsburgh, Pa.) for 7 h, and stored in 10% zinc Formalin until examined. The knees and hind paws were bisected longitudinally, embedded in paraffin, cut into 6-mm sections, placed on glass sides, and stained with hematoxylin and eosin. The hind legs were randomly selected from groups for histopathologic examination.

Statistics. Plethysmograph values were tested by analysis of variance. The Fisher least-significant-difference test (41) 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

Macrophages exposed to *B. burgdorferi* induced severe destructive arthritis. We determined the ability of macrophages obtained from vaccinated and nonvaccinated hamsters with or without exposure to Formalin-treated *B. burgdorferi* in vitro to induce arthritis in recipient hamsters after infection with viable *B. burgdorferi* (Fig. 1). Slight swelling was detected in recipients of macrophages incubated with Formalin-treated *B. burgdorferi* within 8 h of infection. The swelling peaked at 21 h and decreased rapidly by day 4 of infection (Fig. 2). Subsequently, a second period of severe destructive arthritis (severe swelling) occurred in these recipients. Swelling peaked 11 days after infection and then gradually declined by day 21. By contrast, recipients of macrophages obtained from vaccinated and nonvaccinated hamsters without exposure to *B. burgdorferi* in vitro developed significantly less swelling 21 h after infection (Fig. 2). In addition, they failed to develop the second cycle of severe hind paw swelling. Similar results were obtained when recipients of macrophages from vaccinated and nonvaccinated hamsters exposed to *B. burgdorferi* in vitro were injected with BSK (Fig. 3). These recipients developed moderate swelling of the hind paws 17 h after infection. Likewise, only slight swelling of the hind paws was detected with recipients of macrophages from vaccinated and nonvaccinated hamsters not exposed to Formalin-treated *B. burgdorferi* and injected with BSK. Spirochetes, however, were recovered from all the recipients challenged with *B. burgdorferi* (Table 1). In other experiments, recipients infused with macrophages obtained from vaccinated and nonvaccinated hamsters with or without exposure to Formalin-treated *B. burgdorferi* in vitro and challenged with nonviable or viable high-passage-number *B. burgdorferi* failed to develop the second cycle of severe destructive arthri-

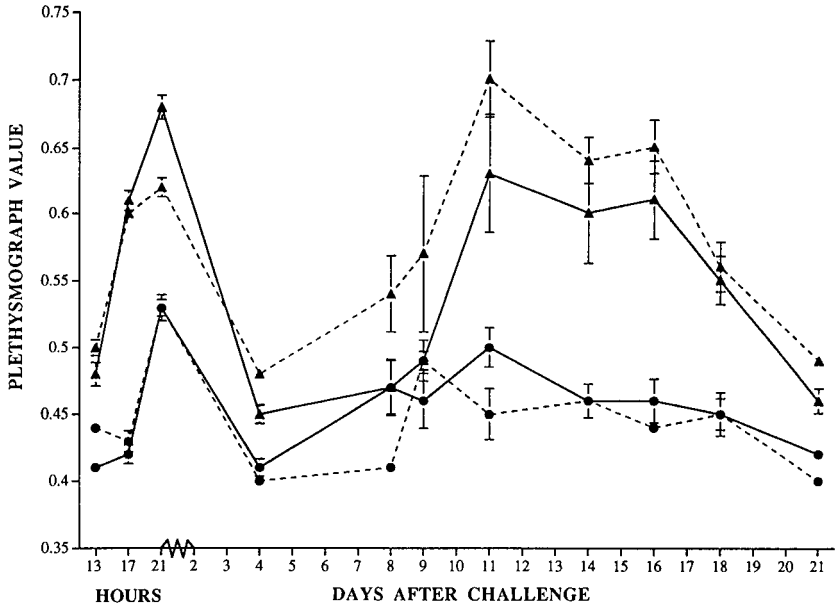

FIG. 2. Development of swelling of the hind paws of naive recipient hamsters infused with macrophages from vaccinated (——) and nonvaccinated (- - - - -) hamsters with (▲) and without (●) exposure to Formalin-inactivated *B. burgdorferi* in vitro. Hamsters were then challenged with viable *B. burgdorferi*. There were three hamsters per group, and this experiment was repeated seven times.

tis. When these experiments were repeated (seven times), similar results were obtained.

Subsequently, recipients were infused with 3×10^3, 3×10^4, 3×10^5, 3×10^6, 6×10^6, or 9×10^6 macrophages exposed to Formalin-treated spirochetes in vitro and then challenged with viable *B. burgdorferi*. Swelling of the hind paws was detected with recipients of 10^6 macrophages or more within 8 h of infection (Fig. 4). The swelling rapidly decreased, and a second cycle of severe destructive arthritis developed approximately 5 days after infection. Maximum swelling occurred with recipients of 9×10^6 macrophages. Significantly less swelling was detected with recipients of 6×10^6 and 3×10^6 macrophages. No statistically significant swelling of the hind paws was detected with recipients of 10^5 macrophages or less. Likewise,

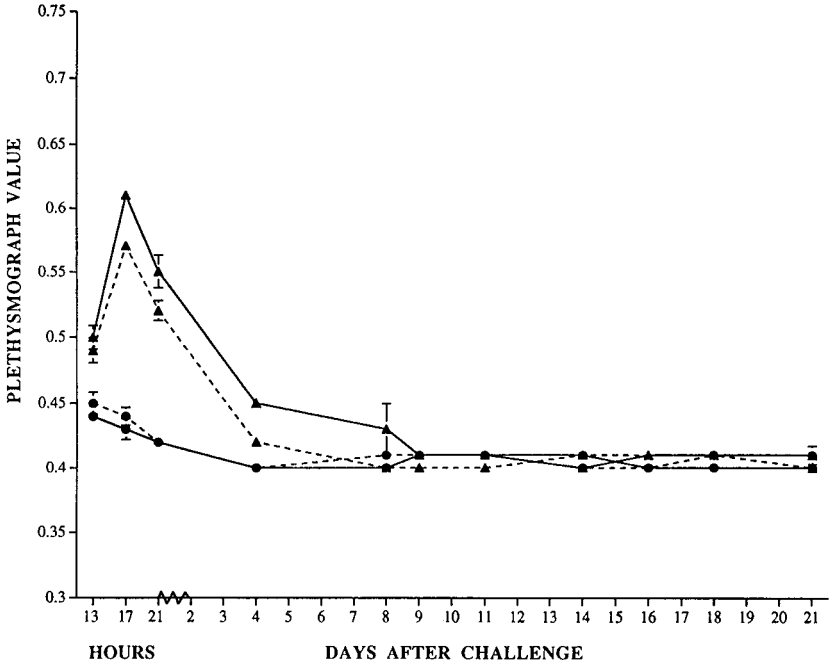

FIG. 3. Development of swelling of the hind paws of naive recipient hamsters infused with macrophages from vaccinated (——) and nonvaccinated (· · · · · ·) hamsters with (▲) and without (●) exposure to Formalin-inactivated *B. burgdorferi* in vitro. Hamsters were then injected with BSK medium. There were three hamsters per group, and this experiment was repeated three times.

TABLE 1. Isolation of *B. burgdorferi* sensu stricto isolate 297 from tissues of recipients of macrophages obtained from vaccinated and nonvaccinated hamsters with or without incubation in vitro with Formalin-inactivated *B. burgdorferi*

Tissue	No. of cultures positive[a]			
	Vaccinated		Nonvaccinated	
	With *B. burgdorferi*	Without *B. burgdorferi*	With *B. burgdorferi*	Without *B. burgdorferi*
Bladder	3	3	3	3
Spleen	3	2	2	3

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

recipients of 3×10^6 macrophages exposed to Formalin-treated *B. burgdorferi* in vitro and injected with BSK failed to develop the second cycle of severe hind paw swelling. When these experiments were repeated (three times), similar results were obtained.

Flow cytometric examination. We also examined the suspensions of macrophages obtained from vaccinated hamsters for contamination with T lymphocytes, specifically $CD4^+$ T lymphocytes (Fig. 5). No $CD4^+$ T lymphocytes were detected in the suspensions of macrophages.

Histopathology of hind paw swelling. An erosive and destructive arthritis was detected in the hind paws of recipients infused with 9×10^6 macrophages exposed to Formalin-treated *B. burgdorferi* in vitro and then challenged with the Lyme spirochete (Fig. 6A). There was extensive thickening of the synovial lining and subsynovial tissues in the periarticular regions of the tibiotarsal and intertarsal joints (Fig. 6A). The periarticular soft tissues also showed an extensive mononuclear inflammatory infiltrate that extended and focally destroyed skeletal muscle. In addition to the periarticular changes, there was severe synovial and subsynovial inflammation that progressed to pannus formation which destroyed and penetrated trabecular bone (Fig. 6A). The resulting arthropathy was so fulminate that the tibiotarsal joint was fused and rendered nonfunctional. The severity of these histopathologic manifestations was dependent on the concentration of macrophages transferred. Hamsters infused with lesser concentrations of macrophages (3×10^6) from vaccinated (Fig. 6B) and nonvaccinated (Fig. 6C) hamsters exposed to Formalin-treated *B. burgdorferi* and then infected with the Lyme spirochete showed milder histopathologic manifestations. By contrast, recipients of macrophages from vaccinated (Fig. 6D) and nonvaccinated (Fig. 6E) hamsters not exposed to *B. burgdorferi* and then challenged showed only minimal hypertrophy and inflammation. A cellular infiltrate of mononuclear cells was present in subsynovial areas (Fig. 6D and E). However, the joint spaces were free of significant histopathologic alterations. Similarly, control recipients that received macrophages obtained from either vaccinated or nonvaccinated hamsters exposed or not exposed to *B. burgdorferi* and injected with BSK developed no observable histopathologic alterations.

DISCUSSION

Several investigations (13, 17, 21–23, 29, 42) have provided indirect evidence that macrophages are involved in the pathogenesis of Lyme arthritis. Elevated levels of macrophage-associated cytokines such as interleukin-1 (7, 17, 21, 22) and tumor necrosis factor alpha (14, 30) have been detected after infection with *B. burgdorferi*. Both interleukin-1 and tumor necrosis factor alpha can activate osteoclastic cartilage and bone reabsorption that may facilitate the development of Lyme arthritis (2, 10, 45). Ma et al. (29) also showed that *B. burgdorferi* stimulated the production of nitric oxide in murine macrophages. Nitric oxide has been associated with the induction of other arthritic diseases (31, 46). Other studies (40), however,

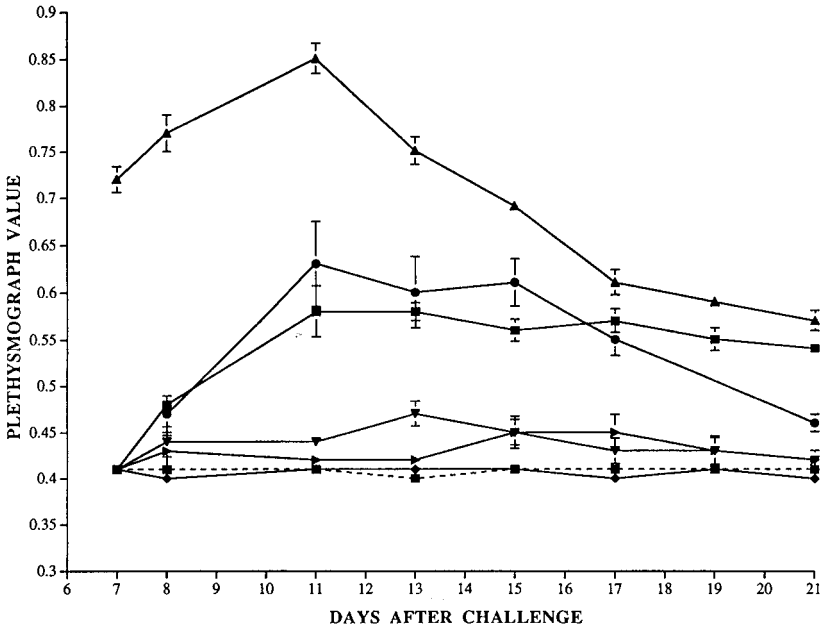

FIG. 4. Development of swelling of the hind paws of naive recipient hamsters infused with 9.0×10^6 (▲), 6.0×10^6 (●), 3.0×10^6 (■), 3.0×10^5 (▼), 3.0×10^4 (▶), or 3.0×10^3 (◆) macrophages exposed to Formalin-inactivated *B. burgdorferi* in vitro. Hamsters were then challenged with *B. burgdorferi* (——) or injected with BSK medium (- - - - -). There were three hamsters per group, and this experiment was repeated three times.

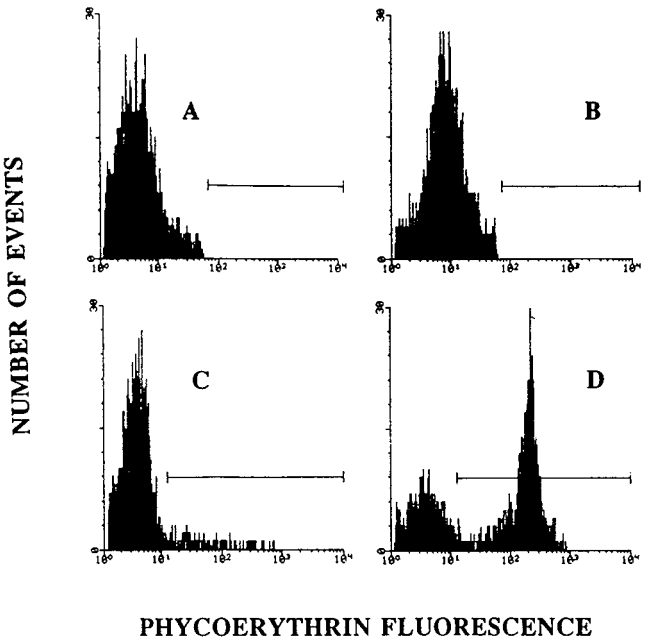

FIG. 5. Flow cytometric analysis of suspensions of macrophages for contamination with $CD4^+$ T lymphocytes. Macrophages (A and B) and T lymphocytes (C and D) were incubated in the presence (B and D) or absence (A and C) of phycoerythrin-conjugated rat anti-[L3T4, mouse (CD4)] antibody, which has specificity for the CD4 (L3T4) molecule on murine and hamster T lymphocytes. Suspensions of macrophages were free of $CD4^+$ T lymphocytes.

have failed to confirm these findings or have not shown the direct effect of the modulator on the induction of Lyme arthritis.

In this study, we showed that macrophages play a direct role in the induction of severe destructive Lyme arthritis. When macrophages were incubated with *B. burgdorferi* in vitro and then injected into recipient hamsters challenged with the Lyme spirochete, severe destructive arthritis developed. Severe swelling of the hind paws of recipients was detected 8 days after infection, peaked on day 11, and gradually decreased. Histopathologic examination confirmed that macrophages induced severe destructive arthritis. The severity of the arthritis was dependent on the number of *B. burgdorferi*-exposed macrophages transferred. In addition, the severe destructive arthritis was evoked only if viable *B. burgdorferi* organisms were used for challenge of the recipients.

The induction of severe destructive arthritis was due to macrophages. We previously showed (24) that severe destructive Lyme arthritis could also be induced in recipients with *B. burgdorferi*-specific T lymphocytes obtained from hamsters vaccinated with a whole-cell preparation of Formalin-inactivated *B. burgdorferi* organisms in alum. Contamination of the macrophage preparations with *B. burgdorferi*-specific T lymphocytes could have occurred. Several lines of evidence, however, suggest that contaminating *B. burgdorferi*-specific T lymphocytes were not responsible for the induction of severe destructive arthritis. Lim et al. (24) showed that induction of severe destructive Lyme arthritis required 5×10^6 or more *B. burgdorferi*-specific T lymphocytes. We consistently induced severe destructive Lyme arthritis with preparations containing fewer than 3×10^6 macrophages. In addition, flow cytometric examination of preparations of macrophages from vaccinated hamsters were free of $CD4^+$ T lymphocytes, the T cells previously shown to be responsible for the induction of Lyme arthritis (25). More importantly, severe destructive Lyme arthritis was induced with macrophages obtained from nonvaccinated hamsters. No *B. burgdorferi*-specific T lymphocytes were present to contaminate the macrophage preparations used for the adoptive transfer experiments.

Clinical manifestations of severe destructive Lyme arthritis occurred 8 days after recipient hamsters were infused with macrophages incubated with *B. burgdorferi* in vitro and then challenged with the Lyme spirochete. Lim et al. (24) also showed that approximately 8 days were required before clinical manifestations of severe destructive Lyme arthritis developed in recipients of *B. burgdorferi*-specific T lymphocytes. This finding suggests that cooperation between macrophages and T lymphocytes is required before severe destructive arthritis develops. We hypothesize that transferred macrophages promote the recruitment and accumulation of endogenous T lymphocytes within the tissues of the hind paws of recipient hamsters. The presentation of borrelial antigens by macrophages exposed to Formalin-inactivated spirochetes results in the activation of recruited T lymphocytes, especially $CD4^+$ T lymphocytes (25). Activation of T lymphocytes further stimulates macrophages to release cytokines, enzymes, or other factors that potentiate the severe arthritic manifestations. In support of this hypothesis, macrophages not exposed to Formalin-inactivated spirochetes failed to induce severe destructive Lyme arthritis, despite challenge of recipients with *B. burgdorferi*. These macrophages had no opportunity to process and present borrelial antigens to nonprimed endogenous T lymphocytes.

It is interesting that macrophages incubated with *B. burgdorferi* in vitro induced significant swelling of the hind paws of recipient hamsters shortly after transfer of cells and infection with *B. burgdorferi*. The swelling peaked at 21 h after infection and rapidly decreased. Although recipients of macrophages not incubated with *B. burgdorferi* developed swelling of the hind paws, the severity of swelling was significantly less. This first cycle of swelling of the hind paws may be due to release of proinflammatory cytokines by adoptively transferred macrophages, especially those macrophages incubated with *B. burgdorferi*. This first cycle of swelling of the hind paws was not influenced by the in vivo challenge with *B. burgdorferi*. If challenge of recipients of macrophages with or without prior exposure to Formalin-inactivated *B. burgdorferi* was delayed for approximately 24 h, swelling of the hind paws still occurred. It has been shown that human, murine, and bovine macrophages are stimulated to produce large amounts of interleukin-1 by *B. burgdorferi* in vitro (7, 17, 21, 22, 29). In addition, murine macrophages can be stimulated to produce large amounts of tumor necrosis factor alpha by *B. burgdorferi* (13, 29). These and other cytokines released by adoptively transferred macrophages may be responsible for the initial cycle of swelling detected in recipients of macrophages with or without exposure to *B. burgdorferi*. It should be noted, however, that only macrophages incubated with *B. burgdorferi* could induce the second period of severe destructive arthritis after recipients were challenged with viable *B. burgdorferi.*

We also showed that severe destructive arthritis could be evoked only in recipient hamsters infected with viable low-passage-number *B. burgdorferi*. When recipients of macrophages exposed to Formalin-inactivated spirochetes were challenged with high-passage-number or killed *B. burgdorferi*, severe destructive arthritis failed to develop. These results confirmed our previous finding (22) that high-passage-number or killed low-passage-number *B. burgdorferi* could not induce arthritis in the hind paws of highly susceptible irradiated hamsters. This finding suggests that viable low-passage-number *B. burgdorferi* possess a factor(s) that triggers a unique set of specific immune

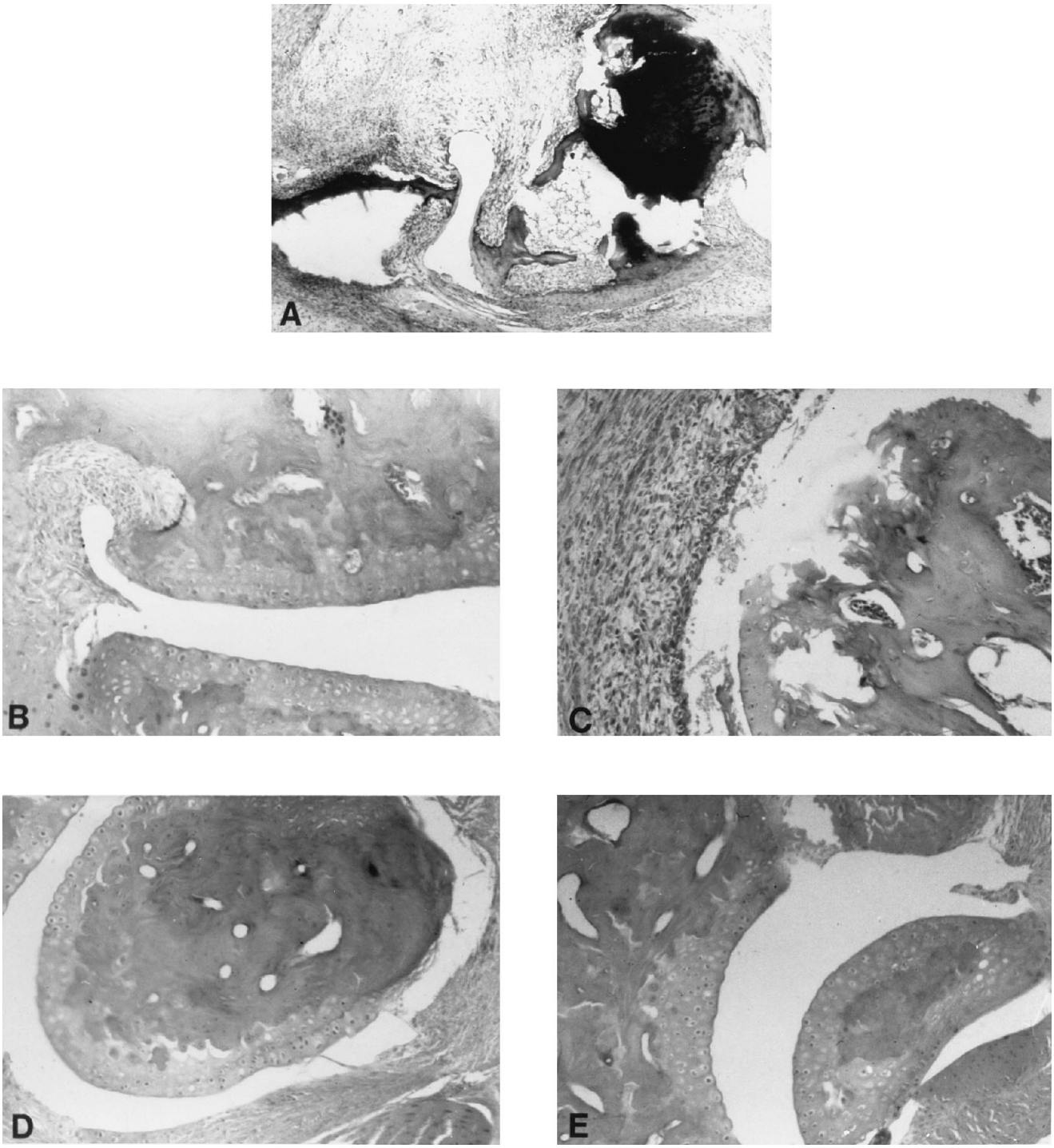

FIG. 6. Histopathology of the hind paws of naive recipient hamsters 21 days after infusion with 3.0×10^6 macrophages from vaccinated (B and D) and nonvaccinated (C and E) hamsters with (B and C) and without (D and E) exposure to Formalin-inactivated *B. burgdorferi* in vitro. Severe destructive arthritis was detected in recipients of macrophages exposed to Formalin-inactivated *B. burgdorferi* (B and C) and challenged with the Lyme spirochete. The severity of joint destruction increased when higher concentrations (9.0×10^6) of macrophages were used (A). Arthritis was not detected in recipients of macrophages without exposure to Formalin-inactivated *B. burgdorferi* in vitro (D and E) and challenged with *B. burgdorferi*. Hamsters were randomly selected for histopathologic examination.

responses responsible for the induction of severe destructive arthritis. It is also possible that live *B. burgdorferi* is processed differently by macrophages than killed or high-passage-number *B. burgdorferi*. Additional studies are needed to determine the specific components of low-passage-number *B. burgdorferi* that evoke the manifestations of arthritis and influence the presentation of processed borrelial antigens to T cells. Presently, we are determining if live and killed *B. burgdorferi* cells are processed differently.

Although macrophages have been shown to phagocytize *B.*

burgdorferi (8, 9, 16, 32–34) and play a significant role in the induction of severe destructive arthritis, they are not the limiting factor in controlling the infectivity of *B. burgdorferi*. When recipients of macrophages exposed to *B. burgdorferi* in vitro were challenged with the Lyme spirochete, spirochetes were readily recovered from their tissues during the period of severe destructive arthritis. Lim et al. (24) also showed that *B. burgdorferi*-specific T lymphocytes could not eliminate spirochetes from recipients exhibiting severe destructive arthritis. Collectively, these results suggest that macrophages and T lymphocytes, although directly involved in the induction of severe destructive Lyme arthritis, play only a minor role in elimination of spirochetes from the host. Considerable evidence, however, suggests that borreliacidal antibodies are directly involved in eliminating *B. burgdorferi*. We demonstrated that borreliacidal antibody (38) eliminated *B. burgdorferi* from the host and could kill the spirochete in vitro (28). We also showed that human serum containing borreliacidal antibody adoptively protected hamsters from subsequent infection with *B. burgdorferi* (12). These results and the results of other investigators (14, 15, 37) show that humoral immunity plays a major role in controlling *B. burgdorferi* infection.

In conclusion, we have demonstrated that macrophages obtained from hamsters exposed to Formalin-inactivated *B. burgdorferi* induced a severe destructive arthritis in the hind paws of recipient hamsters when infected with *B. burgdorferi*. We further demonstrated that the severity of the arthritis was dependent on the concentration of macrophages transferred and the state of borrelial antigen presentation. Additional experiments are needed to determine if macrophages and T lymphocytes interact synergistically to induce the severe arthritic manifestations. These studies are important and will further characterize the cell interactions and immunologic mechanisms responsible for the induction of Lyme arthritis. Ultimately this research will facilitate the identification of approaches that may modulate the immune response to circumvent the induction and development of Lyme arthritis.

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