INFECTION AND IMMUNITY, Aug. 1997, p. 3100–3106 Vol. 65, No. 8
0019-9567/97/$04.00+0

Effects of *Ixodes scapularis* and *Borrelia burgdorferi* on Modulation of the Host Immune Response: Induction of a TH2 Cytokine Response in Lyme Disease-Susceptible (C3H/HeJ) Mice but Not in Disease-Resistant (BALB/c) Mice

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Received 19 March 1997/Returned for modification 24 April 1997/Accepted 14 May 1997

Previous studies have demonstrated that both *Ixodes scapularis* saliva and *Borrelia burgdorferi* antigens modulated lymphokines and monokines in vitro. The studies presented here were designed to delineate the role of *I. scapularis* and *B. burgdorferi* in modulation of the host immune response in vivo. Infestation of C3H/HeJ mice with infected *I. scapularis* resulted in an up regulation of IL-4 as early as 8 days after tick infestation, while the levels of T helper cell type 1 (TH1) cytokines, interleukin-2 (IL-2) and gamma interferon (IFN-γ), were significantly decreased by days 10 to 12. In contrast, the cytokine profile of BALB/c mice exposed to infected nymphal ticks resulted in only transient alterations in IL-4, IL-2, and IFN-γ production throughout a 12-day period postinfestation. Although the IL-10 level was elevated in both C3H/HeJ and BALB/c mice infested with infected nymphal ticks, no significant difference in the levels of IL-10 was noted between the mouse strains. Flow-cytometric analysis demonstrated increases in the numbers of splenic B-cell and $CD4^+$ lymphocytes in C3H/HeJ but not BALB/c mice exposed to infected ticks. Cell depletion experiments with C3H/HeJ mice demonstrated that $CD4^+$ cells were the sole producers of IFN-γ and IL-10 while both $CD4^+$ and $CD8^+$ splenocytes contributed to the production of IL-2 and IL-4. These findings suggest that B and $CD4^+$ splenocytes are activated, increase in number, and produce a polarized TH2 response in C3H/HeJ mice exposed to infected *I. scapularis*. Given that C3H/HeJ mice are susceptible to Lyme disease and the initial TH2 polarization is not evident in BALB/c mice, effective control of this response may have ramifications for spirochete transmission in vivo.

Lyme disease, caused by the spirochete *Borrelia burgdorferi* and transmitted by the tick *Ixodes scapularis*, is now the most commonly reported vector-borne disease in the United States (4). Clinical manifestations of this disease range from acute erythema migrans occurring days to weeks after the tick bite to chronic arthritis and neuralgia in approximately 10% of people who did not receive or respond to antibiotic treatment (32). The increased incidence and morbidity of this disease syndrome have led to a search for an effective vaccine and/or prevention strategy.

Interaction between the arthropod vector, *I. scapularis*, and the host has profound implications for the eventual transmission of a vector-borne pathogen like *B. burgdorferi*. *I. scapularis* feeding and resultant modulation of the host immune response greatly influence the infectivity and dissemination of the spirochete (21, 26). Systemic dissemination of the tick-inoculated spirochete is delayed in the host for 7 to 10 days (30). Nevertheless, early host immune effector mechanisms are ineffective in clearing this organism. This may be due in part to the regulation of spirochete outer surface proteins (Osps) during the tick feeding process (2, 5, 29). *B. burgdorferi* OspA, which is important for the generation of an effective humoral response against resident spirochetes within the tick midgut, is down regulated during nymphal attachment and feeding, greatly limiting the humoral response to this protein during the earliest phases of spirochete inoculation in the mammalian host (2, 5, 28, 29). Likewise, the effect of tick saliva may contribute to the relatively ineffective immune response against the spirochete after tick inoculation (21, 33).

Ixodid saliva and *B. burgdorferi* greatly modulate a number of proinflammatory responses within the host (6, 22, 23, 34). Ramachandra et al. (23) demonstrated reductions in murine macrophage activation in vitro, including reductions in the ability of macrophages to produce interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α). Likewise, the production of the T helper cell (TH)-associated cytokines, interleukin-2 (IL-2) and gamma interferon (IFN-γ), was also greatly suppressed in lymphocytes exposed to salivary gland lysates in vitro (24). This data was corroborated by Urioste et al. (33), who demonstrated that *I. scapularis* saliva directly inhibited T-cell proliferation in response to both concanavalin A (ConA) and phytohemagglutinin, down regulated the production of IL-2 by T cells in a concentration-dependent manner, and inhibited nitric oxide production by macrophages. The immunosuppressive effects of saliva and/or the tick-feeding process were apparent during the period of tick infestation and for 4 to 5 days after engorgement, since salivary gland extracts collected throughout this 9-day period suppressed T-cell proliferation and cytokine production in vitro (24). Similar studies done by Ganapamo et al. demonstrated reduced T-cell prolif-

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eration and reductions in IL-5 production in BALB/c mice infested with *Ixodes ricinus*, the principal vector of Lyme disease in Europe (11). In these studies, the authors demonstrated a concomitant rise in IL-10 production in these mice as well as a lymphocytosis in response to lipopolysaccharide. The authors' conclusions from these studies, which were done with only uninfected *I. ricinus*, were that the decreases in T-cell responses to ConA within the draining lymph nodes of tick-infested mice were due to an increase in TH2 cytokine levels, most notably IL-10 (11).

The cumulative effect of these events would be the down regulation of the initial inflammatory response to spirochete inoculation, specifically in terms of IFN-γ regulation of antigen presentation, cytotoxic T-cell responses, and immunoglobulin production. Moreover, both IFN-γ and TNF-α up regulate macrophage antimicrobial activity via the induction of intracellular nitric oxide production (13), a crucial factor in the intracellular killing of several macrophage-tropic pathogens (12). Considering that antigen-presenting cells, like macrophages and dendritic cells, are cellular targets of *B. burgdorferi* infection (9, 18), down regulation of any of these proinflammatory factors would play a crucial role in promoting the initial dissemination of spirochete infection.

The purpose of the studies presented here was to evaluate the effect of feeding by the natural vector on systemic cytokine production in vivo, within a murine model of Lyme borreliosis. To accomplish this, we evaluated the effects of uninfected versus infected *I. scapularis*, placed first on mice shown to be susceptible to the development of clinical Lyme disease (C3H/HeJ) and then compared to animals that are clinically resistant (BALB/c) (1). In this manner, we attempted to evaluate the effect of the vector, *I. scapularis*, in comparison with the influence of the pathogen, *B. burgdorferi*, on the early modulation of both the TH1 and TH2 immune responses in vivo. The results of these studies, which demonstrate a clear bias toward a TH2 immune response in susceptible mice, may affect future prevention strategies for Lyme disease as well as other *Ixodes*-borne zoonoses like human granulocytic ehrlichiosis.

MATERIALS AND METHODS

Mice. Virus-free 6- to 8-week-old C3H/HeJ and BALB/c mice were obtained from the Jackson Laboratory (Bar Harbor, Maine). They were maintained in group cages and were sacrificed at the end of these studies by exposure to carbon dioxide gas.

Maintenance of tick colonies and infection of mice by tick bite. *I. scapularis* larvae used in these studies were derived from adult ticks originally harvested in Westchester County, N.Y. These larvae were fed on ICF (CDC strain) mice previously infected with *B. burgdorferi* B31 and maintained at 21°C and 97% humidity. The resultant nymphs, 3 to 4 months postmolting, were used to infect mice by the natural route of tick exposure. The rate of infection within the tick colony was greater than 80% as previously determined (19). Ticks not harboring *B. burgdorferi*, originally obtained from the Saint Croix State Park, Minn., were maintained in the laboratory in a separate colony at 21°C. These ticks were determined to be negative for *B. burgdorferi* by dissection, culture, and dark-field microscopy as previously described (19). In addition, pools of negative ticks were analyzed for the presence of granulocytic ehrlichiae (HGE) by a nested PCR assay shown to specifically amplify GE 16S rRNA gene sequences (17). All ticks examined were negative for HGE by qualitative PCR. In all cytokine studies, each mouse was exposed to eight nymphal ticks and the ticks were allowed to feed to repletion over a 72- to 96-h period.

Preparation of splenocytes for cytokine production. Spleens from groups of three to six animals (total of nine per time point) were harvested on days 4 through 12 after the tick infestation. Individual spleens were teased apart between the frosted ends of two microscope slides, and a single-cell suspension of mononuclear cells was isolated by Ficoll-Hypaque gradient centrifugation (NycoPrep 1.077; NycoMed Pharma AS, Oslo, Norway). After being washed three times in phosphate-buffered saline (PBS), the cells were suspended at 5×10^6 cells per ml of complete media consisting of RPMI 1640 supplemented with 10% fetal calf serum, 2% glutamine, 2% sodium bicarbonate (7.5%, vol/vol), 2% essential amino acids, 1% nonessential amino acids, 1% pyruvate, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 2×10^{-5} M 2-mercaptoethanol. A total of 5×10^6 cells were then placed in individual wells of a 24-well plate (no. 3524; Costar Corp., Cambridge, Mass.) and stimulated with 2 μg of ConA (Boehringer, Mannheim, Germany) per ml or sonicated low-passage *B. burgdorferi* B31 at 10 to 30 μg/ml. Supernatants from these cultures were harvested at either 24 h poststimulation to analyze for IL-2 or 72 h poststimulation to determine IFN-γ, IL-4, and IL-10 production. The supernatants were frozen at −70°C until use.

Quantification of TH1 and TH2 cytokines. IL-2, IFN-γ, IL-4, and IL-10 were quantified by an enzyme-linked immunosorbent assay as specified by the manufacturer (Pharmingen, San Diego, Calif.) with slight modifications. Briefly, 100 ng of antigen-binding antibody was used in these assays, splenocyte supernatants were run at either a 1:5 or 1:10 dilution in blocking buffer, and 25 ng of biotin-labeled detection antibody was used for each cytokine. After extensive washing, horseradish peroxidase-labeled streptavidin (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) was plated at 1:2,000 in blocking buffer and incubated for 30 min, and the plates were developed with 3,3′,5,5′-tetramethylbenzidine in the presence of hydrogen peroxide (Kirkegaard & Perry Laboratories) for 8 min. Development in the wells was stopped with 2.5 N sulfuric acid, and the absorbance at 450 nm was determined spectrophotometrically for each well. Each experimental sample was run in triplicate wells, and the percentage of the control cytokine was determined by dividing the experimental value (nanograms per milliliter) by control values (supernatants harvested from splenocytes derived from mice that had not been infested with ticks) and multiplying by 100. ELISA capture and detection monoclonal antibody pairs used in these studies were as follows: IL-2, clones JES6-1A12 and JES6-5H4; IFN-γ, R464A2 and XMG1.2; IL-4, BVD4-1D11 and BVD6-24G2; and IL-10, MP6-XT22 and BVD6-24G2 (Pharmingen). Spontaneous release of either TH1 or TH2 cytokines from unstimulated cells (ConA-negative control wells) accounted for no more than 0.1% of the cytokine levels for each time point.

Immunophenotyping of lymphocyte subsets. Splenocytes (5×10^5) were washed once with PBS containing 0.1% sodium azide and 2% fetal calf serum. A fluorescein isothiocyanate-conjugated monoclonal antibody (Pharmingen) specific for B lymphocytes ($CD45^+$/B220; clone RA3-6B2), $CD4^+$ T cells (L3T4; clone RM4-5), or $CD8^+$ T cells (Ly-2; clone 53-6.7) was added, and the mixture was incubated for 1 h at 4°C. The cells were then washed three times with PBS and suspended in PBS containing sodium azide and fetal calf serum for flow-cytometric analysis. Surface labeling was analyzed with a Coulter EPICS Profile II flow cytometer equipped with a no. 488 argon laser. A total of 10^4 cells were analyzed by electronic bit map gating determined by forward- and log side-scatter patterns. Analysis gates were set so that <1% of all cells were labeled with fluorescein isothiocyanate-conjugated isotype control antibody.

In vivo depletion of lymphocyte subsets. Anti-$CD4^+$ (clone GK1.5; rat immunoglobulin G2b [IgG2b]) and anti-$CD8^+$ (clone 116-13.1; mouse IgG2b) monoclonal antibodies were injected intraperitoneally on days 3 and 7 (1 mg/mouse/injection) prior to tick infestation. Depletion of $CD4^+$ and $CD8^+$ T cells was monitored by flow cytometry as described above. Normal rat IgG2b (anti-TNP) was administered as an isotype control in negative control groups of animals.

Statistical analysis. Significant differences in the mean levels of cytokine production were determined by Student's *t*-test. *P* values less than 0.05 were considered significant.

RESULTS

Immune modulation of C3H/HeJ mice by *I. scapularis* and *B. burgdorferi*. C3H/HeJ mice were exposed to either eight nymphal *I. scapularis* ticks which harbored *B. burgdorferi* or eight nymphal ticks which were negative for the Lyme disease spirochete. Spleens from these mice were harvested at 2-day intervals, ranging from 4 to 12 days after tick infestation. Splenocytes were stimulated with either sonicated *B. burgdorferi* or ConA, and supernatants were analyzed for specific cytokine production. Because *B. burgdorferi*, which was used at between 10 and 30 μg/ml, could only consistently stimulate the production of IFN-γ and IL-10, all results discussed below represent ConA stimulation only. As demonstrated in Fig. 1A, the amount of IL-2 that could be produced from spleen cells derived from C3H/HeJ mice exposed to infected nymphs progressively decreased to 17% of control values (831.7 ng/ml) over a 12-day period. In contrast, mice infested with negative nymphs produced levels of IL-2 which declined to 55% of control values by day 6 but recovered to normal values (5,769 ng/ml [$P < 0.000009$] compared to infected nymphs) by day 12 postinfestation. IFN-γ production in C3H/HeJ mice exposed to infected nymphs was suppressed to 60% of control values by day 4 postinfestation (Fig. 1B) and continued to decline to a low of 49% (94.1 ng/ml) of control values by day 12. When the

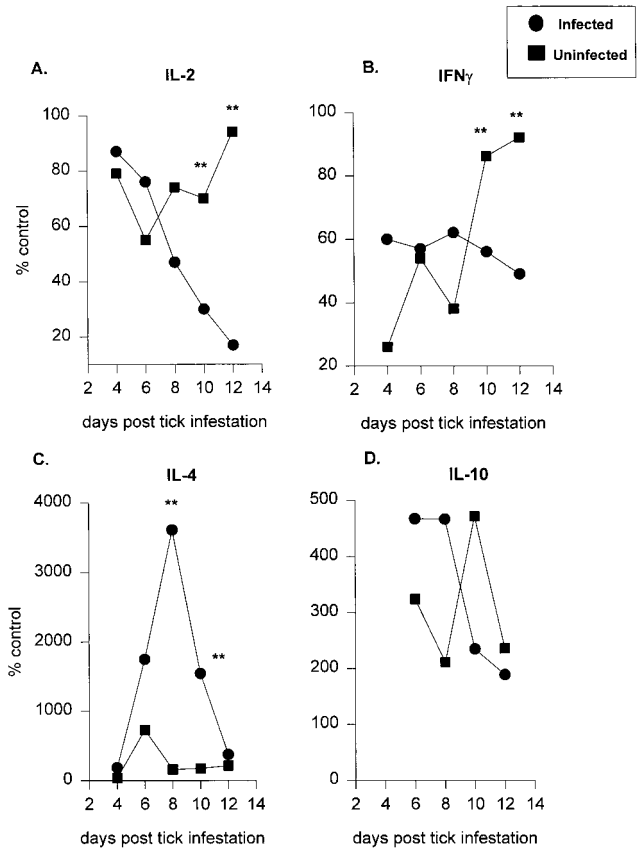

FIG. 1. TH1 (IL-2 plus IFN-γ)-associated and TH2 (IL-4 plus IL-10)-associated cytokine production by splenocytes harvested from C3H/HeJ mice infested with *B. burgdorferi*-infected (circles) or uninfected (squares) nymphal ticks. Splenocytes were harvested from individual mice on the days indicated and stimulated with ConA. Cytokine levels in supernatants were then quantitated by antigen capture ELISA. % control is equal to the cytokine level (nanograms per milliliter) in the experimental group divided by levels obtained from naive (unexposed) mice and multiplied by 100. ** indicates statistically significant ($P < 0.05$) differences between experimental groups. Nine animals were analyzed per time point.

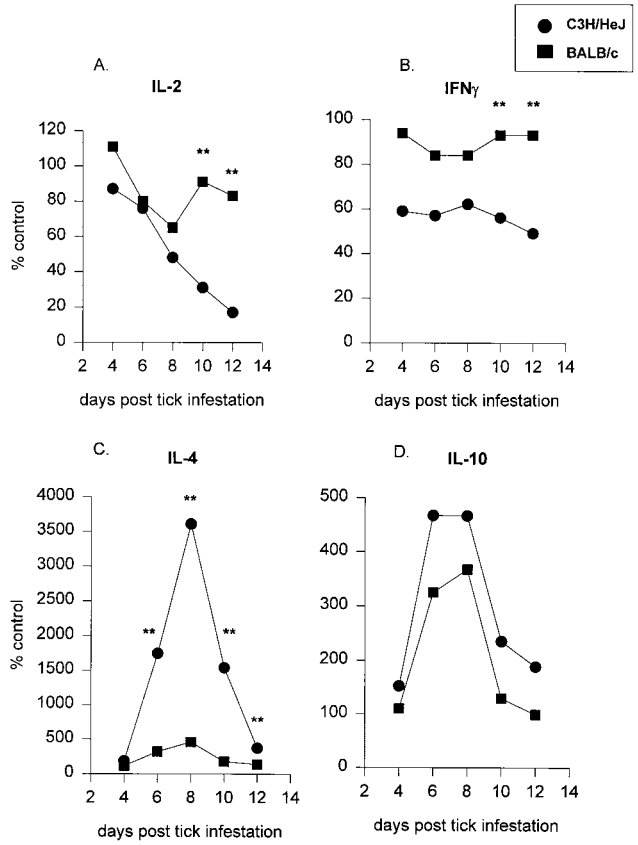

FIG. 2. TH1 (IL-2 plus IFN-γ)-associated and TH2 (IL-4 plus IL-10)-associated cytokine production by splenocytes harvested from C3H/HeJ (circles) and BALB/c (squares) mice infested with *B. burgdorferi*-infected nymphal ticks. Splenocytes were harvested from individual mice on the days indicated and stimulated with ConA. Cytokine levels in supernatants were quantified by antigen capture ELISA. % control is equal to the cytokine levels (nanograms per milliliter) in experimental groups divided by levels obtained from syngeneic naive mice and multiplied by 100. ** indicates statistically significant ($P < 0.05$) differences between experimental groups. Nine animals were analyzed per time point.

mice were exposed to uninfected nymphs, the process of tick feeding suppressed IFN-γ production to a low of 26% (47.3 ng/ml on day 8), but recovery to 92% of control values ($P < 0.024$ compared to infected nymphs) occurred by day 12 (Fig. 1B). In contrast, production of the TH2 cytokines, IL-4 and IL-10, was significantly up regulated when C3H/HeJ mice were exposed to either infected or uninfected nymphs (Fig. 1C and D). Exposure to infected nymphs induced a rapid rise in IL-4, reaching a peak level of 3,600% of control values (11,728 pg/ml [$P < 0.0090$] compared to uninfected nymphs) by day 8 and decreasing toward baseline levels by day 12. Exposure to uninfected nymphs resulted in a rapid rise in the IL-4 level to 723% of control values by day 6 postinfestation, and the level remained elevated throughout the entire 12-day period. No significant difference between exposure to infected and uninfected nymphs was noted on day 12 postinfestation (214 versus 374% of control values [$P > 0.190$]). Exposure to infected nymphs resulted in a significant rise in IL-10 production by days 6 to 8 postinfestation (740 and 460 pg/ml, respectively) with a decrease toward normal values by day 12. Although the peak of the curve appeared to be delayed in mice exposed to uninfected nymphs, there was no significant difference in IL-10 levels from days 6 to 12 in mice exposed to uninfected ticks (Fig. 1D). Thus, stimulation of high levels of IL-10 in C3H/HeJ mice after exposure to *I. scapularis* did not appear to be dependent on the presence of *B. burgdorferi* within the tick.

Modulation of BALB/c versus C3H/HeJ immunity with exposure to *B. burgdorferi*-infected nymphs. As described above for C3H/HeJ mice, all BALB/c mice in these experiments were exposed to eight *B. burgdorferi*-infected nymphs, which were allowed to feed to repletion. Splenocytes were harvested at time points from days 4 to 12 after tick infestation and were stimulated with mitogen, and supernatants were subsequently analyzed for cytokine production. As noted in Fig. 2, the TH1 cytokines, IL-2 and IFN-γ, were profoundly suppressed, while TH2-associated cytokine production was significantly enhanced in C3H/HeJ over BALB/c mice. In contrast to C3H/HeJ mice, in which IL-2 levels declined to 17% of control values, only a transient decrease in IL-2 levels was noted in BALB/c mice, where production declined to 65% of control values (52,205 ng/ml) by day 8 but recovered to normal levels by day 12 postinfestation. Likewise, IFN-γ production remained normal in BALB/c mice exposed to infected ticks, with only a slight decline to 83% of control values (400 ng/ml) noted by day 8 postinfestation. In contrast, C3H/HeJ mice exposed to

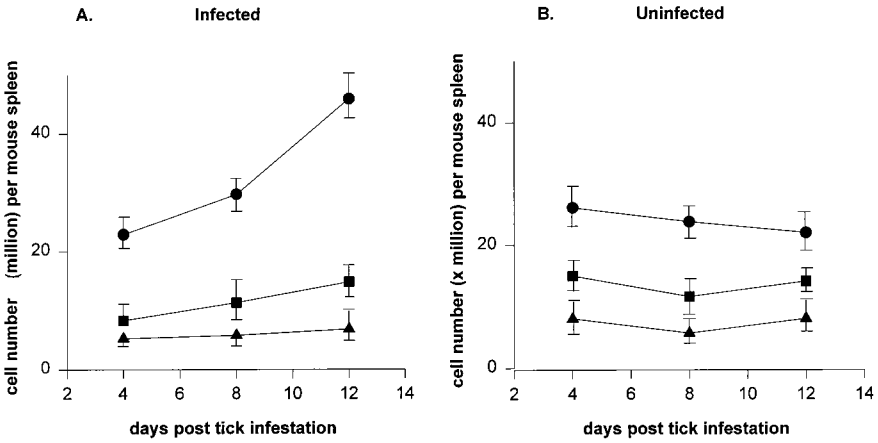

FIG. 3. Flow-cytometric enumeration of splenic lymphoid populations in C3H/HeJ mice infested with *B. burgdorferi*-infected (A) or uninfected (B) nymphal ticks. Splenocytes were harvested on the days indicated and labeled with monoclonal antibody specific for B lymphocytes (circles), $CD4^+$ lymphocytes (squares), or $CD8^+$ cells (triangles). Error bars represent the standard error of the mean for each time point. Five animals were analyzed per time point.

infected ticks produced significantly less IFN-γ throughout the entire 12-day period after tick infestation, although the reduction was statistically significant only on days 10 to 12 postinfestation (Fig. 2B, $P < 0.0048$ on day 10 and $P < 0.0010$ on day 12). In terms of TH2 cytokine production (Fig. 2C), the presence of *B. burgdorferi* in nymphs induced high IL-4 levels in BALB/c mice by day 8 postinfestation (460% of control values; 11.1 pg/ml), which were significantly lower ($P < 0.035$) than those produced in C3H/HeJ mice (3,800% of control values). In terms of IL-10 production (Fig. 2D), no significant difference was noted between BALB/c and C3H/HeJ mice, since peak elevations in both mouse strains occurred on days 6 to 8 postinfestation and returned to just above normal values by day 12. Thus, the complete cytokine profile noted in BALB/c mice exposed to infected nymphs appeared analogous to that in C3H/HeJ mice exposed to uninfected nymphal ticks.

Splenocyte lymphoid populations responsive to *I. scapularis* infestation in C3H/HeJ mice. Splenocytes were harvested from C3H/HeJ mice exposed to either infected or uninfected nymphal ticks, and the total cell numbers were quantified by flow cytometry. Figure 3 demonstrates that mice exposed to infected nymphs displayed a 109% rise in the total number of B cells (22×10^6 versus 46×10^6 cells) over a 12-day period after tick infestation compared to mice exposed to uninfected nymphs, in which the B-cell counts were reduced 14%, from 28×10^6 to 24×10^6 cells by day 12. Likewise, an 88% increase in the number of $CD4^+$ cells (8×10^6 to 15×10^6 cells) was noted in mice exposed to infected nymphs relative to C3H/HeJ mice infested with uninfected ticks (12×10^6 to 12.5×10^6 cells), where cell numbers remained constant. No significant rise in total $CD8^+$ cell numbers was demonstrated in mice exposed to either infected or uninfected nymphs. Cellular profiles detected in BALB/c mice exposed to infected *I. scapularis* were similar to those in C3H/HeJ mice infested with uninfected nymphal ticks (data not shown). Thus, splenocytes in C3H/HeJ mice exposed to infected ticks responded to a rise in IL-4 levels with expansions of both the B and $CD4^+$ cellular compartments.

Cytokine production in *I. scapularis*-infested C3H/HeJ mice depleted of $CD4^+$ and $CD8^+$ lymphocytes. Splenocytes were harvested from C3H/HeJ mice 8 days after tick infestation and stimulated for TH1 and TH2 cytokines as described above. In these experiments, mice were previously treated with monoclonal antibody to deplete specific lymphoid populations 7 days before tick infestation. As noted in Fig. 4B, the source of IFN-γ in these mice was completely dependent on the presence of $CD4^+$ T cells. There was no significant difference in IFN-γ production in mice receiving anti-$CD8^+$ or control Ig. However, for IL-2 (Fig. 4A), both $CD4^+$ and $CD8^+$ T cells contributed to IL-2 production, since mice receiving anti-CD8 antibody produced 46% less IL-2 than did animals receiving control Ig (6,246 versus 13,609 ng/ml). Likewise, in terms of TH2 cytokine production, both $CD4^+$ and $CD8^+$ lymphocytes participated in the production of IL-4 (Fig. 4C). Mice receiving anti-$CD4^+$ antibody produced 6.5 ng/ml, mice which received anti-$CD8^+$ antibody produced 27.2 ng/ml, and mice which were treated with control Ig produced, on average, 57.6 ng/ml of IL-4. Again animals in which $CD8^+$ T cells were depleted produced only 47% of the level of IL-4 produced by the animals receiving control Ig. In terms of IL-10 production (Fig. 4D), elimination of $CD4^+$ cells abolished IL-10 production. However, animals treated with anti-$CD8^+$ antibody produced only 48% of the IL-10 produced by animals receiving control Ig, indicating that other cell types play a role in the release of IL-10 in these mice.

DISCUSSION

The purpose of these studies was to evaluate the role of the vector, *I. scapularis*, and the pathogen, *B. burgdorferi*, in modulating the immune response of the murine host after tick infestation. By concentrating on responses produced by host splenocytes, we investigated the systemic effects of tick feeding. Our results demonstrate that the systemic immune response of the murine host is greatly biased toward a TH2 response during the earliest period of tick infestation and feeding. This polarization toward a TH2 cytokine profile in mice was variable, depending on the relative genetic susceptibility to Lyme disease in the affected host. C3H/HeJ mice, which were shown previously to be highly susceptible to clinical Lyme disease (1, 31, 35), were unable to produce adequate levels of the TH1-associated cytokines, IL-2 and IFN-γ, while production of the TH2-associated cytokines, IL-4 and IL-10, was grossly up regulated compared to that in naive mice. In contrast, BALB/c mice, which were shown previously to be more resistant to the development of clinical disease (1, 35), demonstrated minimal

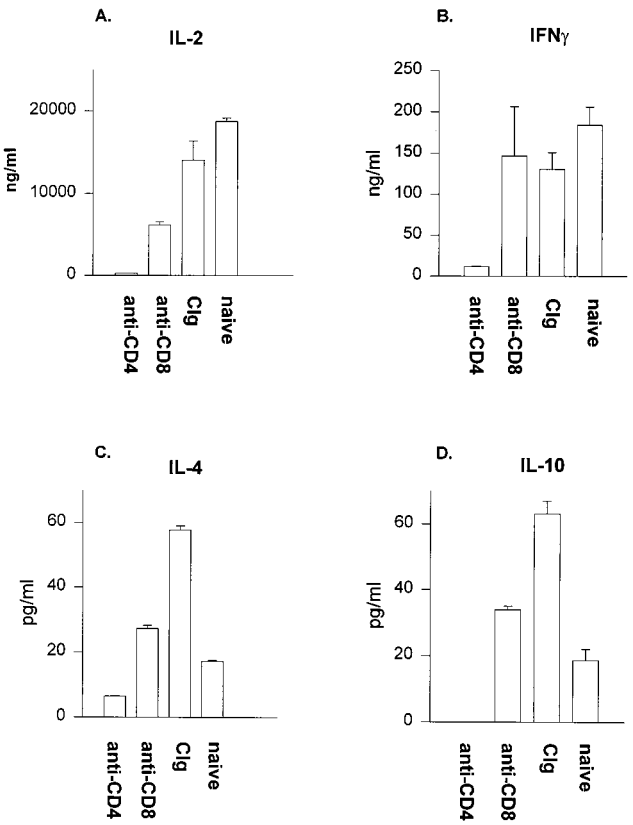

FIG. 4. TH1 (IL-2 plus IFN-γ)-associated and TH2 (IL-4 plus IL-10)-associated cytokine production by splenocytes harvested from C3H/HeJ mice depleted of $CD4^+$ and $CD8^+$ lymphocytes. Individual mice (five per group) received either anti-$CD4^+$, anti-$CD8^+$, or isotype control antibody 7 days prior to exposure to *B. burgdorferi*-infected nymphal ticks. Splenocytes were then harvested 8 days after tick infestation and stimulated with ConA. Supernatants were harvested and analyzed by antigen capture ELISA for specific cytokines; "naive" refers to animals that had not been infested with nymphal ticks prior to splenocyte harvest. Error bars represent the standard error of the mean for each experimental group.

suppression of IFN-γ and IL-2 and only minimal elevations in IL-4 levels, which returned to normal values 12 days after tick infestation. In fact, cytokine regulation in BALB/c mice, infested with infected *I. scapularis*, mirrored that demonstrated for C3H/HeJ mice exposed to uninfected ticks.

Flow-cytometric analysis of splenocytes in C3H/HeJ mice reflected this cytokine polarization, in that both the B-cell and $CD4^+$ lymphocyte compartments increased in number within the spleens of mice exposed to infected *I. scapularis*. These studies suggest that murine B and $CD4^+$ splenocytes, in response to tick feeding and the initial transmission of *B. burgdorferi*, are activated, expand in number, and produce high levels of TH2-associated cytokines in vivo. That *B. burgdorferi* inoculation can potentiate B-cell activation and the release of inflammatory cytokines has been demonstrated previously (34). Yang et al. (34) demonstrated that syringe inoculation of cultured *B. burgdorferi* induced the proliferation of B cells in regional lymph nodes and a persistent and generalized increase in Ig levels, most of which was not directed against *B. burgdorferi* (34). This polyclonal B-cell activation was sevenfold more evident in disease-susceptible (C3H/HeJ) mice than in disease-resistant (BALB/c) mice (34). Likewise, several investigators have implicated the lipid moiety of OspA as the mediator of B-cell mitogenic activity as well as a generalized activator of macrophages in vitro (16, 20, 36). Whether the lipid moiety of OspA plays a role in influencing the initial spirochete load and resultant pathogenesis in target tissues when inoculated by the natural vector has not been demonstrated. However, given that OspA is down regulated during the earliest phases of tick inoculation and no humoral response to OspA is generated within the host (2, 28), its relevance in natural transmission is debatable. Because there may be other *B. burgdorferi*-associated antigens responsible for B- and $CD4^+$-cell activation in C3H/HeJ mice, studies are under way in our laboratory to identify unique antigens responsible for lymphocyte activation in response to vector-transmitted infection.

Although this is the first study to compare both TH1 and TH2 cytokines in vivo in response to both infected and uninfected *I. scapularis*, similar cytokine polarization has been demonstrated by using the primary European vector of Lyme disease, *Ixodes ricinus*. Ganapamo et al. (10) demonstrated increased IL-4 production in parallel with a decline in IFN-γ production by lymphocytes of mice exposed to uninfected *I. ricinus* nymphal ticks. Likewise, in a follow-up study which demonstrated a rise in IL-10 and IL-5 levels with multiple tick infestations, it was concluded that a decrease in T-cell proliferation and suppression of IL-2 and IFN-γ production was due to a concomitant rise in IL-10 levels (11). Our studies indicate that both IL-4 and IL-10 are up regulated in response to tick infestation and transmission of *B. burgdorferi* in C3H/HeJ mice, in parallel with effective suppression of IL-2 and IFN-γ production. Data from our studies also demonstrated that IL-10 production is increased in disease-resistant as well as disease-susceptible mice, indicating that suppression of TH1-associated cytokines induced by *I. scapularis* in this model is not necessarily due to a precipitous rise in IL-10 levels.

The observation that TH1 cytokines are down regulated during the initial spirochete transmission period in C3H/HeJ mice is significant in that previous studies demonstrated that exogenous delivery of the TH1 cytokines, IFN-γ and IL-2, given at the time of tick feeding, could suppress spirochete transmission by *I. scapularis* (37). In these studies, the spirochete-free status of these mice was maintained despite a lack of humoral response to OspA and OspB (37). Previous studies have indicated that the resultant infection and the development of arthritis in mice might be antibody independent (15) and therefore independent of a specific humoral response to OspA and OspB (7, 8). In fact, recent studies by Busch et al. (3) demonstrated a role for $CD8^+$ cytotoxic T cells in the control of Lyme arthritis. In these studies, major histocompatibility complex class I-restricted lysis of *B. burgdorferi*-infected targets could be demonstrated and cloned from human patients only after resolution of the clinical disease (3). All of these studies implicate a major role for cellular immunity in the control of spirochete transmission, especially derived from the natural vector. Our observations regarding the TH2 polarization in C3H/HeJ mice indirectly supports a role for IFN-γ and IL-2 in controlling initial spirochete transmission. In fact, recent studies performed in this laboratory, in which *B. burgdorferi*-pulsed dendritic cells were injected into naive mice prior to tick infestation, ultimately reversed the TH2 polarization in C3H/HeJ mice by enhancing the endogenous release of both IFN-γ and IL-2 during tick infestation. This cytokine reversal led to a block of *I. scapularis*-transmitted *B. burgdorferi* in these mice, underscoring the importance of maintaining TH1 cytokine production at the time of tick feeding (unpublished observations).

Our observations, obtained with vector-transmitted *B. burgdorferi*, are in direct contrast to work published by Keane-Myers et al., in which IL-4 was shown to enhance host resis-

tance to syringe-inoculated *B. burgdorferi* in C3H/HeN mice while increases in IFN-γ production were associated with an increased spirochete burden in target tissues and susceptibility to development of arthritis (14, 15). Likewise, Rao and Frey demonstrated that a cloned $CD4^+$ cell line, derived from mice primed with *B. burgdorferi* antigen and elaborating TH2 cytokines, was able to transfer resistance to infection by needle inoculation into BALB/c mice (25). The contrast in results noted between these studies and those reported here clearly implicate the need for studies with the natural vector to evaluate the immunopathogenesis of this disease process. In this way, the host would not be initially biased toward a humoral response against specific outer surface proteins like OspA, which have potent mitogenic effects and are clearly not recognized early in vector-transmitted disease (20, 28, 36).

The use of the natural vector, *I. scapularis*, suggests that both the vector and the pathogen, *B. burgdorferi*, may have evolved similar mechanisms to manipulate and evade the host immune response. Our data indicate that the process of feeding by uninfected ticks in the susceptible host (C3H/HeJ mice) biases the host toward a TH2 response, which is then exaggerated by the presence of *B. burgdorferi* in the tick. Tick saliva is a potent down regulator of the TH1-associated cytokines, IL-2 and IFN-γ (24, 33) as well as macrophage-associated nitric oxide (24), while *B. burgdorferi* appears to promote the release of IFN-γ, IL-1, and TNF-α (16). Although these studies have demonstrated that *B. burgdorferi* can induce IFN-γ production in vitro (16), our in vivo analysis demonstrates that in the interplay between vector and pathogen modulation of the host, the factors associated with tick feeding (saliva) synergize with *B. burgdorferi* to disarm the susceptible host's initial TH1 response needed to control spirochete transmission after inoculation in the skin. This would suggest that immunization strategies aimed at disarming the ability of both the vector and the pathogen to control the initial cytokine response by the host might be successful at blocking the transmission of *B. burgdorferi* at the vector-host interface (27). To that end, studies in this laboratory have been designed to identify both tick-associated and *B. burgdorferi*-associated antigens that would serve to rearm the host cellular immune response at the time of tick feeding, in order to reverse tick modulation and ultimately control the dissemination of the spirochete.

ACKNOWLEDGMENTS

We thank Christine Happ for expert technical assistance and Kathy Orloski for statistical analysis of the cytokine data.

This work was supported, in part, by the Association of State and Territorial Public Health Laboratory Directors fellowship program for emerging infectious diseases.

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Editor: R. E. McCallum