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Borrelia burgdorferi-Pulsed Dendritic Cells Induce a Protective Immune Response against Tick-Transmitted Spirochetes

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***Borrelia burgdorferi*-pulsed dendritic cells and epidermal cells were able to initiate the production of anti-outer surface protein A (OspA) antibody in vitro with normal T and B cells from either BALB/c or C3H/HeJ mice. Inhibition of anti-*B. burgdorferi* antibody production was observed after 3 days, but not after 2 days, of exposure of the antigen-presenting cells to tumor necrosis factor alpha ± granulocyte-macrophage colony-stimulating factor. Furthermore, splenic dendritic cells pulsed in vitro with live *B. burgdorferi* spirochetes and then adoptively transferred into naive syngeneic mice mediated a protective immune response against tick-transmitted spirochetes. This protection appeared not to be due to killing of spirochetes in the feeding ticks, since ticks fed to repletion on *B. burgdorferi*-pulsed dendritic cell-sensitized mice still harbored live spirochetes. Western blot analysis of the sera collected from dendritic cell-sensitized mice demonstrated that the mice responded to a limited set of *B. burgdorferi* antigens, including OspA, -B, and -C compared to control groups that either had received unpulsed dendritic cells or were not treated. Finally, mice in the early stage of *B. burgdorferi* infection were able to develop anti-OspA antibody following injection with *B. burgdorferi*-pulsed dendritic cells. Our results demonstrate for the first time that adoptive transfer of *B. burgdorferi*-pulsed dendritic cells induces a protective immune response against tick-transmitted *B. burgdorferi* and stimulates the production of antibodies specific for a limited set of *B. burgdorferi* antigens in vivo.**

Borrelia burgdorferi, the agent of Lyme disease, is transmitted by *Ixodes* ticks (4, 33, 34). The clinical manifestations of the disease include erythema migrans, arthritis, carditis, and neurologic disorders. The humoral response to *B. burgdorferi* transmitted by tick bite is characterized by the lack of an antibody (Ab) response to two major outer surface proteins (OspA and OspB) in the early stage of *B. burgdorferi* infection (12, 27). However, Ab specific for some epitopes on OspA and OspB can protect the host against a homologous challenge with the Lyme disease spirochete (6, 7).

It has been shown that the outcome of the immune response of the host to microorganisms can be determined in part by the antigen-presenting cell (APC) type involved in the processing and presentation of antigens (11, 39). In individuals infected with the spirochetes by tick bite, the bacteria are injected into the skin and subsequently disseminate to other tissues (30). Thus, the APCs in the skin (e.g., dendritic cells [DCs]) may regulate the immune response against tick-transmitted microorganisms. DCs are professional APCs specialized to initiate primary T-cell immune responses (35). Previous studies have stressed the significance of different types of APCs in the immune response against *B. burgdorferi*. Bone marrow-derived murine macrophages and the DC line D2SC/1 were shown to support primary and secondary T-cell responses to lipidated OspA (1). However, it was found that only bone marrow-derived macrophages, but not D2SC/1, efficiently present antigens from intact spirochetes to naive or in vivo-primed T cells. In addition, it has recently been reported that human DCs were also able to phagocytose and process live *B. burgdorferi* spirochetes (10). Moreover, blood-derived DCs and dermal DCs were found to harbor the spirochetes, which suggests that DCs contribute to T-cell priming in humans infected with *B. burgdorferi*. However, the role of DCs in the regulation of the immune response against tick-transmitted *B. burgdorferi* has not been investigated.

We report in this study that both murine Langerhans cell (LC)-enriched epidermal cells (ECs) and splenic DCs pulsed with live spirochetes mediate an anti-*B. burgdorferi* Ab response in vitro. Furthermore, mice sensitized with DCs pulsed in vitro with live spirochetes showed a selective Ab response to spirochetal antigens and were protected against a subsequent challenge with tick-transmitted *B. burgdorferi*.

MATERIALS AND METHODS

Mice. Female BALB/c and C3H/HeJ mice 8 to 12 weeks old were purchased from The Jackson Laboratory (Bar Harbor, Maine).

Ticks. Nymphal *Ixodes scapularis* ticks infected with *B. burgdorferi* B31 were reared as previously described (22) and kept at 21°C with saturating humidity until use. The *B. burgdorferi* infection rate for individual nymphal cohorts was over 90% (22).

***Borrelia* strain.** Low-passage *B. burgdorferi* (passage 7) B31 cells were cultured in Barbour-Stoenner-Kelly (BSK) II medium at 33°C (2) and grown to late log phase (10^8/ml) for in vitro antigen pulsing.

APCs. Splenic DCs were isolated as previously described (37). Briefly, spleen cell suspensions (3×10^7/ml) were obtained by teasing spleens with the frosted ends of microscope slides. The cell suspension was separated with dense bovine serum albumin (BSA) columns (density, 1.080 g/ml), and low-density cells at the interface were harvested. DCs were further enriched by plastic adherence and overnight incubation at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Loosely adherent cells were harvested and were determined to contain 70 to 80% DCs, as judged by typical DC morphology under phase-contrast microscopy and specific staining with anti-mouse CD11c.

EC suspensions were prepared as previously described (36) with certain modifications. Briefly, ears were washed in 70% ethanol and air dried, and the two sides were peeled apart. Dermal sides were floated on 0.05% trypsin–1 mM EDTA and incubated for 1 h at 37°C. Epidermal sheets were peeled from the underlying dermis with curved sterile forceps, trimmed into small pieces, and

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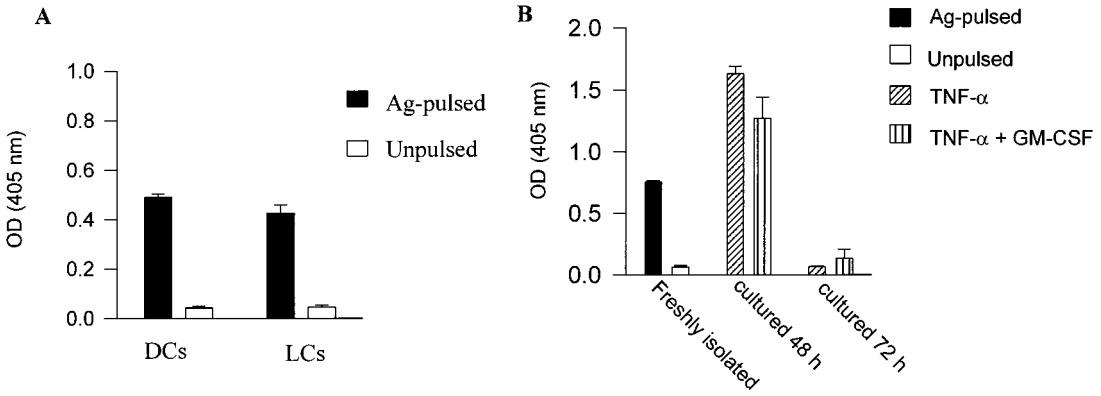

FIG. 1. In vitro anti-*B. burgdorferi* Ab production. (A) Anti-OspA Ab (IgG plus IgM) production in vitro with *B. burgdorferi*-pulsed DCs or LC-enriched ECs from BALB/c mice as APCs (Ag). Ab production was monitored by ELISA as described in Materials and Methods. Mixtures of accessory cell-depleted normal T and B cells from normal BALB/c mice were obtained after passing spleen cell suspensions over Sephadex G-10 columns. (B) Anti-*B. burgdorferi* Ab production in vitro with TNF-α ± GM-CSF-treated DCs from BALB/c mice as APCs. The APCs were exposed for 48 or 72 h to TNF-α ± GM-CSF (10 ng of each per ml). Cells were washed before being pulsed with live *B. burgdorferi* spirochetes. The values represent the means ± standard errors of triplicate cultures. The figure is representative of four independent experiments. OD, optical density.

transferred to a phosphate-buffered saline (PBS) solution containing 2 mg of dispase (GIBCO) per ml. After 20 min of incubation at 37°C, the EC suspension was filtered through a 40-μm-pore-size nylon mesh, washed, and fractionated with a dense BSA column to obtain low-density cells containing 20 to 40% NLDC 145^+ LCs by flow cytometric analysis.

Cell culture for antibody production. Cells were cultured in complete medium, which contained RPMI 1640 (GIBCO BRL Laboratories, Gaithersburg, Md.) supplemented with 10% heat-inactivated fetal calf serum (Hyclone Laboratories, Logan, Utah), 1 mM sodium pyruvate (GIBCO), 0.1 mM nonessential amino acids (GIBCO), 25 mM HEPES (GIBCO), 0.05 mM 2-mercaptoethanol (GIBCO), and 1% penicillin–streptomycin (GIBCO).

The in vitro production of Ab was performed as previously described (19) with several modifications. All incubations were done at 37°C in a humidified atmosphere of 95% air and 5% CO_2. APCs (10^5 per well) were incubated in flat-bottom microculture plates with live spirochetes (1:10 ratio of APCs/spirochetes) in antibiotic-free complete medium for 15 to 18 h and then washed before addition of a mixture of normal T and B cells. Accessory cell-depleted normal splenic T and B cells (approximately 30% $CD4^+$, 13% $CD8^+$, and 49% $B220^+$ by flow cytometric analysis) were isolated by two passages on Sephadex G-10 columns (Pharmacia, Alameda, Calif.) as previously described (20). Cells were added at 10^6 per well and incubated for 5 days with antigen-pulsed APCs. Culture supernatant was collected, centrifuged at 400 × *g*, and stored at −20°C. In several experiments, APCs were cultured with recombinant mouse tumor necrosis factor alpha (rmTNF-α) and/or granulocyte-macrophage colony-stimulating factor (GM-CSF) (10 ng of each/well) (GIBCO) for 2 or 3 days before being pulsed with live *B. burgdorferi* spirochetes.

ELISA. Anti-*B. burgdorferi* Ab levels were determined by enzyme-linked immunosorbent assay (ELISA) by a standard indirect procedure (27) with some modifications. Briefly, recombinant lipidated OspA (kindly provided by J. Frantz, Pfizer, Inc., Lincoln, Nebr.) or *B. burgdorferi* lysate was used to coat Immulon 2 plates (Dynatech, Chantilly, Va.) at 100 ng/well and 1 μg/well, respectively. The wells were blocked with Tris-buffered-saline (TBS) (20 mM Tris base, 150 mM NaCl, 5 mM KCl [pH 7.4]), 0.1% Tween 20, and 3% BSA and sequentially incubated with culture supernatant and alkaline phosphatase-conjugated goat anti-mouse immunoglobulin G (IgG) and IgM (H+L) (1:2,000) (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Md.). The color was developed with *p*-nitrophenyl phosphate (Kirkegaard and Perry Laboratories, Inc.) as a substrate. ELISA results were expressed as the mean value of the A_{405} (minus the mean value of the negative control wells). Absorbance greater than 0.2 was considered positive.

In vivo protection studies. Freshly isolated splenic DCs were pulsed with *B. burgdorferi* as described above, washed extensively in PBS to remove unprocessed spirochetes by differential centrifugation (400 × *g* for 10 min), and then injected (1×10^5 to 5×10^5) intravenously into syngeneic mice. The DC preparation was routinely checked for spirochete contamination by dark-field microscopy to ensure the purity of transferred DCs. Two groups of control mice received the same number of unpulsed DCs and PBS, respectively. Ten days later, mice were challenged with 10 *B. burgdorferi*-infected *I. scapularis* nymphal ticks per mouse. Mice were bled 10 days after DC transfer and 21 days after tick infestation. Infection with *B. burgdorferi* was monitored by culture of ear biopsy specimens in BSK II medium 3 weeks after tick feeding (31). Ten to 15 days after feeding to repletion, *I. scapularis* ticks were crushed in BSK II medium, and the cultures were examined weekly for a period of 3 weeks for the presence of live spirochetes (22).

Western blot analysis. For immunoblotting, 80 μg of *B. burgdorferi* lysate in Laemmli's sample buffer was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (4% stacking and 12% resolving gels) and transferred onto nitrocellulose by use of the Mini-Protean II system (Bio-Rad Laboratories, Hercules, Calif.). After being blocked with 3% BSA in TBS, the membrane was incubated overnight at 4°C with mouse sera diluted 1/100 in TBS–0.05% Tween 20, followed by incubation with alkaline phosphatase-conjugated (Kirkegaard and Perry Laboratories, Inc.) goat anti-mouse IgG + IgM (H+L) diluted 1/1,000 in the same buffer for 1 h. The membrane was developed with a 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium reagent (Kirkegaard and Perry Laboratories, Inc.). Several washes were performed between all incubations. Monoclonal Abs specific for OspA, OspB, OspC, and flagellin (Harlan Laboratories, Indianapolis, Ind.) were used as positive controls.

Flow cytometry. T and B cells isolated from Sephadex G-10 columns were directly stained in PBS–0.5% BSA for 30 min on ice with phycoerythrin-conjugated anti-CD4 (clone H129.19), fluorescein isothiocyanate (FITC)-conjugated anti-CD8α (clone 53.-6.7), and FITC-conjugated anti-CD45R/B220 (clone RA3-6B2) (PharMingen, San Diego, Calif.). Rat anti-mouse CD16/CD32 (clone 2.4G2) (PharMingen) was first used to block nonspecific binding. Splenic DCs were stained with biotinylated anti-mouse CD11c (clone HL3 [PharMingen]), followed by FITC-conjugated avidin. Epidermal LCs were stained with the monoclonal antibody NLDC-145 (Bachem Bioscience, Inc., King of Prussia, Pa.), followed by goat anti-rat IgG-FITC. Cells were analyzed on an EPICS Profile cell sorter (Coulter Corp., Miami, Fla.). At least 10,000 events were scored.

RESULTS

DCs induce the production of anti-OspA Ab in vitro. *B. burgdorferi* organisms transmitted via tick bite reside in the skin for several days before disseminating to specific target organs (30). Therefore, to explore the hypothesis that skin DCs or splenic DCs might play a role in eliciting a specific Ab response to *B. burgdorferi* antigen, the following studies were conducted. Splenic DCs or LC-enriched ECs were pulsed with live *B. burgdorferi* spirochetes and were used to stimulate a mixture of syngeneic normal T and B cells from either BALB/c or C3H/HeJ mice. Aliquots of the culture supernatant were removed, and levels of anti-OspA Abs were measured. As shown in Fig. 1A, anti-OspA Abs were generated when DCs and LC-enriched ECs from BALB/c mice were used as APCs. The possibility that this response may be attributed to LPS is unlikely, because T and B cells isolated from the lipopolysaccharide-resistant mouse strain C3H/HeJ were also able to support the production of anti-OspA Ab in vitro (data not shown). DCs cultured for 48 h with rmTNF-α ± GM-CSF before being pulsed with *B. burgdorferi* enhanced production of *B. burgdorferi*-specific Ab (Fig. 1B). In contrast, DCs cultured for 72 h in the presence of rmTNF-α ± GM-CSF significantly inhibited

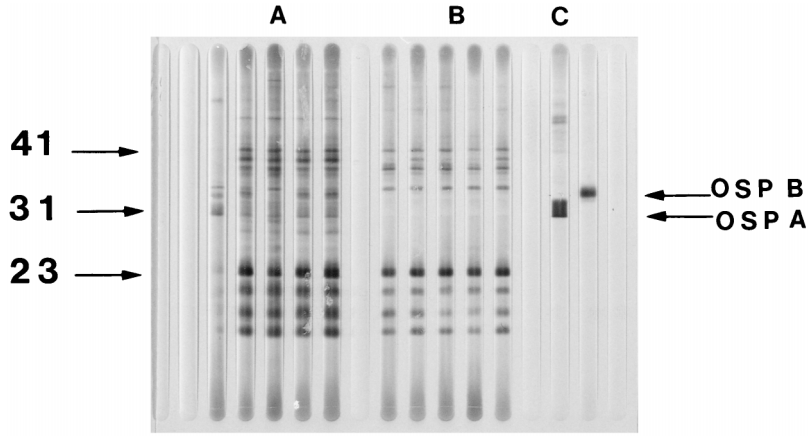

FIG. 2. Representative Western blot analysis (IgG plus IgM) of serum samples from BALB/c mice exposed to *B. burgdorferi* by tick bite prior to injection with DCs. Five mice were injected intravenously with 10^5 *B. burgdorferi*-pulsed DCs per mouse (A), and five mice received a similar concentration of nonpulsed DCs (B). (C) Anti-OspA- and -OspB-positive control. Numbers to the left indicate molecular mass markers in kilodaltons. The figure is representative of four independent experiments.

anti-*B. burgdorferi* Ab production in vitro (Fig. 1B). The same phenomenon was seen when LC-enriched ECs were used as APCs (data not shown).

Antigen-pulsed DCs elicit an anti-OspA Ab response in mice with early *B. burgdorferi* infection. Mice infected with *B. burgdorferi* via tick bite do not develop detectable levels of Ab against OspA until late in infection (12, 27). To test whether T and B cells in tick-infected mice could be primed for anti-OspA Ab production, we adoptively transferred *B. burgdorferi*-pulsed splenic DCs into syngeneic BALB/c recipients that had been infected 6 weeks earlier by tick bite. Western blot analysis of sera of mice collected before sensitization with DCs showed no detectable Ab against OspA (Fig. 2B), which is in agreement with previous studies (12, 27). In contrast, following injection of *B. burgdorferi*-pulsed DCs, the sera of mice reacted with a 31-kDa protein by Western blotting (Fig. 2A) and with recombinant lipidated OspA by ELISA (data not shown). However, the mice remained infected with *B. burgdorferi* after DC transfer (data not shown).

Antigen-pulsed DCs elicit a protective immune response against tick-transmitted spirochetes in normal mice. The ability of DCs to present spirochetal antigens in vitro (Fig. 1A) and to elicit a humoral response in *B. burgdorferi*-infected mice prompted us to examine whether *B. burgdorferi*-pulsed DCs could prime naive mice and induce protective immunity to tick-borne *B. burgdorferi* infection. In two separate studies, BALB/c and C3H/HeJ mice receiving syngeneic DCs pulsed with live *B. burgdorferi* spirochetes in vitro were protected against tick-transmitted spirochetes (Table 1), because culture of ear biopsy specimens from DC-sensitized mice remained negative, while positive cultures were derived from untreated mice and mice treated with unpulsed DCs (Table 1).

Ten days after DC sensitization, the sera of mice treated with *B. burgdorferi*-pulsed DCs were tested by Western blotting and reactivity to *B. burgdorferi* proteins migrating in the 20-kDa, 23-kDa (OspC), 31-kDa (OspA), and 34-kDa (OspB) ranges was demonstrated, while some faint bands were noted in the 37- and 60-kDa ranges (Fig. 3A). Three weeks postchallenge with *B. burgdorferi*-infected ticks, the Western blot profile from the sera of mice injected with *B. burgdorferi*-pulsed DCs revealed four major bands migrating in the 20-kDa, 23-kDa (OspC), 31-kDa (OspA), and 34-kDa (OspB) ranges (Fig. 4A). Sera from nonpulsed DC-treated mice and untreated mice did not react with OspA and OspB and showed similar Western blot profiles (Fig. 4B and C).

Thus, in our study, *B. burgdorferi*-pulsed DCs elicited Abs against a restricted set of *B. burgdorferi* antigens. For example, Abs to the 41-kDa (flagellin) and 15- to 18-kDa ranges of proteins, readily detected upon infection with *B. burgdorferi* (Fig. 4B and C), were not produced by sensitization with *B. burgdorferi*-pulsed DCs (Fig. 3A). Finally, we also found that ticks fed on *B. burgdorferi*-pulsed DC-treated mice still harbored live spirochetes, as demonstrated by our ability to culture *B. burgdorferi* from replete ticks (Table 1).

DISCUSSION

We investigated the role of specialized APCs, splenic DCs, and LCs in the initiation of the immune response against *B. burgdorferi*. Our in vitro studies demonstrated that both DCs and LCs were able to support the production of anti-OspA Ab. However, the production of anti-*B. burgdorferi* Ab was significantly inhibited after a 3-day exposure of the APCs to rmTNF-α ± GM-CSF. Therefore, *B. burgdorferi* processing and/or presentation by DCs could be inhibited by these cytokines in vitro. TNF-α and GM-CSF were shown in previous studies to induce the maturation of DCs in vitro (15, 29).

TABLE 1. Injection of C3H/HeJ mice with *B. burgdorferi*-pulsed DCs induces protection against challenge with *B. burgdorferi*-infected ticks

Mouse treatment group	No. of infected mice/total no. of mice[a]	Presence of live spirochetes in culture of ticks[b]	Reactivity to[c]:		
			OspA	OspB	Flagellin
B. burgdorferi-pulsed DCs	0/10	+	+	+	−
Nonpulsed DCs	10/10	+	−	−	+
Untreated	10/10	+	−	−	+

[a] Ear biopsy specimens were taken and cultured in medium for 3 weeks as described in Materials and Methods.

[b] Replete ticks were homogenized in BSK II medium, and the cultures were examined weekly for 3 weeks for the presence of live spirochetes.

[c] Reactivity of mouse sera to *B. burgdorferi* antigens analyzed by Western blotting. The same results were obtained with BALB/c mice.

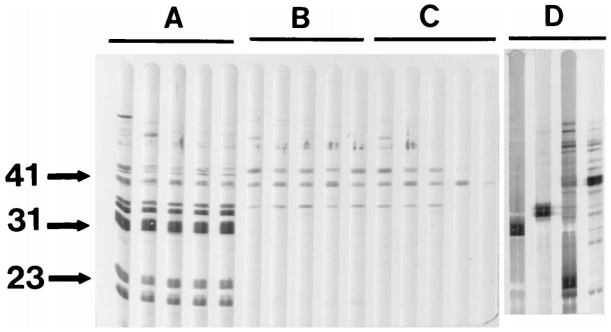

FIG. 3. Representative Western blot analysis (IgG plus IgM) of serum samples from C3H/HeJ mice 10 days after transfer with *B. burgdorferi*-pulsed DCs. Five mice were injected with *B. burgdorferi*-pulsed DCs (A), five mice received nonpulsed DCs (B), and five mice were left untreated (C). (D) Positive control with anti-OspA, -OspB, -OspC, and -flagellin monoclonal Abs from left to right. Numbers to the left indicate molecular mass markers in kilodaltons. The figure is representative of seven independent experiments.

Mature DCs were no longer able to process native antigens in vitro as opposed to freshly isolated, immature DCs (28). Both LCs and splenic DCs were shown to undergo similar phenotypic and functional changes in vitro (14). Our observations may have in vivo relevance with regard to the initial inflammatory response at the tick feeding site. In previous studies, TNF-α was detected at the tick attachment site 12 h post-tick attachment (21), suggesting that cytokines produced locally at the tick attachment site may have profound influence on the function of skin LCs. Considering that *Ixodes* ticks remain attached for at least 36 h before the spirochetes are injected into the skin (23), local production of TNF-α, as well as other cytokines, which precedes the deposition of the spirochetes, may impair the ability of LCs to process and present native antigens transmitted by the feeding tick by several mechanisms. First, a partial depletion of LCs at the tick feeding site is likely to occur. This is supported by previous findings showing the involvement of TNF-α in providing signals for migration of LCs to lymphoid tissue. Depletion of up to 30% of LCs was reported 2 h after injection of rmTNF-α into ear skin of mice (5). Secondly, in addition to its ability to induce the migration and maturation of LCs (15, 29), loss of immunogenic peptides by LCs after exposure to TNF-α has been reported (17). Finally, downregulation of the function of LCs by the saliva of the tick cannot be excluded with respect to the established immunosuppressive role of some proteins in the tick saliva (38). Whether one or several mechanisms are at work in our system remains to be elucidated.

Previous studies have demonstrated that antigen-pulsed DCs can prime naive T cells and also enhance the Ab response to soluble proteins in vivo (3, 32). The studies presented here demonstrated that DCs pulsed with live spirochetes in vitro and then adoptively transferred into naive syngeneic mice were able to induce a protective immune response against tick-transmitted spirochetes. The possibility of coinjection of intact *B. burgdorferi* spirochetes during DC transfer was unlikely because of the selective Ab response observed in DC-sensitized mice. For example, Abs against 15- and 18-kDa proteins, readily detected with naturally transmitted spirochetes (Fig. 4), were not present in mice sensitized with *B. burgdorferi*-pulsed DCs (Fig. 3 and 4). Protection of mice against transmission of *B. burgdorferi* by the natural vector was associated with selective production of Ab against *B. burgdorferi* antigens, specifically OspA, OspB, OspC, and p20. This observation raises the distinct possibility of a selective processing of *B. burgdorferi* antigens by DCs, although further studies are needed to elucidate the mechanisms underlying this phenomenon. A distinct set of proteases involved in the degradation of *B. burgdorferi* antigens within DCs may provide an explanation for this phenomenon, as previously described in another experimental system (25).

Abs against OspA, -B, and -C were shown to mediate a protective immune response against *B. burgdorferi* infection by blocking the transmission of the spirochetes by ticks (9, 13). In the study outlined here, blocking of transmission of spirochetes appeared qualitatively different from that reported previously (6, 9); that is, we found that ticks fed on mice sensitized with *B. burgdorferi*-pulsed DCs still harbored live spirochetes, as demonstrated by positive cultures derived from replete ticks. It is possible that anti-OspC Abs are involved in the protection we observed. Mice actively immunized with OspC were shown to be protected against homologous challenge with spirochetes in spite of the lack of direct cytolytic activity of the anti-OspC Abs generated (24). Those authors suggest that a complement-independent mechanism of protection, such as enhancement of opsonization, may operate in that system. A protective immune response against tick-transmitted *B. burgdorferi*, independent of killing of the spirochetes within the ticks, was also reported in mice actively immunized with recombinant OspC (13). The precise mechanism of protection in *B. burgdorferi*-pulsed DC-sensitized mice has not been fully elucidated. However, *B. burgdorferi*-pulsed DCs delivered to mice that had been previously naturally infected with *B. burgdorferi* did not change the spirochetal load despite the different antigenic profile (e.g., the presence of anti-OspA Ab) observed. This observation suggests that the immune response induced by *B. burgdorferi*-pulsed DCs protected the mice against *B. burgdorferi* infection only during the initial phase of interaction between the tick and its host.

Given the ability of DCs to induce both a primary T-cell response and to enhance antigen-specific Ab in vivo (3, 32), the mechanisms of protection against *B. burgdorferi* infection in mice sensitized with *B. burgdorferi*-pulsed DCs may involve both cellular and humoral components of the immune response. Likewise, both antibody-dependent and antibody-inde-

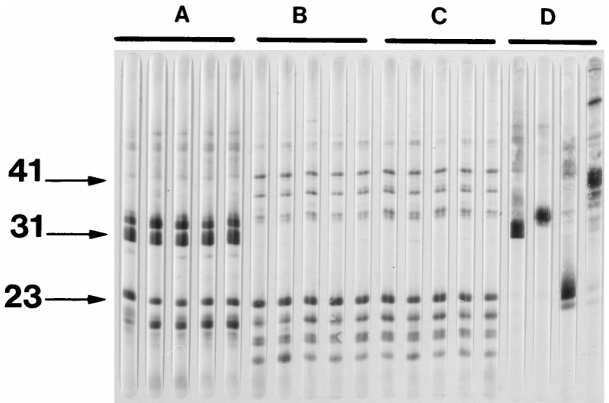

FIG. 4. Representative Western blot analysis (IgG plus IgM) of serum samples from C3H/HeJ mice 3 weeks after challenge with *B. burgdorferi*-infected ticks. Five mice received *B. burgdorferi*-pulsed DCs and were challenged with *B. burgdorferi*-infected ticks 10 days later (A), five mice received nonpulsed DCs followed by challenge with ticks 10 days later (B), and five mice were challenged with ticks (C). (D) Positive control with anti-OspA, -OspB, -OspC, and -flagellin monoclonal Abs from left to right. Numbers to the left indicate molecular mass markers in kilodaltons. The figure is representative of seven independent experiments.

pendent mechanisms were previously demonstrated to operate in immunity against Lyme disease (16). These studies demonstrated that treatment with recombinant interleukin-4 significantly reduced the severity of *B. burgdorferi* infection in an Ab-independent manner. In addition, adoptive transfer of a *B. burgdorferi*-specific $CD4^+$, Th2 T-cell clone was able to mediate protective immunity to experimental *B. burgdorferi* infection (26), implicating a role for T cells and their related cytokines in the development of a protective immune response against *B. burgdorferi.*

In conclusion, our results demonstrate for the first time that adoptive transfer of *B. burgdorferi*-pulsed DCs stimulates the production of specific Ab against a limited set of *B. burgdorferi* antigens in vivo and induces a protective immune response against tick-transmitted *B. burgdorferi.*

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