Cutting Edge: CD1d Deficiency Impairs Murine Host Defense Against the Spirochete, *Borrelia burgdorferi*

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*J Immunol* 2000; 165:4797-4801; doi: 10.4049/jimmunol.165.9.4797
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# Cutting Edge: CD1d Deficiency Impairs Murine Host Defense Against the Spirochete, *Borrelia burgdorferi*

Hemant Kumar,* Alexia Belperron, * Stephen W. Barthold, † and Linda K. Bockenstedt‡*

| CD1 molecules can present microbial lipid Ag to T cells, suggesting that they participate in host defense against pathogens. In this study, we examined the role of CD1d in resistance to infection with the Lyme disease spirochete, *Borrelia burgdorferi* (Bb), an organism with proinflammatory lipid Ag. Bb infection of CD1d-deficient (CD1d<sup>−/−</sup>) mouse strains normally resistant to this pathogen resulted in arthritis. Pathology correlated with an increased prevalence of spirochete DNA in tissues and enhanced production of Bb-specific IgG, including IgG to Ag rapidly down-modulated on spirochetes in vivo. CD1d<sup>−/−</sup> mice exhibited high-titer Bb-specific IgG2a, an isotype commonly induced in disease-susceptible mice but not in the disease-resistant control mice in this study. These results show that CD1 deficiency impairs host resistance to a spirochete pathogen, and are the first example of a mutation that imparts Bb-resistant mice with the Ab and disease profile of a susceptible mouse strain. The Journal of Immunology, 2000, 165: 4797–4801. |

T he CD1 family of transmembrane glycoproteins comprises a unique group of antigen-presenting molecules that bind and present lipid Ag (1). Several CD1 genes exist in humans (CD1a, -b, -c, -d, and -e) and in mice (CD1d1 and CD1d2), all of which display limited polymorphism and Ag binding pockets that favor the presentation of hydrophobic motifs. CD1 molecules have been shown to bind foreign lipid Ag from infectious pathogens and marine sponge as well as self phospholipid Ag molecules have been shown to bind foreign lipid Ag from infecting pockets that favor the presentation of hydrophobic motifs. CD1 exist in humans (CD1a, -b, -c, -d, and -e) and in mice (CD1d1 and CD1d2), all of which display limited polymorphism and Ag binding pockets that favor the presentation of hydrophobic motifs. CD1 molecules have been shown to bind foreign lipid Ag from infectious pathogens and marine sponge as well as self phospholipid Ag (1–6). CD1-restricted T cells have been identified and may serve a variety of functions that range from surveying against autoimmune and tumor development (reviewed in Ref. 1) to participating in the host response to infectious pathogens (6–11). Although such studies strongly implicate CD1-mediated immunity in protection against intracellular pathogens, a role for CD1 molecules in host defense against extracellular microbial agents has not yet been established.

Mouse CD1d is constitutively expressed on antigen-presenting cells and thymic epithelium (12), where it is believed to play a role in selection of NK T cells based on the absence of this subset in CD1d<sup>−/−</sup> mice (13–15). However, the highest level of expression is found on splenic marginal zone B (MZB)<sup>3</sup> cells, a minor subset of B cells that are a source of Ab to T-independent Ag (12, 16). This noncirculating B cell subset is confined to the spleen in a region adjacent to the marginal sinuses, where it is in a position to sample bloodborne Ag (17, 18). Whether CD1d facilitates MZB cell activation and/or production of Ab by this cell population is unknown.

The Lyme disease spirochete, *Borrelia burgdorferi* (Bb), is an example of a pathogen for which Ab to Bb lipoproteins, and not T cells, are critical for early protective immunity (19). In the mouse model of Lyme borreliosis, protective Ab are directed toward Bb outer surface membrane lipoproteins, which are recognized B cell mitogens (20). Passive immunization of infected B cell-deficient (μMT) or SCID mice with immune serum can reduce spirochete numbers in tissues and attenuate arthritis, which is a principal disease manifestation in this model (19). This protective and disease-modulating immunity can arise independently of conventional T cell helper function, as has been shown by the presence of effective immunity in mice deficient in the B cell costimulatory molecule CD40 ligand expressed on T cells (21) or lacking MHC class II and CD4<sup>+</sup> T cells (22).

Bb lipoproteins and lipopeptides activate innate immune cells and may be the major spirochete component leading to disease manifestations (23). The lipid moiety, common to all spirochete lipoproteins, is formed by the posttranslational attachment of three palmitoyl residues to the N-terminal glycosylcysteine residue of the polypeptide chain (24). This N-terminal tripalmitoyl cysteine moiety shares structural similarity to lipid and glycolipid Ags from other microorganisms which are known to bind CD1 molecules (1). Because Bb contains lipoprotein Ags that have the potential to bind CD1, Bb infection could provide a model for assessing the role of CD1d in the response to extracellular bloodborne pathogens.

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1 This work was supported by National Institutes of Health Grants AR42637 and AI 38339, and the Arthritis Foundation.

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3 Abbreviations used in this paper: MZB, marginal zone B; Bb, *Borrelia burgdorferi*; NMS, normal mouse serum; Bb, C57BL/6; B6, C57BL/6; cN40, cloned Bb strain N40; LCMV lymphocyte choriomeningitis virus; GT, glutathione transferase.

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for which Ab, but not T cells provide host immunity. In this study, we examined the immune responses and evolution of disease in CD1d-deficient (CD1d-/-) mice experimentally infected with Bb.

Materials and Methods

Mice

C57BL/6 (B6) x 129 (B6129) CD1d-/- mice (F2 generation) produced by targeted disruption of the CD1d1 gene were the kind gift of Dr. Luc van Kaer (Vanderbilt University School of Medicine and the Howard Hughes Medical Institute, Memphis, TN; Ref. 15). B6.CD1d-/- mice (N7-N9 generation), derived from ES line of 129/Ola origin and used after six to eight backcrosses to B6, were generously provided by Dr. Albert Bendelaar (Princeton University, Princeton, NJ; Ref. 25). Control mice included age-matched heterozygote littermates and B6129 CD1d+/+ (F2 generation), Taconic Laboratories, Germantown, NY). Mice were housed and fed in microisolator cages according to Yale University institutional animal care and use guidelines.

Infection of mice

Low passage, cloned Bb strain N40 (cN40) spirochetes were expanded in modified Barbour-Stoenner-Kelly medium. Infection status of mice was evaluated at the day 1 time point (pooled data, Fisher exact test, p < 0.0001).

PCR of Bb DNA

DNA was isolated from urinary bladders according to standard methods (28). The Bb plasmid encoded ospA gene and the eukaryotic tubulin gene were amplified by PCR in a total reaction volume of 50 μl containing 1 μg DNA template, 25 μl each primer, 20 mM MgCl2, and 0.5 μl Taq (Qiagen, Valencia, CA). The following primer pairs were used: Osp A sense primer 5’AAAACAGGGTTCACTGACACATTCG3’ and anti-sense primer 5’CAACTGCTGACCCCTCTAATTTGGTGCC3’; tubulin sense primer 5’GGGCCCCTCTCTTGTATGGGCTTGGACCAAA3’ and antisense primer 5’CAGGCTGGTCAATGTGGCAACCAGATCGGT3’. Amplification of targeted DNA sequences was performed on the Robocycler Gradient 40 (Stratagene, La Jolla, CA) with 40 cycles of denaturation at 95°C for 1.5 min, annealing at 60°C for 45 s and amplication at 74°C for 1.5 min, followed by a 4-min extension at 73°C. For tubulin amplification, PCR was conducted for 35 cycles with denaturation at 94°C for 45 s, annealing at 60°C for 45 s and amplification at 74°C for 1.5 min, followed by a 10-min extension at 74°C. Amplified products were separated by 2% agarose gel electrophoresis and visualized by ethidium bromide staining.

ELISA and immunoblot

ELISA was performed using microtiter plates (ICN Biomedicals, Aurora, OH) coated with 2.5 μg/well Bb lysate or the indicated purified recombinant Bb proteins (29). Recombinant lipopolysaccharide-binding protein A (lpp-DpbPα, a gift from MedImmune, Gaithersburg, MD), recombinant delipidated Osp A produced as a fusion protein with glutathione transferase (GT)-Osp A or cleaved from GT (rOsp A), and recombinant delipidated GT-Osp B were purified as described (30–32). Bb Ab endpoint-titers in serial 2-fold serum dilutions were determined by ELISA using alkaline phosphatase-conjugated anti-mouse IgM, IgG, or IgG isotypes and the Vector Elite visualization kit (Vector Laboratories, Burlingame, CA). OD values > 2 SD from the mean of negative samples were considered positive. For immunoblot analysis, proteins were separated by SDS-PAGE and transferred to nitrocellulose (33). Seven-day-infected mouse sera were used at 1:50 dilution, whereas 14-day-infected mouse sera were used at 1:100 dilution. Where indicated, Osp A mAb VIIC3.78 (34), Osp B mAb VIIB10.36 (31), or mouse polyclonal antisera to lppDpbPα-N40 (32) were incubated with individual nitrocellulose strips to mark the location of specific Bb Ab. Bound Bb Ab were detected using alkaline phosphatase-conjugated goat anti-mouse IgG (Vector Laboratories) and visualized with 5-bromo-4-chloro-3-indolyl phosphate p-toluidine and nitroblue tetrazolium substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD).

Results

CD1d deficiency renders disease-resistant mice susceptible to arthritis

C57BL/6 (B6) and 129 mice are highly resistant to disease manifestations associated with Bb infection, and intercrosses of these mouse strains remain disease-resistant (35). We examined the influence of CD1d molecules in knockout mice on these resistance backgrounds after intradermal inoculation with 10^6 cN40. As expected, infected control mice were resistant to disease and showed no joint or soft tissue abnormalities at any of the three time points (4, 7, and 14 days) analyzed. In contrast, within 7 days CD1d-/- mice developed arthritis that progressed in severity by day 14 (Table I). Joint infiltrates were typical of murine Lyme arthritis, with soft tissue edema and neutrophilic infiltrates of tendon sheaths and joint synovium (Ref. 35; data not shown). Acute inflammation was also present on histopathologic examination of the skin inoculation site in CD1d-/- but not similarly infected control mice at infection days 4, 7, and 14. In dose-response studies, CD1d-/- mice seroconverted to Bb at 10-fold lower inoculum than control mice (data not shown).

Spirochete DNA is more prevalent in urinary bladders of CD1d-/- mice

PCR amplification of Bb DNA was performed to assess whether spirochetes disseminate from skin to urinary bladders at a different rate in the absence of CD1d. DNA for the Bb ospA gene was more prevalent in urinary bladders of 7-day-infected CD1d-/- mice when compared with infected control mice (Table II). In one experiment, Osp A DNA could be detected as early as day 4 in CD1d-/- mice. Blood cultures were negative in both groups of

Table I. Bb-infected CD1d-/- mice are susceptible to arthritis

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Infection day 4</th>
<th>Infection day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>B6129.CD1d-/-</td>
<td>0/5</td>
</tr>
<tr>
<td>2</td>
<td>B6129.CD1d-/-</td>
<td>0/5</td>
</tr>
<tr>
<td>1</td>
<td>B6129.CD1d-/-</td>
<td>0/5</td>
</tr>
<tr>
<td>2</td>
<td>B6129.CD1d-/-</td>
<td>0/5</td>
</tr>
</tbody>
</table>

* Amplified PCR products were visualized by agarose gel electrophoresis using ethidium bromide. Results are reported as the number of urinary bladders testing positive over the total number of samples tested. Statistical significance was achieved at the day 7 time point (pooled data, Fisher exact test, p = 0.003).
Table III. Reciprocal end point ELISA titers for IgG isotypes in Bb-infected CD1d<sup>−/−</sup> vs control mouse sera<sup>a</sup>

<table>
<thead>
<tr>
<th></th>
<th>IgG1</th>
<th>IgG2a&lt;sup&gt;b&lt;/sup&gt;</th>
<th>IgG2b</th>
<th>IgG3</th>
<th>Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-day-infected B6.CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>50</td>
<td>&lt;50</td>
<td>50</td>
<td>50</td>
<td>Bb lysate</td>
</tr>
<tr>
<td>7-day-infected B6.CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>800</td>
<td>6400</td>
<td>3200</td>
<td>800</td>
<td>LppDbpA</td>
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<tr>
<td>14-day-infected B6.CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>800</td>
<td>&lt;50</td>
<td>3200</td>
<td>3200</td>
<td></td>
</tr>
<tr>
<td>7-day-infected B6.CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>100</td>
<td>&lt;50</td>
<td>200</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>7-day-infected B6.CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>400</td>
<td>6400</td>
<td>3200</td>
<td>3200</td>
<td></td>
</tr>
<tr>
<td>14-day-infected B6.CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>800</td>
<td>&lt;50</td>
<td>3200</td>
<td>3200</td>
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</tr>
<tr>
<td>7-day-infected B6.CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>&lt;50</td>
<td>&lt;50</td>
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<tr>
<td>7-day-infected B6.CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>400</td>
<td>3200</td>
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<sup>a</sup> Serum end point ELISA titers were determined for the indicated IgG isotypes using either 2.5 μg/well Bb lysate, 2.5 μg/well LppDbpA, or 2.5 μg/well GT-Osp A. Results depicted were derived from a single mouse/group and are representative of results from two experiments using five mice/group. Values for serum from 14-day-infected B6.CD1d<sup>−/−</sup> mice were provided for comparison. Differences among isotype titers derived from B6.CD1<sup>−/−</sup> and B6.CD1d<sup>−/−</sup> mice were significant (Student’s t test, p < 0.001).

<sup>b</sup> The isotype-specific secondary Ab binds both IgG2a and IgG2c, which share 84% sequence homology at the amino acid level.
Spirochete lipoprotein expression and the rate at which the immune system clears lipoprotein Ag may be critical parameters determining extent and duration of disease. In this study, absence of CD1d permitted the development of typical Lyme arthritis in mice normally resistant to Bb-induced disease. Although an increased pathogen burden could account for these findings, spirochete numbers in joints do not always correlate with arthritis severity (38). An alternative explanation for our findings is that Bb lipoproteins may not be eliminated as readily in the absence of CD1d. Bb-infected CD1d−/− mice developed IgG to Osp A, a lipoprotein dominantly expressed on cultured spirochetes but rapidly lost in vivo (33). Mice only seroconvert to Osp A when inoculated with large numbers of spirochetes (39), suggesting that transiently expressed lipoproteins may be efficiently removed by phagocytes and Ab without the development of detectable serum Ab; in the absence of CD1d, such clearance mechanisms may be impaired. Both spirochete phenotypic change and Osp A Ag clearance must occur rapidly in the control mice used in the present study, because neither IgM nor IgG directed against Osp A could be detected in their serum at any time.

Although CD1d-deficiency rendered the disease-resistant mice used in this study susceptible to enhanced IgG and pathology similar to Bb-susceptible mice, it remains unclear why CD1d is not sufficient to prevent disease in the latter group. The mechanism through which CD1d impacts on Bb infection and disease in the mice used in this study may involve CD1d presentation of Bb lipid Ag to NK T cells. Indeed, Bb lppDbpA but not delipidated DbpA can compete with α-galactosylceramides for CD1d-binding sites and inhibit activation of a CD1d-restricted NK T cell hybridoma (L. Bockenstedt, K. Benlagha, A. Bendelac, unpublished observations), providing evidence that CD1d can bind lipidated Bb Ag. However, distal effects of NK T cells on CD4+ T cell and 2 cell subset differentiation cannot explain our findings. B6 mice that lack either αβ T cells (TCR α−/− mice) (3) or TCR β−/−δ− (40) or MHC class II (22) do not exhibit more severe arthritis (22), and interruption of the B7/CD28 T cell costimulatory pathway (29) or absence of CD40 ligand (21) does not enhance arthritis.

Because Ab that arise in the absence of T cell help are sufficient to protect against Bb infection, we were intrigued by the fact that CD1d is constitutively expressed at high levels on splenic MZB cells, a source of inducible Ab to T-independent Ag. CD1d or CD1d-restricted NK T cells could facilitate the production of Bb-specific Ab by the MZB cell population. Paradoxically, CD1d−/− mice produced higher titer Ab to Bb Ag early after infection, suggesting that MZB cell Ab can be induced. Rapid production of these Ab may be required within the first hours to days of infection to limit spirochete burden and to eliminate pro-inflammatory Ag. This period was not examined in our study.

Our results parallel the effects of absence of natural Ab on experimental infection of mice with lymphocyte choriomeningitis virus (LCMV). I.v. injection of LCMV into B6 μMT mice leads to increased viral burden, enhanced CTL responses, and loss of splenic architecture when compared with similarly infected control mice (41). In that system, natural Ab and complement are important for trapping LCMV Ag in the marginal zone of the spleen. In the absence of Ab to initially control pathogen burden, stronger T cell responses are elicited resulting in secondary pathology. In the case of Bb infection, an impaired early host defense leads to the induction of stronger Ab responses, but this occurs too late to prevent disease.

Previously, the role of CD1 in the host response to infectious agents had been best characterized for intracellular pathogens. Our study is the first demonstration that CD1d deficiency impairs resistance to disease due to an extracellular pathogen. Further work is in progress to elucidate CD1d-mediated immune events during Bb infection.

Note added in proof. The immunological changes noted in Bb-infected CD1d−/− mice are both spirochete dose and mouse age dependent, as has been shown for disease-susceptible mouse strains (27).

Acknowledgments

We are grateful to Drs. Joseph Craft, Charles Janeway, Mark Manulis, and Ruth Montgomery for helpful review of the manuscript and to Debbie Beck and Jailing Mao for excellent technical assistance.

References


