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Cutting Edge: Anti-CD1 Monoclonal Antibody Treatment Reverses the Production Patterns of TGF-β2 and Th1 Cytokines and Ameliorates Listeriosis in Mice[1]

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Protection against intracellular bacteria by T cells is regulated by Ag-presenting molecules, which comprise classical MHC class I molecules, MHC class II molecules, and nonclassical MHC class Ib molecules. The role of CD1 molecules, which are structurally similar to classical MHC class I gene products, but less polymorphic, is not understood so far. We show that CD1 surface expression increased on APC in *Listeria*-infected mice. The in vivo treatment with anti-CD1 mAb reduced TGF-β2 levels and concomitantly increased secretion of the proinflammatory cytokine TNF, the Th1 cell promoting cytokine IL-12, and the Th1 cell cytokine IFN-γ at the onset of listerial infection. These findings point to a regulatory role of CD1-reactive cells in the immune response against listeriosis. ***The Journal of Immunology,*** **1999, 162: 6955–6958.**

In listeriosis, which is caused by the intracellular bacterium, *Listeria monocytogenes,* CD8 T cells are central to protection (1). In addition to the highly polymorphic, classical MHC class Ia molecules, nonclassical MHC class Ib molecules play a role in listeriosis (2). Beside MHC class I and class II, the CD1 gene products belong to a third class of presentation molecules for T cell Ags (3). The human CD1 system consists of two groups based on sequence similarities (3). Group 1 CD1-restricted T cells recognize unusual ligands such as glycolipids of mycobacteria, emphasizing a unique role of these T cells in immunity to intracellular bacteria (4, 5). According to sequence similarities, murine CD1 (mCD1)[4] molecules are exclusively of the CD1 group 2 (6). However, recent data show mCD1 expression on hemopoietic cells (7, 8). Unusual T cells that express the NK1.1 marker (NK T cells) are restricted by CD1 molecules, and CD1 mutant mice are deficient in NK T cells and lack the early CD3-induced IL-4 production (9–11). Accordingly, it has been speculated that NK T cells perform immune regulatory functions in infections (12).

With the availability of mAbs against mCD1 molecules, it is now possible to study CD1 expression during infection and to gain further insights into their functional properties. In our approach to these issues, we infected mice with *Listeria* and examined CD1 expression and the effects on listeriosis after treatment with anti-CD1-specific mAbs.

Materials and Methods

Mice

C57BL/6 mice were kept under specific pathogen-free conditions at the animal facilities of the University of Ulm, and experiments were performed with 8- to 10-wk-old mice of either sex. Animals were age and sex-matched in a given experiment.

Abs

Spleen cells were stained with the following Abs: anti-L3T4 (CT-CD4; Medac, Hamburg, Germany), anti-Lyt2 (CT-CD8α; Medac), anti-CD3 (clone 145-2c11; a kind gift from J. Bluestone), anti-Ly5 (B220) (clone RA3-6B2; Medac), anti-TCR-αβ (clone H57-597; a kind gift from R. Kubo), anti-TCR-γδ (clone GL-3; kindly provided by L. Lefrancois), anti-NK1.1 (clone PK136; American Type Culture Collection, Manassas, VA), anti-CD11c (clone HL-3; PharMingen, San Diego, CA), anti-MAC1 (clone M1/70, American Type Culture Collection), anti-Fc-receptor (clone 2.4G2; kindly provided by M. Lamers), anti-$H2D^b$ (clone B22249; kindly provided by G. Hämmerling), anti-$H2I\text{-}A^b$ (clone AF6-120.1; PharMingen), and anti-CD1 (clone 1B1; PharMingen). Abs were either PE or FITC conjugated or biotinylated. Biotinylated Abs were detected with streptavidin-RED670 (Life Technologies, Grand Island, NY).

For in vivo experiments, anti-CD1-producing hybridomas 1B1 (rat IgG2b) or 20H2 (rat IgG1; a kind gift from A. Bendelac (13)) were cultured, and mAb were purified on a protein G-Sepharose affinity column (Pharmacia, Piscataway, NJ) before use.

Flow cytometric analysis

Staining of cell surface markers was performed as described elsewhere (7). Cells were analyzed in a FACScan (Becton Dickinson, Mountain View. CA) using Cell Quest software (Becton Dickinson). Both anti-CD1 mAb,

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[4] Abbreviations used in this paper: mCD1, murine CD1; p.i., postinfection.

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1B1 and 20H2, were tested for interference and no cross-blocking was detected (data not shown).

Bacterial infection of mice

Mice were infected i.v. with 5×10^3 *L. monocytogenes*. For anti-CD1 mAb treatment, 0.5 mg of either 1B1 or 20H2 mAb were injected i.p. simultaneously with *L. monocytogenes*. Rat γ-globulin (11 mg/ml; Jackson ImmunoResearch, West Grove, PA) was used as control Ab. Survival was monitored over 14 days after infection with 8×10^3 *L. monocytogenes* and mAb treatment.

Determination of cytokines

Single-cell suspensions of spleen cells from *L. monocytogenes*-infected mice, either rat γ-globulin or anti-CD1 (20H2) treated, were prepared at different time points postinfection (p.i.), restimulated, and culture supernatants were screened for cytokine production, i.e., IFN-γ, IL-12, and TNF by double-sandwich ELISA as described (14). TGF-β2 determination was performed by a TGF-β2-ELISA kit (Promega, Madison, WI). TGF-β1 measurements resulted in high media background, prohibiting conclusive results.

Statistics

Significant differences in survival time were analyzed by a log-rank test for curve comparison using a Graph Pad Prism computer program (GraphPad Software; Becton Dickinson). In all cases, $p < 0.05$ was considered to be significantly different.

Results

Increased CD1 surface expression during listeriosis

Constitutive surface expression of mCD1 molecules on almost all lymphocytes as well as on monocytes and macrophages has been described (7). We followed mCD1 cell surface expression in mice during listeriosis (day 0 to day 7 p.i.) by staining distinct cell populations with mAb against CD1. In naive mice, $B220^+$ cells, $CD3^+$ cells, $CD11c^+$ cells, MAC-1^+ cells, and $NK1.1^+$ cells expressed CD1 molecules on their cell surface (Fig. 1). CD1 expression was increased primarily on APC, as well as on NK cells during listeriosis (Fig. 2*A*). In contrast, CD1 expression on T cells remained virtually stable throughout infection. Our findings that CD1 expression was elevated mainly on APC during infection could mean that CD1 molecules play a role in Ag presentation during listeriosis.

Outcome of listeriosis in anti-CD1 mAb-treated mice

Mice were infected with *Listeria* and treated simultaneously with control Ab or anti-CD1 mAb 20H2 or 1B1. The in vivo treatment with these mAb did not affect CD1 expression on spleen cells of infected mice (Fig. 2*B*). CD1 expression in both groups was not significantly different (Student's *t* test) despite a trend toward reduced CD1 expression in anti-CD1-treated animals. Moreover, in vivo treatment with mAb did not deplete corresponding cell populations, because cell numbers in both groups were comparable (data not shown). The γδ T cell population was also not changed (59% $CD1^+$ γδ cells in rat γ-globulin-treated mice vs 62% $CD1^+$ γδ cells in anti-CD1-treated mice at day 1 p.i. and 76% vs 80% at day 3 p.i., respectively). Variations in the NK T cell population could not be determined, because total numbers in spleen were <1%. Listeriosis was ameliorated in anti-CD1-treated mice (Fig. 3). We conclude that CD1 molecules interact with cells that influence the course of listeriosis. The most likely explanation would be that this is due to changes in the early cytokine pattern.

Cytokine secretion in Listeria*-infected mice treated with anti-CD1 mAb*

Proinflammatory cytokines, in particular TNF-α, the Th1 cell-promoting cytokine IL-12, and the Th1 cytokine IFN-γ are crucial for protection against listeriosis, while Th2 cytokines counteract protective immunity (1, 8). The regulatory cytokine TGF-β has an ambiguous role in listeriosis, although TGF-β often curtails ongoing immune responses (15, 16). Fig. 4 reveals that during listeriosis IFN-γ, IL-12, and TNF secretion were increased in anti-CD1-treated mice as compared with controls. In contrast, TGF-β2 production was decreased and this was not due to changes in cell numbers of single-cell populations. We conclude that diminished TGF-β2 secretion favored production of a protective cytokine response to *Listeria*, thus ameliorating listeriosis.

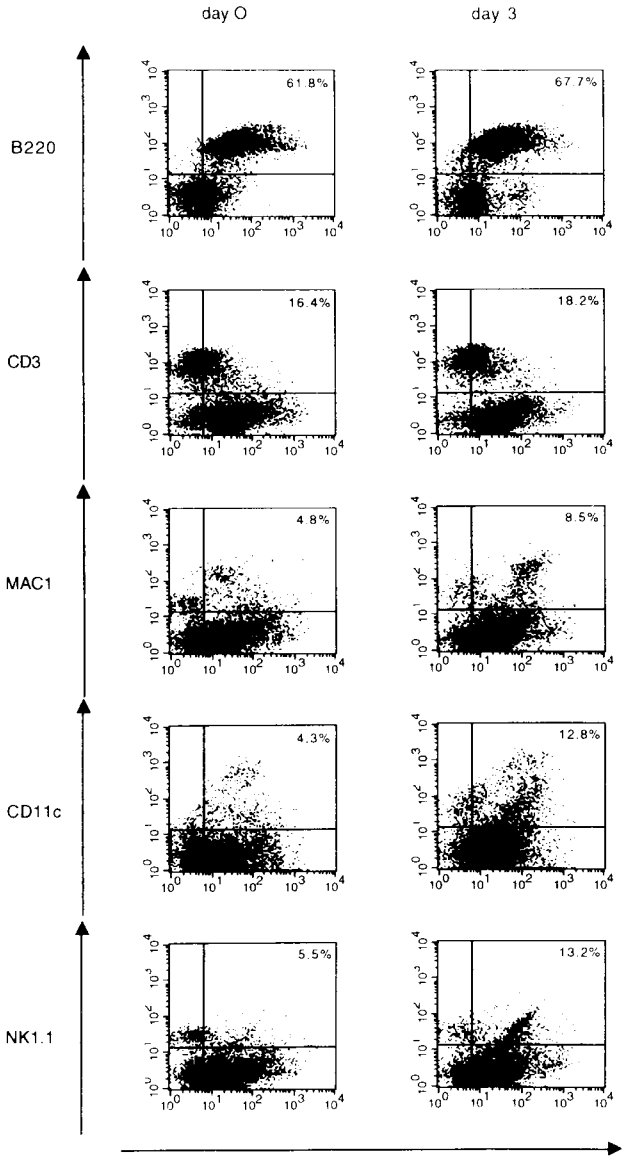

FIGURE 1. CD1 expression on different spleen cell populations during listeriosis. At day 0 and day 3 of *L. monocytogenes* infection, spleen cells were stained for B220 (clone RA3-6B2), CD3 (clone 145-2c11), MAC-1 (clone M1/70), CD11c (clone HL-3), NK1.1 (clone PK136), and CD1 (clone 1B1) expression. Unspecific staining was blocked by the addition of anti-Fc receptor mAb (clone 2.4G2). Experiments were performed six times with similar results.

Discussion

The results presented above support the notion that mCD1 molecules are involved in the control of listeriosis. A role in Ag presentation of CD1 molecules can be deduced from the observation that CD1 expression increased during listerial infection and that this augmentation was most prominent on APC. Decreased IL-2

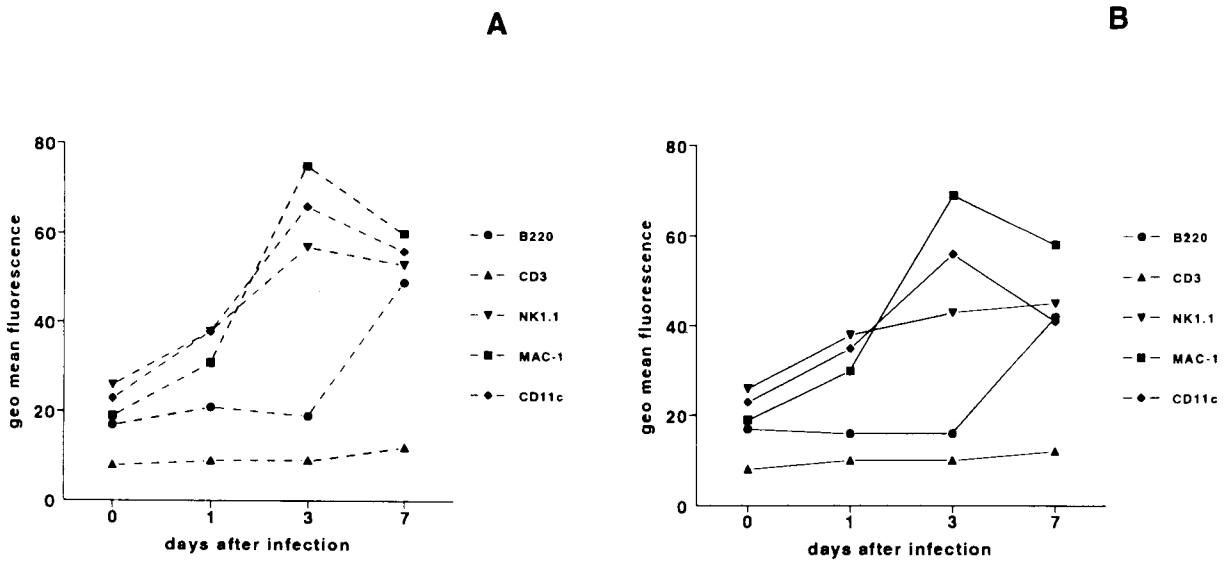

FIGURE 2. Mean fluorescence of CD1 expression on spleen cell populations during listeriosis. Infected mice were either left untreated (*A*), or CD1 (clone 20H2) treated (*B*). Spleen cells were stained for B220, MAC-1, NK1.1, CD3, CD11c, and CD1 (see Fig. 1). Geometric mean fluorescence was determined by gating on single populations and CD1 expression. Nonspecific staining was blocked by addition of anti-Fc receptor mAb. Experiments were performed five times with comparable results. In vivo treatment with anti-CD1 mAb 1B1 gave similar results (data not shown).

production of CD1-restricted T cell hybridomas in the presence of anti-CD1 mAb has been described (17). We also found in vitro blocking by anti-CD1 mAbs of cytokine production by *Listeria*-specific T cells and autoreactive hybridoma cells (data not shown). Yet, our data reveal amelioration of listeriosis in mice treated with anti-CD1 mAb.

In listeriosis, protection is mediated by Th1 cytokine-secreting cells, while Th2 cytokines play a subordinate role. The role of TGF-β in listeriosis is so far insufficiently understood (15). In several infections, protective immune responses are regulated by TGF-β, which controls both Th1 and Th2 cytokine secretion, and is therefore referred to as a Th3 cytokine (18, 19). TGF-β is produced by multiple cells of the leukocyte lineage, including lymphocytes, macrophages, and dendritic cells, and it acts in an autocrine and paracrine mode (16). TGF-β impairs the capacity of macrophages to produce IL-12 and to express CD40, and thus inhibits IL-12-mediated Th1 responses (20). In contrast, TGF-β limits immunopathological consequences of infection, and hence may play a role in the termination of the anti-infective immune response after pathogen eradication (18).

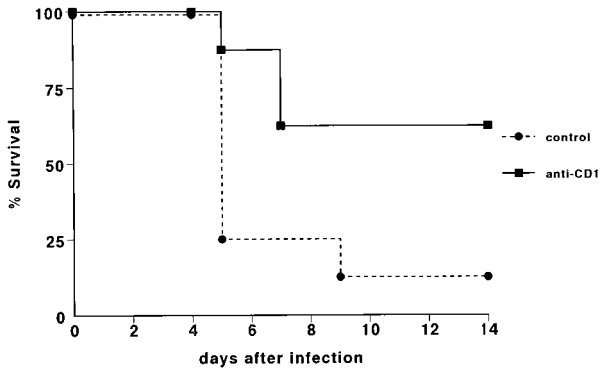

FIGURE 3. Survival of *Listeria*-infected mice treated with anti-CD1 mAb. Mice were infected with 8×10^3 *L. monocytogenes* and one group of animals received 0.5 mg anti-CD1 mAb (■) or 0.5 mg rat γ-globulin (●) as control. Survival was monitored over 14 days ($p = 0.0264$). Numbers of animals per group, $n = 8$. Experiments were performed three times with comparable results.

In anti-CD1 mAb-treated mice, increased secretion of the protective cytokines TNF, IL-12, and IFN-γ was paralleled by decreased TGF-β2 production soon after listerial infection. This could mean that TGF-β produced by the innate immune system controls Th1 cytokine production. Thus a decrease in TGF-β could lead to elevated Th1 production and therefore better protection. Most likely candidates that could regulate anti-listerial immune responses include NK T cells and $\gamma\delta$ T cells (1). CD1 restriction has been demonstrated for the majority of NK T cells (9). Upon primary activation by CD1 molecules, these cells produce IL-4 and IFN-γ, and CD1 deficiency results in impaired IL-4 production (10, 11, 21). NK T cells also secrete TGF-β and express immunosuppressive activities (22). Another potential target are $\gamma\delta$ T cells. Like NK T cells, $\gamma\delta$ T cells can produce TGF-β (23). They participate in protection against listeriosis, but so far no restriction element for $\gamma\delta$ T cells in murine listeriosis has been identified (1). Therefore, further investigation of CD1-specific ligands of microbial origin recognized by murine T cells is necessary.

Independent of the exact cellular source of TGF-β, our data reveal that anti-CD1 mAb treatment interferes with TGF-β2 production and up-regulates secretion of TNF, IL-12, and IFN-γ. We assume that the up-regulation of protective cytokines was a consequence of TGF-β down-modulation. As a result, anti-CD1 mAb treatment ameliorated listeriosis. Thus, our report provides first evidence for a regulatory role of CD1 in antilisterial immunity.

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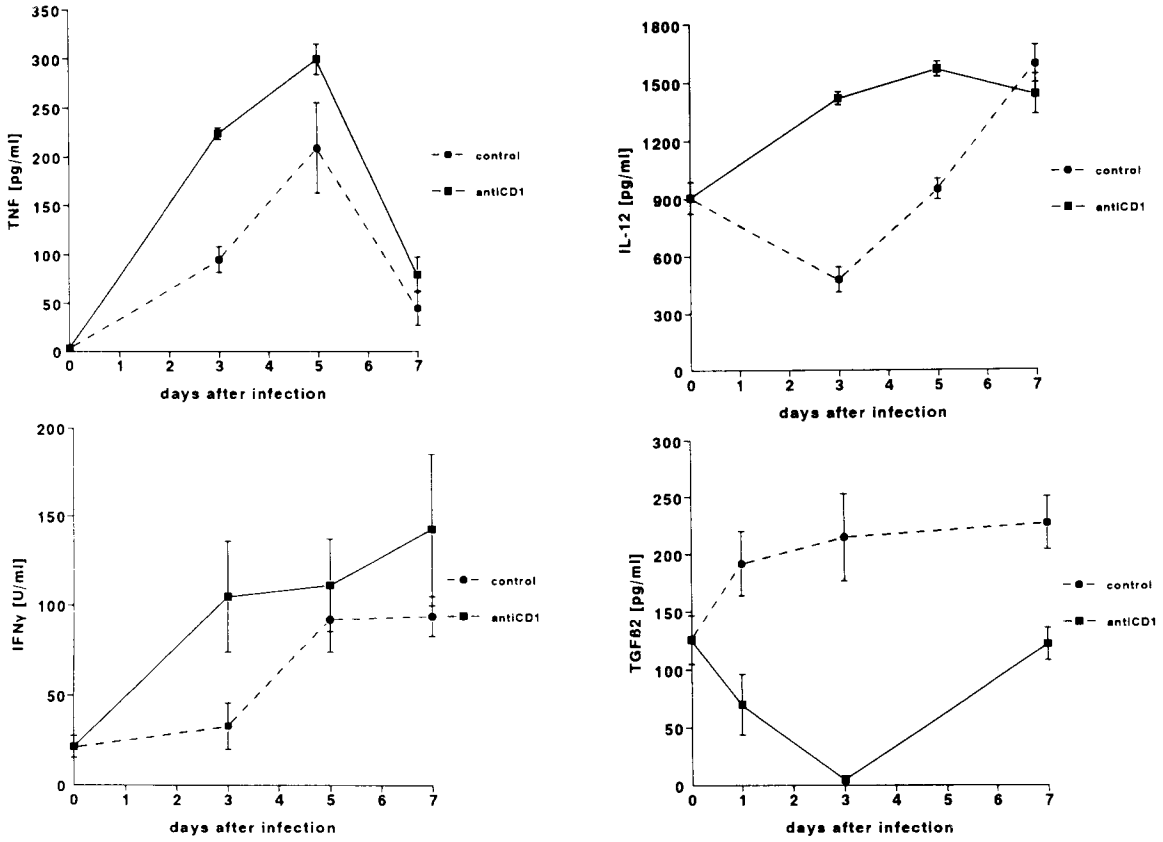

FIGURE 4. Cytokine production in *Listeria*-infected mice treated with anti CD1 mAb. At indicated days p.i., cytokine production by spleen cells from control mice (■) or treated with anti-CD1 mAb (20H2) (●) was determined by ELISA. Experiment was one representative of three.

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