Direct CD1d-Mediated Stimulation of APC IL-12 Production and Protective Immune Response to Virus Infection In Vivo

Simon C. Yue, Michael Nowak, Angela Shaulov-Kask, Ruojie Wang, Dominic Yue, Steven P. Balk and Mark A. Exley

*J Immunol* 2010; 184:268-276; Prepublished online 30 November 2009;
doi: 10.4049/jimmunol.0800924
http://www.jimmunol.org/content/184/1/268

---

**References**
This article cites 75 articles, 42 of which you can access for free at:
http://www.jimmunol.org/content/184/1/268.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Direct CD1d-Mediated Stimulation of APC IL-12 Production and Protective Immune Response to Virus Infection In Vivo

Simon C. Yue, Michael Nowak,1 Angela Shaulov-Kask,2 RuoJie Wang,3 Dominic Yue,4 Steven P. Balk, and Mark A. Exley

CD1d-restricted NKT cells rapidly stimulate innate and adaptive immunity through production of Th1 and/or Th2 cytokines and induction of CD1d+ APC maturation. However, therapeutic exploitation of NKT cells has been hampered by their paucity and defects in human disease. NKT cell–APC interactions can be modeled by direct stimulation of human APCs through CD1d in vitro. We have now found that direct ligation with multiple CD1d mAbs also stimulated bioactive IL-12 release from CD1d+ but not CD1d knockout murine splenocytes in vitro. Moreover, all of the CD1d mAbs tested also induced IL-12 as well as both IFN-γ and IFN-α in vivo from CD1d+ but not CD1d-deficient recipients. Unlike IFN-γ, CD1d-induced IFN-α was at least partially dependent on invariant NKT cells. Optimal resistance to infection with picornavirus encephalomyocarditis virus is known to require CD1d-dependent APC IL-12–induced IFN-γ as well as IFN-α. CD1d ligation in vivo enhanced systemic IL-12, IFN-γ, and IFN-α and was protective against infection by encephalomyocarditis virus, suggesting an alternative interpretation for previous results involving CD1d “blocking” in other systems. Such protective responses, including elevations in Th1 cytokines, were also seen with CD1d F(ab′)2 in vivo, whereas an IgM mAb (with presumably minimal tissue penetration) was comparably effective at protection in vivo as well as cytokine induction both in vivo and in vitro. Although presumably acting immediately “downstream,” CD1d mAbs were protective later during infection than the invariant NKT cell agonist α-galactosylceramide. These data indicate that NKT cells can be bypassed with CD1d-mediated induction of robust Th1 immunity, which may have therapeutic potential both directly and as an adjuvant. The Journal of Immunology, 2010, 184: 268–276.

Abbreviations used in this paper: EMCV-D, diabetogenic encephalomyocarditis virus; iNKT, invariant NKT; KO, knockout; WT, wild type.

1Current address: Medizinische Klinik III-Venuesburg, Universitaetsklinikum Bonn, Bonn, Germany.
2Current address: Allergy and Infectious Diseases, University of Washington, Seattle, WA.
3Current address: AVEO Pharmaceuticals, Cambridge, MA.
4Current address: Imperial College of Science, Technology, and Medicine, London, United Kingdom.

Received for publication March 20, 2008. Accepted for publication October 21, 2009.

This study was supported by grants from the National Institutes of Health (R01 DK066917 to M.A.E.), the Dana-Farber/Harvard Cancer Center Specialized Program in Research Excellence in Prostate Cancer (P50 CA90381 to S.P.B. and M.A.E.), the Prostate Cancer Foundation (to S.B.), the Prostate Cancer Research Fund (to S.B.), and the Hershey Family Prostate Cancer Research Excellence in Prostate Cancer (P50 CA90381 to S.P.B. and M.A.E.), the Dana-Farber/Harvard Cancer Center Specialized Program in Research Excellence in Prostate Cancer (P50 CA90381 to S.P.B. and M.A.E.), the Prostate Cancer Foundation (to S.B.), the Prostate Cancer Research Fund (to S.B.).

Address correspondence and reprint requests to Dr. Mark A. Exley, Hematology/Oncology Division, Beth Israel Deaconess Medical Center, RW 663, 330 Brookline Avenue, Boston, MA 02215. E-mail address: mexley@bidmc.harvard.edu

Copyright © 2009 by The American Association of Immunologists, Inc. 0002-1767/10S16.00

APCs, including B cells, thymocytes, and rodent (but not human) T cells and hepatocytes (6–10). Surface CD1d is inducible on these and certain other cell types, in some cases from intracellular stores (1, 6–10). Some CD1d-restricted T cells use a unique invariant Ag TCR α-chain rearrangement (murine Vα14Jα18) with restricted TCR β-chain repertoire (invariant NKT [iNKT] cells) (1–8). These and other distinct CD1d-restricted T cell subsets can positively or negatively regulate ongoing as well as naive adaptive immune responses through rapid production of large amounts of Th1- and/or Th2-type cytokines, including IFN-γ and IL-4, respectively (1–8, 11–13). A specific iNKT cell glycolipid Ag, α-galactosylceramide (α-GalCer), was originally derived from marine sponge in an anticancer drug screen (1, 5, 6), and there are subsequently identified related bacterial analogues and other lipids, which include a first candidate endogenous ligand (14–20). Activation of iNKT cells by α-GalCer, widely used to exploit iNKT cells in vivo in rodents, induces a rapid mixed Th1/Th2 systemic cytokine pattern and transient stimulation of both the innate and the adaptive immune systems, including NK cells (1–8).

Physiologically, CD1d-restricted T cells can augment or inhibit Th1 responses, including antitumor, autoimmune, and antipathogen responses, through a variety of mechanisms depending on context (1–8, 21–28). The positive or negative contribution of CD1d-restricted T cells in Th1-like immune responses to pathogens depends upon the individual pathogen and resistance mechanisms involved. In particular, CD1d-restricted T cells appear to contribute to resistance against specific viral infections but not others (22, 23, 25, 26, 28–40), and there is evidence for antiviral roles of human iNKT (41, 42). Optimal resistance to picornavirus diabetogenic encephalomyocarditis virus (EMCV-D) requires IL-12, IFN-γ, NK cells, and CD1d-restricted T cells (30, 33, 39). Similar results have been reported with HSVs (34, 35), although this may be strain- or dose-specific (38). EMCV resistance...
involves the CD1d-dependent sequential induction of IL-12 and type 1 and 2 IFNs, leading to both innate and adaptive immune responses with NK and T cell activation (33, 39). CD1d-restricted T cells also appear to stimulate CD8 T cell responses against respiratory syncytial virus (32), but the reverse has been found in the case of lymphocytic choriomeningitis virus (31), and immunity to certain viruses as well as other infections appears to be CD1d-independent (26, 31, 36–38, 43–45). Also consistent with a critical role for NKT cells in resistance to specific viral and bacterial infections, multiple cases of MHC-like suppression of CD1d expression and Ag presentation to NKT cells by infections have been uncovered (46–53). In contrast, several unrelated infections, including low-dose HSV-1, coxsackievirus CBV3, HCV, and Listeria, can lead to upregulation of local tissue CD1d (54–57), which could be reflective of immune-surveillance and/or alternative pathogen countermeasures. Consistent with these activities, α-GalCer is transiently prophylactically protective against a wide variety of pathogens in rodent models (1–6, 25, 26, 28, 30, 36, 58, 59), irrespective of physiological involvement of iNKT cell or other CD1d-restricted T cell populations in resistance.

Despite the potential for therapeutic exploitation of CD1d-restricted T cells, clinical progress to date has been hampered by the relative paucity of the iNKT cell subset in humans (6, 8, 22, 23, 41, 42). Interestingly, CD1b, CD1c, or CD1d (but not CD1a) Ab cross-linking can activate CD1d cells (60, 61). We found that direct CD1d ligation can model human iNKT cell activation of APCs, leading to bioactive IL-12 production (62). We now show that ligation of murine CD1d with multiple mAbs (IgM or IgG) is similarly active at inducing bioactive IL-12, IFN-γ, as well as IFN-α release, both in vitro and in vivo. Interestingly, of the three, IFN-α only was partially dependent upon iNKT cells. Furthermore, we find that CD1d mAb treatment can alleviate acute EMCV-D disease in vivo. The protective treatment response was also found with CD1d mAb F(ab')2 fragments, and protection was associated with increased systemic IL-12, IFN-γ, and IFN-α. iNKT cell agonist α-GalCer has been shown to be protective in EMCV-D and murine CMV infections (30, 36). However, α-GalCer exacerbated viral disease when given on 1 d postinfection or later. In contrast, CD1d mAb administration was protective up to 2 d into infection. Collaboratively, we have also found that CD1d mAb can induce antitumor responses in vivo, which are at least partially dependent upon IL-12 and IFN-γ (63, 64). Together, therefore, direct CD1d ligation (either alone or with other stimuli) provides a potential Th1-type therapeutic option in infectious disease as well as cancer. Significantly, previous reports of CD1d Ab “blocking” CD1d-restricted T cell function in vivo (65–71) are reinterpreted based upon these data.

**Materials and Methods**

*Reagents and measurement of splenocyte cytokines in vitro*

Rat anti-mouse CD1d IgG mAbs 1B1 (BD Pharmingen, San Diego, CA), HB323 (American Type Culture Collection, Manassas, VA), 19G11 (kindly provided by A. Bendelac, University of Chicago, Chicago, IL), 3C11 (a protein A as well as protein G binding rat IgM (72) or control isotype mAbs (BD Pharmingen) were bound to 96-well plates indirectly for correct orientation and to obscure Fc portions from inhibitory FcRs via protein G, as previously described for human CD1d mAb (62). Control stimuli were LPS (1 μg/ml) or α-GalCer (0.2 μg/ml). Approximately 1 x 10^6 splenocytes per well of 96-well plates were used. p70 IL-12, IFN-γ, and IFN-α release were determined in triplicate by ELISA (Ab pairs, Endogen, Rockford, IL; kits from R&D Systems, Minneapolis, MN). Limit of detection was ~1 pg/ml. Results are shown with standard deviations.

*CD1d mAb in vivo, EMCV-D infection, treatment, and disease measurement*

Mice were i.p. given 50 μg of each intact CD1d mAb, 3C11, HB323, 19G11, and 1B1, and 30 μg of F(ab')2 (prepared according to the kit manufacturer’s directions; Pierce, Rockford, IL), with isotype controls LPS (100 μg) or α-GalCer (2 μg). Serum for measurement of cytokines in vivo was diluted 1:10 for assay, and values were corrected following ELISA as above. Data are means with SD or for individual animals, as shown. Approximately 5–7 wk-old male Th1-dominant relatively virus-resistant wild-type (WT) C57BL/6 or more sensitive N12 C57BL/6J CD1d knockout (KO) mice deficient in both CD1d genes (33; Stock No. 000881; to be available at The Jackson Laboratory, Bar Harbor, ME) or lacking only iNKT cells (30; Jn18/Jn18 KO mice, N10) or 10-wk-old more sensitive Th2-biased male WT BALB/c mice were used. Mice were infected with 500 PFU of EMV-D, essentially as previously described (30, 33, 39). Briefly, glucose tolerance tests were performed 5–7 d post-infection (depending on extent of paralysis) by injection of 2 g/kg glucose, and blood was collected 1 h later with glucose oxidase inhibitors for analysis by the glucose basic glucometer (Lifescan, Mountain View, CA). Encephalitis was assessed by semiquantitative parasitic score (30, 33); 1 = no paralysis (to indicate number of animals per group), 2 = weakness in one limb, 3 = one completely paralyzed limb, 4 = weakness in two limbs, 5 = paralysis of two limbs, 6 = paralysis of three or more limbs.

**Results**

*Cytokine responses of mice splenocytes to CD1d-mediated stimulation in vitro*

We previously found that CD1d* human monocytes and dendritic cells produce large amounts of IL-12 in response to CD1d mAb ligation (56). Therefore, splenocyte cultures were similarly tested for bioactive p70 IL-12 production in response to stimulation with plate-bound CD1d mAbs or controls. Splenocytes from C57BL/6 mice produced substantial IL-12 at 24 h in response to two distinct CD1d IgG mAbs, 1B1 and 19G11, when presented on protein-G–coated plates, and the levels further increased over 3 d (Fig. 1A, not shown). In contrast, isotype control mAbs did not produce IL-12 levels above background. Importantly, IL-12 levels induced by both CD1d mAbs were comparable to those induced by LPS (Fig. 1A). Similarly, IL–12 levels of WT splenocytes but not those of CD1d KO cultures were markedly increased by a third CD1d mAb, IgM 3C11, with a mean of ~400 pg/ml (Fig. 1B), comparable to the other CD1d mAbs (Fig. 1A, 1B), LPS (Fig. 1A, 1B), and to iNKT cell ligand α-GalCer (Fig. 1B). Consistent with activation of IL-12 production, substantial IFN-γ was also induced in CD1d-mAb–stimulated WT and not CD1d KO cultures (Fig. 1C). CD1d-stimulated IFN-γ production was comparable to that induced by α-GalCer, although less than that produced in response to LPS.

Therefore, these studies demonstrate efficient and specific induction of murine bioactive IL-12 and downstream activation of IFN-γ production in vitro by CD1d mAbs of IgG or IgM isotypes. Further studies with the 1B1 CD1d mAb versus isotype control confirmed these in vitro findings and, interestingly, extended the results of CD1d ligation of splenocytes to marked specific stimulation of potent antiviral type 1 interferon (Fig. 1D–F).

*Cytokine responses to CD1d-mediated stimulation in vivo*

To determine whether CD1d mAbs could similarly stimulate CD1d* APCs in vivo, mice were treated with CD1d mAbs and sera were tested for cytokine production (Fig. 2A–C). Remarkably, intact CD1d IgM mAb 3C11 (Fig. 2A) as well as CD1d IgG mAb 1B1 (Fig. 2D) could specifically induce substantial systemic bioactive p70 IL-12 production. Notably, no such induction was noted from CD1d KO mice, thereby eliminating the possibility of selective LPS contamination of the CD1d mAb and further demonstrating specificity. However, mice lacking only the iNKT cell population but retaining intact CD1d- and CD1d-restricted “non-invariant” NKT cells (Jn18 KO mice) were able to mount strong IL–12 responses, as expected. Comparable IFN-γ responses were also found to be specifically induced by CD1d mAbs in WT and Jn18 KO mice, although not in CD1d KO animals (Fig. 2B).
Interestingly, however, although WT mice produced a strong IFN-α response to CD1d mAb and CD1d KO mice (as expected) did not, Jα18 KO mice made only very modestly increased IFN-α relative to isotype controls (Fig. 2C). Jα18 KO mice also had a slightly higher baseline response (Fig. 2C). These results may indicate that unlike for IFN-γ reciprocal iNKT responses (other than IFN-γ itself) are required for optimal IFN-α induction. In further experiments, CD1d mAb was compared with other control stimuli in vivo. As above, CD1d mAb but not isotype control mAb stimulated systemic IL-12, IFN-γ, and IFN-α production from WT mice. Control stimuli LPS and α-Galcer were also active in WT mice (Fig. 2D–F). However, there was no effect of CD1d mAb or α-Galcer in CD1d KO mice (Fig. 2D–F), demonstrating a lack of LPS in the CD1d mAb preparations. Only
LPS-stimulated cytokines from CD1d KO mice were comparable to WT (Fig. 2D, 2F).

Decreased susceptibility of mice treated with CD1d mAb to EMCV-D infection in vivo

CD1d mAb could induce IL-12 from human APCs in vitro (62) and murine IL-12 along with type 1 and type 2 IFNs both in vitro (Fig. 1) and systemically in vivo (Fig. 2). Therefore, mice challenged with EMCV were treated with CD1d mAb to determine whether such activation of APCs could occur during infection in vivo and whether such treatment could be protective, as previously shown for α-Galcer (30). Representative results from a series of EMCV-D infections are summarized in Figs. 3–5. Incidence of abnormal glucose tolerance hyperglycemic response in relatively resistant 7-wk-old (at day of infection) C57BL/6J WT male mice was specifically eliminated by CD1d mAb treatment (Fig. 3A). As previously shown (30, 33), CD1d KO mice had significantly higher incidence and severity of hyperglycemia (Figs. 3A and 4A). Similarly, CD1d mAb also specifically eliminated the relatively low incidence of mild paralysis in this series of infections (Figs. 3B and 4B).

Next, WT EMCV-D-infected animals were treated either with IgM CD1d mAb 3C11 alone or with a second CD1d mAb, IgG2b 1B1, compared with appropriate isotype controls. Again, either or both CD1d mAbs eliminated the relatively modest frequency of blood glucose increases observed (Fig. 3C). The severe paralytic disease in these younger more sensitive 5-wk-old WT mice was also partially but significantly ameliorated by CD1d mAb 3C11 in severity, with reduced incidence (Fig. 3D). Although a combination of two CD1d mAbs also eliminated the modest level of hyperglycemia in this series of infections (Fig. 3C), they appeared to only marginally reduce paralysis severity, and incidence was actually as high as that in isotype-control–treated mice (Fig. 3D), which might represent a competitive effect.

Table I summarizes experiments assessing the protective effect of two CD1d mAbs. As shown previously (30, 33), CD1d KO mice were more susceptible to EMCV infection than WT mice in both diabetes and paralysis (Table I). Notably, IgM CD1d mAb 3C11 could significantly and specifically reduce incidence of diabetes and paralysis (Table I). IgG CD1d mAb 1B1 induced a similar level of paralysis protection as did 3C11, although was less active on a glucose tolerance test (Table I). Finally, when analyzed in aggregate with built-in experiment-to-experiment variability, α-Galcer had only a modest effect in this series (Table I), although statistically significant protection was seen in individual experiments (see below).

Increased production of cytokines by mice treated with CD1d mAb during EMCV-D infection

Resistance to EMCV depends on IL-12–induced IFN-γ, which can be produced by CD1d-restricted T cells (30, 33). To determine whether the protective effects of CD1d mAb treatment in vivo during EMCV-D infection reflected similar cytokine induction to that seen with uninfected animals in vivo (Fig. 2), further EMCV infections were performed and glucose responses, paralysis scores, and serum cytokines were determined (Fig. 4). CD1d mAb specifically reduced incidence of disease, as could α-Galcer (Fig. 4A, 4B). Fig. 4C–E show that both CD1d mAb and α-Galcer also stimulated substantial systemic production of bioactive IL-12, IFN-γ, and IFN-α from EMCV-infected WT mice. As previously reported, CD1d KO mice infected with EMCV were defective in cytokine responses (33, 39).

Protection of mice with CD1d intact mAb versus F(ab’)2 against EMCV-D infection in vivo

The above results showed that intact CD1d mAbs could reduce disease in mice challenged with EMCV. It was possible that distinct CD1d mAbs recognizing separate epitopes could have different efficacies and that the presence of Fc portions, which could bind to inhibitory FcRs in vivo, reduced efficacy of the treatment.

**FIGURE 3.** Decreased disease following treatment of virus infection with anti-CD1d mAb in vivo. Male C57BL/6J mice were infected with EMCV-D and followed as described previously (30, 33). Fifty micrograms of anti-CD1d mAbs or isotype control mAbs were given once per animal 1 d prior to infection. Results show individual animal glucose tolerance test and paralysis score data. A and B, 3C11 anti-CD1d eliminating mild diabetes (A) and paralysis (B) of relatively resistant 7-wk-old mice to naturally normal levels of uninfected controls. CD1d KO mice included for comparison in the same experiment. C and D, Anti-CD1d mAbs 3C11, 1B1 and 3C11 or isotype control mAbs reduced diabetes (C) as well as severe paralysis (D) of more sensitive 5-wk-old mice. As previously described (30, 33), graph lines shown define hyperglycemia as values more than three times SD over the mean value of uninfected controls. For glucose data, lines indicate frank hyperglycemic values. For paralysis results, a line separates values above baseline (defined as “1” to show the number of mice) with no paralysis (uninfected or cured). *p < 0.05.
Therefore, several CD1d mAbs were compared and divalent mAb F(ab′)2 were prepared and used compared with the corresponding intact mAb during EMCV infection in vivo (Fig. 5). Fig. 5A shows that a CD1d mAb F(ab′)2 as well as all three intact CD1d mAbs tested suppressed EMCV induced paralysis when treated on the day of infection. Glucose tolerance testing revealed that CD1d mAb F(ab′)2 and the intact CD1d mAb IgG could also modestly reduce hyperglycemia incidence in this experiment, although to a lesser extent (Fig. 5B) than paralysis (Fig. 5A).

In a further experiment (Fig. 5C), two different CD1d mAb F(ab′)2s were directly compared with isotype and isotype F(ab′)2, this time treating on day 1 postinfec tion. The results confirmed and extended the previous data and showed that two independent CD1d mAbs could protect against EMCV infection. Again, there was little difference in efficacy between intact mAb and F(ab′)2 (Fig. 5C).

**FIGURE 4.** Anti-CD1d mAb reduces disease and enhances cytokines following virus infection in vivo. Male C57BL/6 WT or CD1d KO mice were infected with EMCV-D and followed as in Fig. 3. Results show individual animal data. A, Glucose tolerance test. B, Paralysis score. Fifty micrograms of anti-CD1d mAb 1B1 or isotype control mAb or 2 μg of α-galactosylceramide was given once per animal on day of infection, reducing diabetes (A) and paralysis incidence of 6 wk-old mice (B), measured at 7 d. Lines shown define hyperglycemia or values above baseline (defined as “1” to show mice) of no paralysis (uninfected animals). The same groups of mice as shown in Fig. 4A, 4B were tested for serum cytokines. CD1d mAb could specifically induce elevated systemic Th1 cytokine levels. Results show individual animal day 7 serum p70 IL-12 (C), IFN-γ (D), and IFN-α (E) ELISA data (lines indicate control uninfected and untreated animal baseline values).

Table 1. Mean and median glucose tolerance and paralysis scores for EMCV infections of sensitive 5–6-wk-old C57BL6 mice

<table>
<thead>
<tr>
<th></th>
<th>Glucose Tolerance Test</th>
<th>Paralysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Median</td>
</tr>
<tr>
<td>CD1d KO</td>
<td>490</td>
<td>551</td>
</tr>
<tr>
<td>WT/Control</td>
<td>302</td>
<td>290</td>
</tr>
<tr>
<td>WT/CD1 3C11 (IgM)</td>
<td>263</td>
<td>278</td>
</tr>
<tr>
<td>WT/CD1 1B1 (IgG)</td>
<td>291</td>
<td>236</td>
</tr>
<tr>
<td>WT/α-Galcer</td>
<td>279</td>
<td>254</td>
</tr>
</tbody>
</table>

Results are from two to up to six available independent experiments. p values are for comparisons shown. Arrows define comparisons being made.
6A, 6B) without enhancing protective IL-12 relative to no virus or to virus plus isotype control mAb (not shown). A more severe increase in disease was found with treatment on subsequent days (Fig. 6A, 6B).

FIGURE 6. Treatment of established virus infection with α-Galcer. Two micrograms of α-Galcer or vehicle were given per animal before infection (day −1) or on days 1–3 of EMVC-D infection of BALB/c mice as shown. Infected animals were monitored for disease as in Fig. 3, with lines indicating abnormal value limits. Results show individual animal glucose tolerance test results (A) and paralysis scores (B), measured on day 7 postinfection with percentage incidence of abnormal glucose results or paralysis. Normoglycemic values were defined as within three times the SD of the mean value of PBS-injected uninfected controls.

Discussion
The increased susceptibility of CD1d KO mice to EMVC-D and other infections (22, 23, 25, 26, 30, 33) as well as against tumors (1, 2, 5, 21, 23, 24, 27) has identified roles for CD1d-restricted
T cells in protective Th1 immune responses in vivo. Resistance to EMCV-D, as with multiple other viruses, is dependent upon early IFN-γ production and IL-12 induction leading to NK cell activation (22, 23, 25, 26, 28, 33, 36). Consistent with these observations, α-Galcer is at least prophylactically protective against EMCV-D and many other infections, and in vitro studies with α-Galcer have shown that IL-12 production by dendritic cells is stimulated by iNKT cell IFN-γ production and NKT–APC CD40–CD154 interactions (1–6, 73, 74). Taken together, these observations suggest that the rapid physiological activation of dendritic cells to produce IL-12 in response to specific acute infections may be particularly dependent on CD1d-restricted T cells.

We recently found that direct CD1d stimulation can induce activation and maturation of human dendritic cells and monocytes in vitro (62). We have now found that murine CD1d ligation can also induce bioactive IL-12 both in vitro and in vivo as well as apparently mimetic physiological IL-12–dependent responses in vivo. As expected, CD1d KO mice did not respond to CD1d mAb and iNKT-deficient mice responded with normal levels of both IL-12 and IFN-γ production. Interestingly, however, Jx18 KO mice IFN-α levels were intermediate, suggesting that downstream of IL-12 there is indirect amplification of IFN-α responses by iNKT cells, possibly through plasmacytoid dendritic cells, which in rodents are CD1d+ (6) and which may sensitize by upregulating CD1d in Listeria infection in response to IFN-β (57), or directly from iNKT cells, as previously shown for IFN-β from CD4+ iNKT cells (75).

Single IgG or IgM mCD1d mAbs were comparably effective; apparently IgM mAbs were able to activate APCs systemically to induce IL-12 and provide protection. Multiple CD1d mAbs to different epitopes had similar effects on IL-12 induction in vitro. However, combining two mAbs in vitro actually reduced this protective effect. This could reflect the presence of inhibitory FcRs on monocytc cells, which were minimized through binding CD1d mAb via protein G in vitro, or antagonistic effects of multiple mAbs on CD1d signaling. Similarly, two mAbs were actually somewhat less effective than one in vivo, where Fc-mediated clearance may have been more important. Preparation of therapeutic quantities of IgG F(ab′)2 presumably resulted in circumventing FcR-dependent issues but did not result in a significant increase in protective effect in vivo. Instead, IgM 3C11 was as potent or better than the IgG mAbs, intact or as F(ab′)2. This may reflect the potency of multivalent IgM ligation resulting in as strong effects systemically or IgG dilution into tissue.

Previous studies have shown that systemic injection of CD1d mAbs can alter CD1d-restricted T cell–dependent immunity (65–71). In some cases, no doubt, blocking of such responses may be the primary explanation, as also contributing where type 2/non-invariant NKT cells can suppress antitumor responses (63, 64). However, the ability of CD1d mAbs to induce potent systemic Th1 responses clearly has the potential to directly influence such responses independently of CD1d-restricted T cells.

A physiological role for CD1d-restricted T cells appears to rapidly integrate signals from CD1d, cytokines, and the innate immune system and to influence both the innate response and the decision for Th1- or Th2-type adaptive responses based upon the nature of the Ag challenge. There are relatively few iNKT cells in humans, and these are further reduced and defective in a wide range of chronic diseases (6, 8, 22, 23, 41, 42). Bypassing iNKT cells via direct therapeutic modulation of CD1d, feasibly in the context of other appropriate innate immune signals, may therefore be an approach to optimize natural and vaccine-induced antipathogen and antitumor Th1-biased immune responses.

Acknowledgments

Drs. A. Bendelac and S. Porcelli kindly provided some of the CD1d mAb. We thank Dr. N. Bigley for generously providing her expertise and EMCV-D for us to set up the virus in vivo challenge system in our laboratory as well as our colleagues, especially Drs. M. Brenner, S. Porcelli, M. Smyth, J. Stein-Streilein, and S.B. Wilson for suggestions and advice, and Kirin Pharma for α-Galcer.

Disclosures

S.C.Y., S.P.B, and M.A.E. have a joint patent application on induction of cytokines by direct CD1d stimulation, currently licensed to NKT Therapeutics, with which S.P.B. and M.A.E. have consulting agreements.

References


