

Natural Killer T Cells in Mucosal Homeostasis

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ABSTRACT: The mucosal-associated lymphoid tissues (MALT), including the gut-associated lymphoid tissues, are a tightly regulated environment. In fact, it might be stated that on the basis of studies from animal models of inflammatory bowel disease (IBD), the major means of peripheral regulation of immune responses in the intestine is not necessarily from processes such as deletion or anergy, but more likely from the controls imposed upon responses due to the activities of a variety of regulatory subsets of cells. One type of regulatory cellular subset that has recently gained attention is the subset of T cells that are associated with CD1d-restricted responses. Recently, CD1d-restricted T cells have been increasingly appreciated to play a significant role in mucosal tissues of the intestine and lung, for example. Insights from these studies have clearly elevated these cells to particular importance in the regulation of a variety of infectious and inflammatory conditions, such as those associated with idiopathic IBD. In this review, we focus on recent observations on the characteristics of CD1d-restricted pathways in mucosal compartments, after a brief introduction into the biology of CD1d and CD1d-restricted T cells.

KEYWORDS: mucosal homeostasis; inflammatory bowel disease; CD1; CD1d; natural killer T (NKT) cells; epithelial cells; antigen-presenting cells

INTRODUCTION

CD1d is a member of the CD1 gene family,^{1,2} which consists of five genes, CD1a to CD1e. Four of these genes (CD1a to CD1d) encode functional proteins, whereas the CD1e gene has not been associated with a detectable product to date.³ The CD1 gene family can be divided into two groups, CD1a to CD1c and CD1d, based on sequence homology.³ Whereas humans contain genes on chromosome 1 for CD1a to CD1d, rodents express only a CD1d homologue. In fact, the human CD1d isoform is more similar to mouse and rat CD1d than it is to the other human CD1 isoforms.³

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All CD1 genes have an MHC class I-like structure both genetically and biochemically.⁴ In this regard, the CD1 genes contain exons 2 to 4, encoding domains $\alpha 1$, $\alpha 2$, and $\alpha 3$, that generate a protein product that is associated with $\beta 2$ -microglobulin. The recent solution of the X-ray crystallographic structure of CD1d⁵ and, more recently, CD1a,⁶ have confirmed earlier predictions from cellular studies by Brenner and Porcelli that the CD1-related molecules play a role in presenting a variety of glycolipid antigens to unique subsets of T cells. In particular, the glycolipid antigens presented by the CD1a to CD1c proteins have been shown to be a variety of lipoglycan antigens that derive from mycobacteria and other bacteria.⁷ The glycolipid antigens presented by CD1d are less well defined. Whereas a model glycolipid antigen derived from marine sponge, α -galactosylceramide (α GalCer), has been shown to be presented by CD1d to unique groups of CD1d-restricted T cells,⁸ the endogenous antigens that are presented by CD1d to CD1d-restricted T cells, either *in vitro* or *in vivo*, are less clear but do include such molecules as the phospholipid phosphatidylethanolamine.⁹ It also remains unclear whether exogenous glycolipid antigens derived from the microbial universe can also be presented by CD1d, with most information suggesting that the CD1d-specific antigens are derived from host lipids. This, in particular, emphasizes the significant autoreactivity that has been identified in the CD1 system.

The types of T cells that have been shown to be restricted by CD1d, both in mouse and human, include either those that have an invariant T cell receptor (TCR)- α chain or those that have a semidiverse group of TCR- α chains.¹⁰

Characteristics of the first group of T cells, the so-called invariant natural killer T (iNKT) cells, include the following: These T cells express an invariant TCR- α chain that results from the rearrangement of TCR- α gene products to contain only canonical germ line sequences. In rodents, this invariant TCR- α chain comprises a V $\alpha 14$ gene product in contiguity with a J $\alpha 18$ gene product.¹¹ The human homologue for this invariant TCR- α chain is the result of the rearrangement of a V $\alpha 24$ gene segment to J αQ gene segment.¹¹ Because invariant T cells often carry NK cell markers on the cell surface, in addition to the TCR/CD3 complex, they are considered to be natural killer T (NKT) cells. Invariant NKT cells either express CD4 or are double negative; in fact, forced expression of CD8 in the thymus causes deletion of these particular cells. Although these cells carry an invariant TCR- α chain, the TCR- α chains pair with a wide variety of TCR- β chains that predominantly use V $\beta 8.2$, V $\beta 7$, or V $\beta 2$.¹¹ As a result, although iNKT cells express an invariant TCR- α chain, owing to the association of this invariant TCR- α chain with a wide variety of TCR- β chains, these cells are, in fact, quite polymorphic. This may explain the wide variety of biologic processes that these cells are able to regulate, as described in more detail below. The iNKT subset is the major subset that responds to α GalCer.⁸ Indeed, virtually all α GalCer reactivity maps to the iNKT subset of CD1d-restricted T cells.

In addition to iNKT cells, recent studies in both mouse and human have identified a perhaps larger group of CD1d-restricted T cells that contain a much more diverse TCR repertoire.¹²⁻¹⁴ The so-called semidiverse NKT cells are also either CD4 or double negative in mouse and, perhaps, CD8, CD4 or double negative in humans with a preferential usage of particular TCR- α and - β chains.^{12,14} Specifically, semidiverse iNKT cells use either V $\alpha 3.2$ conjoined to a J $\alpha 9$ gene segment or a V $\alpha 8$ gene segment, together with a V $\beta 8$ gene segment.¹² The glycolipid antigens that restrict these T cells are less clear, but they are defined as CD1d-reactive owing to the

ability to detect restriction to CD1d when costimulation is provided and/or autoreactivity is evident in cellular model systems.¹²

Finally, there is a much larger number of NKT cells that are not CD1d-restricted but are restricted to either MHC class I or MHC class II.¹⁵ These cells are considered NKT cells because of the expression of the NK1.1 marker on the cell surface, a marker that recently has come to be appreciated as one frequently associated with T cell activation.¹⁶ These non-CD1d-restricted NKT cells have a very diverse array of TCRs and express CD4, CD8, or are double negative.¹⁵

The vast majority of biologic functions that have been assigned to CD1d-restricted T cells to date are those associated with the iNKT subset of cells.¹⁰ A unique feature of the regulatory functions of iNKT cells is the fact that they are able to regulate a wide variety of cell types and, thus, subsequent cellular processes owing to their involvement at the earliest points of the immune response.¹⁷ iNKT cells have a very special relationship with dendritic cells (DCs) through expression of CD1d on the DC.¹⁷ This interaction between the DC and the iNKT cell leads to regulation of both subsets through secretion of IL-12 by the DC and secretion of IFN- γ , for example, by the iNKT cell that is, in turn, regulated by CD40 on the DC and CD40 ligand on iNKT.¹⁷ Once activated by CD1d on the antigen-presenting cell (APC), the iNKT cell is able to regulate a wide variety of cell types, both through cell-cell contact and through secretion of a large number of cellular mediators.¹⁷ In fact, the iNKT cell predominantly has a T helper 0 (Th0) phenotype when stimulated through its TCR-CD3 complex.^{18–21} Recent studies with human iNKT cells suggest that there might be polarization of these cells, with IFN- γ production primarily associated with the double-negative subset, and Th0 associated with the CD4⁺ subset.¹⁸ Such polarization is less evident with rodent iNKT cells but may indeed be present. iNKT cells have been shown to express Fas ligand on the cell surface and to exert cytolytic activity through the secretion of perforin and granzyme,²² and thus to regulate tumor cells and perhaps DCs, which, in turn, would have significant effects on subsequent immune responses.²³ In fact, through these cytolytic properties, iNKT cells may not only play a role in antitumor immunity but may also, through the cytolytic removal of DC1 cells, lead to potent effects on subsequent immune responses and downregulation of immune responses, in particular.^{23,24} Through the secretion of MIP1, iNKT cells regulate neutrophils.²⁵ Through signals that are yet to be defined, iNKT cells are well known to regulate NK cells and B cells. In fact, iNKT cell activation may be the most potent mediator of NK cell mobilization during the course of an immune response.¹⁰ Finally, through the secretion of a wide range of cytokines and chemokines, including IL-4, IL-10, IL-13, TGF- β , and IFN- γ , iNKT cells have the potent ability to immune deviate conventional T cells to Th1 or Th2.¹⁰ With that said, it is also well known that the absence of CD1d-restricted T cells due to deletion of the CD1d gene, for example, does not abrogate a Th2 response,^{26–28} indicating that CD1d is one of many regulatory pathways that are involved in immune deviation of conventional T cells.

As indicated in the introductory comments, these properties of iNKT cells are likely to be relevant to mucosal homeostasis and, perhaps more importantly, to the regulation and/or pathogenesis of a variety of disease processes. The MALT is constantly bombarded by a variety of foreign antigens, which require tight regulation of immune responses along the epithelial cell surface. Using IBD as a model, immune responses that are characterized by either Th1 or Th2 cytokine production can be

associated with chronic inflammation.^{29,30} Therefore, regulation of conventional T cell responses is particularly important. Given the aforementioned comments on the biology of iNKT cells, it is not surprising that iNKT cells have recently been shown to play an important role in a variety of processes related to the mucosal immune system.^{31,32} In particular, a variety of cellular subsets that bear CD1d on the cell surface, including intestinal epithelial cells (IECs), DCs, and B cells, reside within these compartments. Similarly, iNKT cells have been detected in mucosal compartments, such as the epithelium, albeit at very low proportions.^{32,33} Although iNKT cells have been identified in the epithelial compartments by the use of CD1d-tetramers, little information has been obtained on the semidiverse group of CD1d-restricted T cells as yet. However, recent studies in the oxazolone colitis model and in humans with ulcerative colitis (UC) suggest that this form of T cell may be particularly important, as discussed further below.³¹ The next section of this chapter will focus on evidence that supports a role for CD1d-restricted pathways in the regulation of several MALTs, most notably in the intestine and in the lung.

REGULATION OF MUCOSAL INFLAMMATION BY iNKT CELLS

IBD appears to be a dysregulated mucosal immune response to a subset of bacterial antigens in a genetically susceptible host.^{29,30,34} These concepts are largely based upon recent studies in a variety of animal models of colitis, which require the presence of a normal microbial ecology in conjunction with some perturbation of the MALT either through genetic manipulation or the administration of a variety of exogenous agents, including those that break down the intestinal barrier.³⁰ All of these models of IBD suggest that the mucosal inflammation is distilled from a final common pathway that generates either excess Th1 or excess Th2 cytokine production, either of which will cause the development of IBD-like immunopathology. Both Th1 and Th2 inflammation are, in turn, tightly regulated by cytokines derived from a variety of both B cells and T cells. The latter include both CD4⁺CD25⁺ T cells, the so-called T regulatory cells, which secrete TGF- β and/or IL-10. The recent appreciation that iNKT cells are decreased in a variety of autoimmune conditions (as well as, perhaps, IBD), and the fact that iNKT cells can regulate autoimmune diseases such as allergic autoimmune encephalitis and diabetes mellitus in animal models,¹⁷ originally suggested that iNKT cells may have a similar regulatory effect in IBD.

One of the earliest studies to test this hypothesis was performed in the dextran sodium sulfate (DSS) colitis model.³⁵ In this model, the administration of DSS leads to disruption of the epithelial barrier and activation of macrophages, leading to a severe colonic inflammation that is often associated with significant mortality. DSS colitis can be established in a T cell-deficient environment, in either SCID or Rag-deficient animals. However, studies from others have shown that although the generation of colitis is T cell independent, T cells clearly play an important role in the perpetuation and possibly, as shown below, the regulation of inflammation associated with the administration of DSS.

To determine whether iNKT cells are able to regulate colitis, either C57BL/6J (wild-type), CD1d-deficient, or Rag2-deficient mice were exposed to 2.5% DSS *ad libitum* in the presence of either the agonist glycolipid antigen, α GalCer, or a

nonagonist (nonfunctional) glycolipid analogue of α GalCer, α ManCer.³⁵ These studies showed that treatment of wild-type mice with α GalCer, but not α ManCer, prolonged survival after administration of DSS.³⁵ This was also associated with an amelioration of clinical symptoms (body weight loss) or signs (evidence of gastrointestinal bleeding).³⁵ The protective effect of α GalCer was dependent on iNKT cells in wild-type mice because it was abrogated by the deletion of these cells by monoclonal antibodies.³⁵ Moreover, the protective effect of α GalCer was iNKT cell-dependent because α GalCer activity was lost in either Rag-deficient or CD1d-deficient mice.³⁵ Specifically, the survival of wild-type mice was prolonged by the administration of α GalCer but not α ManCer.³⁵ The survival score of the α ManCer-treated group was identical to that of either Rag-deficient or CD1d-deficient mice administered α GalCer. Finally, to prove that these effects were due to iNKT cells, when iNKT cells were obtained from wild-type mice primed with α GalCer, they were able to adoptively transfer protection to Rag-deficient mice.³⁵ In contrast, iNKT cells from wild-type mice that had been primed with α ManCer were unable to confer protection against DSS administration.³⁵ Although the basis for the amelioration of DSS colitis by iNKT cells was not determined initially, subsequent studies have shown that the regulation of DSS colitis by iNKT cells was the result of the production of Th2 and perhaps regulatory cytokines.³⁶ Specifically, intraperitoneal injection of a glycolipid, OCH, that has previously been shown to preferentially induce production of Th2 cytokines by iNKT cells resulted in an amelioration of colitis in wild-type mice exposed to DSS but not in $J\alpha 18$ -deficient mice.³⁶ This protection from colitis in wild-type mice was associated with induction of IL-4 and IL-10.³⁶ In addition, $J\alpha 18$ -deficient mice exhibited significantly increased colitis in comparison with wild-type mice.³⁶ Taken together, these studies in the DSS colitis model suggested that certain iNKT cells are associated with the intestine and have a unique ability to preferentially secrete Th2 cytokines and/or regulatory cytokines, which are able to either immune deviate away from Th1 bias or regulate colitis.

More definitive evidence that CD1d-restricted pathways are involved in mucosal regulation associated with intestinal inflammation came from the following series of studies in hapten-mediated colitis: Instillation of several different haptens is able to induce colitis. Specifically, administration of either trinitrobenzene sulfonic acid (TNBS) or oxazolone to mice, either with or without prior skin sensitization, induced colitis that is predominantly characterized by a Th1 response in certain animal strains with the former hapten, or Th2 in certain animal strains with the latter.³⁰ Recent studies suggest that in some strains, such as Balb/C, oxazolone-induced colitis is a process generated by both Th1 and Th2 cytokines.³⁰ Similarly, C57BL/6J mice develop an oxazolone-induced colitis that is a mixed Th1- and Th2-mediated process, in contrast to SJL/J mice, which, as originally described, generate a predominantly Th2-mediated colitis. With this in mind, we studied colitis induced by oxazolone in CD1d-deficient mice. These studies showed that both CD1d-deficient and $J\alpha 18$ -deficient mice were resistant to oxazolone-induced colitis, in comparison to wild-type C57BL/6J mice.³¹ Similarly, administration of a neutralizing anti-CD1d antibody was able to achieve a similar protection of wild-type mice from the development of immunopathology associated with oxazolone administration.³¹ These studies suggested that CD1d-restricted T cells acted as provocateurs in the generation of colitis. However, other studies suggested that the ability of iNKT cells to produce or drive colitis was the result of the preferential production of Th2 cyto-

kines.^{31,37} Previous studies in the SJL/J mouse strain had shown that oxazolone colitis in this genetic background is caused by the production of IL-4.³⁷ Studies with both lamina propria mononuclear cells (LPMCs) and mesenteric lymph nodes in the C57BL/6J strain showed that oxazolone administration was also associated with a very high production of IL-4 early in the course of colitis (days 2 to 4) and is superseded by a very large production of IL-13 (days 4 to 7).³¹ This production of IL-13 was shown to be biologically important, as oxazolone colitis could be prevented by neutralization of IL-13 activity through intraperitoneal injection of an IL-13-receptor $\alpha 2$ -Fc fusion protein.³¹ Evidence that IL-13 responsible for the generation of colitis was derived from iNKT cells was obtained from observations showing that stimulation of LPMC by either anti-CD3 + CD28 or L cells transfected with CD1d, but not untransfected L cells, in the presence of α GalCer, induced significant production of IL-4 and, to a greater extent, IL-13.³¹ Taken together, the observations that (1) deletion of either CD1d or iNKT cells, or neutralization of IL-13, ameliorated colitis, and (2) activation of LPMCs by CD1d-restricted antigens induced IL-13 suggest that CD1d-restricted T cells act as effector cells in the development of Th2-mediated colitis in the oxazolone model.³¹ Thus, it would appear that Th2-associated colitis is driven by iNKT cells. This is consistent with recent studies in Th2-associated inflammation of the lungs such as asthma, which is also iNKT cell mediated.³²

Recent studies have advanced the notion that iNKT cells are protagonists in the development of Th2-associated inflammation and have provided additional insights into the regulation of these processes. Specifically, whereas the Th1-associated IBD-like inflammation characteristic of Crohn's disease is the result of DC-mediated production of IL-12, which functions as a master cytokine in driving Th1 responses, the factors presumably secreted by DCs in response to iNKT cells in Th2-associated immunopathology are less well defined.³⁸ One possibility is that, like the Th1 inflammation caused by DC production of IL-12, DCs secrete an IL-12-related factor that drives iNKT cells to regulate conventional T cell production of excess Th2 cytokines. This particular notion has, in fact, been investigated and a novel IL-12 p40-like molecule, Epstein-Barr virus-induced gene 3 (EBI3), has been identified as a potential factor in this effect.³⁹

In addition to the IL-12 molecule, a heterodimer that consists of a p40 chain and a p35 chain,⁴⁰ recent studies have identified a variety of other IL-12 family members. These include IL-23, a heterodimer consisting of the p40 chain, IL-12, and a novel p19 chain, and IL-27, a novel heterodimer formed by EBI3 and p28.^{40,41} Recent studies suggest that whereas IL-27 acts very early in the immune response to support the development of Th1 responses through interactions with an orphan cytokine receptor, WSX-1, the subsequent perpetuation of the Th1 response is mediated by IL-12, and its maintenance through effects on T memory cells by IL-23.⁴⁰⁻⁴² However, in addition to the association between EBI3 and p28, it is clear from other studies that EBI3 may associate either with itself as a homodimer or with other factors, as yet to be defined, to produce other effects such as the possible induction of Th2 responses. These effects have recently been suggested by investigations in models of mucosal inflammation, and may include a role for EBI3 in regulating Th2 pathways (in addition to Th1 pathways) through effects on the iNKT cell.³⁹

Recently, EBI3-deficient mice have been generated.³⁹ Although these mice have normal numbers of conventional T cells and B cells and have no obvious immuno-

pathology, they maintain a reduced number of iNKT cells in the liver as well as in the spleen in comparison with control mice, as evidenced by CD1d tetramer staining of CD3-positive cells.³⁹ Moreover, CD1d-restricted iNKT cells from EBI3-deficient mice secrete lower levels of the Th2 cytokine, IL-4, but not the Th1 cytokine, IFN- γ , when stimulated with a model glycolipid antigen, α GalCer.³⁹ Specifically, when iNKT cells were obtained from EBI3-deficient animals, low levels of IL-4 secretion were stimulated, in comparison with quantities of IFN- γ secreted, when these cells were exposed to CD1d-transfected B cells in the presence of α GalCer.³⁹ Similarly, serum IL-4 was lower in EBI3 mice that received α GalCer intravenously than in comparable wild-type mice.³⁹ In other studies, it was shown that these defects in IL-4 production by iNKT cells were due not only to the quantitative defect in iNKT cells, but presumably also to a defect in DC regulation of iNKT cell responses (unpublished observations). This defect in Th2 cytokine production by iNKT cells was clinically meaningful in that EBI3-deficient mice were protected from the development of oxazolone-associated colitis.³⁹ In contrast, the response of EBI3-deficient mice to TNBS was similar to that of wild-type mice.³⁹ These results further support the concept that iNKT cells are major drivers in the development of oxazolone-associated colitis. Moreover, the results in the EBI3-deficient environment suggest that EBI3 is a major regulator of this iNKT cell-mediated response and, ultimately, colitis.³⁹ This protection from pathologic injury to oxazolone in the context of EBI3 deficiency was, importantly, associated with diminution in not only clinical scores as defined by body weight loss, but also the degree of pathology and the production of IL-4, but not IFN- γ , in the EBI3-deficient mice.³⁹ Specifically, the absence of EBI3 was associated with decreased IL-4 production by the LPMCs and diminished nuclear translocation of GATA-3, a major nuclear transcription factor associated with Th2 cytokine production.³⁹

These studies suggest that the CD1d-restricted T cell, notably the iNKT cell subset, is a major effector cell in the development of Th2-associated colitis. This, in turn, suggests that an EBI3-secreting APC, such as a DC, (1) regulates both the quantity and function of iNKT cells that may directly cause colitis through the secretion of Th2 cytokines, or (2) regulates the ability of the iNKT cell to cause cytotoxicity at target cells such as intestinal epithelial cells (IEC), or (3) modulates the quantities of Th2 cytokine production by conventional T cells. This contrasts with the ability of DCs, through the production of IL-12, to regulate Th1-mediated colitis and, ultimately, production of IFN- γ and TNF- α . The molecular form of EBI3 that is associated with this biologic response remains undefined. It is unlikely to be IL-27, based on the major role of this cytokine in regulating proliferation of naive T cells and promoting Th1 cytokine production.⁴¹ It may very well be the case that the proliferative effect of IL-27 on iNKT cells may play such a role. It is possible, however, that EBI3 causes these effects in Th2 cytokine production either through its molecular association with itself as a homodimer or with other molecules, such as either the p35 chain of IL-12 or other as yet to be defined molecules. Nonetheless, these studies show how CD1d-restricted pathways can regulate mucosal inflammation and prove the biologic importance of CD1d-restricted T cells in the generation of mucosal inflammation. These concepts derived from studies in Th2-deviated animals may apply to analogous human disease, namely, ulcerative colitis (UC).⁴³ Increased EBI3 expression has been found in patients with active UC.^{43,44} In contrast, EBI3 expression is relatively normal in Crohn's disease (CD), similar to that in normal subjects,

as evidenced by several different quantitative RT PCR analyses.^{43,44} This contrasts again with the production of IL-12 in humans, where the expression is increased in CD but not in UC.⁴⁵ The production of EB13 in human UC appears also to be primarily associated with DCs. Moreover, recent preliminary studies have shown that LPMCs from patients with CD secrete significant quantities of IFN- γ , in contrast to UC patients who secrete very high concentrations of IL-13 and IL-5 but, interestingly, not IL-4, suggesting a modified Th2 response in human UC. This production of IL-13 and IL-5 is derived from CD1d-restricted T cells and, perhaps, predominantly from the semidiverse subset of human CD1d-restricted T cells within the LP (I.F. and W.S., unpublished observation). This contrasts somewhat with studies in mouse models and deserves further investigation. Nonetheless, this does suggest that CD1d-restricted T cell pathways play a role not only in mouse models of colitis, but also in the human condition.

REGULATION OF MUCOSAL COLONIZATION BY BACTERIAL PATHOGENS

A characteristic feature of iNKT cells is their ability to secrete both Th1 and Th2 cytokines. This property is presumably due to the heterogeneity of these cells and/or the variation in glycolipid antigens to which they respond, as well as other factors, including the characteristics of costimulatory molecules on the cell surface of the stimulating APC at the time of activation. This may also account for the ability of iNKT cells to both promote and downregulate inflammation, and to participate in such disparate biologic processes as regulation of Th1 and/or Th2 inflammation.¹⁰ Further evidence that iNKT cells and, thus, CD1d-restricted antigen presentation pathways are operative in mucosal tissues has been obtained from studying clearance of *Pseudomonas aeruginosa* from the lungs.⁴⁶

Previous studies on the pathogenesis of *Pseudomonas* airway infection have identified alveolar macrophages and their products as well as neutrophils as important mediators of anti-*Pseudomonas* immunity.⁴⁷ Alveolar macrophages have been shown to secrete chemokines such as MIP-2 and KC, which activate neutrophils through the CXCR2 receptor.⁴⁷ Depletion of macrophages, neutralization of MIP-2, or neutralization of CXCR2 have all been shown to abrogate *Pseudomonas* infection within the lung.⁴⁷

The importance of CD1d-restricted T cells in these processes was recently established through experiments in a *P. aeruginosa* model lung system.⁴⁶ In these experiments, Balb/C or C57BL/6J mice, Rag-deficient mice, or CD1d-deficient mice were inoculated with a clinical strain of *P. aeruginosa* (strain D4) and the lung tissues, blood and peritoneal macrophages examined before or after different experimental manipulations, including administration of α GalCer (or the nonfunctional glycolipid analogue, α ManCer) or neutralizing anti-CD1d antibody.⁴⁶ Studies showed that CD1d-deficient animals exhibited reduced clearance of *P. aeruginosa* from lung tissues.⁴⁶ Similar observations were made when wild-type Balb/C mice were treated with an anti-CD1d monoclonal antibody 24 h after inoculation with the bacterial pathogen.⁴⁶ The reduced ability of CD1d-deficient animals to clear *P. aeruginosa* was observed in mice from both a C57BL/6J or Balb/C background, consistent with the nonpolymorphic nature of CD1d. It was associated with a decrease in MIP-2 pro-

duction and, as a corollary, neutrophil recruitment into bronchoalveolar lavage fluid (BALF) at 6 h after induction of the *Pseudomonas* infection.⁴⁶ These studies indicate that *P. aeruginosa* clearance is CD1d-dependent and is mediated through macrophage regulation of MIP-2 production, which results in neutrophil recruitment. In the absence of CD1d, MIP-2 production and neutrophil recruitment are abrogated.⁴⁶

To confirm and extend these results, wild-type mice were treated with α GalCer or the nonfunctional glycolipid analogue, α ManCer.⁴⁶ In these studies, activation of CD1d and NKT cell pathways by α GalCer enhanced *P. aeruginosa* clearance in the lungs.⁴⁶ Interestingly, in the setting of activation of the CD1d-restricted pathway by α GalCer, clearance of *P. aeruginosa* was shown to be independent of neutrophil recruitment into the BALF.⁴⁶ The rapid clearance of *P. aeruginosa* in these circumstances was associated with rapid recovery from pneumonia, despite evidence of tissue inflammation within hours of infection. In contrast to the rapid resolution of inflammation seen in α GalCer-treated mice, similar animals that received α ManCer had very severe pneumonia by 24 h after *P. aeruginosa* infection.⁴⁶ In addition, α GalCer treatment of wild-type mice was noted to activate peritoneal macrophages within 30 min of α GalCer administration.⁴⁶ This effect was presumably based on the ability of α GalCer to activate iNKT cells, in that direct treatment of peritoneal macrophages with α GalCer did not result in increased peritoneal macrophage activation and, thus, clearance of *P. aeruginosa* through phagocytosis.⁴⁶ As shown by

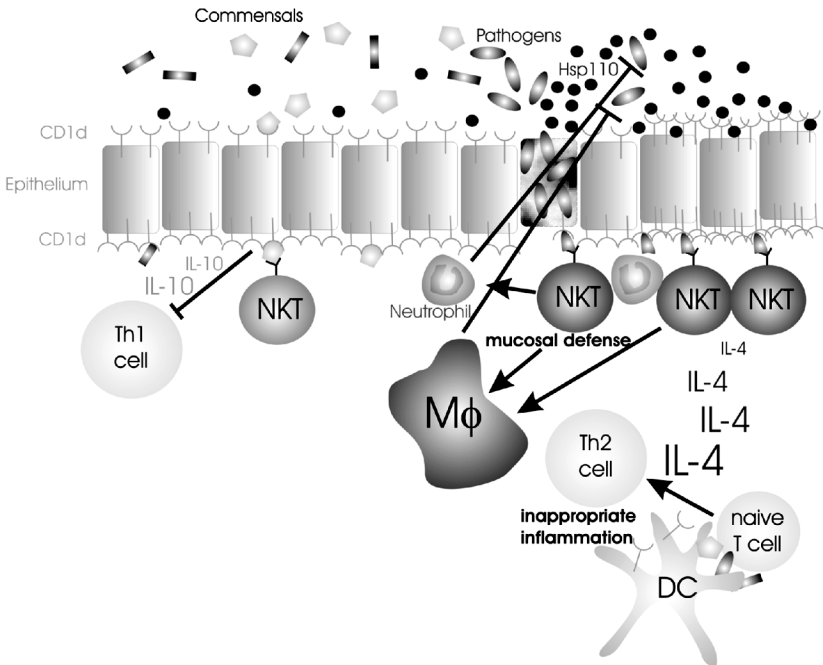


FIGURE 1. Proposed model of CD1d function in the intestinal epithelium.

CD1d tetramer staining in BALF, α GalCer also caused significant augmentation of CD1d-restricted T cells.⁴⁶ Interestingly, normal BALF contained a small number of iNKT cells that increased dramatically by almost a thousandfold after α GalCer treatment in direct proportion to the number of conventional T cells after *P. aeruginosa* instillation.⁴⁶ This was associated with high levels of IFN- γ and TNF- α in BALF after α GalCer treatment.⁴⁶ In addition, alveolar macrophages of α GalCer-treated mice had strongly enhanced phagocytosis of *P. aeruginosa* within one hour of colonization.⁴⁶

Taken together, these studies indicate that CD1d-restricted T cells play a key role as sentinels within mucosal tissues monitoring and reacting to mucosal colonization by bacterial pathogens. Specifically, observations in CD1d-deficient animals suggest that CD1d-restricted pathways are an endogenous antimicrobial defense in the lung and perhaps other mucosal tissues. In animals given α GalCer, these observations suggest that once activated, the CD1d-restricted T cell arms the mucosal immune system by regulating macrophage phagocytosis of microbial pathogens. These studies further indicate that iNKT cells activated early during the immune response regulate downstream effector cells, such as macrophages and neutrophils, critical to mucosal defense. These observations in α GalCer-treated animals may present a paradigm for the role of mucosal immunity in preventing colonization of the intestine by *P. aeruginosa*, in the absence of alterations in either intestinal morphology or epithelial barrier function. Consistent with this, CD1d-deficient mice exhibit a decreased ability to clear mucosal colonization by *P. aeruginosa* from the intestine (E.E.S.N. & R.S.B., unpublished observations).

THE INTESTINAL EPITHELIAL CELL, A NOVEL ANTIGEN-PRESENTING CELL TYPE IN MUCOSAL TISSUES

The comments above show that CD1d and CD1d-restricted pathways are operative in both promoting inflammation and providing defense. A number of different cell populations expressing CD1d that might provide these functions are present at mucosal surfaces. These include dendritic cells, macrophages, B lymphocytes and, importantly, epithelial cells.⁴⁸ Over the past decade, increasing evidence has suggested that epithelial cells in the intestine and, it is likely, other organ systems may very well play an important role in regulating CD1d-restricted pathways. The remainder of this review will focus on evidence to support a role for CD1d-restricted antigen presentation by epithelial cells (FIG. 1).

Immunohistochemical studies have shown that CD1d is expressed by intestinal epithelial cells in both humans⁴⁹ and rodents (mice and rats).⁵⁰ In rats, CD1d protein expression occurs primarily in the villous epithelial cells, with transcription most evident in the crypt cells. Recent studies have confirmed these results in humans (personal observation, L. M.). In addition to IECs, CD1d has been found to be expressed on epithelial cells of the bile ducts and skin.⁵¹ Additional morphologic evidence that IECs express CD1d is derived from an observation that when α GalCer is injected into mice, it localizes very specifically to IECs; such localization is not observed in CD1d-deficient animals.³⁵

The biochemical expression of CD1d on IECs is unique.^{4,48} Two isoforms have been identified. The major isoform is a bona fide 48-kDa glycoprotein, consistent with the known molecular weight of CD1d, that consists of a polypeptide backbone of approximately 35 kDa with four N-linked carbohydrate side chain modifications.^{48,49,52} This 48-kD glycoprotein is associated with β_2 -microglobulin (β_2 M) and has been shown by selective cell surface biotinylation to be localized to both apical and basal cell surfaces.⁴⁸ A smaller proportion of CD1d, approximately 10% or less of the CD1d associated with the IEC, has been identified as a novel, 37-kDa isoform that is not associated with β_2 M and is nonglycosylated.^{48,53–55} This isoform of CD1d is restricted to the apical cell surface of the IEC; there is no evidence of expression on the basal cell surface. The 37-kDa isoform is characterized by a novel posttranslational modification that consists of hydroxylation of proline residues.⁵⁵ This is consistent with the observed association of CD1d with protein disulfide isomerase (PDI), an endoplasmic reticulum resident protein that itself associates with prolyl-4 hydroxylase and other ER proteins.⁵⁵

CD1d on the IEC is functional, as neutralization of CD1d neutralizes the activation of peripheral blood T cells by mouse and human IEC lines, and freshly isolated IECs are capable of CD1d-restricted antigen presentation. This was shown by the following observations: The IEC line MODE-K, which is an immortalized but not transformed mouse cell line derived from normal mouse small intestine, exhibits dose-dependent presentation of α GalCer but not β GalCer, as interpreted by the production of murine IL-2 by the iNKT cell hybridoma, DN32.D3.⁵⁶ Similarly, freshly isolated human IECs and the human IEC line T84 transfected with human CD1d exhibit dose-dependent presentation of α GalCer to the DN32 cell line.⁵⁶ This presentation of α GalCer is CD1d restricted because it is inhibited by a monoclonal antibody specific for human CD1d.⁵⁶ The CD1d-restricted antigen presentation also exhibits polarity: when T84 cells transfected with CD1d are grown in polarized monolayers, CD1d-restricted antigen presentation is evident primarily on the basal cell surface and less so on the apical cell surface.⁵⁶ This is consistent with the normal localization of intestinal intraepithelial lymphocytes (iIEL) to this anatomic space. However, in contrast to CD1d-restricted antigen presentation by specialized APCs, IECs are incapable of processing glycolipid antigens, as shown by modeling with glycolipid analogues of α GalCer. Whereas Gal(α 1–6)GalCer does not require processing for presentation on CD1d, Gal(α 1–2)GalCer requires processing by lysosomal galactosidases.⁵⁷ Unlike B cells, which are capable of processing and presenting these modified glycolipid antigens, IECs cannot present such glycolipid antigens that require processing.⁵⁶ These studies indicate that CD1d on IECs is functional and capable of CD1d-restricted antigen presentation.

Altogether, it is likely that IECs are a novel, CD1d-restricted APC type. A characteristic feature of specialized APCs that express CD1d is their ability to communicate in a bidirectional fashion with the CD1d-restricted T cell through the expression of ligands on the APC, such as CD40, and counter-ligands on the CD1d-restricted T cells, such as CD40 ligand.^{10,17} Such interactions lead to the expression of IL-12, for example. A similar attribute is likely to be ascribed to IECs as a CD1d-restricted APC, in view of the observation that when CD1d is ligated on the IEC, it is able to induce significant IL-10 secretion by the IEC.⁵⁸ The quantities of IL-10 that are secreted by the IEC are sufficient to abrogate the permeability defect induced by IFN- γ .⁵⁸ Because IFN- γ is a major proinflammatory cytokine secreted by

iIELs and is a potent cytokine upregulating CD1d on the IEC,⁵⁹ IL-10 induced by CD1d ligation on IECs may promote the preservation of epithelial barrier function during the course of proinflammatory events, as in IBD and mucosal infections. CD1d may function together with a novel CEA-like molecule, gp180, to provide these effects.^{60,61}

Finally, CD1d on IECs seems to be uniquely regulated. Very little is known about the regulation of CD1d on any cell type. However, recent studies in IECs have shed important light on IECs and perhaps other CD1d-restricted APCs. Earlier studies indicated that IFN- γ , but not other cytokines, is capable of regulating CD1d on IECs.⁵⁹ A notable characteristic of CD1d expression in the intestine is the increased expression with freshly isolated epithelial cells versus epithelial cell lines.⁶² This suggests that factors are present *in vivo* that are uniquely capable of regulating CD1d expression. Recently, in testing this hypothesis, it was discovered that IEC expression of CD1d is induced by aqueous components from the normal human and murine milieu.⁶² These factors were shown to be proteinaceous in character, and thus sensitive to heat denaturation, and to be of large molecular weight (>100 kDa).⁶² It was shown that these components exhibited activity within the intestinal lumen on multiple IEC lines and could be identified in the luminal milieu of germ-free mice, indicating a likely eukaryotic origin, rather than a prokaryotic one. This was confirmed when purification and tryptic digest sequencing identified the biologic activity as heat shock protein (HSP)-110.⁶² Indeed, HSP-110 could be identified in the luminal aqueous milieu, the biologic activity of the luminal components could be neutralized by an antibody specific for HSP-110, and recombinant HSP-110 added to epithelial cell lines was able to induce CD1d expression.⁶² Perhaps most interestingly, the cellular origin of the HSP-110, as shown by immunohistochemistry, appears to be the IEC itself.⁶² IECs of the human small and large intestine express significant amounts of HSP-110.⁶² These studies indicate an autocrine pathway of CD1d regulation controlled by the release into the luminal milieu of HSP-110, which is able to act upon a cellular receptor, presumably apical in origin, to induce CD1d. The molecular nature of this putative apical receptor remains to be defined. However, it is well known that several heat shock proteins, including HSP-70 and HSP-90, are able to bind a variety of putative HSP receptors, potentially including CD36, CD40, and Toll-like receptors (TLR)-4 and TLR-2, among others. Ligation of these receptors induces an activation pathway that upregulates cell surface molecules and induces the secretion of cytokines such as IL-12, TNF- α , and IL-1 β . Presumably, HSP-110 acts similarly on IECs and includes in its repertoire the upregulation of CD1d.⁶² Thus, CD1d expression on the epithelial cell may very well be involved in the maintenance of mucosal homeostasis as well as the regulation of mucosal inflammation and antimicrobial defense through its ability to regulate and/or be regulated by HSP-110.

CONCLUSION

The MALTs in intestine and lung are uniquely responsible for managing a variety of environmental antigenic exposures, including exposure to microbial pathogens. A unique feature of these mucosal surfaces is the expression of CD1d on novel APCs, such as epithelial cells, and the presence of CD1d-restricted T cells that are operative

in managing a variety of events by promoting mucosal inflammation and, at the same time, providing resistance against pathogenic microbial exposure. The relationship between CD1d-restricted pathways and a variety of different mucosal-related immunopathologies may, on the one hand, appear paradoxical. On the other hand, however, they are consistent with the pleiotropic functions of these novel antigen-presenting pathways in managing a variety of biologic processes, and with the cell types associated with these pathways. The association of CD1d-restricted pathways in promoting IBD, in particular, may illustrate their role in mucosal infections that cause inappropriate inflammation. Providing insights and defining additional molecular details of these CD1d-restricted pathways on epithelial surfaces will likely be extremely informative for understanding mucosal immunobiology and for designing novel therapeutic strategies for treating numerous clinical conditions of these organs.

REFERENCES

1. CALABI, F. & C. MILSTEIN. 1986. A novel family of human major histocompatibility complex-related genes not mapping to chromosome 6. *Nature* **323**: 540–543.
2. MARTIN, L.H., F. CALABI & C. MILSTEIN. 1986. Isolation of CD1 genes: a family of major histocompatibility complex-related differentiation antigens. *Proc. Natl. Acad. Sci. USA* **83**: 9154–9158.
3. DASCHER, C.C. & M.B. BRENNER. 2003. Evolutionary constraints on CD1 structure: insights from comparative genomic analysis. *Trends Immunol.* **24**: 412–418.
4. BLUMBERG, R.S., D. GERDES, A. CHOTT, *et al.* 1995. Structure and function of the CD1 family of MHC-like cell surface proteins. *Immunol. Rev.* **147**: 5–29.
5. ZENG, Z., A.R. CASTANO, B.W. SEGELKE, *et al.* 1997. Crystal structure of mouse CD1: An MHC-like fold with a large hydrophobic binding groove. *Science* **277**: 339–345.
6. ZAJONC, D.M., M.A. ELSLIGER, L. TEYTON & I.A. WILSON. 2003. Crystal structure of CD1a in complex with a sulfatide self antigen at a resolution of 2.15 Å. *Nat. Immunol.* **4**: 808–815.
7. GUMPERZ, J.E. & M.B. BRENNER. 2001. CD1-specific T cells in microbial immunity. *Curr. Opin. Immunol.* **13**: 471–478.
8. KAWANO, T., J. CUI, Y. KOEZUKA, *et al.* 1997. CD1d-restricted and TCR-mediated activation of valpha14 NKT cells by glycosylceramides. *Science* **278**: 1626–1629.
9. GUMPERZ, J.E., C. ROY, A. MAKOWSKA, *et al.* 2000. Murine CD1d-restricted T cell recognition of cellular lipids. *Immunity* **12**: 211–221.
10. KRONENBERG, M. & L. GAPIN. 2002. The unconventional lifestyle of NKT cells. *Nat. Rev. Immunol.* **2**: 557–568.
11. LANTZ, O. & A. BENDELAC. 1994. An invariant T cell receptor alpha chain is used by a unique subset of major histocompatibility complex class I-specific CD4+ and CD4-8- T cells in mice and humans. *J. Exp. Med.* **180**: 1097–1106.
12. BEHAR, S.M., T.A. PODREBARAC, C.J. ROY, *et al.* 1999. Diverse TCRs recognize murine CD1. *J. Immunol.* **162**: 161–167.
13. RIESE, R.J., G.P. SHI, J. VILLADANGOS, *et al.* 2001. Regulation of CD1 function and NK1.1(+) T cell selection and maturation by cathepsin S. *Immunity* **15**: 909–919.
14. PARK, S.H., A. WEISS, K. BENLAGHA, *et al.* 2001. The mouse CD1d-restricted repertoire is dominated by a few autoreactive T cell receptor families. *J. Exp. Med.* **193**: 893–904.
15. EBERL, G., R. LEES, S.T. SMILEY, *et al.* 1999. Tissue-specific segregation of CD1d-dependent and CD1d-independent NK T cells. *J. Immunol.* **162**: 6410–6419.
16. SLIFKA, M.K., R.R. PAGARIGAN & J.L. WHITTON. 2000. NK markers are expressed on a high percentage of virus-specific CD8+ and CD4+ T cells. *J. Immunol.* **164**: 2009–2015.
17. WILSON, S.B. & T.L. DELOVITCH. 2003. Janus-like role of regulatory iNKT cells in autoimmune disease and tumour immunity. *Nat. Rev. Immunol.* **3**: 211–222.

18. GUMPERZ, J.E., S. MIYAKE, T. YAMAMURA & M.B. BRENNER. 2002. Functionally distinct subsets of CD1d-restricted natural killer T cells revealed by CD1d tetramer staining. *J. Exp. Med.* **195**: 625–636.
19. KADOWAKI, N., S. ANTONENKO, S. HO, *et al.* 2001. Distinct cytokine profiles of neonatal natural killer T cells after expansion with subsets of dendritic cells. *J. Exp. Med.* **193**: 1221–1226.
20. MATSUDA, J.L., L. GAPIN, J.L. BARON, *et al.* 2003. Mouse V alpha 14i natural killer T cells are resistant to cytokine polarization in vivo. *Proc. Natl. Acad. Sci. USA* **100**: 8395–8400.
21. LEE, P.T., K. BENLAGHA, L. TEYTON & A. BENDELAC. 2002. Distinct functional lineages of human V(alpha)24 natural killer T cells. *J. Exp. Med.* **195**: 637–641.
22. KANEKO, Y., M. HARADA, T. KAWANO, *et al.* 2000. Augmentation of Valpha14 NKT cell-mediated cytotoxicity by interleukin 4 in an autocrine mechanism resulting in the development of concanavalin A-induced hepatitis. *J. Exp. Med.* **191**: 105–114.
23. YANG, O.O., F.K. RACKE, P.T. NGUYEN, *et al.* 2000. CD1d on myeloid dendritic cells stimulates cytokine secretion from and cytolytic activity of V alpha 24J alpha Q T cells: a feedback mechanism for immune regulation. *J. Immunol.* **165**: 3756–3762.
24. STROMINGER, J.L., M.C. BYRNE & S.B. WILSON. 2003. Regulation of dendritic cell subsets by NKT cells. *C. R. Biol.* **326**: 1045–1048.
25. KAWAKAMI, K., N. YAMAMOTO, Y. KINJO, *et al.* 2003. Critical role of Valpha14+ natural killer T cells in the innate phase of host protection against *Streptococcus pneumoniae* infection. *Eur. J. Immunol.* **33**: 3322–3330.
26. SMILEY, S.T., M.H. KAPLAN & M.J. GRUSBY. 1997. Immunoglobulin E production in the absence of interleukin-4-secreting CD1-dependent cells. *Science* **275**: 977–979.
27. CHEN, Y.H., N.M. CHIU, M. MANDAL, *et al.* 1997. Impaired NK1+ T cell development and early IL-4 production in CD1-deficient mice. *Immunity* **6**: 459–467.
28. MENDIRATTA, S.K., W.D. MARTIN, S. HONG, *et al.* 1997. CD1d1 mutant mice are deficient in natural T cells that promptly produce IL-4. *Immunity* **6**: 469–477.
29. BLUMBERG, R.S., L.J. SAUBERMANN & W. STROBER. 1999. Animal models of mucosal inflammation and their relation to human inflammatory bowel disease. *Curr. Opin. Immunol.* **11**: 648–656.
30. STROBER, W., I.J. FUSS & R.S. BLUMBERG. 2002. The immunology of mucosal models of inflammation. *Annu. Rev. Immunol.* **20**: 495–549.
31. HELLER, F., I.J. FUSS, E.E. NIEUWENHUIS, *et al.* 2002. Oxazolone colitis, a Th2 colitis model resembling ulcerative colitis, is mediated by IL-13-producing NK-T cells. *Immunity* **17**: 629–638.
32. AKBARI, O., P. STOCK, E. MEYER, *et al.* 2003. Essential role of NKT cells producing IL-4 and IL-13 in the development of allergen-induced airway hyperreactivity. *Nat. Med.* **9**: 582–588.
33. PARK, S.H., K. BENLAGHA, D. LEE, *et al.* 2000. Unaltered phenotype, tissue distribution and function of Valpha14(+) NKT cells in germ-free mice. *Eur. J. Immunol.* **30**: 620–625.
34. BLUMBERG, R.S. & W. STROBER. 2001. Prospects for research in inflammatory bowel disease. *JAMA* **285**: 643–647.
35. SAUBERMANN, L.J., P. BECK, Y.P. DE JONG, *et al.* 2000. Activation of natural killer T cells by alpha-galactosylceramide in the presence of CD1d provides protection against colitis in mice. *Gastroenterology* **119**: 119–128.
36. UENO, Y., M. SUMII, S. TANAKA, *et al.* 2003. OCH, a newly synthetic glycolipid, enhances protective immunity against mouse DSS-induced colitis in the presence of Valpha14 natural killer T cells [abstract]. *Gastroenterology* **124**: A-36.
37. BOIRIVANT, M., I.J. FUSS, A. CHU & W. STROBER. 1998. Oxazolone colitis: A murine model of T helper cell type 2 colitis treatable with antibodies to interleukin 4. *J. Exp. Med.* **188**: 1929–1939.
38. NEURATH, M.F., S. FINOTTO & L.H. GLIMCHER. 2002. The role of Th1/Th2 polarization in mucosal immunity. *Nat. Med.* **8**: 567–573.
39. NIEUWENHUIS, E.E., M.F. NEURATH, N. CORAZZA, *et al.* 2002. Disruption of T helper 2-immune responses in Epstein-Barr virus-induced gene 3-deficient mice. *Proc. Natl. Acad. Sci. USA* **99**: 16951–16956.

40. TRINCHIERI, G., S. PFLANZ & R.A. KASTELEIN. 2003. The IL-12 family of heterodimeric cytokines: new players in the regulation of T cell responses. *Immunity*. **19**: 641–644.
41. PFLANZ, S., J.C. TIMANS, J. CHEUNG, *et al.* 2002. IL-27, a heterodimeric cytokine composed of EB13 and p28 protein, induces proliferation of naive CD4(+) T cells. *Immunity* **16**: 779–790.
42. PFLANZ, S., L. HIBBERT, J. MATTSO, *et al.* 2004. WSX-1 and glycoprotein 130 constitute a signal-transducing receptor for IL-27. *J. Immunol.* **172**: 2225–2231.
43. OMATA, F., M. BIRKENBACH, S. MATSUZAKI, *et al.* 2001. The expression of IL-12 p40 and its homologue, Epstein-Barr virus-induced gene 3, in inflammatory bowel disease. *Inflamm. Bowel. Dis.* **7**: 215–220.
44. CHRIST, A.D., A.C. STEVENS, H. KOEPPEN, *et al.* 1998. An interleukin 12-related cytokine is up-regulated in ulcerative colitis but not in Crohn's disease. *Gastroenterology* **115**: 307–313.
45. PODOLSKY, D.K. 2002. Inflammatory bowel disease. *N. Engl. J. Med.* **347**: 417–429.
46. NIEUWENHUIS, E.E., T. MATSUMOTO, M. EXLEY, *et al.* 2002. CD1d-dependent macrophage-mediated clearance of *Pseudomonas aeruginosa* from lung. *Nat. Med.* **8**: 588–593.
47. CHEUNG, D.O., K. HALSEY & D.P. SPEERT. 2000. Role of pulmonary alveolar macrophages in defense of the lung against *Pseudomonas aeruginosa*. *Infect. Immun.* **68**: 4585–4592.
48. BALK, S.P., S. BURKE, J.E. POLISCHUK, *et al.* 1994. Beta 2-microglobulin-independent MHC class Ib molecule expressed by human intestinal epithelium. *Science* **265**: 259–262.
49. BLUMBERG, R.S., C. TERHORST, P. BLEICHER, *et al.* 1991. Expression of a nonpolymorphic MHC class I-like molecule, CD1D, by human intestinal epithelial cells. *J. Immunol.* **147**: 2518–2524.
50. BLEICHER, P.A., S.P. BALK, S.J. HAGEN, *et al.* 1990. Expression of murine CD1 on gastrointestinal epithelium. *Science* **250**: 679–682.
51. CANCHIS, P.W., A.K. BHAN, S.B. LANDAU, *et al.* 1993. Tissue distribution of the nonpolymorphic major histocompatibility complex class I-like molecule, CD1d. *Immunology* **80**: 561–565.
52. BALK, S.P., P.A. BLEICHER & C. TERHORST. 1989. Isolation and characterization of a cDNA and gene coding for a fourth CD1 molecule. *Proc. Natl. Acad. Sci. USA* **86**: 252–256.
53. KIM, H.S., J. GARCIA, M. EXLEY, *et al.* 1999. Biochemical characterization of CD1d expression in the absence of beta2-microglobulin. *J. Biol. Chem.* **274**: 9289–9295.
54. SOMNAY-WADGAONKAR, K., A. NUSRAT, H.S. KIM, *et al.* 1999. Immunolocalization of CD1d in human intestinal epithelial cells and identification of a beta2-microglobulin-associated form. *Int. Immunol.* **11**: 383–392.
55. KIM, H.S., S.P. COLGAN, R. PITMAN, *et al.* 2000. Human CD1d associates with prolyl-4-hydroxylase during its biosynthesis. *Mol. Immunol.* **37**: 861–868.
56. VAN DE, W.Y., N. CORAZZA, M. ALLEZ, *et al.* 2003. Delineation of a CD1d-restricted antigen presentation pathway associated with human and mouse intestinal epithelial cells. *Gastroenterology* **124**: 1420–1431.
57. PRIGOZY, T.I., O. NAIDENKO, P. QASBA, *et al.* 2001. Glycolipid antigen processing for presentation by CD1d molecules. *Science* **291**: 664–667.
58. COLGAN, S.P., R.M. HERSHBERG, G.T. FURUTA & R.S. BLUMBERG. 1999. Ligation of intestinal epithelial CD1d induces bioactive IL-10: critical role of the cytoplasmic tail in autocrine signaling. *Proc. Natl. Acad. Sci. USA* **96**: 13938–13943.
59. COLGAN, S.P., V.M. MORALES, J.L. MADARA, *et al.* 1996. IFN-gamma modulates CD1d surface expression on intestinal epithelia. *Am. J. Physiol.* **271**: C276–C283.
60. CAMPBELL, N.A., H.S. KIM, R.S. BLUMBERG & L. MAYER. 1999. The nonclassical class I molecule CD1d associates with the novel CD8 ligand gp180 on intestinal epithelial cells. *J. Biol. Chem.* **274**: 26259–26265.
61. YIO, X.Y. & L. MAYER. 1997. Characterization of a 180-kDa intestinal epithelial cell membrane glycoprotein, gp180. A candidate molecule mediating t cell-epithelial cell interactions. *J. Biol. Chem.* **272**: 12786–12792.
62. COLGAN, S.P., R.S. PITMAN, T. NAGAISHI, *et al.* 2003. Intestinal heat shock protein 110 regulates expression of CD1d on intestinal epithelial cells. *J. Clin. Invest* **112**: 745–754.