LECTURE

Characterization of CD1d in mucosal immune function: an immunotherapeutic target for inflammatory bowel disease

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Abstract. In addition to the classical MHC class I and class II molecules, human intestinal epithelial cells also express nonclassical MHC class I-like molecules on their cell surface. CD1d is a non-polymorphic MHC-like molecule whose expression is mainly localized to the epithelial cells of the gastrointestinal tract. The biochemical structure of CD1d on intestinal epithelial cells (IECs) exists in two forms: a 37-kD nonglycosylated, β_2-microglobulin (β_2M) independent and a 48-kD glycosylated, β_2M dependent form. Immunolocalization studies suggest that the 37-kD nonglycosylated form of CD1d is limited to the apical cell surface whereas the 48–50-kD glycosylated, β_2M dependent form of CD1d is expressed both on the apical and the basolateral surfaces. The β_2M association with CD1d seems to be important in regulating the pattern of glycosylation and the localization of CD1d within the cell based upon studies of the structure of CD1d in a transfected model cell line and in polarized epithelial cell monolayers. The functional role of intestinal CD1d remains unknown. However, based upon *in vitro* studies of the antigens presented by human CD1d and mouse CD1d, CD1d expressed on IECs likely presents a very hydrophobic glycolipid molecule possibly from the cell wall of bacteria or host cells. The processed-lipid antigen presented by CD1d may then involve a yet-to-be-identified subpopulation of the resident, oligoclonal αβ TCR $CD8^+$ intestinal intraepithelial lymphocyte (iIEL) T cells. Subsequently, these T cells would be very important in regulating the local immune response by producing cytokines and recruiting other immune modulating cells to destroy infected cells, regenerate normal IECs, and possibly downregulate activated T cells to maintain mucosal integrity. (Keio J Med 50 (1): 39–44, March 2001)

Key words: CD1d, mucosal immunity, inflammatory bowel disease, NK-T cells

Introduction

Human inflammatory bowel disease (IBD) is a complex disorder of unknown origin that seems to represent the complex interactions between three factors involving genetic susceptibility, environmental triggers and immune dysregulation. More than 50 years of intense scientific investigation world wide, together with recent rapid insights derived from immunologic studies in humans and animal models, have converged in the following, unifying hypothesis. IBD represents the dysregulated mucosal immune response to some antigen in a genetically susceptible host which is modified by a variety of potential environmental factors leading to a chronic inflammatory response that is manifest as tissue injury and clinical symptoms.[1] Although the genetic basis for this disease is unknown, recent genome-wide scans of humans with IBD have revealed potentially confirmed loci on chromosomes 12 and 16.[2] Although the specific genetic elements that are associated with IBD have not yet been identified on these putative loci, based upon what is known to date, it can be predicted that these genetic associations likely are involved in T cell regulation and cytokine production, which impact

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upon the mucosal immune response, and genes associated with mucosal barrier function and/or repair of a disrupted barrier, which would impact on the access of the subepithelial immune system to antigens contained within the lumen. Although the antigens that are putatively involved in the pathogenesis of IBD are unknown, a major breakthrough in our understanding of this disease is the appreciation that the antigen is likely derived from components contained within the normal luminal microbiota. As such, IBD represents an inappropriate immune response to some component of the normal microbiota in a genetically susceptible host. Stated another way, in the genetically susceptible host, antigens from the normal microbiota may be perceived as if they were a pathogen. Major support for such a concept has come from a wide variety of animal models.[3] A number of genetic perturbations in mice lead to chronic inflammation similar to that observed in human IBD. In virtually all of these models that have been studied to date, which can be characterized as either associated with perturbations of T cell regulation or alterations in mucosal barrier function and/or repair, colitis is observed only in the presence of the enteric microbiota. Germ-free animals do not, in fact, develop colitis in almost all of the animal models studied.[3]

Such notions of IBD pathogenesis focus significant attention on the functions of the intestinal epithelial cell (IEC) which, together with the intestinal intraepithelial lymphocyte (iIEL), are the first cells to come in contact with luminal antigens. These cells regulate transcytosis of macromolecules across the epithelium and paracellular permeability associated with barrier function and function in immunosurveillance and local immunoregulation. This has led to the appreciation that the IEC can function as an important component of the mucosa-associated lymphoid tissue. It is clear from numerous studies that the IEC participates actively in innate immunity as manifest by its abilities to function in antimicrobial defense, produce inflammatory mediators and regulate leukocyte traffic, can actively regulate barrier function through the autocrine and paracrine function of immune mediators (e.g. IL-10, TGF-β, IL-15) and participate in the immunosurveillance and immunoregulatory functions associated with adaptive immunity.[4]

MHC Class I-Related Molecules in Immune Functions of Epithelium

One class of molecules that is prominently involved in the immune functions of the human epithelium is the major histocompatibility class I-related molecules. In general, these molecules are characterized by a similar structure that consists of a 43–48-kD heavy or α-chain that is glycosylated and in non-covalent association with the 12-kD β2-microglobulin (β_2M) molecule. In general, the MHC class I-related α-chain consists of three extracellular domains, α1, α2 and α3, encoded by discrete exons. The MHC class I-related α-chains are type 1 glycoproteins with a transmembrane region and a short cytoplasmic tail. The MHC class I-related molecules can be divided into three types: the classical MHC class I molecules which are encoded on the MHC locus within human chromosome 6 (HLA-A, B and C), the MHC class I-related or class Ib molecules that are encoded by genes linked to the MHC locus on human chromosome 6 (HLA-E, F, G, H (*Hfe*)) and the MHC class I-related gene chain A (MICA), and the MHC class I-related or class Ib genes which are encoded outside of the MHC locus on other chromosomes (FcRn, zinc α-2 glycoprotein, MR1, CD1 and the protein C receptor).[5] Whereas the classical class I molecules are characterized by significant allelic diversity, a major distinguishing feature of the so-called MHC class Ib molecules, both MHC linked and unlinked, is their relative lack of allelic diversity, which causes them to be considered as non-polymorphic. The MHC class Ib molecules can be thus distinguished from the classical MHC class I molecules by their relative lack of polymorphism and their restricted cell and tissue distribution relative to the ubiquitously expressed classical MHC class I molecules.

MHC class I-like molecules are prominently displayed on human IECs. These include CD1d, which is encoded on chromosome 1 and is involved in the presentation of hydrophobic, glycolipid antigens to specific subsets of T cells, the neonatal Fc receptor for IgG (FcRn) which is encoded on chromosome 19 and which functions in the bidirectional transport of human IgG across the epithelial cell, the MICA which is encoded on chromosome 6 and is involved in the stress recognition of IECs by Vδ1$^+$ T cells, HLA-H (*Hfe*) which is encoded on chromosome 6 and involved in the regulation of iron transport across the epithelium, and HLA-E which is encoded on chromosome 6 and involved in the presentation of leader peptides from distinct types of MHC class I molecules to killer inhibitory receptors (CD94/NKG2) which is expressed on natural killer cells and subsets of T cells and regulates their activity. The remainder of this review will focus on the immunobiology of the CD1 molecules on the human intestinal epithelium.

Human CD1

The human CD1 locus on chromosome 1 contains five genes: CD1a–e. These genes can be divided into two types based upon nucleotide and deduced amino acid sequence homologies.[6] The so-called type 1 CD1 gene products (CD1a, b, c and e) have been described in humans, sheep and guinea pigs. The so-called type 2

CD1 molecules includes a single member, CD1d, which is observed in humans, rabbits, mice and rats. Notably, the type 1 CD1 molecules are absent from rodents, which only express the CD1d gene product. Interestingly, CD1 molecules in a given class are most homologous to members of that class such that human CD1d is more homologous to rodent CD1d than it is to the human CD1a, b and c molecules. This fact, in particular, suggests a conservation of function across the evolution of species. The X-ray crystallographic structure of CD1d has recently been solved.[7] Similar to the classical MHC class I molecules, the α1 and α2 domains of CD1d forms a groove that is comprised of two α-helices and a floor composed of a series of β-pleated sheets. However, in contrast to the groove of the classical class I molecule, which is wide and shallow and is competent to bind small 9–10 amino acid peptides for presentation to $CD8^+$ T cells, the groove of CD1d is narrow and deep, which is competent to bind large hydrophobic molecules such as glycolipids. In fact, the electrostatic surface charge of the CD1d-containing groove is neutral, consistent with this function in binding glycolipid antigens. CD1d thus functions to bind glycolipid antigens for presentation to distinct subsets of T cells.

The glycolipid antigens that have been identified to date to bind CD1d have similar structure and consist of α-glycosylated phytosphingosines. Current models suggest that the lipid core of these glycolipids are bound within the two pockets of the CD1d groove with a hydrophilic cap formed by the sugar moiety facing out into the aqueous milieu for recognition by the T cell receptor (TCR) of distinct subsets of T cells.[8] One model glycolipid antigen that is capable of binding CD1d and which has been quite useful in characterizing the function of CD1d is a molecule called α-galactosylceramide (α-GalCer). This glycolipid was originally isolated from marine sponge by Kirin Pharmaceuticals.[9] This glycolipid consists of a ceramide moiety that is characterized by two acyl chains of 26 and 18 carbons in length, respectively, in α-linkage with a galactose residue. Using α-galactosylceramide as a tool, it has been shown that the lipid components of α-GalCer are bound to CD1d on an antigen-presenting cell and presented to a distinct subset of T cells called Natural Killer-T (NK-T) cells which recognize the hydrophilic galactose moiety (Fig. 1). NK-T cells are characterized by their use of an invariant TCR that is characterized as Vα14Jα281 in association with particular TCR β chains (Vβ8) and the expression of certain natural killer cell markers such as CD161. Similar types of T cells have been identified in humans.[6] When activated, NK-T cells rapidly express a TH0 phenotype and are therefore thought to play an important role in the initiation and regulation of immune responses. It is important also to recognize that when an NK-T cell and CD1d-bearing antigen presenting cell (APC) communicate with each other, activation occurs of not only the NK-T cell, but also of the APC. The signals generated in the latter cell type are, as of yet, poorly defined.

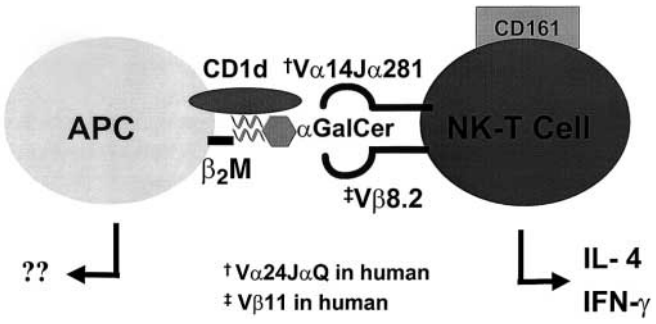

Fig. 1 CD1d on an antigen presenting cell (APC) presents α-galactosylceramide to Natural Killer-T (NK-T) cells. [ref. 8]

CD1d in the Human Intestine

A prominent tissue site for CD1d expression is the intestinal epithelium. We first detected CD1d in mouse intestinal epithelial cells in 1990.[10] Although our initial report could not be confirmed by others, more recently it has, in fact, been confirmed in rats and by us, in humans and functionally in mice (*vide infra*).[11,12] In rats, CD1d transcription is quite low and limited to the crypt epithelium with protein primarily detectable in villous epithelium.[11] As noted, we have identified CD1d expression in human IECs, which, similar to rat IECs, transcribe CD1d at very low levels. CD1d transcription in human intestinal epithelium has been shown by us to be regulated by γ-IFN[13] and able to activate peripheral blood T cells in IEC-peripheral blood T cell co-cultures.[14] Such activation is dependent on CD1d and gp180, a carcinoembryonic antigen-related molecule identified by Mayer and colleagues on IECs.[15] CD1d expression on human IECs is polarized (Fig. 2). CD1d is expressed apical and predominantly lateral as defined by confocal microscopy with CD1d-specific antibodies and selective cell surface biotin labeling studies.[16] Biochemically, CD1d is observed on human IECs in two forms: a 48-kD glycosylated form, which is expressed in association with β_2M and distributed apical and basal, and a novel 37-kD form, which is restricted apical, has a tendency to trimerize, and is expressed without any evidence of N-linked carbohydrate side-chain modifications or association with β_2M.[17,18] The 37-kD form appears to be derived from a novel post-translation modification that involves hydroxylation of CD1d on proline residues due to an interaction between CD1d and prolyl-4 hydroxylase within the endoplasmic reticulum (unpublished data).

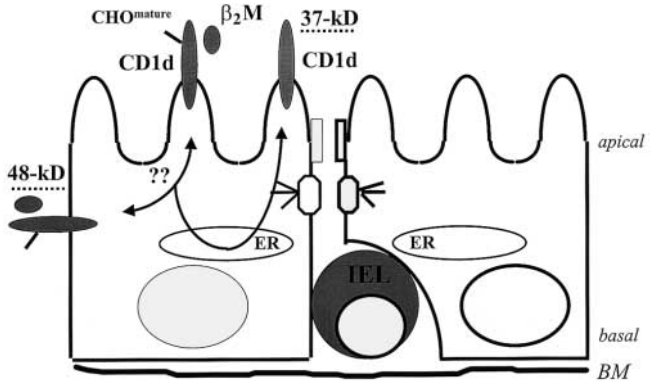

Fig. 2 Biochemical Structure of CD1d on human intestinal epithelial cells. CHO^{mature}, mature N-linked carbohydrate; IEL, intraepithelial lymphocyte; β_2M, β_2-microglobulin. [refs. 12, 16, 17, 18]

Functions of CD1d in Intestine

A major question that arises is, what is the function of CD1d and how does it relate to the binding of glycolipid antigens and interactions with NK-T cells? One hypothesis that arises from the biochemical data is that CD1d functions in the processing and presentation of glycolipid antigens, perhaps derived from the lumen, to local T cells. To begin to elucidate this, we have recently investigated CD1d in a dextran sodium sulfate (DSS) colitis model. As is well known, DSS placed *ad libidum* in the drinking water induces colitis with non-inflammatory dropout of IECs as one of the earliest changes suggesting that the primary event in this model is at the level of the IEC. We therefore examined the effects of α-GalCer and its non-functional analogue, α-mannosylceramide, in the DSS colitis model. Mice were administered 2.5% DSS *ad libidum* and either α-GalCer or α-ManCer at a dose of 100 μg/kg IP in wild-type C57BL/6J, $CD1d^{-/-}$ or Rag-$2^{-/-}$ mice. Mice were monitored for daily weights, diarrhea, bleeding scores and survival. These studies showed that wild-type mice are protected from colitis associated with DSS administration by α-GalCer but not α-ManCer.[19] This protection was observed both clinically, as defined by evaluation of daily weight, diarrhea, bleeding scores and survival, and histopathologically. In addition, the protective effect of α-GalCer is dependent on the presence of CD1d and NK-T cells, since it was lost in the $CD1d^{-/-}$ and Rag-$2^{-/-}$ animals. In the absence of CD1d, NK-T cells do not develop in the thymus and CD1d is not present on peripheral antigen presenting cells. Similarly, in the absence of Rag-2, T cells, including NK-T cells, do not develop. Finally, the protective effect of α-GalCer was likely mediated by activated NK-T cells since it was lost with NK-T cell depletion by antibody and gained with the transfer of α-GalCer, but not α-ManCer, activated NK-T cells to Rag-$2^{-/-}$ mice.

To begin to get at the mechanism(s) of this protection, the sites of localization of the parenterally administered α-GalCer were evaluated. α-GalCer labeled with a fluorescent dye was injected parenterally into either wild-type or $CD1d^{-/-}$ mice. After four hours, colonic tissues were obtained and analyzed by confocal microscopy. These studies showed that the green fluorescence, consistent with α-GalCer, was detectable predominantly in the surface epithelium of the mouse colon of the wild-type but not $CD1d^{-/-}$ animals, indicating that CD1d in this cellular location, was likely binding the labeled lipid.[19]

We therefore focused our attention on the function of CD1d in the IEC. We sought to model the effects of CD1d ligation as might occur through glycolipid antigen binding or cross-linking of CD1d by a CD1d-restricted T cell. To do so, we transfected the human IEC line T84 by retroviral-mediated gene transfer with either the wild-type CD1d cDNA or the CD1d DNA that had been mutated to have a non-functional cytoplasmic tail.[20] The CD1d cytoplasmic tail encodes a tyrosine-based motif that consists of YXXZ; a well-known sorting motif that is associated with tyrosine phosphorylation characterized by tyrosine-amino acid-amino acid-hydrophobic amino acid. By swapping the cytoplasmic tail of CD1d with the cytoplasmic tail of CD1a that does not encode this motif, the cytoplasmic tail of CD1d can be rendered non-functional. These studies showed that antibody cross-linking of CD1d on human IECs induced CD1d tyrosine phosphorylation since phosphorylation of CD1d was observed in the wild-type but not the mutant. Moreover, substitution of the CD1a cytoplasmic tail, which lacks the YXXZ motif, resulted in a dominant-negative phenotype in that even phosphorylation of the endogenously expressed CD1d was not observed. The tyrosine phosphorylation triggered by CD1d specifically induced IL-10 production by IECs but not the production of pro-inflammatory cytokines such as IL-8 or TNF. This production of IL-10 initiated by CD1d cross-linking was tyrosine phosphorylation dependent since it was totally blocked in a dose-dependent manner by genestein, a non-specific inhibitor of tyrosine kinase activity. Finally, the epithelial-derived IL-10 initiated by CD1d cross-linking was able to attenuate γ-interferon- (γ-IFN) induced permeability effects. We thus hypothesize that cross-linking of CD1d on an IEC leads to the production of powerful barrier-enhancing and immunoregulatory cytokines such as IL-10, which are able to abrogate or antagonize the proinflammatory and barrier disruptive consequences of cytokines such as γ-IFN (Fig. 3). Notably, γ-IFN is a cytokine that is extremely important to the pathogenesis of TH-1 responses often associated

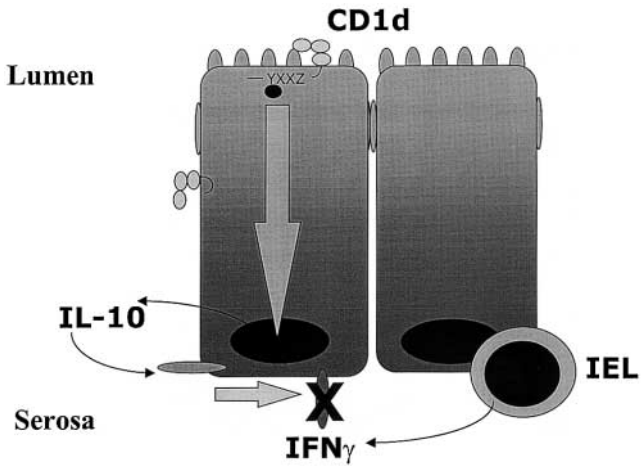

Fig. 3 Activation of CD1d on intestinal epithelial cells induces production of barrier promoting and down-regulatory cytokines. [refs. 19, 20]

with IBD and is a cytokine that is commonly expressed by most intraepithelial lymphocytes after activation.[3]

These data raised the question of how the activation of IEC regulatory functions by CD1d on the IEC might occur. There are two possibilities. One is that they are NK-T cell dependent. In this model, the presentation of glycolipid antigen to regional NK-T cells would lead to cross-linking of CD1d on the IEC and cross-linking of the TCR/CD3 complex on the NK-T leading to activation of both cell types. Alternatively, it is possible that activation of the IEC by glycolipid antigen may not require NK-T cells and thus be NK-T independent. In this model, CD1d is hypothesized to function in a novel manner as a signaling receptor. We have therefore begun to test these two hypotheses. In preliminary studies, we have found that α-GalCer can directly block the barrier effects of γ-IFN using a transfected T84 cell line as a model system. In addition, we have observed that human and mouse IECs can present α-GalCer, but not α-ManCer, to model NK-T cell clones in a dose-dependent fashion resulting in the production of cytokines such as IL-2 and IL-10. This presentation of α-GalCer to human and mouse IECs to model NK-T cells occurs in a CD1d-restricted fashion because it can be blocked by anti-human CD1d and anti-mouse CD1d specific monoclonal antibodies, respectively. These studies suggest that activation of CD1d signaling pathways on IECs may be both NK-T dependent and independent.

Such studies also raised the question as to whether NK-T cells can be detected among normal human intestinal intraepithelial lymphocytes. Human iIEL have been characterized in the past by us and others and found to consist of an oligoclonally expanded population that express on more than 95% of the lymphocytes TCR-αβ, CD8-αβ, CD45RO, αEβ7, CD-69, CD-101 and BY55.[21–22] Notably absent is the CD25 marker, indicative of the IL-2 receptor α-chain, and CD28, an important costimulatory molecule for most conventional T cells. Such phenotypic characteristics suggest that iIEL are memory cells in a novel state of activation that are restricted to the basolateral surface of the epithelium due to the expression of a unique constellation of integrins for the recognition of a limited number of antigens in the context of MHC class I-related molecules. With these considerations in mind, we have recently stained normal human iIEL by multi-parameter flow cytometry utilizing antibodies specific for the invariant TCR-chain characteristic of CD1d-restricted T cells, CD8 characteristic of iIEL, and CD161 characteristic of NK-T cells. Utilizing this methodological approach, we have been able to reproducibly detect a small subset of T cells within the human iIEL population consistent with the presence of NK-T cells. Specifically, T cells expressing the invariant TCR, CD8 and CD161 can be detected in this compartment. The immunoregulatory functions of this small subset of cells remains to be established. It is important to recognize that the spectrum of CD1d-responsive T cells is increasingly expanding to include not only the classical NK-T cell, but also other T cell subsets that do not express either the characteristic TCR-α or β chain of this cell type.[8] Thus, where human IEL fit into this rubric is unknown.

Summary

In summary, CD1d is expressed in a polarized manner in biochemically distinct forms on human and rodent IECs. Functional studies in rodents would suggest that activation of CD1d-NK-T-related pathways leads to the generation of immunoregulatory factors that are both anti-inflammatory and barrier-enhancing; characteristics that would be quite beneficial to the perturbations associated with IBD. Our studies would suggest that activation of CD1d on IECs in either an NK-T-dependent or -independent fashion leads to the production of cytokines such as IL-10, which can directly regulate IEC barrier function in an autocrine manner. It is therefore possible that glycolipid antigens, perhaps derived from luminal microbial antigens, may either directly stimulate CD1d on IECs or indirectly stimulate IECs through presentation of these glycolipid antigens to local CD1d-restricted T cells such as the NK-T cell. These studies further suggest that NK-T cells may be a novel type of immunoregulatory cell relevant to IBD pathogenesis.[23] How these insights relate to immunotherapeutic approaches in IBD remains to be established. However, it is becoming increasingly clear that ration-

Table 1 Inflammatory Bowel Disease: Rationalizing Therapy [adapted from ref. 1]

Target	Mechanisms	Therapeutic
Antigen	Eliminate and/or prevent uptake of pathogenic bacterial strain and/or antigens	Antibiotic or Probiotic, therapeutic vaccine, increase epithelial cell barrier function
T Cell/APC Interactions	Block excess costimulation	Anti-CD80/86, Anti-CD40L
Cytokines & Membrane Receptors	Block pro-inflammatory & increase anti-inflammatory cytokines	Anti-TNF, Anti-IL-12, IL-10, TGF-β, Blockade of NF-κB or activate regulatory cells (NK-T, Tr1, T_H3)
Cellular Recruitment	Block T cell homing/endothelial cell addressins	Anti-α4β7, anti-MadCAM-1
Inflammatory Mediators	Block activity of proteases & ROM	Metalloprotease inhibitors, Antioxidants
Epithelial Repair & Healing	Increase epithelial proliferation & barrier function	IL-11, Trefoil Factors, KGF

alizing IBD therapy around the sequence of immunologic events associated with IBD pathogenesis is extremely important (Table 1).

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