Lysosomal Localization of Murine CD1d Mediated by AP-3 Is Necessary for NK T Cell Development

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The presentation of lipid and glycolipid Ags to T cells is mediated through CD1 molecules. In the mouse and rat only a single isoform, CD1d, performs these functions, while humans and all other mammals studied have members of both group I (CD1a, -b, and -c) and group II (CD1d) isoforms. Murine CD1d contains a cytoplasmic tyrosine-based sorting motif that is similar to motifs recognized by adapter protein complexes that sort transmembrane proteins. Here we show that the adaptor protein complex, AP-3, directly interacts with murine CD1d and controls its targeting to lysosomes. AP-3 deficiency results in a redistribution of CD1d from lysosomes to the cell surface of thymocytes, B cell-depleted spleenocytes, and dendritic cells. The altered trafficking of CD1d in AP-3-deficient mice results in a significant reduction of NK1.1+ TCR-β+ and CD1d tetramer-positive cells, consistent with a defect in CD1d self-Ag presentation and thymocyte-positive selection. The AP-3 complex has recently been shown to associate with the human CD1b isoform, which has an intracellular distribution pattern similar to that of murine CD1d. We propose that lysosomal sampling may be so critical for efficient host defense that mice have evolved mechanisms to target their single CD1 isoform to lysosomes for sampling lipid Ags. Here we show the dominant mechanism for this trafficking is mediated by AP-3. The Journal of Immunology, 2003, 171: 4149–4155.

CD1 molecules are composed of transmembrane MHC class I-like heavy chains that noncovalently associate with β2-microglobulin and mediate the presentation of nonpeptide lipid and glycolipid Ags to T cells. They are separated into two groups, group I (CD1a, -b, and -c) and group II (CD1d) molecules, based on nucleotide and amino acid sequence similarity (1). Species differences in expression are also striking, since both group I and II CD1 molecules are present in humans, while CD1d is the only isoform expressed in murid rodents. The group I CD1 molecules are best characterized for their ability to present foreign mycobacterial lipids (2–4) to T cells with diverse TCRs. In contrast, while the physiological lipids presented by CD1d remain enigmatic, both human and murine CD1d-restricted T cells are likely to recognize self-lipid Ags in vivo and recognize α-galactosylceramide (α-GalCer) in experimental systems (5–9). CD1d-restricted T cells have proven functionally significant, since they secrete copious amount of IL-4, IL-10, and IFN-γ and also display cytotoxic effects (10–13).

Besides differences in Ag-presenting capacity and T cell activation, the CD1 isoforms follow discrete intracellular trafficking routes that correlate with their ability to intersect with the lipid Ags that each presents. The CD1b, CD1c, and CD1d isoforms contain a cytoplasmic tyrosine-based sorting motif, YXXZ (Y = tyrosine, X = any amino acid, Z = bulky hydrophobic amino acid), that mediates trafficking to appropriate intracellular compartments. Interaction with AP-2 may be important in their internalization from the plasma membrane (14). The AP-3 complex has been shown to be important in the receptor-mediated trafficking of proteins such as lysosome-associated membrane protein 1 (LAMP-1) and CD63 that are targeted to lysosomes (15, 16). Recently, the tyrosine-based sorting motif of CD1b has been shown to interact with AP-3 and to be critical for the localization of CD1b to late endosomes and lysosomes (17, 18). Although CD1c has a similar cytoplasmic tyrosine motif, it does not associate with AP-3 and primarily localizes to early recycling endosomes, with only a small fraction distributed in lysosomes (18–21). Similarly, human CD1d (hCD1d) displays limited lysosomal localization and lacks the ability to bind AP-3 in yeast two-hybrid assays (18). CD1a, which does not contain a cytosolic sorting motif, traffics exclusively through early recycling endosomes (22). Consistent with the differences in CD1 isoform localization is the dependence of vesicular acidification for effective Ag presentation by CD1b, which traffics to lysosomes, but not for CD1a, which traffics through early endosomes (19, 22, 23).

Abbreviations used in this paper: α-GalCer, α-galactosylceramide; DC, dendritic cells; h, human; LAMP-1, lysosome-associated membrane protein 1; m, murine.
Like human CD1b (hCD1b), murine CD1d (mCD1d) molecules are prominently expressed in LAMP-1+ lysosomes, and redistribution from lysosomes to the plasma membrane had been observed for cytoplasmic tail-truncated forms of both molecules. Furthermore, cytosolic tail modifications that alter trafficking of hCD1b and mCD1d impair their ability to present foreign microbial Ags. CD1b and CD1d tail deletion resulted in loss of recognition by foreign Ag-reactive T cells and the majority of invariant CD1d-reactive T cells, respectively (5, 17, 24, 25). These findings led us to speculate that the intracellular distribution of mCD1d appeared to more closely resemble that of hCD1b than that of hCD1d. This suggested a possible mechanism for rodents to compensate for the absence of CD1a, -b, and -c molecules. Here, we show that mCD1d, like hCD1b, interacts with the adaptor protein AP-3 via its cytoplasmic tail tyrosine-based sorting motif that controls the localization of mCD1d to lysosomes. This interaction is critical for the development and maintenance of CD1d-specific NK T (NKT) cells. Pearl mice, which have a 125-aa deletion in the gene encoding the β3A subunit of the AP-3 complex (26–28), and mice deficient in the β3A subunit of the AP-3 complex by homologous recombination (AP-3β3A−/−) (16) were found to have significant defects in CD1d intracellular localization and NKT cell number. AP-3 deficiency causes redistribution of mCD1d from lysosomes resulting in increased cell surface expression on thymocytes, dendritic cells (DC), and B cell-depleted splenocytes. Despite a higher level of cell surface expression, the altered CD1 trafficking appeared to impair CD1d Ag presentation and positive selection in the thymus, since a significant reduction of NKT and CD1d tetramer-positive cells was noted in spleen, thymus, and liver. Thus, the murine group II CD1d isoform accomplishes critical trafficking to and sampling of lysosomes by adopting adaptor protein interactions noted for the human group I CD1b isoform.

Materials and Methods

Mice

Pearl mice on the C57BL/6 background and age- and sex-matched, wild-type C57BL/6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME). AP-3β3A−/− deficient mice on the C57BL/6 background were provided and previously characterized by Dr. S. Mansour (16). Heterozygotes were mated to generate homozygous AP-3β3A−/− mutants identified by PCR (16) and coat color and were compared with their heterozygote, age- and sex-matched littermates. Mice were used between 8–20 wk of age. Mice were maintained according to institutional animal care and use committee guidelines.

Flow cytometry and Abs

Single-cell suspensions were prepared from thymus, spleen, and liver (after perfusion with 30 cc of PBS). Splenocyte, thymocyte, and liver cell suspensions in RPMI medium were centrifuged over a layer of Histopaque (Sigma-Aldrich, St. Louis, MO). Mononuclear cells were harvested from the interface. To obtain splenic DC, teased spleen tissue was digested with collagenase (1 mg/ml; type IV, Sigma-Aldrich) for 1 h at 37°C. Splenic DC were isolated using CD11c magnetic microbeads (Miltenyi Biotec, Germany) as per manufacturer’s instructions. Bone marrow derived DC were generated as previously described (29). Purity of the DC populations was assessed by flow cytometry using hamster anti-mouse CD11c (HL3)-FITC and rat anti-mouse I-A/I-E (M5/114,15.2)-PE Abs (Abs) partially quenched with unconjugated rat anti-mouse I-A/I-E Abs (BD PharMingen, San Diego, CA). B cells were depleted from splenocytes with CD19 magnetic microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer’s instructions.

To block FcγRs, cells were blocked with 2.4G2-purified mAb. Cells were subsequently stained with optimal concentrations of conjugated mAbs and their appropriate isotype controls for 20 min at 4°C in flow cytometry buffer (PBS with 1 mg/ml of BSA (Sigma-Aldrich), 2 mM EDTA, and 0.05% NaN3), washed, and analyzed using a FACSscan flow cytometer and CellQuest software (BD Biosciences, San Jose, CA). Fluorochrome-conjugated Abs to pan-TCR-β-FITC, NK1.1-PE, CD8α-PE, CD1d-PE, CD3-CyChrome, CD22-FITC, rat IgG2b-PE control, and human IgG-CyChrome control were obtained from BD PharMingen. Abs to CD19-FITC and mouse IgG2a-PE control were obtained from Caltag (Burlingame, CA) and ebioscience (San Diego, CA), respectively. Abs to CD3 (2C11), CD4 (GK1.5), and CD8 (53.6.7) were generated from American Type Culture Collection (Manassas, VA) hybridomas, purified and conjugated to Alexa 488 (Molecular Probes, Eugene, OR) according to the manufacturer’s instructions.

CD1d-α-GalCer tetramer staining

Murine CD1d tetramers complexed to Alexa 488 (Molecular Probes) were constructed as previously described (6, 30). α-GalCer was synthesized from commercially available α-l-lyxose according to a modification of the previously reported procedures (31), employing different protecting groups and removal and coupling strategies. The complete synthesis will be reported elsewhere (G. S. Besra and S. Maltsev, unpublished observations). The key features were the use of tert-butylidiphenyl silyl ether and benzotriazole protecting groups and removal therein. The sugar-coupling procedure employed the intermediate protected azido-sphingosine, followed by reduction, N-acetylation, and final removal of protecting groups. The final material α-GalCer was in excellent agreement with 1H-nuclear magnetic resonance, 13C-nuclear magnetic resonance, and mass spectral data previously reported (31). Lyophilized lipid was dissolved in DMSO at a concentration of 100 μg/ml at 37°C by agitation and was sonicated before Ag loading. α-GalCer-loaded or DMSO mock-loaded CD1d tetramers and fluorochrome-conjugated mAbs were incubated with cells blocking with 2.4G2 mAb.

Yeast two-hybrid assays

The GAL4-μ3A and GAL4βd-human CD1a, CD1b, and CD1d two-hybrid constructs were generated as previously described (18). GAL4βd-murine CD1d was generated similarly by ligation of the GAL4βd construct with synthetic dsDNA encoding the entire cytoplasmic domain of murine CD1d. The constructs were confirmed by DNA sequencing. Saccharomyces cerevisiae strain HF7c was transformed with the GAL4-μ3A construct together with each of the GAL4βd-CD1 tail constructs. Subsequently, the transformed cells were inoculated in histidine-deficient medium, and their growth was assessed by measuring OD600 at various time points as previously described (15).

Immunogold-labeled transmission electron microscopy and quantitation

Wild-type and pearl splenic and bone marrow-derived DC, purified as described above, were fixed with 2% paraformaldehyde (Polysciences, Warrington, PA). Samples were prepared for ultrathin cryosectioning as previously described (32). Cryosections were labeled with rat anti-mouse CD1d mAb (19G11, gift from Dr. A. Bendelac) for 60 min and secondarily incubated with rabbit anti-rat Ab (DAKO, Carpinteria, CA) and protein A conjugated to 15 nm of gold (EM Laboratory, Utrecht University, Utrecht, The Netherlands). Sections were analyzed on a Tecnai electron microscope (Phillips Electronic Instruments, Mahwah, NJ). Quantitation of immunogold-labeled lysosomes and membranes was performed as previously described (33, 34).

Generation of chimeric CD1d molecule plasmid constructs

A CDNA encoding a chimeric mCD1d molecule with the hCD1d tail was generated by PCR using mouse CD1d in pSRS-neo as template DNA. The primers used were 5′-GGG GCC GCC CGC TAT GCG GTA CCT ACC ATG GCT GTT-3′ (sense) and 5′-GGC CTC GAG TTC CCT CCA GAT GAT GAG TAC-3′ (antisense). Following digestion with NotI and XhoI, the PCR product was cloned into pBluescript KS (Stratagene, La Jolla, CA) and sequenced. The inserted cDNA construct was then cut out by digestion with NotI and XhoI, and recloned into pcDNA3.1 for transfection.

Conifocal microscopy and immunofluorescence labeling

HeLa cells grown on coverslips were transfected with plasmids encoding wild-type mCD1d or the chimeric construct by calcium phosphate precipitation as previously described (35). Two days after transfection the cells were fixed, permeabilized, and stained intracellularly as previously described (18). Staining with Abs against murine CD1d (15F7; American Type Culture Collection) and LAMP-1 (36) (a gift of Dr. Minoru Fukuda, The Burnham Institute, La Jolla, CA) was followed by incubation with FITC-conjugated donkey anti-rat IgG and Texas Red-conjugated donkey anti-rabbit IgG Abs (Jackson ImmunoResearch Laboratories, West Grove, PA). Conifocal microscopy was performed on the labeled cells with a
TCS-NT confocal laser scanning microscope (Leica, Deerfield, IL) fitted with krypton and argon lasers as previously described (22).

**Results**

Murine, but not human, CD1d binds the AP-3 μ3A subunit and mediates lysosomal localization

One mechanism by which differential protein sorting occurs involves the selective recognition of nearly similar tyrosine-based sorting signals in the cytoplasmic tails of transmembrane proteins. Recently, we showed that the AP-3 complex selectively recognizes hCD1b, but not other hCD1 isoforms (hCD1a, -c, or -d). This interaction with the AP-3 complex plays a critical role in the lysosomal localization and function of hCD1b (14, 18). In contrast, hCD1d fails to bind the AP-3 complex, resulting in a much smaller lysosomal pool of hCD1d molecules. Since mCD1d, unlike hCD1d, has been demonstrated to colocalize extensively with LAMP-1 (5) we sought to determine the mechanism by which mCD1d might accomplish its critical lysosomal trafficking. Therefore, a yeast two-hybrid system was employed to determine whether the tyrosine-based motif in the cytoplasmic tail of mCD1d was capable of interacting with AP-3. The GAL4 transcription activation domain was fused to the μ3A subunit of human AP-3, which is 99% homologous to murine AP-3, and was coexpressed in yeast cells with the GAL4 DNA binding domain fused to the cytoplasmic tyrosine based sequences derived from either human or mouse CD1d. Specific interactions were identified by growth of the transformed yeast cells in histidine-deficient medium. As previously demonstrated, no growth was detected in histidine-deficient medium for yeast cells expressing the hCD1d tail (18). Surprisingly, yeast cells expressing the mCD1d tail showed efficient growth in histidine-deficient medium, suggesting a specific interaction of the μ3A subunit of AP-3 with murine, but not human, CD1d tail (Fig. 1).

To directly evaluate the role of the CD1d tail in intracellular localization in vivo, wild-type mCD1d and a chimeric mCD1d molecule in which the cytoplasmic tail was swapped with the hCD1d tail were expressed in HeLa cells by transfection, and their localization was compared using confocal microscopy. Wild-type mCD1d molecules were prominent in peripherally distributed vesicles, as was LAMP-1, an endogenous marker for lysosomes. Electronic merging of the images revealed that the mCD1d-containing vesicles and the LAMP-1-containing vesicles were substantially colocalized, confirming the prominent localization of wild-type mCD1d molecules in lysosomes (Fig. 2C). In contrast, the chimeric mCD1d:hCD1d tail molecules showed markedly reduced colocalization with LAMP-1 (Fig. 2F) and were expressed more prominently on the cell surface than wild-type mCD1d. Thus, the efficient targeting of mCD1d to lysosomes required the sorting motif present in the mCD1d tail and was significantly impaired by replacement with the hCD1d tail. These findings suggest that the

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**FIGURE 1.** Murine CD1d, but not hCD1d, binds to the adaptor complex AP-3. Yeast cells were transformed with the GAL4ad-μ3A construct together with GAL4bd-mCD1d, GAL4bd-hCD1d, GAL4bd-hCD1b as a positive control, or GAL4bd-hCD1a as a negative control. Cells were inoculated in histidine-deficient medium. Specific interactions between the constructs were detected by the ability of the transformed cells to grow in the absence of histidine, as determined by measuring the OD_{600} at the indicated time points. Murine CD1d and hCD1b specifically bound to AP-3, whereas hCD1d did not.

**FIGURE 2.** Replacement of murine with human CD1d tail fails to deliver murine CD1d to the lysosomal compartment. HeLa cells transfected with wild-type mCD1d (top panels) or chimeric mCD1d with hCD1d tail (bottom panels) were labeled intracellularly with Abs against mCD1d (A and D, green) and the lysosomal marker LAMP-1 (B and E, red) and examined by confocal microscopy. The two images were superimposed to detect the intracellular compartments expressing both CD1d and LAMP-1 (C and F, yellow). Scale bars (5 μm) are in the lower left of the merge panel.
ability of mCD1d molecules to bind to AP-3 and the lack of interaction with AP-3 by hCD1d molecules may be the main molecular explanation for the differential intracellular localization of the CD1d molecules in these two species.

**CD1d expression at the cell surface is increased in AP-3-deficient mice**

Functional experiments have shown that the lysosomal trafficking of hCD1b and mCD1d are important determinants of their ability to present Ags to T cells. Given that the data presented here indicated a role for AP-3 in localization of mCD1d, we next examined the expression and function of CD1d in AP-3-deficient *pearl* mice. Importantly, we noted a marked increase in the cell surface expression of mCD1d on *pearl* thymocytes, splenic DC, and splenic T cells (Fig. 3). The effect of AP-3 deficiency on mCD1d expression was most pronounced on DC and B cell-depleted splenocytes, where the fold increases, respectively, in the mean fluorescence intensity over that in wild-type cells were 2.8 ± 0.2 and 2.6 ± 0.1. Further, there was a 1.8 ± 0.2-fold increase in CD1d mean fluorescence intensity in *pearl* thymocytes over that in wild-type cells. Interestingly, there was no increase in the cell surface expression of mCD1d on B cells, including splenic mCD1d*high* B cells (data not shown). The basis for B cells differing from DC, T cells, and thymocytes in altered mCD1d expression is not known. The marked increase in CD1d cell surface expression on DC, T cells, and thymocytes is consistent with a redistribution of CD1d from intracellular lysosomal compartments to the plasma membrane as a result of the absence of their interaction with the AP-3 complex (see below). These findings were confirmed in another AP-3-deficient strain, AP-3b1 LN knockout mice generated by homologous recombination, which were compared with their heterozygote littermates (data not shown).

**Altered trafficking of CD1d molecules in AP-3-deficient mice**

Previous studies have shown that the lysosome-related organelle proteins, LAMP-1, LAMP-2, CD63, tyrosinase, and hCD1b, have altered trafficking patterns in AP-3-deficient cells (15, 16, 18). The altered trafficking of these proteins in AP-3-deficient cells resulted in their redistribution to the plasma membrane. Given the difficulties involved in using lysosomal markers such as LAMP-1, which is misrouted in AP-3 deficiency (16), in colocalization experiments, transmission electron microscopy was used to assess the cellular distribution of CD1d in wild-type and *pearl* splenic CD11c⁺ and bone marrow-derived DC. Immunogold-labeled mCD1d was observed on plasma membrane, lysosomes, recognized by characteristic morphology, and, in some small vesicles, probably endocytic recycling vesicles. Consistent with the increase in cell surface expression observed by flow cytometry, there was a 2.3-fold increase in mCD1d plasma membrane staining in *pearl* bone marrow-derived DC compared with wild-type cells. There was a reciprocal 2.9-fold reduction in mCD1d lysosomal labeling consistent with redistribution of mCD1d to the plasma membrane in AP-3-deficient mice (Fig. 4 and Table I). In splenic CD11c⁺ DC, which have higher levels of cell surface mCD1d by flow cytometry than bone marrow-derived DC, the redistribution of mCD1d to the plasma membrane was even more dramatic, with a 5.8-fold increase in mCD1d plasma membrane labeling in *pearl* mice compared with wild-type mice, respectively (1.56 ± 0.315 vs 0.27 ± 0.045 gold particles/µm plasma membrane).

**Reduction of NKT number in AP-3-deficient mice**

Next we examined the possibility that interactions with AP-3 might impair CD1d function despite its higher cell surface expression. Since CD1d expression is required for the development of...
NKT cells, as demonstrated in CD1d-deficient mice (37, 38), we determined whether the alteration in CD1d trafficking observed in AP-3-deficient cells resulted in impaired NKT cell development. Thus, NKT cell numbers were determined by staining with both anti-NK1.1 Ab- and α-GalCer-loaded CD1d tetramers. NK1.1+ TCRβ+ cells in pearl mice were reduced in thymus and spleen, consistent with a defect in positive selection and self-Ag presentation (Fig. 5A). There was, on the average, a 60% reduction in NK1.1+ TCRβ+ thymocytes in pearl compared with wild-type mice. NK1.1+ TCRβ+ splenocytes were reduced, on the average, by 41% in pearl mice. Liver NK1.1+ TCRβ+ cells in pearl mice were reduced, on the average, by 30% compared with those in wild-type mice. There was a corresponding reduction in α-GalCer-loaded CD1d tetramer-stained cells, which are a major subset of NKT cells in thymus and spleen (Fig. 5B). Tetramer-positive cells in thymus and spleen in pearl mice were reduced, on the average, by 55 and 43%, respectively, compared with wild-type mice. These findings are consistent with an important role for the adaptor complex AP-3 in CD1d intracellular trafficking that alters the ability of CD1d self-Ags to be presented for positive selection of CD1d-reactive T cells in the thymus or to sustain their maintenance in peripheral compartments.

Discussion

The efficient processing and presentation of many foreign and self-Ags by Ag-presenting molecules such as MHC class II and CD1d requires their intersection in specialized lysosomal compartments. Murine CD1d contains a tyrosine-based sorting motif and the MHC class Iβ2mβ1 complex contains di-leucine-based sorting motifs that are targeting signals recognized by adaptins to direct intracellular trafficking (39, 40). Differences within the sorting motif as well as the amino acid residues adjacent to the motif in the cytoplasmic tail are important in conferring specificity for adaptor protein interactions (41–43). Secondary effects on CD1d localization may involve association between human and murine CD1d with MHC class II and invariant chain complexes in the late endosomal pathway. This interaction was independent of the cytoplasmic tail of CD1d and may represent a secondary trafficking pathway into the endocytic system (44–46). The alteration of CD1d expression, localization, and function in AP-3-deficient mice would be independent of invariant chain and MHC class II trafficking as trafficking of both these molecules have been shown to be AP-3 independent (47, 48). Here, we identify and delineate the critical role of the adaptor complex AP-3 in CD1d trafficking and function.

The AP-3 adaptor complex is an important regulator of trans-Golgi network and lysosomal trafficking. Localization of prototypic lysosomal proteins such as LAMP-1 and CD63 to lysosomes has been shown to be AP-3 dependent (15, 16). Recent studies have demonstrated a role for AP-3 in the intracellular trafficking of hCD1b, while no role was observed in hCD1d trafficking (14, 18). Murine CD1d is the only isoform found in the mouse for which two CD1d genes, designated CD1D1 and CD1D2, have been identified. In the C57BL/6 strain the CD1D2 gene, which has not been shown to have a role in NKT cell development, is a pseudogene secondary to a frameshift mutation (49, 50). Murine CD1d has a similar intracellular distribution pattern to hCD1b, raising the question of whether AP-3 may play a role in its trafficking. In pearl and AP-3b1LN mice, CD1d cell surface expression was significantly elevated in thymocytes, B cell-depleted splenocytes, and

![FIGURE 5.](http://www.jimmunol.org/Downloadedfromhttp://www.jimmunol.org)
splenic CD consistent with an important role for AP-3 in CD1d trafficking. Interestingly, however, mCD1d expression was not increased on B cells. In wild-type mice, B cells have significantly higher cell surface expression levels of mCD1d than thymocytes or T cells. B cell mCD1d cell surface expression levels are approached in AP-3 deficiency by thymocytes and T cells. These findings suggest that AP-3 does not play a significant role in CD1d trafficking in B cells and that intracellular trafficking pathways and adaptins may play different roles in different cell types. Alternatively, AP-3 may be expressed at lower levels in murine B cells vs other cell types. These data also raise the possibility that the repertoire of Ags that mCD1d in B cells encounters may be different from that in other cell types such as DC or thymocytes. 

Consistent with the increase in cell surface CD1d expression observed by flow cytometry in 

\[ \text{pearl} \] cells, pearl DC had increased plasma membrane and reduced CD1d localization in lysosomes noted by immunogold-labeled transmission electron microscopy analyses. The redistribution of CD1d to the plasma membrane, however, was only partial, suggesting that there are additional AP-3-independent pathways for lysosomal targeting of CD1d, as also noted for LAMP-1 and CD63. Recent studies have demonstrated an association between CD1d and invariant chain, raising this as a potential mechanism for the lysosomal targeting of a subset of CD1d molecules. Pearl and AP-3b1\W mice, which have intact MHC class II expression and function, may provide a tool for refined dissection of the role of invariant chain in CD1d trafficking and function. 

We suggest that lysosomal Ag sampling may be essential for the Ag presentation function of mCD1d. In humans, CD1b reveals the potential mechanism for the lysosomal targeting of a subset of CD1d molecules. Pearl and AP-3b1\W mice, which have intact MHC class II expression and function, may provide a tool for refined dissection of the role of invariant chain in CD1d trafficking and function. 

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