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Failure of Trafficking and Antigen Presentation by CD1 in AP-3-Deficient Cells

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Summary

Endocytosed microbial antigens are primarily delivered to lysosomal compartments where antigen binding to MHC and CD1 molecules occurs in an acidic and proteolytically active environment. Signal-dependent delivery to lysosomes has been suggested for these antigen-presenting molecules, but molecular interactions with vesicular coat proteins and adaptors that direct their lysosomal sorting are poorly understood. Here CD1b but not other CD1 isoforms bound the AP-3 adaptor protein complex. In AP-3-deficient cells derived from patients with Hermansky-Pudlak syndrome type 2 (HPS-2), CD1b failed to efficiently gain access to lysosomes, resulting in a profound defect in antigen presentation. Since MHC class II traffics normally in AP-3-deficient cells, defects in CD1b antigen presentation may account for recurrent bacterial infections in HPS-2 patients.

Introduction

In order to detect infection efficiently, antigen-presenting molecules are elaborately directed to distinct intracellular compartments where they can intersect with and bind foreign antigens. Peptide antigens generated in the cytosol, such as those derived from virus-infected cells, are transported through the TAP transporter into the lumen of the endoplasmic reticulum (ER). The peptide antigens are then sampled in the ER by MHC class I molecules that are largely retained in the ER until peptide binding occurs (Heemels and Ploegh, 1995). MHC class I-peptide complexes then traffic to the cell surface to activate $CD8^+$ T cell responses. In contrast, microbes and their products present in the extracellular space are endocytosed and delivered to late endosomes and lysosomes for degradation into peptides. Subsequently, these peptides are sampled by MHC class II molecules that are targeted to these compartments by the associated invariant chain (Cresswell, 1994). MHC class II-peptide complexes then traffic to the cell surface and stimulate $CD4^+$ helper T cells to produce immunoregulatory cytokines and induce specific humoral responses. Thus, MHC class I and class II molecules coordinately orchestrate immune responses against the peptide antigens of pathogens by surveying distinct intracellular compartments.

The immune system has evolved a distinct lineage of antigen-presenting molecules encoded by the *CD1* complex located outside the *MHC* region on chromosome 1 in humans and chromosome 3 in mice (Calabi and Milstein, 1986; Porcelli and Modlin, 1999). The CD1 molecules function to present the universe of lipid antigens to T cells (Beckman et al., 1994; Kawano et al., 1997; Moody et al., 1997; Rosat et al., 1999; Sieling et al., 1995) and appear to mediate distinct but complementary pathways of host defense against infection with pathogens (Moody et al., 2000; Park and Bendelac, 2000; Prigozy and Kronenberg, 1998; Schaible and Kaufmann, 2000; Stenger et al., 1997). In mycobacteria-infected cells, lipids and glycolipids derived from phagosome-resident mycobacteria escape from phagosomes and distribute to other subcompartments of the endocytic system, including early recycling endosomes and lysosomes, to which CD1a and CD1b traffic for sampling antigens, respectively (Beatty et al., 2000; Sugita et al., 2000a). The trafficking routes for these two CD1 isoforms appear to be distinct. CD1a is localized to early recycling endosomes and follows an ARF6-dependent pathway (Sugita et al., 1999). In contrast, CD1b is largely excluded from early recycling endosomes and localizes almost exclusively deep in late endosomes and lysosomes (Prigozy et al., 1997; Sugita et al., 1996).

In contrast to live mycobacteria that reside in phagosomes, dead mycobacteria as well as most extracellular microbes that are phagocytosed by antigen-presenting cells (APCs) are subsequently delivered to the acidic environment of lysosomes (Kaufmann, 1993). Sampling of lysosomes is crucial for effective antigen presentation mediated by MHC class II and CD1b molecules. Both molecules are delivered efficiently to a specialized lysosomal compartment, called the MHC class II compartment or MIIC, where antigen loading is proposed to occur (Peters et al., 1991, 1995b; Sugita et al., 1996). Specific cytoplasmic tail signals that dictate their intracellular trafficking have been identified for both MHC class II and CD1b molecules. Following assembly with the invariant chain (Ii) in the ER, MHC class II molecules are sorted to the MIIC by utilizing two dileucine-based sorting signals present within the cytoplasmic domain of Ii as well as an additional dileucine-based signal in the MHC class II β chain (Bakke and Dobberstein, 1990; Lotteau et al., 1990). In contrast, the short cytoplasmic domain of CD1b contains a tyrosine-based sorting motif (YXXZ, where Y is tyrosine, X is any amino acid, and Z is a bulky hydrophobic amino acid) that is crucial for its localization to the MIIC (Jackman et al., 1998). Surprisingly, CD1c also contains a similar tyrosine-based motif, but its intracellular localization is less focused on lysosomes. In fact, CD1c molecules, while more promiscu-

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ous in their endosomal distribution, are largely found in early recycling endosomes, and only a small fraction of CD1c molecules can distribute to lysosomes (Briken et al., 2000; Schaible and Kaufmann, 2000; Sugita et al., 2000b). This difference in localization of CD1b and CD1c, despite containing similar YXXZ sorting motifs, is underscored by their disparate dependence on vesicular acidification for effective antigen presentation (Briken et al., 2000; Sugita et al., 2000a). We hypothesized that the CD1b and CD1c cytoplasmic tail tyrosine-based sorting motifs might mediate interactions with distinct organellar coats involved in protein sorting. A system of adaptor protein (AP) complexes, AP-1, AP-2, AP-3, and AP-4, has been characterized which binds both transmembrane cargo proteins via tyrosine-based motifs and a variety of peripheral vesicle-associated proteins (Kirchhausen, 1999; Robinson and Bonifacino, 2001). The AP-2 complex has been implicated in the internalization of many proteins from the cell surface since it recognizes a range of tyrosine-based sorting motifs (Honing et al., 1996; Ohno et al., 1995). On the other hand, the concept has emerged that marked selectivity in recognition of tyrosine-based motifs may occur for other adaptor protein complexes, dependent upon the sequence of the space residues (X) in the YXXZ motif as well as flanking residues and distance of the motif from the membrane (Ohno et al., 1998; Rohrer et al., 1996; White et al., 1998).

We hypothesized that the ubiquitous AP-3 adaptor complex might selectively mediate sorting of CD1b to lysosomes. This cytosolic protein complex, composed of the four subunits β3A, δ, μ3A, and σ3 (A or B), was shown to function in protein targeting to lysosome-related organelles, such as melanosomes and platelet granules (Dell'Angelica et al., 2000; Odorizzi et al., 1998). AP-3-deficient mice as well as Hermansky-Pudlak syndrome type 2 (HPS-2) patients with mutations in the *AP-3 β3A* gene exhibited hypopigmentation and platelet dysfunction (Dell'Angelica et al., 1999; Feng et al., 1999; Kantheti et al., 1998; Shotelersuk et al., 2000; Yang et al., 2000). The ability of the μ3A subunit to interact specifically with tyrosine-based motifs contained within cytoplasmic domains of lysosome-resident proteins, such as CD63 and LAMP-1, has been noted, and these proteins traffic aberrantly in AP-3-deficient cells (Dell'Angelica et al., 1999). Here, we show that CD1b but not other CD1 isoforms binds the AP-3 complex. In AP-3-deficient cells, CD1b fails to accumulate in lysosomal antigen binding compartments and is instead mislocalized to the plasma membrane and early endosomes. This failure in CD1b trafficking correspondingly results in its failure to efficiently present microbial lipid antigens to T cells, while other CD1 isoforms function normally. Since MHC class II functions normally in AP-3-deficient cells (Caplan et al., 2000; Sevilla et al., 2001), defects in CD1b antigen presentation may account for bacterial infections in HPS-2 patients.

Results

CD1b and CD63 Cytoplasmic Tails Efficiently Mediate Lysosomal Sorting, but Not the CD1c Tail

Among the four CD1 isoforms expressed in humans, CD1b, CD1c, and CD1d contain tyrosine-based sorting motifs in their cytoplasmic domains. Yet, immunofluorescence microscopic analysis of HeLa cells expressing each CD1 isoform detected noticeable differences between CD1b and other CD1 molecules in their intracellular distribution (Figure 1). As we previously showed (Sugita et al., 1999), whereas CD1a was found in a cluster of vesicles at a juxtanuclear position that represented early recycling endosomes (Figure 1A, green vesicles), CD1b was prominent in more peripherally distributed vesicles (Figure 1D, green vesicles) as was lysosome-associated membrane protein-1 (LAMP-1), an endogenous marker for lysosomes (Figure 1E, red vesicles). Electronic merging of the images revealed that the CD1b-containing vesicles and the LAMP-1-containing vesicles were almost entirely superimposable (Figure 1F, yellow vesicles), confirming the prominent localization of CD1b in lysosomes. CD1c and CD1d were also readily detectable in intracellular vesicles (Figures 1G and 1J, green vesicles, respectively). Unlike CD1b, however, their intracellular distribution only partially overlapped with that of LAMP-1 (Figures 1I and 1L), indicating a markedly dominant lysosomal sorting for CD1b as compared to the other CD1 isoforms that also contained tyrosine-based sorting motifs.

To determine directly the ability of CD1b and CD1c tails to mediate protein sorting to lysosomes, wild-type CD1b and a chimeric CD1b molecule in which CD1b tail was swapped with CD1c tail (CD1b:CD1c tail) were expressed in HeLa cells by transfection, and their intracellular localization was determined by confocal microscopy (Figure 2). The CD1b:CD1c tail chimeric molecules were expressed in intracellular vesicles (Figure 2D, green vesicles), but, unlike wild-type CD1b, which was prominent in LAMP-1-containing vesicles (Figure 2, top panels), most of these vesicles lacked LAMP-1 (Figure 2F, green vesicles indicated with arrowheads). Further, markedly more prominent surface expression was noted for the CD1b:CD1c tail chimeric molecule (Figure 2D) than for wild-type CD1b (Figure 2A). This distribution for the chimeric CD1b containing the CD1c tail was similar to that observed previously for wild-type CD1c, which exhibited a large pool of molecules in early recycling endosomes with a small pool in lysosomes (Sugita et al., 2000b). Indeed, most of the CD1b:CD1c tail-containing intracellular vesicles but not wild-type CD1b-containing vesicles coexpressed the transferrin receptor, a marker for early endosomes (data not shown), suggesting that CD1c tail misrouted the chimeric CD1b molecule to early endosomes. For comparison, another chimeric CD1b molecule with the CD63 tail (CD1b:CD63 tail) was expressed in HeLa cells, and its intracellular distribution was determined. Similar to wild-type CD1b, the CD1b:CD63 tail chimeric molecule was expressed in peripherally distributed vesicles (Figure 2G, green vesicles), and virtually all of the vesicles coexpressed LAMP-1 (Figure 2I, yellow vesicles), demonstrating that the CD1b:CD63 tail chimeric molecule was efficiently delivered to lysosomes. Thus, we concluded that both the CD1b and CD63 tails but not the CD1c tail were efficient in mediating protein sorting to LAMP-1-positive lysosomes.

Of the Four CD1 Isoforms, Only CD1b Binds the AP-3 Adaptor Protein Complex

We reasoned that cytosolic proteins associated with vesicular coats might distinctly bind the CD1b cyto-

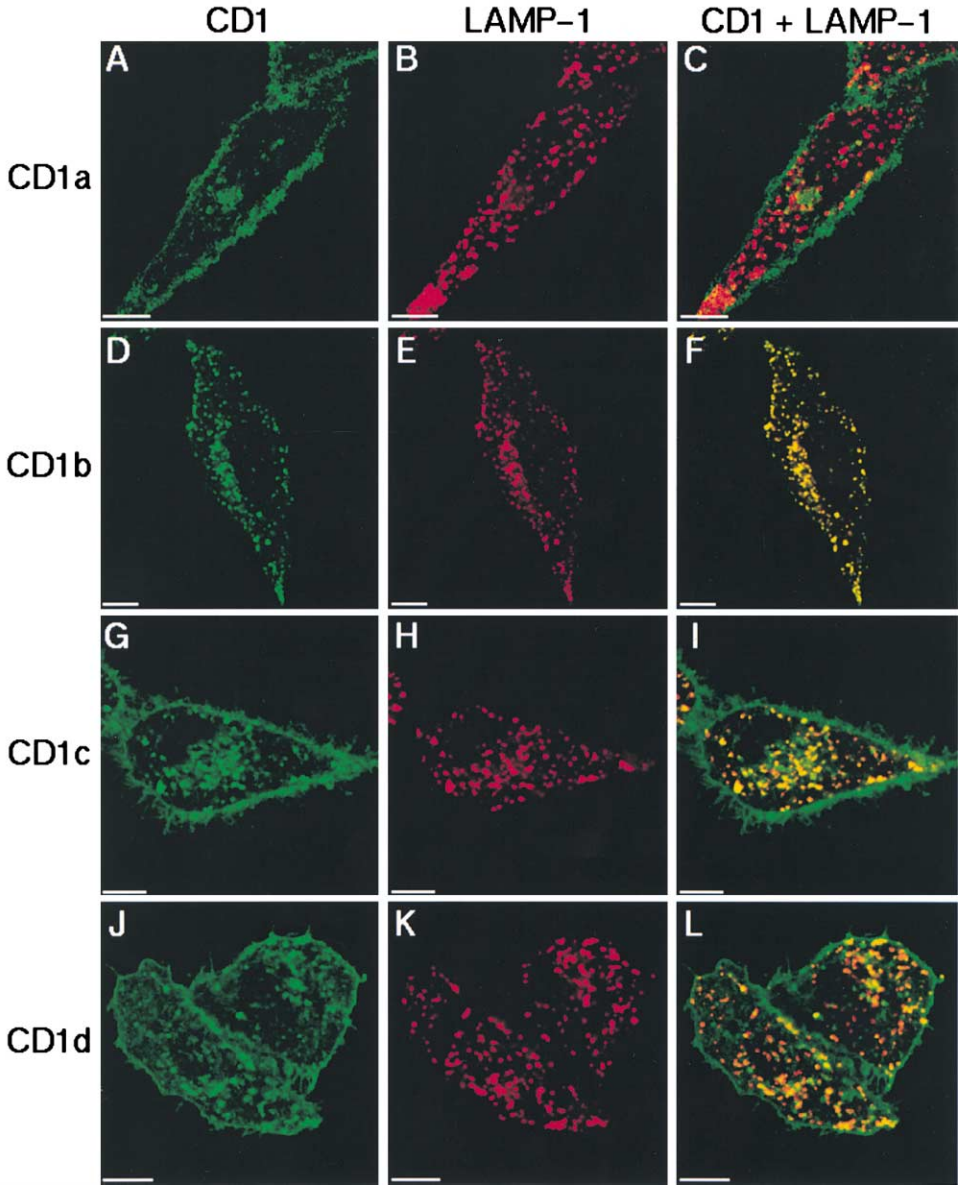

Figure 1. Human CD1 Isoforms Were Differentially Transported to Lysosomes

HeLa cells transfected with either CD1a ([A]–[C]), CD1b ([D]–[F]), CD1c ([G]–[I]), or CD1d ([J]–[L]) were fixed and permeabilized, and then double labeling with antibodies to the appropriate CD1 isoforms (green) and LAMP-1 (red) was performed. The two corresponding images were then electronically merged (CD1 + LAMP-1). Scale bars, 10 μm.

plasmic tail tyrosine-based sorting motif, accounting for its marked delivery to lysosomes. The AP-3 adaptor protein is known to capture selected lysosome-resident integral membrane proteins via interaction between the μ3A subunit of AP3 and cytoplasmic tyrosine-based sequences of the transmembrane proteins (Dell'Angelica et al., 1999). Therefore, we tested the cytoplasmic domains of the four human CD1 isoforms (CD1a, -b, -c, -d) for their ability to bind the μ3A subunit of AP-3, using a yeast two-hybrid approach (Figure 3). The GAL4 transcription activation domain was fused to μ3A and was coexpressed in yeast cells with the GAL4 DNA binding domain fused to the cytoplasmic tyrosine-based sequences derived from CD1a, -b, -c, and -d or from control proteins including CD63, the transferrin receptor (TfR), and LAMP-1. The growth of the transformed yeast cells was evaluated on histidine-supplemented and histidine-deficient plates to identify specific interactions between the AP-3 μ3A subunit and each tyrosine-based motif containing cytoplasmic tail. Yeast cells grew well on histidine-supplemented plates (Figure 3A, left panel, + His), confirming their viability. A specific interaction of CD63 and LAMP-1 but not TfR with the μ3A subunit was demonstrated on histidine-deficient plates (Figure 3A, right panel, − His), consistent with their previously reported specificities. Importantly, among the four CD1 isoforms tested, only CD1b but not the others showed a striking ability to bind the μ3A subunit of the AP-3 complex in this yeast two-hybrid system (Figure 3A, right panel, − His). These results suggested that

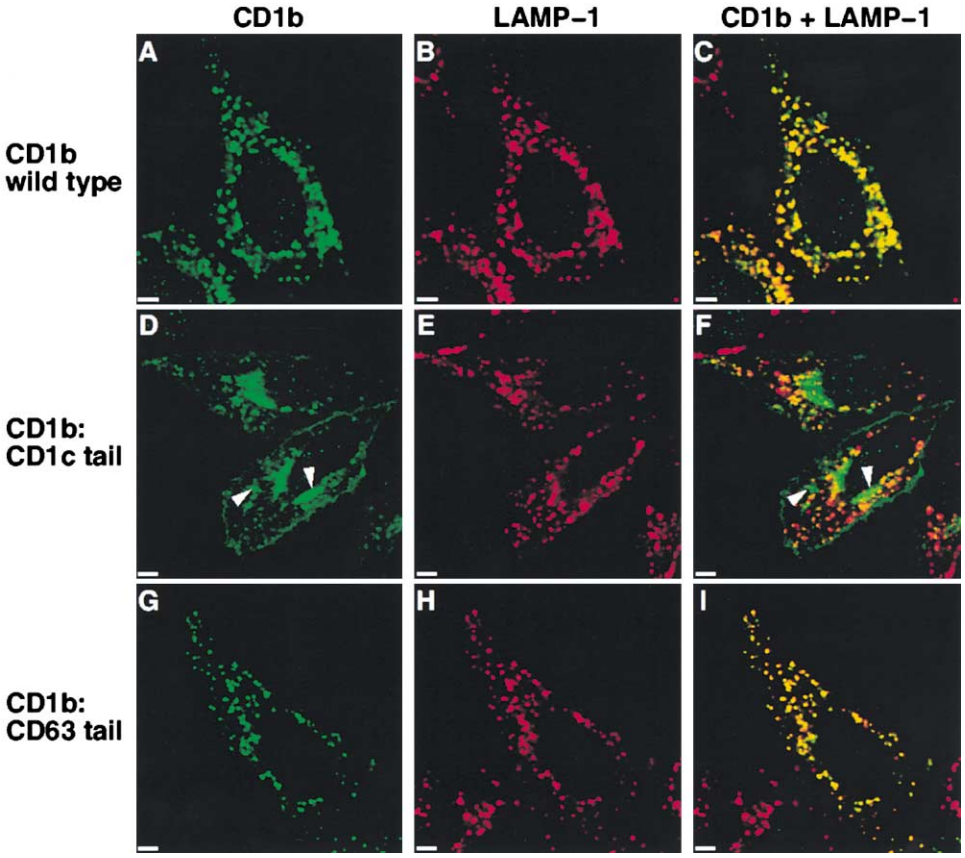

Figure 2. CD63 Tail but Not CD1c Tail Was Substituted for CD1b Tail to Deliver CD1b to LAMP-1$^+$ Lysosomes

HeLa cells transfected with either wild-type CD1b (top panels), CD1b:CD1c tail (middle panels), or CD1b:CD63 tail (bottom panels) were double labeled with antibodies to CD1b ([A], [D], and [G]; green) and LAMP-1 ([B], [E], and [H]; red) and analyzed by confocal microscopy. The two corresponding images were then superimposed to detect any cellular compartments expressing both CD1 and LAMP-1 ([C], [F], and [I]; yellow). Scale bars, 5 μm.

CD1b molecules might be selectively delivered to lysosomes via specific interaction with the AP-3 complex.

The amino acid sequence of the cytoplasmic tail tyrosine-based sorting motifs and their flanking amino acid residues are remarkably similar between CD1b (Arg-Arg-Arg-Ser-Tyr-Gln-Asn-Ile-Pro) and CD1c (Lys-

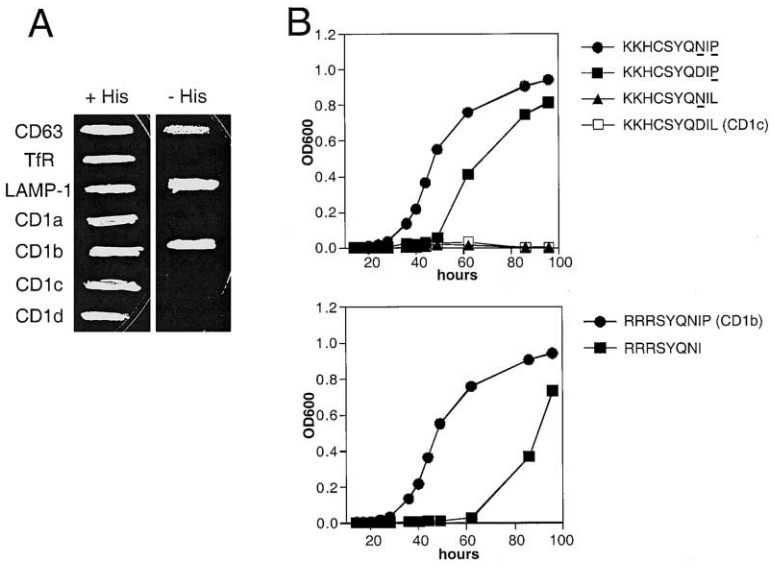

Figure 3. CD1b but Not Other CD1 Isoforms Binds the μ3A Subunit of the AP-3 Complex

(A) Yeast cells were transformed with the GAL4ad-μ3A construct together with either GAL4bd-CD63, GAL4bd-TfR, GAL4bd-LAMP-1, GAL4bd-CD1a, GAL4bd-CD1b, GAL4bd-CD1c, or GAL4bd-CD1d and plated on minimal medium plates with (+ His) or without (− His) histidine. Specific interaction was detected based on the ability of the cotransformed yeast cells to grow in the absence of histidine.

(B) Yeast cells were transformed with the GAL4ad-μ3A construct together with GAL4bd fused with either CD1c-based (top) or CD1b-based (bottom) tail constructs. Their amino acid sequences are shown in the legends, with mutated amino acid residues underlined. The transformed cells were inoculated in histidine-deficient media, and their growth was assessed by measuring OD_{600} at indicated time points.

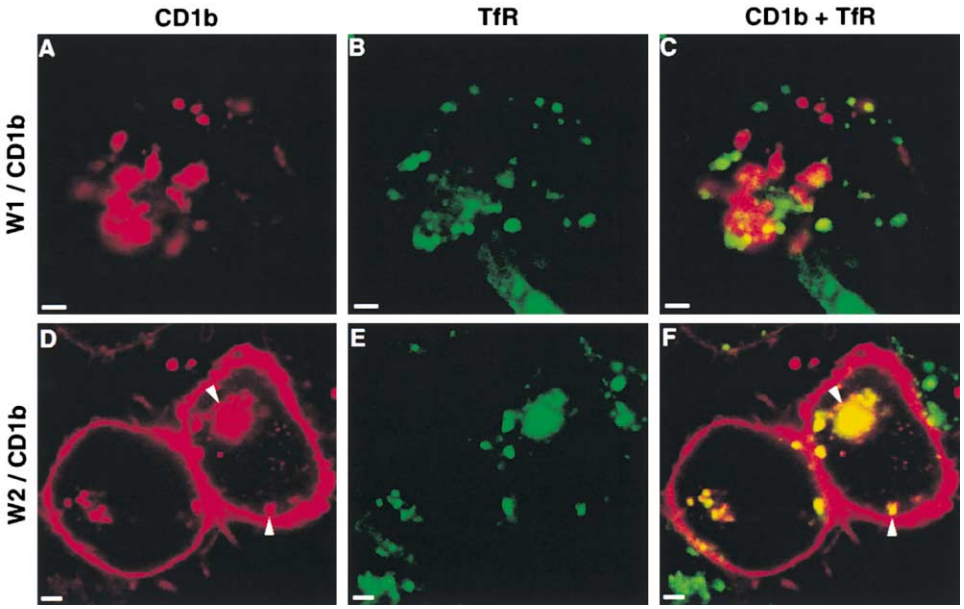

Figure 4. CD1b Molecules Were Redistributed to Early Endocytic Compartments and the Plasma Membrane in AP-3-Deficient Cells

Wild-type (W1) and AP3-deficient (W2) cells were transfected with CD1b, double labeled with antibodies to CD1b ([A] and [D]; red) and to TfR ([B] and [E]; green), and analyzed by confocal microscopy. The two corresponding images were then superimposed to detect intracellular vesicles expressing both CD1b and TfR ([C] and [F]; yellow). Scale bars, 5 μm.

Lys-His-Cys-Ser-Tyr-Gln-Asp-Ile-Leu) (amino acid sequences of the cytoplasmic domains are shown in parentheses with tyrosine-based motifs underlined). Among the C-terminal six amino acid residues, only those at the Y + 2 and Y + 4 positions are different between the two molecules. To appreciate critical residues that might control AP-3 binding, the amino acid residues at these positions of the CD1c tail were mutated either singly or doubly into the corresponding amino acid residues of the CD1b tail (Asp to Asn at Y + 2 and Leu to Pro at Y + 4), and the ability of the mutated sequences to bind the AP3 μ3A subunit was compared by measuring the growth rate of transformed yeast cells (Figure 3B, top panel). No growth was detected in His-deficient media for yeast cells expressing the CD1c tail with the single Asp to Asn mutation at Y + 2 (filled triangle) as well as those expressing the wild-type CD1c tail (open square). In contrast, yeast cells harboring the Leu to Pro mutation at Y + 4, either with (filled circle) or without (filled square) mutation at Y + 2, significantly restored the ability to grow in His-deficient media, suggesting a potential role of the C-terminal proline in positively controlling binding to AP-3. Consistent with this, marked reduction in growth rate was observed for yeast cells expressing the C-terminal proline deleted CD1b tail (Figure 3B, bottom panel, filled square), as compared with the wild-type CD1b tail (filled circle). These observations strongly suggested that the presence or absence of the C-terminal proline residue accounted for differential binding of CD1b and CD1c to AP-3.

CD1b Molecules Are Misrouted in AP-3-Deficient Cells

To confirm that the results from the yeast two-hybrid system were physiologically relevant in mammalian cells, we compared CD1b localization in AP-3-expressing (W1) and AP-3-deficient (W2) cells. W2 is a B-lymphoblastoid cell line derived from a patient with HPS-2 who carries a single nucleotide substitution in one allele of the β*3A* gene and a 63 bp deletion in the other allele of the gene (Dell'Angelica et al., 1999). In these cells, all the AP-3 complexes containing the mutated forms of the β3A subunit fail to assemble and are proteolytically degraded, resulting in markedly reduced levels of functional AP-3 complexes. W1 is a B-lymphoblastoid cell line derived from the father of the patient, who carries the normal β*3A* gene in one allele while having the 63 bp deletion in the other allele (Dell'Angelica et al., 1999). Thus, W1 cells express functional AP-3 complexes and were suitable for comparison with the AP-3-deficient W2 cells.

CD1b was expressed in these cells by transfection, and its intracellular localization was compared. In W1 cells (Figure 4, top panels), the majority of CD1b-containing vesicles (Figure 4A, red vesicles) were separate from TfR-expressing vesicles (Figure 4B, green vesicles) that belonged to the early endocytic system. Consistent with the prominent expression of CD1b in lysosomes, few $CD1b^+$ vesicles were found superimposable with TfR^+ vesicles. In contrast, in AP-3-deficient W2 cells, markedly increased levels of surface expression of CD1b were observed. The $CD1b^+$ vesicles visible intracellularly (Figure 4D, indicated with arrowheads) displayed a vesicular distribution similar to that of the TfR (Figure 4E, green vesicles). By electronically merging the images, it was apparent that the CD1b-containing and the TfR-containing vesicles were almost entirely superimposable (Figure 4F, yellow vesicles). These results suggested that unlike MHC class II that traffics normally in AP-3-deficient cells (Caplan et al., 2000; Se-

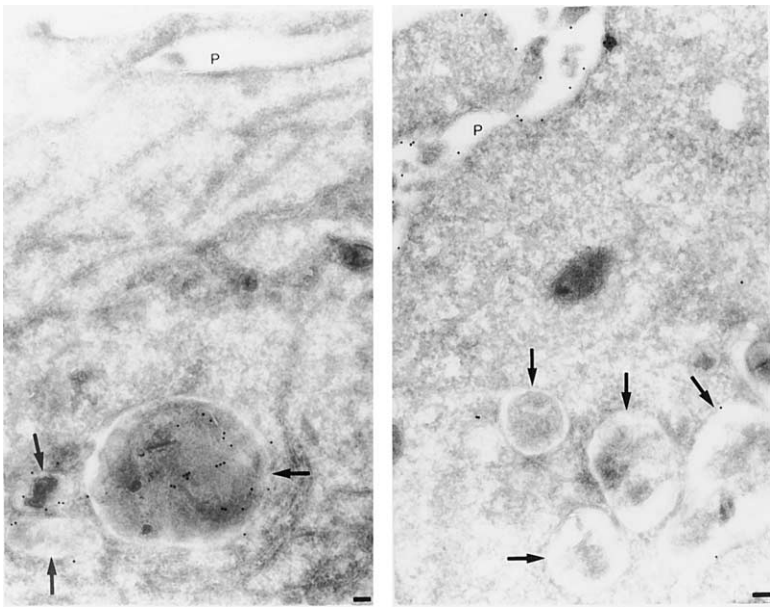

Figure 5. CD1b Molecules Barely Gained Access to Lysosomes in AP-3-Deficient Cells

Immunoelectron micrographs of thin cryosections of W1 (left) and W2 (right) cells transfected with CD1b are shown. In W1 cells, CD1b molecules were prominently expressed in multuvesicular or multilamellar lysosomes (indicated with arrows). In contrast, CD1b molecules are poorly expressed in these compartments (indicated with arrows) of W2 cells while being expressed abundantly on the plasma membrane (P). Scale bars, 50 nm.

villa et al., 2001), CD1b trafficking was selectively altered to early endosomes and to the plasma membrane in AP-3-deficient cells. One cannot readily evaluate lysosomal localization by confocal microscopy detecting colocalization with lysosomal marker proteins, such as LAMP-1, in AP-3-deficient cells because the distribution of these marker proteins is known to be partially altered as a result of AP-3 deficiency (Dell'Angelica et al., 1999). However, since AP-3-deficient cells maintain typical lysosome morphology, we performed immunogold-labeled transmission electron microscopy to locate lysosomes. In W1 cells transfected with CD1b, CD1b was prominently found in compartments with multivesicular or multilamellar morphology, the typical appearance of the lysosome (Figure 5, left, indicated with arrows), while few gold particles were seen on the plasma membrane (P). In contrast, CD1b molecules were detected only rarely in lysosomes of the AP-3-deficient cells (Figure 5, right, indicated with arrows) while being expressed abundantly on the plasma membrane (P). Taken together, these results indicate that the CD1b cytoplasmic tail selectively binds AP-3, and the AP-3 complex plays a dominant role in the efficient transport of CD1b molecules to lysosomes.

CD1b-Dependent Lipid Antigen Presentation Is Defective in AP-3-Deficient Cells

Delivery of CD1b to lysosomes is crucial for its antigen presentation function, since antigen binding to CD1b requires vesicular acidification (Ernst et al., 1998; Sugita et al., 2000b). Thus, it seems likely that AP-3 might play a pivotal role in CD1b-dependent antigen presentation in vivo. To examine this directly, antigen-presentation function of CD1b and CD1c was evaluated in wild-type and AP-3-deficient cells. W1 and W2 cells that were stably transfected with either CD1b (W1/CD1b and W2/CD1b, respectively) or CD1c (W1/CD1c and W2/CD1c, respectively) were utilized as APCs. For detection of antigen presentation, we used T cell receptor (TCR)-deficient Jurkat cells (J.RT3) reconstituted with TCR α and β chains derived from two mycobacterial lipid antigen-specific T cells. LDN5/J.RT3 was obtained by transfection of J.RT3 cells with the TCR α and β chain cDNAs isolated from the LDN5 CD1b-restricted T cell line, whereas CD8-1/J.RT3 was obtained by transfection with the CD8-1 CD1c-restricted T cell line. These T cells produced interleukin-2 (IL-2) in response to specific antigen only when APCs expressed a relevant CD1 isoform (Grant et al., 1999). Despite the fact that wild-type W1/CD1b cells expressed less CD1b proteins than mutant W2/CD1b cells (Figures 6A and 6B), more efficient antigen presentation function was detected for W1/CD1b than for W2/CD1b APCs (Figure 6C, left). T cell activation by the AP-3-deficient W2/CD1b cells was observed only in the presence of the highest antigen concentrations. The impaired antigen-presentation function was not inherent to AP-3-deficient cells, since W2/CD1c cells were more efficient than W1/CD1c cells in presenting CD1c antigens to T cells (Figure 6C, right panel). These results established an AP-3-dependent pathway for antigen presentation by CD1b molecules.

Discussion

Both MHC class II and CD1b molecules intersect lysosomal compartments for efficient sampling of exogenously derived antigens. Signal-dependent vesicular transport to lysosomes has been suggested for these antigen-presenting molecules, but the molecular interactions that precisely direct them remain to be elucidated. Here, we demonstrated that CD1b but not other CD1 isoforms binds the AP-3 adaptor complex that mediates cargo-selective transport to lysosomes. Further, we showed that defects in AP-3 function resulted in impaired antigen presentation by CD1b.

The AP-3 complex interacts with both tyrosine-based and dileucine-based sorting signals (Darsow et al., 1998; Dell'Angelica et al., 1999; Honing et al., 1998; Rapoport

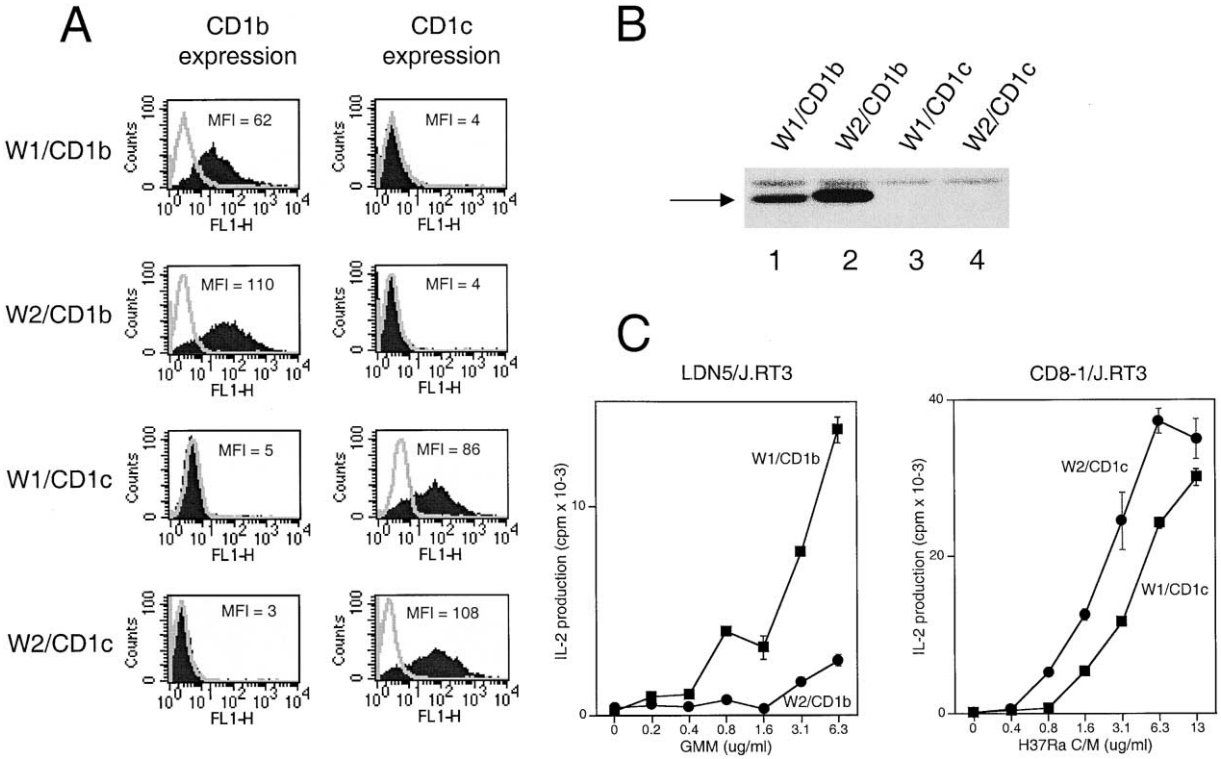

Figure 6. Lipid Antigen Presentation by CD1b but Not by CD1c Was Impaired in AP-3-Deficient Cells

(A) W1 and W2 cells stably transfected with either CD1b (W1/CD1b and W2/CD1b, respectively) or CD1c (W1/CD1c and W2/CD1c, respectively) were labeled with either anti-CD1b (left panels, filled), anti-CD1c (right panels, filled), or a control antibody (unfilled) and analyzed by flow cytometry. Values of the mean fluorescence intensity (MFI) for CD1 staining are provided in each panel.

(B) An equal number (5×10^5) of W1/CD1b (lane1), W2/CD1b (lane 2), W1/CD1c (lane 3), and W2/CD1c (lane 4) cells were lysed in 0.5% Triton X-100, and immunoprecipitation was performed with the BCD1b3.1 anti-CD1b antibody. The immunoprecipitated proteins were deglycosylated and resolved on an SDS-PAGE followed by immunodetection of CD1b heavy chains with the R.b1 rabbit antiserum against CD1b. The band corresponding to the CD1b heavy chain (arrow) was detected on lanes 1 and 2 but not on lanes 3 and 4.

(C) W1/CD1b (squares) and W2/CD1b (circles) were incubated overnight with indicated concentrations of purified glucose monomycolate (GMM) and then fixed with 0.08% glutaraldehyde. The LDN5/J.RT3 cells were cultured with these fixed APCs, and IL-2 production was measured (left panel). To detect CD1c-mediated antigen presentation, W1/CD1c (squares) and W2/CD1c (circles) were incubated overnight with indicated concentrations of the chloroform/methanol (C/M) extract of *Mycobacterium tuberculosis* (strain H37Ra) and then fixed similarly. The CD8-1/J.RT3 cells were cultured with these fixed APCs, and IL-2 production was measured (right panel).

et al., 1998; Vowels and Payne, 1998). Since MHC class II molecules are prominently expressed in lysosomes, and dileucine-based motifs are found in the cytoplasmic domain of MHC class II β chains as well as the associated Ii, a role for AP-3 in MHC class II antigen presentation was considered. However, recent studies in mice and in humans demonstrated that AP-3 is not required for MHC class II trafficking and peptide antigen binding (Caplan et al., 2000; Sevilla et al., 2001). Moreover, except for CD1b, neither the other CD1 isoforms nor MHC class I bind AP-3. Thus, among antigen-presenting molecules, CD1b distinctly utilizes an AP-3-dependent vesicular transport as shown here and also by the BIAcore surface plasmon resonance spectroscopy (Briken et al., 2002). In this respect, it is noteworthy that patients with HPS-2 suffer from recurrent microbial infections (Shotelersuk et al., 2000), raising an interesting possibility that defects in CD1b function may contribute to liability to infections.

In contrast to the selective binding of AP-3 by CD1b, it seems likely that other CD1 isoform tyrosine-based motifs may interact only with AP-2 at the plasma membrane, since recognition of this AP complex occurs for many proteins containing tyrosine-based motifs that are internalized from the plasma membrane. For CD1b, a multistep pathway for internalization to lysosomes may first involve interactions with AP-2 at the plasma membrane, followed by interactions with AP-3 in endosomes that direct CD1b selectively to lysosomal antigen binding compartments. Most CD1c molecules are expressed in vesicles of the early endocytic system (Briken et al., 2000; Sugita et al., 2000b). Without such specific interaction with AP-3, CD1c molecules, like TfR, may follow an early recycling route as a default pathway. Despite the fact that CD1c does not bind AP-3, a small fraction can reach lysosomes in normal cells, and a small fraction of CD1b molecules reach lysosomes in AP-3-deficient cells. Similarly, AP-3-deficient mice show diluted pigmentation due to impaired protein trafficking to the lysosome-related melanosome, but pigmentation is not completely absent. These examples suggest that AP-3-independent pathways for cargo-selective transport to lysosomes must exist, and these other mechanisms may mediate efficient transport of MHC class II-invariant

chain complexes to lysosomes. In this regard, it should be noted that a fraction of CD1b molecules can bind the invariant chain (M.S. and M.B.B., unpublished data), and CD1d is capable of associating with the MHC class II/invariant chain complex (Jayawardena-Wolf et al., 2001; Kang and Cresswell, 2002), raising an interesting possibility that this complex might mediate an AP-3-independent alternative pathway for some CD1 isoforms to reach lysosomes.

In immature dendritic cells (DCs), both MHC class II and CD1b molecules are expressed in the MIIC to sample endocytosed foreign antigens. Once activated by various inflammatory stimuli, DCs undergo a series of striking changes, collectively referred to as DC maturation. This involves dynamic vesicular transport and reorganization of the lysosomal compartments that allow antigen-bound class II molecules to traffic from the MIIC to the cell surface for T cell activation (Cella et al., 1997; Turley et al., 2000). In contrast, CD1b is separated from MHC class II during DC maturation, and a majority of intracellular CD1b molecules are retained in LAMP-1$^+$ lysosomal compartments (X.C. and M.B.B., unpublished data). Thus, it is intriguing to hypothesize that distinct intracellular trafficking of MHC class II and CD1b upon DC maturation may be controlled by their differential binding affinity to AP-3. The present study provides a molecular basis for dissecting pathways for peptide and lipid antigen presentation.

Experimental Procedures

Cell Lines and Antibodies

The J.RT3 cells reconstituted with the mycobacterial lipid antigen-specific, CD1-restricted TCRs (LDN5/J.RT3 and CD8-1/J.RT3) have been described (Grant et al., 1999). These TCR-reconstituted cells were cultured in RPMI 1640 (GIBCO–BRL, Gaithersburg, MD) complete medium (10% heat-inactivated fetal calf serum, 20 mM HEPES, 2 mM L-glutamine, 1 mM sodium pyruvate, 55 μM 2-mercaptoethanol [all from GIBCO–BRL]), containing G418 (GIBCO–BRL) (1 mg/ml) and hygromycin B (GIBCO–BRL) (500 μg/ml). The wild-type (W1) and the AP-3-deficient (W2) human B-lymphoblastoid cell lines have been described (Dell'Angelica et al., 1999). These cells were transfected by electroporation with either CD1b in pCEP4 or CD1c in pCEP4 and cultured in RPMI 1640 complete medium containing hygromycin B (10 μg/ml). The high-expressing cells were collected by fluorescence-activated cell sorting and used for analysis. IL-2-dependent HT-2 cells were cultured in RPMI complete medium supplemented with 1 nM recombinant human IL-2 (Ajinomoto Co., Kawasaki, Japan). HeLa cells were grown in Dulbecco's modified essential medium supplemented with 10% fetal calf serum and 2 mM L-glutamine. The mouse monoclonal antibodies 10H3 (anti-CD1a) (Olive et al., 1984), BCD1b3.1 (anti-CD1b) (Behar et al., 1995), F10/21A3.1 (anti-CD1c) (Beckman et al., 1996), CD1d51 (anti-CD1d) (Exley et al., 1997; Spada et al., 1998), and P3 (negative control) (Sugita and Brenner, 1994), as well as rabbit polyclonal antisera against LAMP-1 (Carlsson et al., 1988) and CD1b (Sugita et al., 1997) have been described. FITC-conjugated mouse monoclonal antibody against TfR was purchased from BD Bioscience Pharmingen (San Diego, CA).

Construction of Plasmids Encoding Chimeric CD1b Molecules

A cDNA encoding the CD1b:CD1c tail chimeric molecule was generated by PCR using CD1b in pSRα-neo as template DNA. Primers used were 5′-GAAACTGTGAGAATTCTC-3′ and 5′-GGTCTAGAG GATGTCCTGATATGAGCAGTGCTTCTTCATATACCATAATGCAAG GCATAG-3′. CD1b in pcDNA3.1(+) was digested with EcoRI and XbaI, and the corresponding fragment was replaced with the PCR product to generate a plasmid encoding the CD1b:CD1c tail protein [CD1b:CD1c tail in pcDNA3.1(+)]. A cDNA encoding the CD1b:CD63 tail chimeric molecule was generated by PCR using CD1b:CD1c tail in pcDNA3.1(+) as template DNA. Primers used were 5′-GAAAC TGTGAGAATTCTC-3′ and 5′-GGTCTAGACTACATCACCTCGTAG CCACTTCTGATACTCTTCATATACCATAATGCAAGGC-3′. CD1b in pcDNA3.1(+) was digested with EcoRI and XbaI, and the corresponding fragment was replaced with the PCR product to generate a plasmid encoding the CD1b:CD63 tail protein. Identity of the constructed plasmids was confirmed by DNA sequencing.

Immunofluorescence Labeling and Confocal Microscopy

HeLa cells were grown on glass coverslips and transfected by a calcium phosphate precipitation method with plasmids encoding wild-type and chimeric CD1b molecules as described previously (Sugita and Brenner, 1995). Two days after transfection, the cells were fixed with 2% formaldehyde in phosphate-buffered saline (PBS) for 10 min at room temperature, permeabilized with 0.2% saponin, and then processed as previously described (Sugita et al., 1999). Briefly, the permeabilized cells were double labeled with the BCD1b3.1 mouse monoclonal antibody against CD1b and rabbit polyclonal antiserum against LAMP-1, followed by incubation with FITC-conjugated donkey $F(ab')_2$ antibody to mouse IgG and Texas red-conjugated donkey $F(ab')_2$ antibody to rabbit IgG (both from Jackson ImmunoResearch Laboratories, Inc.) in PBS containing 5% normal donkey serum. The labeled cells were examined using a Leica TCS-NT confocal laser scanning microscope fitted with krypton and argon lasers as described (Sugita et al., 1999).

W1 and W2 cells stably transfected with CD1b were adhered on glass slides by a cytospin procedure, fixed, and permeabilized as described above. The permeabilized cells were labeled with the BCD1b antibody, followed by incubation with Texas red-conjugated donkey $F(ab')_2$ antibody to mouse IgG, and then double labeled with FITC-conjugated mouse monoclonal antibody to TfR. The cells were examined under confocal microscope as described above.

Yeast Two-Hybrid Assays

The two-hybrid constructs GAL4ad-μ3A, GAL4bd-TfR, GAL4bd-CD63, and GAL4bd-LAMP-1 have been described previously (Dell'Angelica et al., 1999). The constructs for GAL4bd fused to either CD1a, CD1b, CD1c, or CD1d were generated by ligation of the GAL4bd construct with synthetic double-strand DNAs encoding the whole cytoplasmic domain of each of the CD1 isoforms. Their identity was confirmed by DNA sequencing. Transformation of *Saccharomyces cerevisiae* strain HF7c with the above constructs and subsequent growth assays on histidine-supplemented and histidine-deficient agar plates or in histidine-deficient liquid media were performed as described previously (Dell'Angelica et al., 1999).

Immunogold-Labeled Electron Microscopy

W1 and W2 cells transfected with CD1b were fixed with 2% paraformaldehyde and 0.2% glutaraldehyde (both from Polysciences, Inc., Warrington PA) and processed for ultrathin cryosectioning as described (Peters et al., 1995a). Cryosections were incubated with the BCD1b3.1 anti-CD1b antibody for 45 min, washed, and then incubated with protein-A gold (EM Laboratory, Utrecht University) for 30 min. Labeled sections were viewed with a JEOL 1010 electron microscope.

Flow Cytometry and Immunoblotting

Flow cytometric analysis of W1 and W2 transfected with either CD1b or CD1c was performed as described (Pena-Cruz et al., 2001). To detect total cellular expression of CD1b, the cells were lysed in 0.5% Triton X-100 in lysis buffer (150 mM NaCl, 10 mM Tris [pH 7.5], 7.5 mM iodoacetamide, and 1 mM PMSF). Immunoprecipitation was conducted with the BCD1b3.1 anti-CD1b antibody and protein G-Sepharose (Pharmacia, Piscataway, NJ). The immunoprecipitated proteins were deglycosylated by digestion with PNGase F (New England Biolabs, Beverly, MA) according to the manufacturer's instructions. The proteins were then resolved on an SDS-PAGE gel and transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA). The membrane was incubated with the R.b1 rabbit antiserum against CD1b and immunoblotting was performed using horseradish peroxidase-conjugated protein A (Zymed, South San

Francisco, CA) and enhanced chemiluminescence (reagents from DuPont NEN, Boston, MA).

T Cell Transfectants Stimulation Assay

W1 and W2 cells transfected with either CD1b or CD1c were incubated overnight with indicated concentrations of either purified glucose monomycolate (GMM) (for LDN5/J.RT3) (gift of Dr. D.B. Moody, Brigham and Women's Hospital, Boston, MA) or the chloroform/methanol (C/M) extract of *Mycobacterium tuberculosis* (strain H37Ra) (for CD8-1/J.RT3) and washed and fixed with 0.08% glutaraldehyde as described (Sugita et al., 2000b). The TCR transfectant cells (5×10^4/well) were cultured with these fixed APCs in the presence of 10 ng/ml phorbol myristate acetate using 96-well, flat-bottomed microtiter plates (200 μl media/well). Aliquots of the culture supernatants were collected after 24 hr, and the amount of IL-2 released into the supernatants was measured as described (Grant et al., 1999) using the HT-2 indicator cells, whose proliferation is dependent on IL-2.

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References

Bakke, O., and Dobberstein, B. (1990). MHC class II-associated invariant chain contains a sorting signal for endosomal compartments. Cell *63*, 707–716.

Beatty, W.L., Rhoades, E.R., Ullrich, H.J., Chatterjee, D., Heuser, J.E., and Russell, D.G. (2000). Trafficking and release of mycobacterial lipids from infected macrophages. Traffic *1*, 235–247.

Beckman, E.M., Porcelli, S.A., Morita, C.T., Behar, S.M., Furlong, S.T., and Brenner, M.B. (1994). Recognition of a lipid antigen by CD1-restricted alpha beta+ T cells. Nature *372*, 691–694.

Beckman, E.M., Melian, A., Behar, S.M., Sieling, P.A., Chatterjee, D., Furlong, S.T., Matsumoto, R., Rosat, J.P., Modlin, R.L., and Porcelli, S.A. (1996). CD1c restricts responses of mycobacteria-specific T cells. Evidence for antigen presentation by a second member of the human CD1 family. J. Immunol. *157*, 2795–2803.

Behar, S.M., Porcelli, S.A., Beckman, E.M., and Brenner, M.B. (1995). A pathway of costimulation that prevents anergy in CD28- T cells: B7-independent costimulation of CD1-restricted T cells. J. Exp. Med. *182*, 2007–2018.

Briken, V., Jackman, R.M., Watts, G.F., Rogers, R.A., and Porcelli, S.A. (2000). Human CD1b and CD1c isoforms survey different intracellular compartments for the presentation of microbial lipid antigens. J. Exp. Med. *192*, 281–288.

Briken, V., Jackman, R.M., Dasgupta, S., Hoening, S., and Porcelli, S.A. (2002). Intracellular trafficking pathway of newly synthesized CD1b molecules. EMBO J. *21*, 825–834.

Calabi, F., and Milstein, C. (1986). A novel family of human major histocompatibility complex-related genes not mapping to chromosome 6. Nature *323*, 540–543.

Caplan, S., Dell'Angelica, E.C., Gahl, W.A., and Bonifacino, J.S. (2000). Trafficking of major histocompatibility complex class II molecules in human B-lymphoblasts deficient in the AP-3 adaptor complex. Immunol. Lett. *72*, 113–117.

Carlsson, S.R., Roth, J., Piller, F., and Fukuda, M. (1988). Isolation and characterization of human lysosomal membrane glycoproteins, h-lamp-1 and h-lamp-2. Major sialoglycoproteins carrying polylactosaminoglycan. J. Biol. Chem. *263*, 18911–18919.

Cella, M., Engering, A., Pinet, V., Pieters, J., and Lanzavecchia, A. (1997). Inflammatory stimuli induce accumulation of MHC class II complexes on dendritic cells. Nature *388*, 782–787.

Cresswell, P. (1994). Assembly, transport, and function of MHC class II molecules. Annu. Rev. Immunol. *12*, 259–293.

Darsow, T., Burd, C.G., and Emr, S.D. (1998). Acidic di-leucine motif essential for AP-3-dependent sorting and restriction of the functional specificity of the Vam3p vacuolar t-SNARE. J. Cell Biol. *142*, 913–922.

Dell'Angelica, E.C., Shotelersuk, V., Aguilar, R.C., Gahl, W.A., and Bonifacino, J.S. (1999). Altered trafficking of lysosomal proteins in Hermansky-Pudlak syndrome due to mutations in the beta 3A subunit of the AP-3 adaptor. Mol. Cell *3*, 11–21.

Dell'Angelica, E.C., Mullins, C., Caplan, S., and Bonifacino, J.S. (2000). Lysosome-related organelles. FASEB J. *14*, 1265–1278.

Ernst, W.A., Maher, J., Cho, S., Niazi, K.R., Chatterjee, D., Moody, D.B., Besra, G.S., Watanabe, Y., Jensen, P.E., Porcelli, S.A., et al. (1998). Molecular interaction of CD1b with lipoglycan antigens. Immunity *8*, 331–340.

Exley, M., Garcia, J., Balk, S.P., and Porcelli, S. (1997). Requirements for CD1d recognition by human invariant Valpha24+ CD4–CD8-T cells. J. Exp. Med. *186*, 109–120.

Feng, L., Seymour, A.B., Jiang, S., To, A., Peden, A.A., Novak, E.K., Zhen, L., Rusiniak, M.E., Eicher, E.M., Robinson, M.S., et al. (1999). The beta3A subunit gene (Ap3b1) of the AP-3 adaptor complex is altered in the mouse hypopigmentation mutant pearl, a model for Hermansky-Pudlak syndrome and night blindness. Hum. Mol. Genet. *8*, 323–330.

Grant, E.P., Degano, M., Rosat, J.P., Stenger, S., Modlin, R.L., Wilson, I.A., Porcelli, S.A., and Brenner, M.B. (1999). Molecular recognition of lipid antigens by T cell receptors. J. Exp. Med. *189*, 195–205.

Heemels, M.T., and Ploegh, H. (1995). Generation, translocation, and presentation of MHC class I-restricted peptides. Annu. Rev. Biochem. *64*, 463–491.

Honing, S., Griffith, J., Geuze, H.J., and Hunziker, W. (1996). The tyrosine-based lysosomal targeting signal in lamp-1 mediates sorting into Golgi-derived clathrin-coated vesicles. EMBO J. *15*, 5230–5239.

Honing, S., Sandoval, I.V., and von Figura, K. (1998). A di-leucine-based motif in the cytoplasmic tail of LIMP-II and tyrosinase mediates selective binding of AP-3. EMBO J. *17*, 1304–1314.

Jackman, R.M., Stenger, S., Lee, A., Moody, D.B., Rogers, R.A., Niazi, K.R., Sugita, M., Modlin, R.L., Peters, P.J., and Porcelli, S.A. (1998). The tyrosine-containing cytoplasmic tail of CD1b is essential for its efficient presentation of bacterial lipid antigens. Immunity *8*, 341–351.

Jayawardena-Wolf, J., Benlagha, K., Chiu, Y.H., Mehr, R., and Bendelac, A. (2001). CD1d endosomal trafficking is independently regulated by an intrinsic CD1d-encoded tyrosine motif and by the invariant chain. Immunity *15*, 897–908.

Kang, S.-J., and Cresswell, P. (2002). Regulation of intracellular trafficking of human CD1d by association with MHC class II molecules. EMBO J. *21*,, in press.

Kantheti, P., Qiao, X., Diaz, M.E., Peden, A.A., Meyer, G.E., Carskadon, S.L., Kapfhamer, D., Sufalko, D., Robinson, M.S., Noebels, J.L., and Burmeister, M. (1998). Mutation in AP-3 delta in the mocha mouse links endosomal transport to storage deficiency in platelets, melanosomes, and synaptic vesicles. Neuron *21*, 111–122.

Kaufmann, S.H. (1993). Immunity to intracellular bacteria. Annu. Rev. Immunol. *11*, 129–163.

Kawano, T., Cui, J., Koezuka, Y., Toura, I., Kaneko, Y., Motoki, K., Ueno, H., Nakagawa, R., Sato, H., Kondo, E., et al. (1997). CD1d-restricted and TCR-mediated activation of valpha14 NKT cells by glycosylceramides. Science *278*, 1626–1629.

Kirchhausen, T. (1999). Adaptors for clathrin-mediated traffic. Annu. Rev. Cell Dev. Biol. *15*, 705–732.

Lotteau, V., Teyton, L., Peleraux, A., Nilsson, T., Karlsson, L., Schmid, S.L., Quaranta, V., and Peterson, P.A. (1990). Intracellular transport of class II MHC molecules directed by invariant chain. Nature *348*, 600–605.

Moody, D.B., Reinhold, B.B., Guy, M.R., Beckman, E.M., Frederique, D.E., Furlong, S.T., Ye, S., Reinhold, V.N., Sieling, P.A., Modlin, R.L., et al. (1997). Structural requirements for glycolipid antigen recognition by CD1b- restricted T cells. Science *278*, 283–286.

Moody, D.B., Ulrichs, T., Muhlecker, W., Young, D.C., Gurcha, S.S., Grant, E., Rosat, J.P., Brenner, M.B., Costello, C.E., Besra, G.S., and Porcelli, S.A. (2000). CD1c-mediated T-cell recognition of isoprenoid glycolipids in Mycobacterium tuberculosis infection. Nature *404*, 884–888.

Odorizzi, G., Cowles, C.R., and Emr, S.D. (1998). The AP-3 complex: a coat of many colours. Trends Cell Biol. *8*, 282–288.

Ohno, H., Stewart, J., Fournier, M.C., Bosshart, H., Rhee, I., Miyatake, S., Saito, T., Gallusser, A., Kirchhausen, T., and Bonifacino, J.S. (1995). Interaction of tyrosine-based sorting signals with clathrin-associated proteins. Science *269*, 1872–1875.

Ohno, H., Aguilar, R.C., Yeh, D., Taura, D., Saito, T., and Bonifacino, J.S. (1998). The medium subunits of adaptor complexes recognize distinct but overlapping sets of tyrosine-based sorting signals. J. Biol. Chem. *273*, 25915–25921.

Olive, D., Dubreuil, P., and Mawas, C. (1984). Two distinct TL-like molecular subsets defined by monoclonal antibodies on the surface of human thymocytes with different expression on leukemia lines. Immunogenetics *20*, 253–264.

Park, S.H., and Bendelac, A. (2000). CD1-restricted T-cell responses and microbial infection. Nature *406*, 788–792.

Pena-Cruz, V., Ito, S., Oukka, M., Yoneda, K., Dascher, C.C., Von Lichtenberg, F., and Sugita, M. (2001). Extraction of human Langerhans cells: a method for isolation of epidermis-resident dendritic cells. J. Immunol. Methods *255*, 83–91.

Peters, P.J., Neefjes, J.J., Oorschot, V., Ploegh, H.L., and Geuze, H.J. (1991). Segregation of MHC class II molecules from MHC class I molecules in the Golgi complex for transport to lysosomal compartments. Nature *349*, 669–676.

Peters, P.J., Hsu, V.W., Ooi, C.E., Finazzi, D., Teal, S.B., Oorschot, V., Donaldson, J.G., and Klausner, R.D. (1995a). Overexpression of wild-type and mutant ARF1 and ARF6: distinct perturbations of nonoverlapping membrane compartments. J. Cell Biol. *128*, 1003–1017.

Peters, P.J., Raposo, G., Neefjes, J.J., Oorschot, V., Leijendekker, R.L., Geuze, H.J., and Ploegh, H.L. (1995b). Major histocompatibility complex class II compartments in human B lymphoblastoid cells are distinct from early endosomes. J. Exp. Med. *182*, 325–334.

Porcelli, S.A., and Modlin, R.L. (1999). The CD1 system: antigen-presenting molecules for T cell recognition of lipids and glycolipids. Annu. Rev. Immunol. *17*, 297–329.

Prigozy, T.I., and Kronenberg, M. (1998). Presentation of bacterial lipid antigens by CD1 molecules. Trends Microbiol. *6*, 454–459.

Prigozy, T.I., Sieling, P.A., Clemens, D., Stewart, P.L., Behar, S.M., Porcelli, S.A., Brenner, M.B., Modlin, R.L., and Kronenberg, M. (1997). The mannose receptor delivers lipoglycan antigens to endosomes for presentation to T cells by CD1b molecules. Immunity *6*, 187–197.

Rapoport, I., Chen, Y.C., Cupers, P., Shoelson, S.E., and Kirchhausen, T. (1998). Dileucine-based sorting signals bind to the beta chain of AP-1 at a site distinct and regulated differently from the tyrosine-based motif-binding site. EMBO J. *17*, 2148–2155.

Robinson, M.S., and Bonifacino, J.S. (2001). Adaptor-related proteins. Curr. Opin. Cell Biol. *13*, 444–453.

Rohrer, J., Schweizer, A., Russell, D., and Kornfeld, S. (1996). The targeting of Lamp1 to lysosomes is dependent on the spacing of its cytoplasmic tail tyrosine sorting motif relative to the membrane. J. Cell Biol. *132*, 565–576.

Rosat, J.P., Grant, E.P., Beckman, E.M., Dascher, C.C., Sieling, P.A., Frederique, D., Modlin, R.L., Porcelli, S.A., Furlong, S.T., and Brenner, M.B. (1999). CD1-restricted microbial lipid antigen-specific recognition found in the CD8+ alpha beta T cell pool. J. Immunol. *162*, 366–371.

Schaible, U.E., and Kaufmann, S.H. (2000). CD1 molecules and CD1-dependent T cells in bacterial infections: a link from innate to acquired immunity? Semin. Immunol. *12*, 527–535.

Sevilla, L.M., Richter, S.S., and Miller, J. (2001). Intracellular transport of MHC class II and associated invariant chain in antigen presenting cells from AP-3-deficient mocha mice. Cell. Immunol. *210*, 143–153.

Shotelersuk, V., Dell'Angelica, E.C., Hartnell, L., Bonifacino, J.S., and Gahl, W.A. (2000). A new variant of Hermansky-Pudlak syndrome due to mutations in a gene responsible for vesicle formation. Am. J. Med. *108*, 423–427.

Sieling, P.A., Chatterjee, D., Porcelli, S.A., Prigozy, T.I., Mazzaccaro, R.J., Soriano, T., Bloom, B.R., Brenner, M.B., Kronenberg, M., Brennan, P.J., et al. (1995). CD1-restricted T cell recognition of microbial lipoglycan antigens. Science *269*, 227–230.

Spada, F.M., Koezuka, Y., and Porcelli, S.A. (1998). CD1d-restricted recognition of synthetic glycolipid antigens by human natural killer T cells. J. Exp. Med. *188*, 1529–1534.

Stenger, S., Mazzaccaro, R.J., Uyemura, K., Cho, S., Barnes, P.F., Rosat, J.P., Sette, A., Brenner, M.B., Porcelli, S.A., Bloom, B.R., and Modlin, R.L. (1997). Differential effects of cytolytic T cell subsets on intracellular infection. Science *276*, 1684–1687.

Sugita, M., and Brenner, M.B. (1994). An unstable beta 2-microglobulin: major histocompatibility complex class I heavy chain intermediate dissociates from calnexin and then is stabilized by binding peptide. J. Exp. Med. *180*, 2163–2171.

Sugita, M., and Brenner, M.B. (1995). Association of the invariant chain with major histocompatibility complex class I molecules directs trafficking to endocytic compartments. J. Biol. Chem. *270*, 1443–1448.

Sugita, M., Jackman, R.M., van Donselaar, E., Behar, S.M., Rogers, R.A., Peters, P.J., Brenner, M.B., and Porcelli, S.A. (1996). Cytoplasmic tail-dependent localization of CD1b antigen-presenting molecules to MIICs. Science *273*, 349–352.

Sugita, M., Porcelli, S.A., and Brenner, M.B. (1997). Assembly and retention of CD1b heavy chains in the endoplasmic reticulum. J. Immunol. *159*, 2358–2365.

Sugita, M., Grant, E.P., van Donselaar, E., Hsu, V.W., Rogers, R.A., Peters, P.J., and Brenner, M.B. (1999). Separate pathways for antigen presentation by CD1 molecules. Immunity *11*, 743–752.

Sugita, M., Peters, P.J., and Brenner, M.B. (2000a). Pathways for lipid antigen presentation by CD1 molecules: nowhere for intracellular pathogens to hide. Traffic *1*, 295–300.

Sugita, M., van Der Wel, N., Rogers, R.A., Peters, P.J., and Brenner, M.B. (2000b). CD1c molecules broadly survey the endocytic system. Proc. Natl. Acad. Sci. USA *97*, 8445–8450.

Turley, S.J., Inaba, K., Garrett, W.S., Ebersold, M., Unternaehrer, J., Steinman, R.M., and Mellman, I. (2000). Transport of peptide-MHC class II complexes in developing dendritic cells. Science *288*, 522–527.

Vowels, J.J., and Payne, G.S. (1998). A dileucine-like sorting signal directs transport into an AP-3-dependent, clathrin-independent pathway to the yeast vacuole. EMBO J. *17*, 2482–2493.

White, S., Hatton, S.R., Siddiqui, M.A., Parker, C.D., Trowbridge, I.S., and Collawn, J.F. (1998). Analysis of the structural requirements for lysosomal membrane targeting using transferrin receptor chimeras. J. Biol. Chem. *273*, 14355–14362.

Yang, W., Li, C., Ward, D.M., Kaplan, J., and Mansour, S.L. (2000). Defective organellar membrane protein trafficking in Ap3b1-deficient cells. J. Cell Sci. *113*, 4077–4086.