Glycosyl-Phosphatidylinositol Reanchoring Unmasks Distinct Antigen-Presenting Pathways for CD1b and CD1c

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Antigen-Presenting Pathways for CD1b and CD1c

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Human CD1 proteins present lipid and glycolipid Ags to T cells. Cellular trafficking patterns of CD1 proteins may determine the ability of differing isoforms of CD1 to acquire, bind, and present these Ags to T cells. To test this hypothesis, glycosyl-phosphatidylinositol (GPI)-modified variants of CD1b and CD1c were engineered by chimerization with a GPI modification signal sequence derived from decay-accelerating factor (DAF). GPI reanchoring was confirmed by demonstrating the phosphatidylinositol-specific phospholipase C sensitivity of the CD1b·DAF and CD1c·DAF fusion proteins expressed on transfected cell surfaces. Using cytotoxicity and cytokine release assays as functional readouts, we demonstrated that CD1c·DAF is as efficient as native CD1c in presenting mycobacterial Ags to the human CD1c-restricted T cell line CD8-1. In contrast, CD1b·DAF, although also capable of presenting Ag (in this case to the CD1b-restricted T cell line LDN5), was less efficient than its native CD1b counterpart. The data support the idea that CD1c·DAF maintains the capacity to access CD1c Ag-loading compartment(s), whereas CD1b·DAF is diverted by its GPI anchor away from the optimal CD1b Ag-loading compartment(s). This constitutes the first GPI reanchoring of CD1 proteins and provides evidence that CD1b and CD1c have nonoverlapping Ag-presenting pathways, suggesting that these two Ag-presenting molecules may have distinct roles in lipid Ag presentation. The Journal of Immunology, 2000, 165: 1272–1277.

The cd1 family of proteins is made up of nonpolymorphic, transmembrane-anchored Ag-presenting molecules that associate noncovalently with β2-microglobulin (1). Two CD1 isoforms, CD1b and CD1c, present mycobacterial lipid and glycolipid Ags to specific T cell subsets, including CD4+ CD8− and CD4+ CD8+ T cells (2–6). Many of the Ags presented by CD1 molecules possess a structural motif comprised of a hydrophobic head group connected to two hydrophobic aliphatic tails (5, 7). A recent crystal structure of the mouse CD1d1 isoform revealed that the amino-terminal α1 and α2 domains form a hydrophobic groove (8). According to modeling studies, the other CD1 isoforms are predicted to share this putative Ag-binding structure (7). Presumably, the lipid tails of these aliphatic Ags are anchored in the hydrophobic binding groove of CD1 proteins. TCR engagement by CD1 proteins is probably mediated by the amino-terminal α1 and α2 domains of CD1 (9).

The process by which CD1 proteins engage and bind Ags may be distinct for each isoform. Ag presentation of lipid Ags by CD1b is disrupted by glutaraldehyde fixation of cell surfaces before exposure to Ags, suggesting that Ags must be internalized for presentation to occur (10). Furthermore, inhibition of endosomal acidification by chloroquine treatment also blocks CD1b-mediated presentation of lipid Ags to T cells (3, 10). Taken together, this evidence suggests that Ag uptake and transport to acidic endosomal compartments are required for effective presentation by CD1b (5). Much less is known about the Ag-processing requirements of CD1c.

Experimental evidence suggests that tyrosine-based motifs present in the cytoplasmic tails of many CD1 proteins direct their cellular distributions (11, 12). Detailed studies have reported the subcellular distribution of human CD1b, the majority of which is localized inside the cell and is preferentially distributed within late endosomes or lysosomes. At this intracellular site CD1b most likely encounters and binds its cognate Ags (11–14). Localization of CD1b to endosomal compartments is strongly dependent on the tyrosine-based motif in its short cytoplasmic tail. In contrast, preliminary observations indicate that a majority of CD1c, which also contains a tyrosine-based motif in its cytoplasmic tail, is present at the cell surface, with a substantially lower intracellular pool. While a portion of the intracellular CD1c is found in late endosomal compartments, it appears that a larger fraction probably enters early endosomes, from which it may be subsequently recycled back to the cell surface (R. Jackman, V. Briken, and S. Porcelli, unpublished observation). Consequently, although their cellular distributions partially overlap, it has been hypothesized that CD1b and CD1c may survey different intracellular compartments for nonpeptide Ags (12).

Glycosyl-phosphatidylinositol (GPI)4 reanchoring of Ag-presenting molecules has varying, context-dependent effects on their Ag-presenting functions (15–19). Whereas Ag-preloaded, exogenously reincorporated, GPI-modified MHC class I proteins retain

4 Abbreviations used in this paper: GPI, glycosyl-phosphatidylinositol; DAF, decay-accelerating factor; GMM, glucose monomycolate; MFI, mean fluorescence intensity; PI-PLC, phosphatidylinositol-specific phospholipase C; TCM, T cell medium.

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Ag-presenting capacity (20), endogenously expressed, GPI-anchored MHC class I proteins, which are inherently dependent upon intracellular Ag loading, are generally less efficient (16). Such loss of Ag-presenting efficiency has been previously attributed in part to altered intracellular trafficking of the GPI derivatives (15). Ultrastructural localization studies of GPI-anchored proteins have shown a broad distribution within endocytic compartments, with a tendency to localize in early endosomes, sorting endosomes, and recycling endosomes (21, 22). This intracellular distribution places GPI proteins primarily in compartments similar to those proposed for intracellular CD1c, but with only partial overlap for CD1b. A prediction of this intracellular trafficking scenario is that GPI derivatives of CD1c, but not CD1b, should retain strong Ag-presenting functions. The present study addresses this hypothesis by assessing Ag presentation by CD1 molecules bearing GPI anchors.

Materials and Methods

Plasmid construction

The following oligonucleotides were used in construct generation: a) 5'-CCCGGGGTTACCATGCTGCTGCATT, b) 5'-TCTAGAGGATCCGGCTCAGATGCTGATGAGATGTC, d) 5'-CTCGAGATGCTGTTTCTGCAGTTTCTG, e) 5'-CCCGGGGGTACCATGCTGCTGCTGCCATTT, b) 5'-TCTAGAGGATTCAACCCGGTGACATGCTGCTGCCATTT, and g) 5'-CTCGGAATTCGGATGGATGTC. Native CD1b and CD1c expression constructs were generated using cDNAs encoding CD1b and CD1c and provided by Dr. Brian Seed (Harvard University, Cambridge, MA) (23). The human CD1b full-length sequence (encoding Met16 through Pro116) was PCR amplified using primers a and b. The PCR product was digested with KpnI and BanHI and ligated into the corresponding sites in pREP7β (Invitrogen, Carlsbad, CA), an episcopal expression vector previously used by us to transfect C1R cells (16). Similarly, the human CD1c full-length sequence (encoding Met16 through Leu161) was PCR amplified using primers d and e. After digestion with KpnI and BanHI, the PCR product was ligated into the corresponding sites of pREP7β.

To generate GPI-reanchored CD1b and CD1c, chimeric CD1b·DAF and CD1c·DAF constructs were engineered. The human CD1b extracellular domain (encoding Met16 through Trp277) was PCR amplified using primers a and c and was ligated into pT7Blue (Invitrogen), resulting in pCD1b/T7Blue. pCD1b/T7Blue was digested with SmaI and PvuII, and the liberated fragment was ligated into a HindIII-cut, blunted, pDAF/REP7β vector (15), yielding pCD1b·DAF/REP7β. The human CD1c extracellular domain (encoding Met16 through Trp277) was PCR amplified using primers f and g, digested with HindIII, and ligated into the HindIII site of pDAF/REP7β, generating pCD1c·DAF/REP7β. All PCR-amplified DNA sequences were confirmed.

Cell lines

The human B lymphoblastoid cell line Hmy2.C1R (CIR) was provided by Peter Cresswell (Yale University, New Haven, CT). CIR cells were maintained in RPMI 1640 medium (BioWhittaker, Walkersville, MD) supplemented with 10% FCS (Sigma, St. Louis, MO), l-glutamine (Life Technologies, Grand Island, NY), and penicillin/streptomycin (Life Technologies). The T cell lines CD8- and LDN5 (5, 6) were cultured in T cell medium (TCM) composed of RPMI 1640 medium supplemented with 10% FCS (HyClone, Logan, UT), 10 mM HEPES (Life Technologies), penicillin/streptomycin, l-glutamine, 2-ME (Life Technologies), nonessential amino acids (Life Technologies), essential amino acids (Life Technologies), and 1 nM human recombinant IL-2 (Chiron, Emeryville, CA). The T cell lines were restimulated every 10–14 days with mycobacterial sonicates and irradiated CD1γδ monocytes. All cells were cultured at 37°C in humidified 5% CO2 incubators.

Transfections of C1R cell lines

C1R cells were transfected using lipofectin (Life Technologies) according to the manufacturer’s protocol. Transfectants were selected with hygromycin B at a concentration of 300 μg/ml (Calbiochem, La Jolla, CA). For some cell lines, clones with high levels of expression were derived using preparative cell sorting. For some experiments, previously generated CIR transfectants expressing native CD1b or CD1c were used (2).

Flow cytometry

Expression of CD1 Ags by CIR transfectants was assessed by indirect immunofluorescence and flow cytometry. CIR cell transfectants (1 x 10⁶) were immunostained with 50 μl of 10 μg/ml anti-CD1b mAb, BCDB13 (13), or anti-CD1c mAb, F10/21A3 (24). The cells were washed with wash buffer (PBS containing 0.5% BSA and 0.1% sodium azide) and then stained with FITC-conjugated goat anti-mouse Ig (Roche, Indianapolis, IN). After washing, stained cells were analyzed with a FACStar (Becton Dickinson, Mountain View, CA).

Cytotoxicity assays

Effector cell cytotoxic function was assessed by killing assays employing radiolabeled target cells. CIR target cells (1 x 10⁶/ml) in complete medium were labeled with 50–100 μCi of sodium [3H]cholorate (ICN, Irvine, CA) for 2 h at 37°C. The cells were resuspended in TCM and incubated with Ag overnight at 37°C. CD1c targets were loaded with a chloroform/methanol extract of Mycobacterium tuberculosis (6). CD1b targets were loaded with glucose monomycolate purified from Mycobacterium phlei by silicic acid column chromatography (5). Effector and radiolabeled target cells were added in triplicate to wells of a 96-well plate in a total volume of 150 μl and incubated for 4 h at 37°C. Fifty microliters of supernatant from each well was analyzed for [3H]Cr release with a gamma counter. Maximal release was measured by addition of 2% Triton X-100. Percent specific release = 100 x [(experimental − spontaneous release)/(maximal release − spontaneous release)].

INF-γ release assays

Cytokine production by effector T cells was assessed by an INF-γ ELISA. For LDN5 cells, TCM was supplemented with 0.3 ng/ml PMA (Sigma) and 0.1 nM IL-2 (Chiron). For CD8-1 cells, TCM was supplemented with 0.3 ng/ml PMA (Sigma), and 5 x 10⁶ stimulator transfectants were cultured with Ag and 5 x 10⁴ T cells. For a 40-h incubation at 37°C, the supernatants were harvested, and INF-γ levels were determined by ELISA. Briefly, Immulon-4 wells (Dynatech, Chantilly, VA) were coated with 0.95 μg/ml anti-INF-γ Ab (Endogen, Woburn, MA) and blocked with 3% BSA in PBS. Conditioned supernatants were added to each blocked well and incubated at 37°C for 1 h. After washing, 0.5 μg/ml biotinylated anti-INF-γ Ab (Endogen) was added. After incubation for 1.5 h at 37°C, the wells were washed, and streptavidin-HRP (Amersham, Piscataway, NJ) was added. After 0.5 h at 37°C, the wells were washed and p-phenylene-diamine (Sigma) was added. After development, the reaction was stopped with sulfuric acid, and the OD₄₉₀ was measured.

Results

GPI reanchoring of CD1

To study the effects of GPI reanchoring on the Ag-presenting functions of human CD1b and CD1c, GPI derivatives of these proteins were generated. To this end, chimeric cDNAs were assembled in which coding sequences for the extracellular domains of the CD1 isoforms were each linked to the 3’-end sequence of DAF encoding Met16 through Pro116, generating pCD1b·DAF/REP7β and pCD1c·DAF/REP7β as well as expression constructs for native CD1b (pCD1b/REP7β) and CD1c (pCD1c/REP7β) were stably transfected into the EBV-transformed human B lymphoblastoid cell line, CIR, which does not constitutively express either CD1 isoform. Cell surface expression of CD1b and CD1c epitopes on stable, hygromycin B-resistant, CIR transfectants was assessed by indirect immunofluorescence and flow cytometry. BCDB13, an anti-CD1b mAb (13), bound to both pCD1b·DAF/REP7β·C1R and pCD1b/REP7β·C1R transfectants (Fig. 1), but not to pREP7β vector-only transfectants (data not shown). Similarly, CD1c epitopes were detected on both pCD1c·DAF/REP7β·C1R and pCD1c/REP7β·C1R transfectants, but not on a pREP7β·C1R transfectant (data not
immunostain pCD1b/REP7β lines, secondary Ab only. Cells were analyzed on a FACStar flow cytometer (Becton Dickinson), and data are plotted as arbitrary units of log 10 fluorescence and transfectants (G and H). The Ag-presenting capabilities of CD1c were evaluated using the CD1c-restricted T cell line, CD8-1, at a 6:1 E:T cell ratio for 4 h, and specific release of radiolabel was determined. The 51Cr released was measured in a gamma counter. Each point represents the mean of triplicate cultures ± SD. The pCD1c-DAF/REP7β-C1R transfectants presented Ag to CD8-1 cells with an efficiency similar to that of transfectants bearing native CD1c. 51Cr-labeled target cells were cultured with the indicated concentrations of Ag (lipid extract derived from the H37Ra strain of M. tuberculosis) overnight at 37°C. The transfectants were then cocultured with the CD1c-restricted T cell line, CD8-1, at a 6:1 E:T cell ratio for 4 h, and specific release of radiolabel was determined. The 51Cr released was measured in a gamma counter. Each point represents the mean of triplicate cultures ± SD. The pCD1c-DAF/REP7β-C1R and pCD1c/REP7β-C1R transfectants had equivalent levels of CD1c epitope expression. Representative results from one of three experiments are shown.

FIGURE 2. Cytolysis of C1R transfectant target cells by a CD1c-restricted T cell line. pCD1c-DAF/REP7β-C1R transfectants presented Ag to CD8-1 cells with an efficiency similar to that of transfectants bearing native CD1c. 51Cr-labeled target cells were cultured with the indicated concentrations of Ag (lipid extract derived from the H37Ra strain of M. tuberculosis) overnight at 37°C. The transfectants were then cocultured with the CD1c-restricted T cell line, CD8-1, at a 6:1 E:T cell ratio for 4 h, and specific release of radiolabel was determined. The 51Cr released was measured in a gamma counter. Each point represents the mean of triplicate cultures ± SD. The pCD1c-DAF/REP7β-C1R and pCD1c/REP7β-C1R transfectants had equivalent levels of CD1c epitope expression. Representative results from one of three experiments are shown.

FIGURE 1. Flow cytometric analysis of CD1 and DAF epitopes on C1R transfectants. pCD1b·DAF/REP7β and pCD1c·DAF/REP7β (but not pCD1b·REP7β and pCD1c·REP7β) transfectants show loss of detectable epitope after PI-PLC cleavage, as evidenced by the leftward shifts of the staining profiles (note the dotted lines). The CD1b-specific mAb BCD1b3 (A and E) and the human DAF-specific mAb, 2H6 (B and F), were used as primary Abs to immunostain pCD1b/REP7β-C1R (A and B) or pCD1b·DAF/REP7β-C1R transfectants (E and F). The CD1c-specific mAb F10/21A3 (C and G) and the human DAF-specific mAb 2H6 (D and H) were used as primary Abs to immunostain pCD1c/REP7β-C1R (C and D) or pCD1c·DAF/REP7β-C1R transfectants (G and H). Solid lines, primary Ab, secondary Ab, no PI-PLC; dotted lines, primary Ab, secondary Ab, with PI-PLC treatment; dashed lines, secondary Ab only. Cells were analyzed on a FACStar flow cytometer (Becton Dickinson), and data are plotted as arbitrary units of log10 fluorescence intensity vs number of cells.

shown), using the CD1c-specific mAb F10/21A3. Preparative cell sorting was performed to isolate clones with high level cell surface expression of pCD1b·DAF/REP7β, pCD1b/REP7β, and pCD1c/REP7β from their respective transfectant pools. pCD1c·DAF/REP7β-C1R transfectants expressed high levels of CD1c epitope from the outset, and hence did not require selection of high expression clones.

PI-PLC digestion was used to confirm that the chimeric CD1b·DAF and CD1c·DAF proteins were indeed GPI reanchored. The CD1 epitopes on C1R cells transfected with either pCD1b·DAF/REP7β or pCD1c·DAF/REP7β (Fig. 1), but not the native counterparts, were PI-PLC sensitive. Of note, in each instance, CD1 epitope loss was equivalent to the loss of native (GPI-anchored) DAF resident on C1R cell surfaces. Taken together, these findings indicated that GPI reanchoring does not interfere with the synthesis and transit of CD1 proteins to the cell surface.

Ag-presenting function of CD1c·DAF

The Ag-presenting capabilities of CD1c·DAF were evaluated using CD8-1 cells, a CD1c-restricted T cell line that recognizes CD1c-restricted Ags present in the extractable lipid fraction of the H37Ra strain of M. tuberculosis (6). Both pCD1c·DAF/REP7β-C1R and pCD1c/REP7β-C1R transfectants pulsed with an H37Ra lipid extract displayed dose-dependent susceptibility to CD8-1 cell cytolytic effector function (Fig. 2). In contrast, vector-only control transfectants pulsed with Ag were not lysed by CD8-1 cells. Significantly, the pCD1c·DAF/REP7β-C1R transfectant presented Ag as efficiently as the naturally anchored counterpart, inducing equivalent dose-dependent T cell cytotoxicity. Of note, the C1R transfectants used in this experiment had equivalent levels of CD1c epitope expression, as monitored by immunofluorescence and flow cytometry, with pCD1c·DAF/REP7β-C1R transfectants having a mean fluorescence intensity (MFI) of 97, and pCD1c·REP7β-C1R transfectants having an MFI of 110.

As a second functional readout, the relative capacity of the GPI variant to induce Ag-dependent IFN-γ secretion by CD8-1 cells was also assessed. Transfectants bearing CD1c with either GPI or natural polypeptide transmembrane anchors induced equivalent levels of IFN-γ secretion by CD8-1 cells (Fig. 3). No significant IFN-γ secretion was detected in CD8-1 cell cocultures incubated with vector-only transfectants, with or without Ag. Hence, using two different effector responses as readouts of Ag-specific T cell stimulation, the CD1c·DAF variant presented exogenously pulsed, nonpeptide Ag to a T cell line as efficiently as native CD1c.
Ag-presenting function of CD1b \cdot DAF

As previously reported, CD1b presents glucose monomycrolate (GMM), a mycolyl glycolipid derived from mycobacteria to the CD4^+ CD8^- (double-negative) T cell line LDN5 (5). LDN5 was therefore used as a responder to evaluate CD1b \cdot DAF’s Ag-presenting function. Specifically, the capacity of GMM-pulsed C1R transfectants expressing surface CD1b \cdot DAF to stimulate LDN5 cytolytic effector functions was compared with that of C1R transfectants expressing native CD1b. LDN5 lysis of target cells required both Ag and CD1b expression, either as a full-length protein or as a GPI-reanchored protein. Significantly, the pCD1b \cdot DAF/REP7β-C1R transfectant, when pulsed with GMM, was sensitive to LDN5 cytotoxic effector function (Fig. 4), indicating that the GPI-reanchored form of CD1b can be recognized by T cells. However, in contrast to the situation with CD1c, pCD1b \cdot DAF/REP7β-C1R transfectants required a 100- to 1000-fold greater pulsing concentration of GMM to trigger a level of cytolytic activity equivalent to that observed with pCD1b/REP7β-C1R transfectants. More specifically, 0.01 μg of GMM was required to trigger 37% cytotoxicity for CD1b-expressing transfectants, while 1.00 μg of GMM was required to trigger 26% cytotoxicity for CD1b \cdot DAF-expressing transfectants. In this experiment the transfectants had equivalent levels of CD1b epitope expression, as measured by indirect immunofluorescence and flow cytometry, with pCD1b \cdot DAF/REP7β-C1R transfectants having an MFI of 84 and pCD1b/REP7β-C1R transfectants having an MFI of 69. As expected, with or without GMM, C1R cells transfected with pREP7β were not lysed.

The capacity of pCD1b \cdot DAF/REP7β-C1R transfectants to stimulate IFN-γ secretion by LDN5 cells was used as a second readout. When incubated with GMM, both pCD1b \cdot DAF/REP7β-C1R and pCD1b/REP7β-C1R transfectants (expressing equivalent amounts of CD1b epitopes) stimulated IFN-γ secretion by LDN5 cells. The level of induced IFN-γ secretion by LDN5 was significantly lower for the pCD1b \cdot DAF/REP7β-C1R transfectants (Fig. 5), paralleling what was observed with cytotoxicity. As a negative control, no significant IFN-γ secretion was detected in

FIGURE 3. Stimulation of IFN-γ secretion from a CD1c-restricted T cell line by C1R transfectant target cells. CD1c \cdot DAF-expressing and native CD1c-expressing C1R transfectants stimulated equivalent levels of IFN-γ production by CD8-1 cells. C1R transfectants were incubated with an equivalent number of CD8-1 effector T cells and the indicated concentrations of Ag (lipid extract derived from the H37Ra strain of M. tuberculosis). After a 40-h incubation period, the conditioned media were harvested and assayed with an IFN-γ ELISA. Each point represents the mean of triplicate cultures ± SD. The pCD1c/REP7β-C1R transfectant was generated previously (2). Two experiments yielding similar results used a set of pCD1c/DAF/REP7β and pCD1c/REP7β C1R transfectants with equivalent levels of CD1c epitope expression.

FIGURE 4. Cytolysis of C1R transfectant target cells by a CD1b-restricted T cell line. Native CD1b-expressing C1R transfectants more efficiently stimulated cytotoxic effector function from LDN5 cells than did CD1b \cdot DAF-expressing transfectants. ^{51}Cr-labeled target cells were cultured with the indicated concentrations of GMM overnight at 37°C. The transfectants were then cocultured with the CD1b-restricted T cell line, LDN5, at a 25:1 E:T cell ratio for 4 h, and specific release of radiolabel was determined. Released ^{51}Cr was measured in a gamma counter. Each point represents the mean of triplicate cultures ± SD. The pCD1b \cdot DAF/REP7β-C1R and pCD1b/REP7β-C1R transfectants had equivalent levels of CD1b epitope expression. Representative results from one of four experiments are shown.

FIGURE 5. Stimulation of IFN-γ secretion from a CD1b-restricted T cell line by C1R transfectant target cells. Native CD1b-expressing C1R transfectants more efficiently stimulated IFN-γ production by LDN5 cells than did CD1b \cdot DAF-expressing transfectants. C1R transfectants were incubated with an equivalent number of LDN5 effector T cells and the indicated concentrations of GMM. After a 40-h incubation period, the conditioned media were harvested and assayed with an IFN-γ ELISA. Each point represents the mean of triplicate cultures ± SD. The pCD1b \cdot DAF/REP7β-C1R and pCD1b/REP7β-C1R transfectants had equivalent levels of CD1b epitope expression. Representative results from one of three experiments are shown.

FIGURE 5. Stimulation of IFN-γ secretion from a CD1b-restricted T cell line by C1R transfectant target cells. Native CD1b-expressing C1R transfectants more efficiently stimulated IFN-γ production by LDN5 cells than did CD1b \cdot DAF-expressing transfectants. C1R transfectants were incubated with an equivalent number of LDN5 effector T cells and the indicated concentrations of GMM. After a 40-h incubation period, the conditioned media were harvested and assayed with an IFN-γ ELISA. Each point represents the mean of triplicate cultures ± SD. The pCD1b \cdot DAF/REP7β-C1R and pCD1b/REP7β-C1R transfectants had equivalent levels of CD1b epitope expression. Representative results from one of three experiments are shown.
their Ag-presenting functions (11, 27), presumably by diverting them from critical intracellular compartments where Ag loading occurs. Indeed, previous studies have demonstrated the presence of both CD1b and Ag in acidic late endosomal/lysosomal compartments (14). Moreover, initial studies of CD1b-mediated Ag-presenting function demonstrated a requirement for uptake of Ag and endosomal acidification, supporting the idea that late endosomal/lysosomal compartments may be important in Ag loading (3, 10).

In contrast to CD1b, much less is known about the Ag-processing requirements of CD1c-mediated Ag presentation. However, preliminary studies also indicate that mutation of the cytoplasmic domain of CD1c alters its cellular distribution (R. Jackman and S. Porcelli, unpublished observations), although the effect of this on Ag presentation has not yet been carefully assessed.

The present study employs GPI reanchoring as an alternative experimental tool for evaluating the role of transmembrane and cytoplasmic domains of CD1 isoforms in lipid Ag presentation. We (26) and others (25) developed a gene chimerization approach for conferring GPI anchors to any protein of interest. Our laboratory has subsequently reported the successful GPI reanchoring of several immune cell surface molecules, including MHC class I (16, 20). The impetus for these earlier GPI modification efforts was to use GPI derivatives as "protein paints" for cell surface engineering (28, 29). Here we have instead turned to GPI reanchoring as a tool for probing the intracellular trafficking mechanisms that determine Ag loading. By expressing CD1 isoforms as GPI variants for the first time, we were able to functionally differentiate the Ag-processing pathways of CD1b and CD1c.

A principal finding in this study was that CD1c • DAF functions as efficiently as native CD1c at presenting Ags derived from the H37Ra strain of M. tuberculosis to the CD1c-restricted T cell line CD8-1. This comparable efficiency was documented for two separate functional end points: induction of cellular cytotoxicity and IFN-γ secretion. The apparent equivalence in Ag-presenting efficiency between polypeptide-anchored and GPI-reanchored CD1c may be attributable to a similar intracellular distribution of the two proteins. Ultrastructural analyses of GPI-anchored proteins have demonstrated diffuse distribution within the endocytic system, precluding in early endosomes, sorting endosomes, and recycling endosomes (21, 22). It has been suggested that a similar intracellular path may place the majority of CD1c molecules in an Ag-loading environment less acidic than that observed for CD1b (12). The convergence of the intracellular pathways associated with GPI-anchored and native CD1c proteins may explain why substitution of CD1c’s native polypeptide anchor with a GPI anchor does not have a negative impact on its Ag-presenting function (21, 22). It should be noted, however, that cell surface loading of CD1c with lipid Ag has not been studied here and could provide an alternative explanation for the observed CD1c and CD1c • DAF equivalence.

In contrast to the situation with CD1c, CD1b • DAF was considerably less effective than its native counterpart. Once again, the alternative explanation for the observed CD1c and CD1c with lipid Ag has not been studied here and could provide an explanation for the observed CD1c and CD1c • DAF equivalence.

essential for CD1b Ag loading. As such, CD1b • DAF may be substantially diverted from the critical late endosomal compartment, accounting for its diminished Ag-presenting activity. A similar loss of Ag-presenting function was demonstrated for CD1b mutants with altered cytoplasmic tails (11).

Hence, this study provides support for the hypothesis that different CD1 isoforms may survey distinct intracellular compartments for lipid Ags (12). As demonstrated here, an identical GPI anchor selectively attenuates the Ag-presenting function of CD1b, while causing no substantial change in the CD1c Ag-presenting function. This represents the first demonstration that CD1b and CD1c are functionally different as Ag-presenting molecules. Viewed from this perspective, a GPI anchor may alter the intracellular distribution of CD1b, thereby diverting CD1b from its required Ag-loading environment. The fact that CD1c • DAF is as efficient as native CD1c may reflect the fact that the cellular distribution of CD1c is similar to that documented for GPI-anchored proteins. By suggesting that the trafficking pathways of GPI-anchored proteins and CD1c proteins overlap, these functional experiments provide supportive evidence for the proposed endosomal pathway for CD1c (12), wherein it is internalized from the cell surface and travels through early endosomes as it recycles back to the cell surface.

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