Human CD1a Deficiency Is Common and Genetically Regulated


*J Immunol* 2013; 191:1586-1593; Prepublished online 15 July 2013; doi: 10.4049/jimmunol.1300575

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http://www.jimmunol.org/content/suppl/2013/07/15/jimmunol.1300575.DC1

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Human CD1a Deficiency Is Common and Genetically Regulated

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CD1 proteins evolved to present diverse lipid Ags to T cells. In comparison with MHC proteins, CD1 proteins exhibit minimal allelic diversity as a result of nonsynonymous single nucleotide polymorphisms (SNPs). However, it is unknown if common SNPs in gene regulatory regions affect CD1 expression and function. We report surprising diversity in patterns of inducible CD1a expression on human dendritic cells (DCs), spanning the full range from undetectable to high density, a finding not seen with other CD1 isoforms. CD1a-deficient DCs failed to present mycobacterial lipopeptide to T cells but had no defects in endocytosis, cytokine secretion, or expression of costimulatory molecules after LPS treatment. We identified an SNP in the 5′ untranslated region (rs366316) that was common and strongly associated with low CD1a surface expression and mRNA levels (p = 0.03 and p = 0.001, respectively). Using a CD1a promoter-luciferase system in combination with mutagenesis studies, we found that the polymorphic allele reduced luciferase expression by 44% compared with the wild-type variant (p < 0.001). Genetic regulation of lipid Ag presentation by varying expression on human DCs provides a mechanism for achieving population level differences in immune responses despite limited structural variation in CD1a proteins.


Major histocompatibility complex genes are among the most polymorphic in the human genome. For example, the human MHC class I locus consists of three genes, HLA-A, HLA-B, and HLA-C, each of which is represented by >1000 variant alleles (1). This variation is important to achieve population level diversity in the adaptive immune response to pathogens, which coevolve with their human hosts. Within an individual, the breadth of the adaptive immune response is further enhanced by the ability of T cells to rearrange and combine genes encoding Ag receptors. Therefore, MHC allelic diversity and TCR sequence diversity ensure the ability of T cells to recognize a wide array of peptide Ags.

T cells have also evolved the capacity to recognize diverse lipids in the context of CD1 proteins (2, 3). CD1 H chains are homologous to MHC class I and bind noncovalently to β2-microglobulin (4). The human CD1 locus contains five genes (CD1A, CD1B, CD1C, CD1D, and CD1E) clustered on chromosome 1 (5). CD1a, CD1b, CD1c, CD1d, and CD1e proteins differ in which lipid Ags they bind, their patterns of expression on cells, and trafficking within cells (6). Notably, the mouse and rat genomes contain only orthologs of human CD1D (7–9), so mice provide a convenient experimental model for CD1d only. The other CD1 proteins are largely unexplored in the context of human immunology.

Despite their structural homology to MHC class I H chain genes, CD1 genes exhibit limited allelic diversity as a result of nonsynonymous polymorphisms. In early studies, Southern blots revealed largely conserved CD1 sequences among human and inbred mouse strains (5, 7, 10). Human sequence diversity in exon 2 and exon 3, which code for the α-1 and α-2 lipid–Ag-binding domains, respectively, is limited with only two variant alleles in CD1a, CD1d, and CD1e and zero variants in CD1b and CD1c (11, 12). Four other rare variants of CD1e have also been reported in single individuals (13, 14). More recent data derived from The International Haplotyping Project (http://www.hapmap.org) and 1000 Genomes Project (http://1000genomes.org) reveal that common genetic variation exists in the CD1 locus, but this variation is enriched outside of protein coding exons and thus does not alter protein structure and lipid binding. Taken together, these studies suggest that modulation in the T cell response to lipids is not achieved by the diversity of CD1 coding region alleles but might be influenced by genetic variation in noncoding regions of the gene.

The CD1a protein presents lipopeptide Ags to T cells and is expressed on Langerhans cells, thymocytes, and certain subsets of dendritic cells (DCs) (6, 15). Data regarding the importance of CD1a function for human health are limited. Individual T cell clones that recognize host or pathogen derived lipids have been described and provide isolated examples of CD1a-presented lipids (16–18). Mammalian sulfatide is recognized by a CD4+ T cell clone, which produces TNF-α and shows a Th1 phenotype upon TCR engagement (18, 19). Mycobacterial dideoxymycobactin is

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Received for publication March 4, 2013. Accepted for publication June 12, 2013.

This work was supported by the National Institutes of Health (Grants K24AI089794 to T.R.H., K08-AI89938 to C.S., and R01 AI049313 to D.B.M.), the Burroughs Welcome Foundation (to T.R.H. and D.B.M.), the Burroughs Wellcome Fundation (to T.R.H. and D.B.M.), and the Irvington Institute Fellowship Program of the Cancer Research Institute (to C.S.).

C.S., T.H.-M., E.A.-N., M.J.M., T.-Y.C., D.B.M., and T.R.H. designed the experiments. C.S., M.S., R.D.W., and T.-Y.C. conducted all of the experiments except peripheral blood dendritic cell phenotyping, which was performed by T.H.-M. and E.A.-N. C.S., D.B.M., and T.R.H. wrote the manuscript with contributions from all authors. All authors analyzed the data.

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The online version of this article contains supplemental material.

Abbreviations used in this article: DC, dendritic cell; MFI, median fluorescence intensity; SNP, single nucleotide polymorphism; UTR, untranslated region.

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recognized by a CD8+ T cell clone, which produces IFN-γ and IL-2 upon stimulation and is capable of lysing cells infected with *Mycobacterium tuberculosis* (4, 17, 20). More recently, CD1a-autoreactive T cells have been identified in the blood of human donors, in some cases with precursor frequencies as high as 0.1–10% of all peripheral blood T cells (21, 22). These studies have begun to reveal population-level differences in CD1a-restricted T cell responses, but the immunologic mechanisms underlying this variation remain unexplored.

In this study, we report that CD1a deficiency on in vitro–derived DCs is a common phenotype, detected in 15% of study subjects. A common polymorphism in the 5′ untranslated region (UTR) of CD1a is strongly associated with both low surface expression and mRNA levels, and this polymorphism directly regulates gene expression in a promoter-luciferase assay. These studies provide a transcriptional regulatory mechanism for population level differences in T cell responses to lipids Ags that does not depend on non synonymous allelic diversity in the CD1 locus.

**Materials and Methods**

**Human subjects**

The Seattle study group consisted of 122 healthy individuals who provided blood for genotyping, and 33 also provided blood for functional studies. In this cohort, 68 (55%) were female, and self-described ethnic composition was 90 (73%) white, 27 (22%) Asian, and 5 (5%) other.

**Ethics**

All protocols were approved by human subject review committees at the University of Washington.

**Single nucleotide polymorphism selection for genotyping**

We used data from the International Haplotype Mapping Project (http://www.hapmap.org; version 3, release 2) to select single nucleotide polymorphisms (SNPs) within 10 kb of CD1a. We used the CEU population which consists of Utah residents of Northern and Western European ancestry. Haplotype-tagging SNPs with a minimum allele frequency of 4% and R2 cutoff of 0.80 for linkage disequilibrium were identified using Haploview v4.2 (http://www.broad.mit.edu/haploview).

**Genomic techniques**

Genomic DNA was prepared from saliva (Genotek) or peripheral blood (QiaAmp). Multiplex genotyping was performed using allele-specific primer extension on the MassARRAY (Sequenom) platform (23, 24). Single SNP genotyping was performed using the TaqMan SNP Genotyping Assay (Applied Biosystems). Genotypes were confirmed in a subset of individuals by DNA sequencing or genotyping on an alternate platform.

**Quantitative RT-PCR**

RNA was extracted from DCs by TRizol (Invitrogen) and ethanol precipitation. Single-stranded cDNA was generated using Multiscribe reverse transcriptase (Invitrogen). Real-time PCR was performed on StepOnePlus Real Time PCR System (Applied Biosystems) using ABI primer probe sets CD1A-FAM (Hs00233332_m1), CD1C-FAM (Hs00233332_m1), and GAPDH-JOE (402869).

In vitro generation of DCs and phenotyping

Monocytes were isolated from PBMCs by positive selection using CD14 antibodies by staining with CD14-FITC (BioLegend). To examine surface expression of CD1a, monocytes were incubated in RPMI + 10% FCS (Life Technologies) supplemented with 0.2% BSA and 0.09% sodium azide. Abs were CD1a-PE and CD1a-allophycocyanin.

Data was acquired on an FACScanTO (BD Biosciences) equipped with 488 and 633 nm lasers and analyzed using FlowJo v9.3.2 (Tree Star).

**Cellular assays**

To test the capacity of DCs to present lipid Ag to T cells, DCs were generated from cryopreserved monocytes and plated in triplicate in co-culture with T cell clone CD8-2 (E:T 1:2) and the mycobacterial lipopeptide dideoxymycobactin (20). Synthetic dideoxymycobactin (25) was dried under a sterile nitrogen stream, sonicated into media, and added to a final concentration of 10 nM. Cultures were incubated overnight, and supernatants were harvested for IFN-γ ELISA. To test the capacity of DCs to endocytose particulate Ag, DCs were incubated with FITC-conjugated paraformaldehyde-fixed *Escherichia coli* BioParticles (Molecular Probes) at a concentration of 10 mg/ml, FITC-conjugated BSA (Molecular Probes) at a concentration of 10 mg/ml or FITC-conjugated dextran (Sigma-Aldrich) at a concentration of 100 mg/ml for 1 h at either 37˚C or 4˚C as a control. MFI was obtained and fold induction was calculated as MFIPE/MFIcontrol. Abs were CD40-allophycocyanin (Bio-Legend), CD80-PE/Cy7 (BioLegend), CD83-FITC (BioLegend), CD66-PerCP/Cy5.5 (BioLegend), and HLA-DR–APC/Cy7 (BioLegend).

**Statistics**

Statistical analyses were performed using Stata Statistical Software: Release 11 (StataCorp LP, College Station, TX). Simple linear correlation between continuous variables was described using the Pearson r correlation coefficient. The nonparametric Kruskal-Wallis or Mann–Whitney U test was used to compare continuous variables stratified by genotypes. Values for two-tailed hypothesis testing are reported except where specifically noted. Function ‘pval’ was used to calculate R2 measurements of linkage disequilibrium between polymorphisms.

**Cloning and mutagenesis**

Genomic DNA was isolated from whole blood using QIA-Amp (Qiagen). We separately amplified 998 bases and 555 bases proximal to the CD1a translation start site using an Eppendorf Mastercycler Gradient 5331 (Eppendorf). We performed mutagenesis using the QuikChange II Site-Directed Mutagenesis Kit (Strategene). Two sets of primers were used: the first for mutating rs366316 from C to T (C→T) and the second for mutating rs366316 from T to C (T→C). Primers and PCR parameters are detailed in Table II.

Cloning was performed using the pcR.2.1 TOPO TA Cloning Kit according to manufacturer’s instructions (Invitrogen). DNA was extracted from bacterial pellets using the QIAprep Spin Miniprep Kit (Qiagen). Sequencing was performed on an Applied Biosystems 3730XL DNA Analyzer (Applied Biosystems).

**Constructing pGL4 expression vectors**

Wild-type and mutated 5′ UTR sequences as well as the minimal promoter from pGL4.14 [luc2/minimal/Hygro] (Promega) were isolated by digestion with XhoI and HindIII restriction endonucleases (New England Biolabs) and gel purification (QiAquick Gel Extraction Kit). Ligation was performed with T4 Ligase (New England Biolabs) using equal amounts of insert and vector. Endotoxin-free plasmid DNA of these constructs and of the control vectors pGL4.51 [luc2/CMV/Neo], pGL4.14 [luc2/minimal/Hygro], and pGL4.73 [hRlu/SV40] (Promega) was generated using the NucleoBond Xtra Midi EF plasmid preparation kits (Macherey-Nagel).

**Transfection and luminoimetry**

HEK293T cells were plated in 96-well flat-bottom plates (BD Falcon) at a density of 10,000 cells/well in DMEM (Life Technologies) supplemented with 10% FBS (Life Technologies). The next day, cells were transfected with pGL4 expression vectors using X-tremeGENE HP DNA Transfection Reagent (reagent to plasmid DNA ratio 3:1 and 2:1) (Roche Applied Science). After 24 h, cells were lysed using 10 ng/ml or media overnight. Expression of costimulatory markers was assessed by flow cytometry and fold induction for each marker was calculated as MFIPE/MFIcontrast. Abs were CD40-allophycocyanin (Bio-Legend), CD80-PE/Cy7 (BioLegend), CD83-FITC (BioLegend), CD66-PerCP/Cy5.5 (BioLegend), and HLA-DR–APC/Cy7 (BioLegend).

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omotyping of peripheral blood DCs

Whole blood collected in ACD tubes from healthy donors was stained to determine the level of CD1a and CD1c expression on peripheral blood DCs using a previously described method for staining (26). The cells were gated to distinguish DC populations by first gating lymphocytes and monocytes using CD45 and side scatter. Then, monocytes were excluded by gating CD14-negative cells. DCs were distinguished by gating HLA-DR–positive and CD3/CD19/CD20/CD56-negative cells and subsequently excluding any possible contaminating inflammatory monocytes or NK cells by excluding any CD16-positive cells. Plasmacytoid and myeloid DCs were then distinguished by their CD11c and CD123 expression, and CD1a and CD1c expression was examined for each subset as shown.

Results

We measured inducible CD1 surface expression using flow cytometry on monocyte-derived DCs generated after activation with GM-CSF and IL-4. Initial screening of blood bank donors for induction of CD1a, CD1b, and CD1c revealed two donors that induced CD1b and CD1c but not CD1a, a reproducible finding that was not attributable to media or culture conditions such as the lot of FCS or density of cells in culture (data not shown). Therefore, we undertook a more formal analysis of CD1a induction on monocytes in 19 healthy adults. We found >100-fold variability in the absolute expression intensity of CD1a among donors, a pattern that was not linked to variable expression of the other two inducible CD1 isoforms, CD1b and CD1c (Fig. 1A, 1B, Supplemental Fig. 1).

Several lines of evidence indicated that donor-specific factors contributed to varied CD1a surface density rather than the conditions of culture or measurement (27, 28). First, CD1b and CD1c, two other inducible forms of CD1, showed high density among all donors (Fig. 1B, Supplemental Fig. 1). Second, the high or low levels of CD1a were reproduced upon repeated blood collection (r = 0.97; Fig. 1C). Third, low expression of CD1a was consistent over at least three experiments and seen with two mAbs, OKT6 and HI149 (r = 0.97; Fig. 1D), suggesting that low staining was not a result of differences in epitope binding. These data identify a CD1a-specific effect in which we observed high variance of CD1a surface density on DCs among all donors and identified three donors (15%) without detectable CD1a surface protein. We refer to the phenotype of extremely low or absent CD1a surface staining as CD1a deficiency in this study.

Previous studies suggest that the induction of CD1a is particularly sensitive to the presence of IL-4 and time in culture (29, 30). Therefore, we examined the effect of two sources and two concentrations of IL-4 as well as 3 and 6 d of culture. Based on suppression of CD14 expression, we confirmed that monocytes differentiated into DCs in all conditions tested (Fig. 2A–C). Similarly, we observed induction of CD1c in all donors and in all conditions tested (Fig. 2D–F). Among CD1a-sufficient donors, we confirmed increased CD1a expression after 6 d in culture compared with 3 d, but there was no effect of the source or concentration of IL-4. However, among CD1a-deficient donors, neither the source of IL-4, concentration of IL-4, nor extended time in culture could reverse the low expression of CD1a (Fig. 2G–I). Together, these data further confirm that CD1a deficiency is a donor-specific phenomenon that is independent of culture conditions.

The host-specific nature of the effect led us to hypothesize that this phenotype was genetic. Because the expression of CD1b and CD1c did not vary substantially among donors, we considered defects in β2-microglobulin unlikely and instead focused on potential defects near or within the CD1a H chain. There are seven common SNPs located near the CD1a H chain. Only one polymorphism codes for an amino acid substitution, and the rest are located in regulatory or noncoding regions (Table I). Because of the high degree of linkage disequilibrium among these SNPs, all of the major haplotypes of an individual are defined by only three SNPs (Fig. 3A). When we stratified expression levels by genotype, rs411089 and rs366316 but not rs2269714 were associated with CD1a deficiency (Fig. 3B). Of note, rs2269714 codes for one of the two most common allelic variants of CD1A. Our data confirm published studies showing rs2269714 is not associated with defects in surface expression on transfected cells and extends this finding to include DCs (31). The association appeared strongest with the minor homozygous genotypes, so we performed a recessive model analysis and found that the minor homozygous genotypes of rs411089 and rs366316 were associated with low CD1a expression (p = 0.03 and p = 0.01, respectively) (Fig. 3C). By contrast, there was no association between rs2269714 and CD1a expression (p = 0.65; Fig. 3C), and there was no difference in CD1b or CD1c expression when stratified by any SNP (Supplemental Fig. 2A, 2B). Thus, rs411089 and rs366316 are genetic markers for CD1a deficiency.

Having identified donors with CD1a deficiency on in vitro–derived DCs, we next examined the expression of CD1a on peripheral blood DCs ex vivo. It had been previously reported that CD1a is normally expressed on a subset of CD11c+ peripheral blood myeloid DCs (32), though later work revealed that the Ab clone actually

FIGURE 1. Deficiency of CD1a on human DCs. (A) Viable monocyte-derived DCs, identified by high forward and side scatter profiles, were stained with fluorescently conjugated Abs against CD1a, CD1b, and CD1c (dark lines) as well as isotype control Ab (shaded histogram). Shown are representative plots from two donors. (B) MFI of CD1a and CD1c are shown for 19 healthy blood donors. In four donors, CD1a MFI is <10. p value reflects Bartlett’s test for nonhomogeneity of variances. (C) Simple linear correlation between CD1a staining results of sequential blood draws for eight subjects. Data are represented as percent positive cells rather than MFI to adjust for temporal variation in flow cytometry calibration. (D) Simple linear correlation of staining between two Abs (OKT6 and HI149) that bind CD1a.
Therefore, we first used K562 cells that had been stably transfected to express CD1a to validate the specificity of the CD1a staining Ab (Supplemental Fig. 3A). We stained fresh whole blood and examined expression of CD1a and CD1c on plasmacytoid and myeloid DCs (Supplementary Fig. 3B). As expected, we were able to detect CD1c on the surface of myeloid DCs but not on plasmacytoid DCs. However, we were unable to detect CD1a on either DC subset, indicating CD1a is not endogenously expressed on unstimulated circulating DCs. Further experiments were therefore conducted on monocyte-derived DCs.

The identification of three donors with CD1a deficiency allowed mechanistic investigation of the extreme phenotype. First, we evaluated CD1a-sufficient and CD1a-deficient DC presentation of lipid Ag to T cells by coincubating DCs with dideoxymycobactin and Ag-specific T cells. Only CD1a-expressing DCs stimulated the release of IFN-γ from T cells (Fig. 4A). Next, we stimulated DCs with LPS and examined expression of costimulatory molecules as well as secretion of cytokines. Compared to media, LPS stimulation resulted in the expected increase in expression of CD40, CD80, CD83, CD86, and HLA-DR; however, we did not find any difference based on CD1a expression (Fig. 4B, 4C). Similarly, LPS induced the production of IL-12p70, IL-6, and CCL1, though again there was no difference in the analysis stratified by CD1a expression (Fig. 5A, 5B). Finally, we exposed cells to fluorescently conjugated particles to assess endocytic and phagocytic capacity. We observed no difference in the uptake of dextran, BSA, or E. coli based on CD1a expression (Fig. 5C, 5D). Our data show that CD1a-deficient DCs are selectively impaired in their ability to present lipid Ag to T cells, but appear to maintain other important aspects of DC function.

We then sought to determine the mechanism by which an SNP might affect CD1a expression at the cell surface. Previous studies have revealed that most CD1a is localized to the cell surface, and the primary mechanism of CD1a expression at the cell surface is via transcription of new protein rather than altered trafficking (34–36). However, the lack of cell-surface CD1a staining led us to consider the possibility that a trafficking defect could result in the intracellular accumulation of mature CD1a. We stained permeabilized

Table I. List of SNPs in CD1A

<table>
<thead>
<tr>
<th>SNP Name</th>
<th>Position on Chromosome 1</th>
<th>Alleles</th>
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<td>G or A</td>
<td>5' UTR</td>
<td>Unknown</td>
</tr>
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<td>158224282</td>
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<td>5' UTR</td>
<td>Unknown</td>
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<td>Unknown</td>
</tr>
<tr>
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<td>rs389293</td>
<td>158228392</td>
<td>G or A</td>
<td>3'</td>
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</tbody>
</table>

SNPs were tabulated from dbSNP (http://www.ncbi.nlm.nih.gov/SNP) based on human genome assembly GRCh37.p5, build 37.3. SNPs with a minor allele frequency >4% were included.
DCs from CD1a-sufficient and CD1a-deficient donors and noted that intracellular actin was detected in all donors only after permeabilization (Fig. 6A, 6B). Among CD1a-sufficient donors, CD1a staining in permeabilized cells was qualitatively similar to that of unpermeabilized cells. However, among CD1a-deficient donors, there was no additional CD1a detected upon permeabilization (Fig. 6C). These data reveal that the CD1a deficiency phenotype is not the result of a defect in protein trafficking to the cell surface.

In myeloid cells, CD1a expression is an inducible phenomenon that is controlled by transcription, but the regulatory factors involved are poorly understood (36, 37). We found a strong positive linear correlation between CD1a mRNA and surface staining (Fig. 7A). When we stratified CD1a mRNA by genotype, we found that the minor homozygous genotypes of rs411089 and rs366316 were associated with lower transcript level (Fig. 7B), a pattern similar to what we had observed for surface staining (Fig. 3C). Again, there was no association with rs2269714 (Fig. 7B), and the level of CD1c mRNA was not associated with any SNP (Supplemental Fig. 2C). Because rs366316 is located in the 5' UTR of CD1a, we hypothesized that one or more promoter variants might directly regulate CD1a transcription. To study the function of CD1a promoter variants, we developed a promoter-luciferase assay to compare the activity of promoters cloned from a one CD1a-sufficient and one CD1a-deficient subject (Table II). The cloned DNA contains 998 bases composed of 555 bases of the 5' UTR as well as an additional 443 bases upstream of the transcription start site. We found that the CD1a-sufficient promoter construct showed 30-fold higher luciferase expression than the CD1a-deficient promoter construct (p = 0.003) (Fig. 7C). This result was consistent with reduced transcription of CD1a (Fig. 7B) but did not show a causal relationship between rs366316 and gene expression. We analyzed the 998 bp promoter sequence from a total of 3 CD1a-deficient and 6 CD1a-sufficient individuals and found 11 variants, including 8 SNPs, 2 deletions, and 1 insertion (data not shown). However, none of these variants correlated as strongly with CD1a deficiency as rs366316, suggesting this SNP

**FIGURE 3.** Association between CD1A SNPs and expression. (A) Linkage disequilibrium plot of SNPs in CD1A coding region ± 10 kb flanking regions among the Europeans of Caucasian descent population from HapMap. CD1A spans 4132 bases on chromosome 1 and consists of six exons (black squares) and two UTRs (gray squares). Minor allele frequencies (dotted boxes) and linkage disequilibrium as measured by R<sup>2</sup> values (shaded box) are indicated. Three haplotype-tagging SNPs selected for genotyping are emphasized in bold, italic, underlined text. (B) CD1a MFI stratified by SNP genotypes. The bars indicate the median value. The nonparametric Kruskal-Wallis test was used to determine statistical significance for a genotypic model. (C) Recessive model analysis combines SNP genotypes AA and Aa.
might account for the difference in luciferase expression. We repeated the cloning with a focus on the 5′ UTR and performed site-directed mutagenesis of rs366316 (Table II). These constructs allowed us to compare normalized luciferase expression among naturally occurring and mutated 5′ UTR sequences. We found that the T allele showed on average 56% higher expression than the C allele, whether it was naturally occurring (p < 0.001) or the result of mutagenesis (p < 0.001). Further, luciferase expression by the C→T mutant approximated that of the natural T allele, and expression by the T→C mutant substantially reduced promoter activity (Fig. 7D). Our data reveal that the C variant of rs366316 is causally related to reduced luciferase expression in our assay. Because rs366316 is strongly associated with CD1a deficiency, these data suggest rs366316 directly regulates CD1a gene expression.

Discussion

In this study, we report the discovery of three aspects of CD1a function in humans. First, we demonstrate the surprisingly diverse and donor-specific capacity for CD1a induction on DCs. Second, we identify individuals whose DCs lack detectable CD1a expression and are unable to present mycobacterial lipid Ag to T cells. Third, we associate two common SNPs with functional CD1a deficiency and demonstrate that rs366316, which is located in the 5′ UTR of CD1a, is causally associated with gene expression. In the absence of any evidence that rs366316 is in cis with CD1a to affect aspects of DC development and function, these data are consistent with the hypothesis that CD1a deficiency is due to reduced transcription of CD1a.

Because rs366316 is located in the 5′ UTR, it may influence CD1a surface expression by reducing translation. Our data do not exclude this possibility, though we chose to focus on the effect of rs366316 on transcription. We found rs366316 was strongly associated with reduced CD1a mRNA levels as well as reduced luciferase expression in transfection studies. Polymorphisms in the 5′ UTR of a gene can affect gene expression by altering transcription factor binding sites or DNA methylation.
sites or by reducing the stability of the transcript and increasing mRNA degradation (40, 41). Notably, GATA transcription factor binding sites located within the 5' UTR of CD1a but downstream of rs366316 have been shown to affect luciferase expression in a system similar to ours (37). Finally, our data leave open the possibility that multiple SNPs may be involved in regulating CD1a transcription. We show rs366316 is in high linkage disequilibrium with rs411089, which is located in the first intron and also associated with decreased mRNA levels. It is therefore possible that a deficiency haplotype of multiple SNPs within CD1a is collectively responsible for the molecular mechanism of CD1a deficiency.

Our data suggest that donor-specific variation in CD1a expression can modulate CD1a-restricted T cell activation. Recently, high frequencies of CD1a-autoreactive T cells were reported from most but not all healthy blood donors (21, 22). Thus, it is possible that genetically determined variation in CD1a expression could account for difference in frequencies of CD1a-autoreactive T cells. We also found that CD1a-deficient cells had a selective functional deficiency in lipid Ag presentation to T cells. By linking a genetic polymorphism to this phenotype, our data lay the foundation for genetic association studies that seek to elucidate the role of CD1a in human disease susceptibility. Previous studies have attempted to do this with CD1a coding region polymorphisms that are not associated with any functional deficiency. These studies have been underpowered and failed to replicate (42). Instead, we propose that future studies should focus on rs411089 and rs366316. For example, it has been postulated that IL-22 is important for the pathogenesis of psoriasis (43), and it was recently demonstrated that autoreactive CD1a-restricted T cells in the skin produce IL-22 (22). In principle, one could compare the SNP frequency in patients afflicted with psoriasis and compare this to the frequency observed in healthy controls. More detailed

Table II. List of primers used to perform cloning and mutagenesis

<table>
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<tr>
<th>Construct</th>
<th>Primer</th>
<th>Sequence</th>
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<tr>
<td>C→T mutant</td>
<td>Forward</td>
<td>5'-CCAGAGGAAAATGG[AG]GACTGAG-3'</td>
<td>55</td>
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<tr>
<td>Reverese</td>
<td>5'-GATGCTTACTCAGT[CT]CTCTAGG-3'</td>
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<tr>
<td>T→C mutant</td>
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Ta, Annealing temperature.
studies examining the skin lesions from patients with different genotypes would also be possible. Because CD1a also presents mycobacterial lipoproteins to T cells, similar studies could be performed in cohorts of patients with tuberculosis.

Acknowledgments

We thank Marta Janer and Sarah Li at the Institute for Systems Biology (Seattle, WA) for assistance with genotyping.

Disclosures

The authors have no financial conflicts of interest.

References

5. panels were uniformly stained with CD1a and CD8, and CD1a-CD8- cells were negative.
Supplementary Figure 1. CD1 expression on dendritic cells derived from twenty-one healthy blood donors. Data reflect the percentage of cells that stained above an isotype control antibody for CD1a, CD1b, and CD1c. These data were collected independently from that shown in Figure 1. Data are represented as % positive cells rather than MFI to adjust for temporal variation in flow cytometry calibration.
Supplementary Figure 2. Association between CD1b and CD1c expression levels and CD1A SNPs. Percentage of cells gated above isotype control staining stratified by SNPs for (a) CD1b and (b) CD1c. (c) CD1c mRNA levels were normalized to GAPDH and stratified by SNPs.
Supplemental Figure 3. Peripheral blood dendritic cells do not endogenously express CD1a. (a) Validation of CD1a staining antibody (clone HI149, dark line) using K562 cells transfected with either CD1a or CD1c. Shaded histograms indicate isotype control antibody staining. (b) Both myeloid (CD11c+CD123-) and plasmacytoid (CD11c-CD123+) dendritic cells were analyzed for the presence of CD1a and CD1c.