Complete human CD1a deficiency on Langerhans cells due to a rare point mutation in the coding sequence

To the Editor:

The family of CD1 molecules is structurally similar to MHC class I molecules, but the 2 protein families mediate fundamentally different immune functions. MHC class I molecules present peptides to T cells, whereas CD1 molecules present lipids to natural killer T cells and other CD1-restricted T cells. CD1a is highly expressed on human Langerhans cells (LCs), a specialized mononuclear phagocyte that is prevalent in the epithelial cell layer of the skin and mucosal surfaces. Epidermal LCs can function as classical antigen-presenting cells (APCs) to induce naive T-cell responses in draining lymph nodes, but also have a regulatory function in the skin via local induction of regulatory T cells and maintenance of epithelial barrier integrity.1,2 Human dermal dendritic cells (DCs) also express CD1a, but in much lower amounts compared with LCs. CD1a+DCs, which coexpress CD1c, have been shown to efficiently stimulate CD4+ and CD8+ T cells in vitro.3,4 However, immune deficiencies due to selective CD1a defects have not been previously described, and it has proved difficult to dissect the specific role of CD1a in immune regulation.

During the course of a clinical study that involved analysis of APC subsets in human skin biopsies by flow cytometry, we identified a healthy Vietnamese individual, donor 007, who showed complete absence of CD1a expression on skin APCs (Fig 1, A). This case presented an opportunity to study the biological significance of CD1a expression. To check whether LCs were absent altogether in donor 007, we obtained a second skin biopsy, separated the epidermis from the underlying structures, and stained the epidermal tissues with antibodies binding to CD1a and to HLA-DR. Donor 007 LCs displayed intense HLA-DR staining with typical dendritic morphology, but CD1a staining was minimal (Fig 1, B). Flow cytometry analysis showed that only the reference but not donor 007 LCs were HLA-DR+ (data not shown). Intriguingly, we identified a second SNP rs761269454 (Fig E5, C) that differed between donor 007 and nonaffected family members. The rs761269454 T to C conversion results in an amino acid change from Leucine to Proline at position 285 of the CD1a protein, located in the α3 domain of CD1a (Fig 2, A). Interestingly, parent 001 exhibited a double peak at this nucleotide position, suggesting that both the normal and mutant allele were expressed at the mRNA level, resulting in a normal CD1a phenotype at the protein level (Fig E5, B, and Fig E4, A).

We next addressed whether the CD1a deficiency represented a generic expression defect, using monocyte-derived dendritic cells (moDCs) as a model. In keeping with our earlier observations, moDCs from donor 007 showed no surface CD1a expression by flow cytometry or immunohistochemistry (see Fig E1, A, in this article’s Online Repository at www.jacionline.org), in contrast to moDCs derived from a normal healthy control donor. Staining with other anti-human CD1a clones, OKT6 and NA1/34-HLK, showed the same result as staining with clone HI149 (see Figs E2 and E3 in this article’s Online Repository at www.jacionline.org). In addition, no costain with early endosome antigen-1 and CD1a was observed, excluding CD1a accumulation in early endosomes in donor 007 (Fig E1, B).

To address whether the CD1a defect was caused by a mutation in the CD1a gene, we invited the parents and all 4 siblings of donor 007 for a clinical assessment and CD1a expression analysis. Summary clinical information for the family members is presented in Table E1 in this article’s Online Repository at www.jacionline.org. Apart from donor 007’s father, who had severe Parkinson’s disease, the family members were generally healthy and displayed apparently normal skin barrier function and wound healing.

Both parents (001 and 002) and siblings 003, 004, and 006 showed normal CD1a surface expression on skin DCs and/or moDCs by immunohistochemistry and flow cytometry (Fig E4, A, in this article’s Online Repository at www.jacionline.org). However, skin DCs of sibling 005 showed complete absence of surface CD1a expression, similar to donor 007 (Fig E4, B). Blood DC subsets from family members, and from Singaporean healthy controls, were also analyzed by flow cytometry; the absence of CD1a had no impact on the development of blood DC subsets, and did not affect the expression of CD1c and CD1d molecules, excluding an intracellular CD1 protein trafficking defect (Fig E4, C-F).

To establish the genetic cause of the CD1a deficiency, we isolated RNA from moDCs for CD1a mRNA length and sequence analysis (see Fig E5, A, in this article’s Online Repository at www.jacionline.org). The lengths of the CD1a open reading frame from donor 007, from the parents, and from 1 sibling were identical, ruling out a shorter splice variant as the cause of the CD1a expression defect in donor 007. However, sequencing of the mRNA identified a single nucleotide polymorphism (SNP) (rs761269454) (Fig E5, B) that differed between donor 007 and nonaffected family members. The rs761269454 T to C conversion results in an amino acid change from Leucine to Proline at position 285 of the CD1a protein, located in the α3 domain of CD1a (Fig 2, A). Interestingly, parent 001 exhibited a double peak at this nucleotide position, suggesting that both the normal and mutant allele were expressed at the mRNA level, resulting in a normal CD1a phenotype at the protein level (Fig E5, B, and Fig E4, A).

We next isolated whole blood genomic DNA from all family members and sequenced the CD1a gene and 5000 bases upstream and downstream using Illumina MiSeq (see Fig E5, C [Sanger sequencing] and Table E2 [MiSeq] in this article’s Online Repository at www.jacionline.org). Donors 007 and 005 were heterozygous for rs761269454 (Fig E5, C, and Table E2), but expressed only the variant form of CD1a (Fig E5, B), in contrast to parent 001 and sibling 006, who were also heterozygous but expressed both alleles or at least the normal allele, respectively (Fig E4 and Fig E5, B). Intriguingly, we identified a second SNP rs538916791 that introduces a stop codon at amino acid 94 of the CD1a protein. The hereditary distribution of this SNP could explain the CD1a expression pattern: in the presence of the L285P SNP on one allele, the other allele was expressed normally. However, if one allele contained the L285P SNP and the other allele contained the stop codon SNP, for 005 and 007, only the mutant L285P form could be expressed.

To test whether the L285P mutation was sufficient to abrogate surface CD1a expression, we recombinantly expressed both the reference/wild-type and the mutant forms of CD1a in human embryonic kidney cells (a fibroblast cell line) and K562 cells (a granulocytic/monocytic cell line) (Fig 2, B). We chose 2 cell lines to address potential cell-type–specific differences in expression. Flow cytometry analysis showed that only the reference but not the mutant form of CD1a was expressed on the cell surface (Fig 2, B), whereas both forms were transcribed equally (Fig E3). Immunohistochemistry of transfected HEK cells confirmed this
finding (Fig 2, C). Different transfection ratios of normal to mutant CD1a resulted in the expected expression level of normal CD1a and excluded competition at the translational level (Fig 2, B).

In summary, we describe complete CD1a deficiency in 2 apparently healthy Vietnamese adults, and have identified a novel mutation responsible for the expression defect. This did not result in any apparent CD1a-related skin abnormalities, or in systemic immune impairment in either individual.

CD1a-restricted T cells specific for the mycobacterial lipopeptide didehydroxymycobactin can be detected in the blood of tuberculin-positive individuals ex vivo. Besides a potential role of CD1a-restricted T cells in antibacterial responses, presentation of natural skin lipids to CD1a-autoreactive T cells has been suggested to be essential for maintenance of the skin immune barrier. According to this hypothesis, a skin injury causes CD1a-expressing epidermal LCs to activate dermal CD1a-restricted T cells, resulting in IL-22 secretion, which, in turn, helps to repair any epithelial damage. Moreover, the inflammation caused by bee and wasp venom is mediated via CD1a-restricted self-reactive T cells in the skin. These venoms contain phospholipase A2,
which processes skin lipids that are then presented as neoantigens on CD1a, resulting in the activation of CD1a-restricted T cells.8

None of the family members described here had a history of tuberculosis, although all are likely to have been exposed because tuberculosis is endemic in the region. Similarly, there was no apparent difference in the occurrence of common skin infections, or in wound healing, between family members displaying different CD1a expression patterns, and no family members recalled unusual reactions to bee or wasp stings.

These findings suggest that it is unlikely that CD1a surface expression is an essential element in the proposed pathway by which LCs are thought to function to maintain the integrity of the skin immune barrier.

![Recombinant expression of the mutant CD1a-L285P reproduces the in vivo expression defect.](image)

**FIG 2.** Recombinant expression of the mutant CD1a-L285P reproduces the in vivo expression defect. **A,** Structure of the CD1a molecule in complex with a sulfatide (Protein Data Bank 1ONQ). The α domains (pink), β2 microglobulin (blue), and the position of L285P in the α3 subunit (green) are shown. **B,** CD1a surface expression of WT and L285P CD1a-transfected HEK and K562 cells analyzed by flow cytometry 24 hours after transfection. Cells were transfected with the indicated ratios of WT and L285P CD1a plasmid. Bar graphs show means ± SEM of % CD1a-expressing cells measured in 2 independent experiments with total n = 4. **C,** CD1a expression on transfected HEK cells analyzed by immunofluorescence microscopy. HEK, Human embryonic kidney; SSC-A, side scatter-area; WT, wild-type.
Pre-existing anti-PEG antibodies are associated with severe immediate allergic reactions to pegnivacogin, a PEGylated aptamer

To the Editor:

PEGylation is commonly used to extend half-life and limit volume of distribution of an increasing number of nucleic acid, peptide, and small molecule therapeutics. Peginvaccogin is a modified 31-nucleotide RNA aptamer that binds to and inhibits factor IXa conjugated to an inert 40-kd branched methoxypolyethylene glycol polymer. Although early clinical testing did not identify any safety concerns, the phase IIb Randomized, Partially Blinded, Multicenter, Active-Controlled, Dose-Ranging Study Assessing the Safety, Efficacy, and Pharmacodynamics of the REG1 Anticoagulation System in Patients with Acute Coronary Syndromes (RADAR) trial was stopped after 3 allergic reactions. An extensive investigation demonstrated elevated levels of IgG anti-PEG antibodies in the 3 patients with allergic events, suggesting that the PEG moiety, and not the oligonucleotide, was the causative allergic agent. On the basis of previous safety record of PEGylated products, investigators and regulatory authorities agreed that pegnivacogin should undergo additional definitive testing incorporating a risk mitigation and action plan in a phase III trial (Randomized, Open-label, Multi-Center, Active-Controlled, Parallel Group Study to Determine the Efficacy and Safety of the REG1 Anticoagulation System Compared to Bivalirudin in Patients Undergoing Percutaneous Coronary Intervention [REGULATE-PCI]) in which subjects undergoing percutaneous coronary intervention were randomized to pegnivacogin or bivalirudin.

Methodology of the trial, planned biochemical analyses, statistical analyses, and allergy definitions are available in the first and second sections in this article’s Online Repository at www.jacionline.org. REGULATE-PCI was ultimately terminated after enrollment of 3,232 of a planned 13,200 patients after an excess of allergic reactions in pegnivacogin-treated patients.

The incidence and timing of allergic reactions are summarized in Table I. Descriptions of allergies meeting reporting criteria, as judged by the investigators, are provided in the third section in this article’s Online Repository at www.jacionline.org. Assignment to pegnivacogin was associated with a statistically significant increase in allergic reactions. Of the clinical variables assessed, female sex, allergic reactions in the past year, current smoking, and previous percutaneous coronary intervention were associated with severe allergic reactions (see Table E1 in this article’s Online Repository at www.jacionline.org). There was no evidence of altered risk of allergic reactions in patients premedicated with H1 or H2 blockers, corticosteroids, beta blockers, or angiotensin-converting enzyme inhibitors (see Table E2 in this article’s Online Repository at www.jacionline.org).

As stipulated in the risk mitigation and action plan, measurements of complement activation, tryptase release, and anti-PEG IgG antibodies were performed in all patients experiencing allergic reactions within 24 hours of pegnivacogin or bivalirudin treatment. Any additional definitive testing incorporating risk mitigation and action plan was provided by the phase III trial (Randomized, Open-label, Multi-Center, Active-Controlled, Parallel Group Study to Determine the Efficacy and Safety of the REG1 Anticoagulation System Compared to Bivalirudin in Patients Undergoing Percutaneous Coronary Intervention [REGULATE-PCI]) in which subjects undergoing percutaneous coronary intervention were randomized to pegnivacogin or bivalirudin.

# Table I: Incidence of allergic reactions within 24 hours by treatment arm

<table>
<thead>
<tr>
<th>Type of allergic reaction</th>
<th>Bivalirudin (n = 1601)</th>
<th>Peginvaccogin (n = 1605)</th>
<th>Total (N = 3206)</th>
<th>OR (95% CI)</th>
</tr>
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<tbody>
<tr>
<td>Any allergic reaction</td>
<td>10 (0.62)</td>
<td>24 (1.5)</td>
<td>34 (1.06)</td>
<td>2.4 (1.2-5.1)</td>
</tr>
<tr>
<td>Serious allergic reaction</td>
<td>1 (0.06)</td>
<td>10 (0.62)</td>
<td>11 (0.34)</td>
<td>10.0 (1.3-78.5)</td>
</tr>
<tr>
<td>Severe allergic reaction</td>
<td>4 (0.25)</td>
<td>18 (1.12)</td>
<td>22 (0.69)</td>
<td>4.5 (1.5-13.4)</td>
</tr>
<tr>
<td>Nonsevere allergic reaction</td>
<td>6 (0.37)</td>
<td>6 (0.37)</td>
<td>12 (0.37)</td>
<td>1.0 (0.3-3.1)</td>
</tr>
<tr>
<td>Anaphylaxis</td>
<td>1 (0.06)</td>
<td>10 (0.62)</td>
<td>11 (0.34)</td>
<td>10.0 (1.3-78.5)</td>
</tr>
<tr>
<td>Allergic reaction onset &lt; 1 h after study drug dosing</td>
<td>2 (0.12)</td>
<td>16 (1.0)</td>
<td>18 (0.56)</td>
<td>8.1 (1.9-35.1)</td>
</tr>
<tr>
<td>Severe allergic reaction onset &lt; 1 h after study drug dosing</td>
<td>1 (0.06)</td>
<td>12 (0.74)</td>
<td>13 (0.40)</td>
<td>12.1 (1.6-92.8)</td>
</tr>
</tbody>
</table>

Data presented as n (%). Bivalirudin was the reference group in the calculation of OR. OR, Odds ratio.