Human CD141⁺ Dendritic Cells Induce CD4⁺ T Cells To Produce Type 2 Cytokines


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Human CD141⁺ Dendritic Cells Induce CD4⁺ T Cells To Produce Type 2 Cytokines

Chun I. Yu,*¹ Christian Becker,†‡ Patrick Metang,*¹ Florentina Marches,*² Yuanyuan Wang,*§ Hori Toshiyuki,* Jacques Banchereau,‖ Miriam Merad,*# and A. Karolina Palucka*²

Dendritic cells (DCs) play the central role in the priming of naive T cells and the differentiation of unique effector T cells. In this study, using lung tissues and blood from both humans and humanized mice, we analyzed the response of human CD1c⁺ and CD141⁺ DC subsets to live-attenuated influenza virus. Specifically, we analyzed the type of CD4⁺ T cell immunity elicited by live-attenuated influenza virus–exposed DCs. Both DC subsets induce proliferation of allogeneic naive CD4⁺ T cells with the capacity to secrete IFN-γ. However, CD141⁺ DCs are uniquely able to induce the differentiation of IL-4– and IL-13–producing CD4⁺ T cells. CD141⁺ DCs induce IL-4– and IL-13–secreting CD4⁺ T cells through OX40 ligand. Thus, CD141⁺ DCs demonstrate remarkable plasticity in guiding adaptive immune responses. The Journal of Immunology, 2014, 193: 4335–4343.

Dendritic cells (DCs) are an essential component of immune responses through their capacity to capture, process, and present Ags to T cells (1). Their main function is to launch immunity against foreign Ag and maintain the tolerance to self. Ag presentation by immature DCs usually results in immune tolerance because of the lack of costimulatory molecules (2, 3). Activated (mature), Ag-loaded DCs initiate the differentiation of Ag-specific T cells into effector T cells displaying unique functions and cytokine profiles. Research of the past two decades brought about an increased understanding of DC biology and the existence of distinct DC subsets with specific functions (4–6). The functions and cytokine profiles of the different DC subsets to live-attenuated influenza virus are discussed in this review.

Abbreviations used in this article: DC, dendritic cell; ICS, intracellular cytokine staining; i.n., intranasal(ly); LAIV, live-attenuated influenza virus; moi, multiplicity of infection; OKX04L, OKX40 ligand; pol, polyinosinic-polycytidylic acid; TSLP, thymic stromal lymphopoietin.

CD141 (BDCA-3), which are both differentially expressed on circulating classical DCs (8–10). Both CD1c⁺ and CD141⁺ DCs can produce IL-12 upon polyinosinic-polycytidylic acid (poly I:C) stimulation, thereby enabling the generation of IFN-γ–secreting CD4⁺ T cells and the priming of naive CD8⁺ T cells (11, 12). Both CD1c⁺ and CD141⁺ DCs are able to cross-present long peptides of melanoma-tissue-derived Ag to T cell lines (13). However, they also display unique features. Among circulating classical DCs, CD141⁺ DCs uniquely express TLR3, produce very large amounts of IFN-α upon recognition of synthetic dsRNA (11), and, when activated with poly I:C, efficiently cross-prime CD8⁺ T cells (14–20). CD1c⁺ DCs are molecularly equipped to generate Th17 responses in human (12). Furthermore, we (21) recently showed that, although both subsets can expand effector CD8⁺ T cells, CD1c⁺ DCs are uniquely able to drive the differentiation of CD103⁺CD8⁺ mucosal T cells via TGF-β.

Human lung DCs are able to induce different types of CD4⁺ T cell immunity, including Th1, Th2, or Th17 responses to help clear infection (12). However, lung DCs also can mount Th2 responses that contribute to the pathogenesis of allergic asthma (22). Murine studies showed that influenza virus infection leads to maturation of lung DCs, resulting in the presentation of both viral peptides and environmental Ags that have been inhaled concurrently (23). Engagement of TLRs on lung epithelial cells is essential to induce asthma through the production of numerous cytokines, including IL-1α, GM-CSF, thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 (24–26). Although TSLP seems critical under the conditions of high allergen load, IL-1α contributes to asthmatic airway inflammation at a low dose of house dust mite (26). Adoptive-transfer studies concluded that SIRPα⁺ CD11b⁺ lung DCs are the most effective at Th2 priming in the mouse (27, 28). Furthermore, IRF4-dependent DCs drive Th2 responses in the skin (29, 30) and in atopic asthma (31). In contrast to the murine model of asthma, much less is known about the role of human DC subsets in the generation of Th2 cells. In this article, we show that human lung DC subsets differentially regulate CD4⁺ T cell immunity.

Materials and Methods

Abs and reagents

Abs to human CD3 (UCHT1), CD4 (SK3), CD8 (SK1), CD11c (B-ly6), CD19 (B27), lineage mixture 1, OKX40. CD103⁺CD8⁺ mucosal T cells via TGF-β.
ligand (OX40L: IK-1), and TNF (MAb11) were obtained from BD (Franklin Lakes, NJ). Anti-human CD40 (MAB89) Ab was purchased from Beckman Coulter (Brea, CA). Anti-human CD1c (L161) and IL-4 (MP4-25D2) were from BioBioscience (San Diego, CA). Anti-human HLA-DR (L33) and IL-10 (JES3.9D7) were from eBioscience (San Diego, CA). Poly I:C was from InvivoGen (San Diego, CA). Human CD14 (Tuk4) and CD45 (HI30) Abs were from Life Technologies (Carlsbad, CA). Anti-human CD303 (AC144) and CD141 (AD5-14H12) Abs were from Miltenyi Biotec (Auburn, CA). Recombinant human TSLP was from R&D Systems (Minneapolis, MN). Curdlan, PMA, and ionomycin were purchased from Sigma-Aldrich (St. Louis, MO). Trivalent live-attenuated influenza virus (LAIV) vaccine (FluMist; MedImmune, Gaithersburg, MD) was obtained from the Baylor University Medical Center (Dallas, TX) hospital pharmacy.

DC purification

Humanized mice. Humanized mice were generated on the NOD/SCID β2m−/− mice background, as previously described (21). All protocols were reviewed and approved by the Institutional Review Board (IRB 097-053 and 099-076) and the Institutional Animal Care and Use Committee (IACUC A01-005) at Baylor Research Institute (Dallas, TX). At 4 wk posttransplant, humanized mice were vaccinated with LAIV (1/5 of human dose) via intranasal (i.n.) inoculation. Mice were euthanized and harvested at 3 d postvaccination. After flushing out the blood, the lungs were digested with 2 mg/ml collagenase D (Roche Diagnostics, Indianapolis, IN) for 30 min at 37˚C. Single-cell suspensions were made with two frosted slides, and the debris was removed by filtering through a 70-μm cell strainer. Cells were treated first with murine Fc blocker (BD) and human Fc blocker (Miltenyi Biotec) and then stained on ice with fluorochrome-conjugated specific Abs. After washing twice with PBS, DCs were sorted with a FACS Aria using Diva software (both from BD).

Human lungs. Human lung tissues were obtained from patients undergoing lung resection surgery at the Mount Sinai Medical Center (New York, NY) or Baylor University Medical Center (Dallas, TX) after obtaining informed consent. All protocols were reviewed and approved by the Institutional Review Board at Icahn School of Medicine at Mount Sinai (IRB 08-1236-0004) or Baylor Research Institute (IRB 012-101). Care was taken to obtain lung tissue as distant as possible from any primary lesions and anatomic abnormalities and in a way to proportionally represent subpleural (distal) and central (proximal) anatomic regions of

![FIGURE 1. CD141+ DC subset in the lungs initiates Th2 response. Different subpopulations of DCs were sorted from human lungs (A and B) or the lungs of humanized mice at 3 d after i.n. LAIV vaccination (C-E). (A and C) Sorted DCs were stimulated as indicated, and a titrated number of DCs was cocultured with 100,000 allogeneic naive T cells. At day 6, CD4+ T cell proliferation was measured by the percentage of CFSE2 CD4+ T cells. Representative data from more than three independent experiments are shown (two-way ANOVA with Bonferroni posttest). (B and D) Sorted DCs were cocultured with 100,000 allogeneic naive T cells for 6 d. Cytokine production profiles of CFSE2 CD4+ T cells in DC-stimulated T cell cultures (1:100 ratio) after 6 h of PMA and ionomycin restimulation. Representative data from more than three experiments are shown (paired t test). (E) Cytokine-production profiles of IL13+CFSE2 CD4+ T cells in DC-stimulated T cell cultures upon PMA and ionomycin restimulation. Representative dot plots and summary data from more than three experiments are shown.](http://www.jimmunol.org/)

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the lung. Human lung tissue was processed analogously to the humanized mouse protocol.

Human blood. PBMCs were isolated from leukapheresis products using Ficoll-Paque Plus density gradient centrifugation (STEMCELL Technologies, Vancouver, BC, Canada). DCs were enriched using a human Pan-DC Pre-Enrichment Kit (STEMCELL Technologies) before staining for FACS sorting.

DC phenotype
Sorted DCs were stimulated with LAIV (multiplicity of infection [moi] = 2), poly IC (30 μg/ml), Curdlan (100 μg/ml), or TSLP (20 ng/ml) and harvested at 24 h to assess DC maturation phenotype. Cells were treated with Fc blocker (Miltenyi Biotec) and then stained for 30 min on ice with fluorochrome-conjugated specific Abs. After washing twice with PBS, the samples were acquired on a FACS Canto II (BD) and analyzed with FlowJo software (TreeStar, Ashland, OR).

Allogeneic MLR
Naive CD4+ T cells were isolated from PBMCs of healthy volunteers using human T cell enrichment kits (STEMCELL Technologies) and further sorted for CD45RA+CCR7+ T cells (>99% purity). CFSE-labeled naive T cells (100,000 cells) were cocultured with sorted DCs for 6 d. At day 7, T cells were harvested for FACS analysis. For blocking, OK40L, DCs were treated with 50 μg/ml anti-OX40L (IK-5) or control mouse IgG2a isotype Abs and cocultured with naive CD4+ T cells. In CD40L-blocking experiments, 10 μg/ml anti-CD40L (24-31; eBioscience), anti-CD40 (2C3; Bio-Legend), or control mouse IgG1 isotype Ab was used.

Intracellular cytokine analysis
To assess the expression of intracellular cytokines, T cells were stimulated with 50 μg/ml PMA and 1 μg/ml ionomycin for 6 h in the presence of 2 μM monensin (BD), as well as with 1 μg/ml brefeldin A (BD) for the last 4 h. Following T cell stimulation, cells were stained with fluorochrome-conjugated Abs against surface markers at room temperature for 15 min, followed by fixation and permeabilization. They were then stained for intracellular proteins at room temperature for 30 min. After washing twice, the samples were acquired on a LSRII (BD) and analyzed with FlowJo software.

Luminex assay
A total of 50,000 sorted DCs (2.5 × 10^5 cells/ml) was activated with different stimulants, and culture supernatants from a total of 24 h activation were harvested and stored at −20°C for analysis. Cytokine concentrations were calculated using MILLIPLEX Analyst Luminex 100 instrument (both from EMD Millipore, Billerica, MA). All cytokines were measured using a multiplex cytokine measurement kit, as per the manufacturer’s protocol, and analyzed using the BioPlex MILLIPLEX Analyst software, with a five-parameter curve-fitting algorithm applied for standard curve calculations.

Statistical analysis
All statistics and graphs were done with Prism software (GraphPad, La Jolla, CA). Differences in variables between any two groups were analyzed using the Mann–Whitney U test or two-tailed t test. Differences among any three or more groups were analyzed by ANOVA.

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**Results**

**Lung CD141+ DCs induce the differentiation of IL-4– and IL-13–producing CD4+ T cells**

To assess their capacity to control allogeneic naive CD4+ T cell responses, lung DCs were sorted from single-cell suspensions prepared from macroscopically uninvolved normal human lung tissue obtained during surgical resection of lung cancer. Cells were identified by flow cytometry as CD45+CD44+HLA-DR+ CD11c+CD14+ cells with differential expression of CD1c and CD141 (Supplemental Fig. 1) (21). Titrated numbers of CD1c+ or CD141+ DCs were activated with LAIV ex vivo (moi = 2, 1 h) and cocultured with CFSE-labeled, allogeneic, naive T cells for 6 d. Both DC subsets elicited comparable CD4+ T cell proliferation, as assessed by CFSE dilution, at all DC doses (Fig. 1A). CD4+ T cell differentiation was assessed by analyzing cytokine profiles of CFSE+CD4+ T cells using intracellular cytokine staining (ICS) after 6 h of restimulation with PMA and ionomycin. Both CD1c+ and CD141+ DCs elicited IFN-γ–producing CD4+ T cells (Fig. 1B). However, CD141+ DCs were able to simultaneously expand CD4+ T cells producing IL-4 and IL-13 (Fig. 1B, Table I) more efficiently than CD1c+ DCs.

To test whether lung CD141+ DCs that have been exposed to LAIV in vivo also can induce CD4+ T cells producing IL-4 and IL-13, we used a humanized mouse model that we developed earlier (21, 32). Sublethally irradiated NOD/SCID β2m−/− mice were transplanted with 3 × 10^6 CD34+ hematopoietic progenitor cells isolated from G-CSF–mobilized blood of healthy donors. At 4–6 wk posttransplantation, humanized mice were inoculated i.n. with LAIV (1/5 of human dose), and lungs were harvested at day 3. Human DC subsets were sorted as described above (21) and used in a 6-d coculture with CFSE-labeled, allogeneic, naive T cells. Both DC subsets elicited T cell proliferation, as assessed by CFSE dilution, at all DC doses (Fig. 1C). CD141+ DCs were more efficient at inducing T cell expansion than CD1c+ DCs, especially at low DC numbers (Fig. 1C). ICS analysis of proliferating T cells showed a similar pattern of CD4+ T cell differentiation to that observed with human lung DC subsets. Thus, although both DC subsets induce CD4+ T cells producing IFN-γ, CD141+ DCs also expanded CD4+ T cells producing IL-4 and IL-13 (Fig. 1D, Table I). Importantly, CD4+ T cells elicited by CD141+ DCs produced type 2 cytokines, and a fraction of them coproduced TNF-α but not IL-10 (Fig. 1E), suggesting a differentiation toward inflammatory Th2 cells (33).

**Blood CD141+ DCs also induce the differentiation of IL-4– and IL-13–producing CD4+ T cells**

We analyzed blood DC subsets to determine whether CD141+ DCs possess the intrinsic capacity to elicit type 2 cytokine production

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**Table I. ICS profiles of CFSE+CD4+ T cells in lung DC–stimulated T cell cultures**

<table>
<thead>
<tr>
<th></th>
<th>T Cells</th>
<th>DCs</th>
<th>IFN-γ (%)a</th>
<th>IL-4 (%)a</th>
<th>IL-13 (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human lungs</td>
<td>Allo naive CD1c+</td>
<td>CD45RA+</td>
<td>CD141+</td>
<td>24 ± 8.8</td>
<td>13 ± 13</td>
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<tr>
<td></td>
<td>CD45RA+</td>
<td>CD141+</td>
<td>25 ± 9.4</td>
<td>23 ± 12</td>
<td>43 ± 18</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>p = 0.7157</td>
<td>p = 0.0045</td>
<td>p = 0.01</td>
</tr>
<tr>
<td>Hummous lungs</td>
<td>Allo naive CD1c+</td>
<td>CD45RA+</td>
<td>CD141+</td>
<td>36 ± 9.1</td>
<td>10 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>CD45RA+</td>
<td>CD141+</td>
<td>30 ± 8.8</td>
<td>31 ± 18</td>
<td>38 ± 24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p = 0.2492</td>
<td>p = 0.0079</td>
<td>p = 0.0079</td>
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<tr>
<td></td>
<td>Allo naive CD4+</td>
<td>CD45RA+</td>
<td>CD141+</td>
<td>34 ± 8.3</td>
<td>7.7 ± 4.9</td>
</tr>
<tr>
<td></td>
<td>CD45RA+</td>
<td>CD141+</td>
<td>15 ± 6.0</td>
<td>46 ± 6.7</td>
<td>61 ± 9.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p = 0.0186</td>
<td>p = 0.0005</td>
<td>p = 0.0004</td>
</tr>
</tbody>
</table>

Difference between conditions with CD1c+ and CD141+ DCs were measured using the two-tailed t test.

aData are percentage of cytokine-expressing cells (mean ± SD) at 6 h by CFSE CD4+ T cells in lung DC–stimulated T cell cultures (1:100 ratio) upon PMA and ionomycin restimulation.
FIGURE 2. Human blood CD141⁺ DCs preferentially initiate a Th2 response. FACS-sorted human blood DCs were either left untreated (A and B) or stimulated with LAIV for 1 h (C–F) and cocultured with 100,000 allogeneic naive CD4⁺ T cells. (A and C) The CD4⁺ T cell proliferation with titrated number of DCs was measured by the percentage of CFSE² CD4⁺ T cells at day 6. Representative data from more than three independent experiments are shown (two-way ANOVA with Bonferroni posttests). (B and D) Sorted DCs were cocultured with 100,000 allogeneic naive T cells for 6 d. Cytokine-production profiles of CFSE² CD4⁺ T cells in DC-stimulated CD4⁺ T cell cultures (1:100 ratio) upon 6 h of PMA and ionomycin restimulation. Representative data from 10 independent experiments are shown (Mann–Whitney U test). (E) The same type of experiments as in (D) with titrated number of DCs. Representative dot plots from six independent experiments are shown. (F) Cytokine-production profiles of IL-13⁺ CFSE² CD4⁺ T cells in DC-stimulated T cell cultures upon PMA and ionomycin restimulation. Representative dot plots from six independent experiments are shown. (G) Human blood DCs were sorted and stimulated with medium, LAIV (moi = 2), poly I:C (30 μg/ml), TSLP (20 ng/ml), or Curdlan (100 μg/ml). (Figure legend continues)
Representative data from six independent experiments are shown. Both blood DC subsets elicited CD4+ T cell proliferation and IFN-γ–producing CD4+ T cells in all conditions tested, with the capacity to elicit the mixed response with a large fraction of CD4+ T cells. To this end, sorted lineage-hLA-DR+CD11c+ cells differentially expressing CD1c and CD141 (Supplemental Fig. 2) were either left untreated or exposed ex vivo to LAIV (moi = 2, 1 h) and used in cocultures with CFSE-labeled, allogeneic, naive CD4+ T cells. Both blood DC subsets elicited CD4+ T cell proliferation and IFN-γ production without (Fig. 2A, 2B, Table II) or with LAIV activation (Fig. 2C, 2D, Table II), as we found with lung DC subsets. ICS revealed that 10–40% of CD4+ T cells produced IL-4 and IL-13 (Fig. 2B, 2D) when exposed to CD141+ DCs. To assess cytokine secretion, CFSE-CD4+ T cells were expanded by each DC subset were restimulated with anti-CD3/CD28 MicroBeads, and cytokine levels were measured in 6-h supernatants using multiplex bead arrays. In line with ICS data, the highest levels of IL-4 and IL-13 were detected in the supernatant of CFSE-CD4+ T cells expanded by CD141+ DCs (Table II). Furthermore, as few as 300 CD141+ DCs were able to induce 100,000 naive CD4+ T cells to differentiate along the Th2 pathway (Fig. 2E). Finally, only a very minor fraction of CD4+ T cells produced IL-10 (Fig. 2F), suggesting a similar inflammatory Th2-differentiation pathway in CD141+ DCs, regardless of their origin (blood or lung) or activation (T cell or virus driven).

The Th1- and Th2-inducing capacity of CD1c+ and CD141+ DCs appears to be a subject of modulation by only few signals. To this end, we tested selected signals that were shown to activate DCs to induce various Th responses: poly I:C (polarizing IFN-γ–secreting T cells) (34), TSLP (Th2-polarizing stimuli) (35, 37), and Curdlan (Th17-polarizing stimuli) (36). As illustrated in Fig. 2G, CD1c+ DCs activated by LAIV, poly I:C, or Curdlan retained the capacity to elicit IFN-γ–producing CD4+ T cells. Only TSLP exposure enabled them to elicit IL-13-producing CD4+ T cells, consistent with earlier studies (35, 37) (Fig. 2G). Conversely, CD141+ DCs retained the capacity to elicit the mixed response with a large fraction of IL-13–producing CD4+ T cells in all conditions tested, with the exception of the poly I:C-activation signal, which skewed CD4+ T cell responses to very high IFN-γ producers (Fig. 2G).

Thus, CD141+ DCs are able to elicit a mixed CD4+ T cell response with the expansion of both IFN-γ– and IL-4/IL-13–producing CD4+ T cells.

CD1c+ DCs use CD40 signal to inhibit Th2 differentiation

Because CD40-mediated activation enables IL-12 production (38, 39) and subsequent Th1 responses (39, 40), we wondered whether the lack of Th2 differentiation via CD1c+ DCs might be linked to CD40. Indeed, although both subsets upregulated HLA-DR, CD80, and CD86 after LAIV activation (Fig. 3A), CD141+ DCs expressed substantially higher levels of membrane CD40 than did CD1c+ DCs in all conditions (Fig. 3A). Nevertheless, adding neutralizing anti-CD40 Abs to CD141+ DC–T cocultures did not influence their capacity to elicit mixed CD4+ T cell responses with the presence of both IFN-γ– and IL-13–producing T cells (Fig. 3B). Conversely, adding neutralizing anti-CD40 Abs to CD1c+ DC–T cocultures enabled the appearance of IL-13–producing CD4+ T cells (up to 10%; Fig. 3B) with a concomitant (albeit not statistically significant) decrease in IFN-γ–producing CD4+ T cells (Fig. 3B). These results obtained with blood DCs were confirmed using lung DCs harvested from humanized mice. There, both the blockade of CD40L and/or CD40 with neutralizing Abs led to changes in the composition of CD4+ T cells elicited under these conditions by CD1c+ DCs, with up to a 3-fold increase in IL-13–producing CD4+ T cells and a concomitant decrease in IFN-γ–producing CD4+ T cells (Fig. 3C). Thus, CD1c+ DCs use CD40–CD40L interaction to elicit IFN-γ–producing Th1 cells while suppressing the expansion of IL-13–producing Th2 cells.

CD141+ DCs elicit Th2 cells via OX40L

Ox40 expression on DCs induces strong Th2 polarization (35). Neither CD1c+ nor CD141+ DCs showed Ox40 expression upon isolation. However, a 48-h exposure to LAIV or TSLP induced both subsets to express Ox40L (Fig. 4A). The addition of Ox40L-neutralizing Ab to CD141+ DC–T cocultures abrogated the induction of IL-13+ T cells to the levels observed with CD1c+ DCs (Fig. 4B) in several independent experiments (19.1 ± 8.2%, IL-13+ T cells for isotype-treated cultures of CD141+ DCs versus 5.9 ± 2.3%, IL-13+ T cells for anti-OX40L–treated cultures, n = 7, p < 0.001; Fig. 4C). The anti-OX40L treatment did not affect the generation of IFN-γ– T cells (Fig. 4B, 4C). Similar results were observed when DC subsets sorted from the lungs of humanized mice were analyzed (Fig. 4D). Furthermore, the addition of anti-OX40L Ab to cocultures of CD141+ DCs and T cells also blocked the induction of CTH2 expression by CD4+ T cells (Fig. 4E, 5.9 ± 4.2%, for anti-OX40L–treated cultures versus 0.26%, for isotype-treated cultures, n = 3). IL-12 can inhibit Ox40L-mediated Th2 polarization via DCs (35). IL-12p70 was detected in the supernatant of both DC subsets activated by poly I:C, but not LAIV, whereas IFN-α was detected (Fig. 4F). Taken together, our results show that CD141+ DCs preferentially induce a Th2 response via an OX40L-dependent mechanism.

### Table II. Profiles of CFSE-CD4+ T cells in blood DC–stimulated CD4+ T cell cultures

<table>
<thead>
<tr>
<th>Condition</th>
<th>DCs</th>
<th>IFN-γ (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>IL-4 (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>IL-13 (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>IFN-γ (pg/ml)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>IL-4 (pg/ml)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>IL-13 (pg/ml)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>CRTH2* (%)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>GATA-3 (MFI)&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>CD1c+</td>
<td>37 ± 10</td>
<td>5.4 ± 3.9</td>
<td>5.4 ± 3.9</td>
<td>1214 ± 569</td>
<td>44 ± 18</td>
<td>109 ± 42</td>
<td>0.53 ± 0.29</td>
<td>1525 ± 490</td>
</tr>
<tr>
<td></td>
<td>CD141+</td>
<td>31 ± 9.0</td>
<td>25 ± 14</td>
<td>25 ± 14</td>
<td>593 ± 110</td>
<td>1090 ± 788</td>
<td>1433 ± 2010</td>
<td>5.86 ± 4.63</td>
<td>2208 ± 889</td>
</tr>
<tr>
<td></td>
<td>LAIV treated</td>
<td>CD1c+</td>
<td>36 ± 8.5</td>
<td>4.6 ± 3.0</td>
<td>3.8 ± 3.2</td>
<td>1555 ± 609</td>
<td>39 ± 29</td>
<td>102 ± 70</td>
<td>0.59 ± 0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD141+</td>
<td>32 ± 11</td>
<td>18 ± 11</td>
<td>23 ± 12</td>
<td>1057 ± 481</td>
<td>785 ± 412</td>
<td>1371 ± 2039</td>
<td>5.05 ± 2.56</td>
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<td></td>
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<td>p = 0.1856</td>
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<td>p = 0.0556</td>
<td>p = 0.0079</td>
<td>p = 0.0079</td>
<td>p = 0.0003</td>
<td>p = 0.0054</td>
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</table>

<sup>a</sup>Data represent the percentage of cytokine-expressing cells (mean ± SD) at 6 h by CFSE-CD4+ T cells upon PMA and ionomycin restimulation (n = 10). Difference between conditions with CD1c+ and CD141+ DCs was measured by the nonparametric Mann–Whitney U test.

<sup>b</sup>Data represent cytokine production (mean ± SD) in the supernatant at 6 h by CFSE-CD4+ T cells (1 × 10<sup>6</sup> cells/ml) upon anti-CD3/CD8 restimulation at 1:1 ratio (Mann–Whitney U test, n = 5).

<sup>c</sup>Data represent the percentage of CRTH2* cells in CFSE-CD4+ T cells (mean ± SD) of DC-stimulated CD4+ T cell cultures (Mann–Whitney U test, n = 8).

<sup>d</sup>Data represent the mean fluorescent intensity (MFI) of GATA-3 expression in CFSE-CD4+ T cells (mean ± SD) of DC-stimulated CD4+ T cell cultures (two-tailed paired t test, n = 8).

Cytokine-production profiles of CFSE-CD4+ T cells in DC-stimulated CD4+ T cell cultures (1:100 ratio) upon PMA and ionomycin restimulation. Representative data from six independent experiments are shown.
**FIGURE 3.** CD1c⁺ DCs require CD40L signaling to initiate a Th2 response. (A) Human blood DCs were sorted and phenotypes were analyzed prior to (left panels) and after (right panels) stimulation with LAIV (moi = 2) for 24 h. Graphs illustrate surface expression of HLA-DR, CD80, CD86, and CD40 on CD1c⁺ (red lines) or CD141⁺ (blue lines) DCs relative to the isotype control (shaded graph). Data are representative of three donors. (B) LAIV-stimulated human blood DCs were cocultured with CFSE-labeled allogeneic naive CD4⁺ T cells at a 1:100 ratio for 6 d in the presence or absence of CD40L-neutralizing Ab (24-31; 10 μg/ml). Cytokine-production profiles in DC-stimulated T cell cultures after 6 h of PMA and ionomycin restimulation. One representative dot plot is shown. Summary of IFN-γ and IL-13 percentage in CFSE⁺ CD4⁺ T cells is shown (one-way ANOVA with Bonferroni posttest). (C) DCs were sorted from LAIV-vaccinated humanized mouse lungs and cocultured with CFSE-labeled allogeneic naive CD4⁺ T cells at a 1:100 ratio for 6 d. CD40 signaling was blocked using neutralizing anti-CD40L (24-31, 10 μg/ml) or anti-CD40 (5C3, 10 μg/ml), with mouse IgG1 as the isotype control.
FIGURE 4. CD141⁺ DCs initiate a Th2 response through OX40L. (A) Surface OX40L expression on FACS-sorted human blood DCs prior to and after stimulation with LAIV (moi = 2) or TSLP (20 ng/ml) for 48 h by FACS. Data are representative of three donors. (B) Human blood DCs were sorted, stimulated with LAIV (moi = 2), and cocultured with CFSE-labeled allogeneic naive CD4⁺ T cells at a 1:100 ratio for 6 d in the presence or absence of OX40L-neutralizing Ab (IK-5, 50 μg/ml). Cytokine-production profiles in DC-stimulated T cell cultures after 6 h of PMA and ionomycin restimulation. One representative dot plot is shown. (C) Summary of IFN-γ, IL-13, and IL-4 percentage in CFSE² CD4⁺ T cells from seven independent donors is shown (one-way ANOVA with Newman–Keuls multiple-comparison test). (D) The same type of experiments as in (B), with DCs sorted from LAIV-vaccinated humanized mouse lungs. Summary of three independent experiments (one-way ANOVA with Bonferroni posttest). (E) CRTH2 expression of CD4⁺ T cells in CD141⁺ DC–stimulated T cell cultures with or without OX40L blocking. Representative dot plots from four independent experiments are shown. (F) Human blood DCs were sorted and stimulated with LAIV (moi = 2), poly I:C (30 μg/ml), or Curdlan (100 μg/ml). Culture supernatant was analyzed at 24 h after activation for IFN-α and IL-12p70. Summary of three donors.
Using DCs from human lung explants and humanized mice, we revealed the functional plasticity of CD141+ DCs, as illustrated by their capacity to simultaneously elicit Th1 and Th2 responses from a naive polyclonal T cell repertoire. Whether this is driven by functional plasticity at the single-cell level or by the existence of two CD141+ DC subsets remains to be determined. Earlier studies in human and mice showed that a high Ag dose can favor IFN-γ-producing Th1 cells, whereas a low Ag dose or low DC/T ratio favors IL-4–producing Th2 cells (41–43). Our results show that CD141+ DC–driven induction of Th1/Th2 responses is not dependent on their number; however, it is dependent on their expression of OX40L. Indeed, the addition of OX40L–neutralizing Abs to DC–T cell cocultures substantially reduced the ability of CD141+ DCs to induce IL-13–secreting CD4+ T cells. Interestingly, CD141+ circulating DCs were found at significantly higher frequencies in subjects with house dust mite allergy and asthma compared with control subjects (44, 45). Moreover, CD141+ expression in blood leukocytes was higher in children with acute asthma than at convalescence 6 wk later (44). Overall, these results suggest that CD141+ DCs might be involved in the generation of a pathogenic Th2 response.

The expression of OX40L is tightly regulated and can be induced in DCs (46, 47). TSLP can stimulate human blood CD11c+ DCs to express a high level of OX40L (48), and it is overexpressed in many tumors, also was shown to increase OX40L expression on monocyte-derived DCs and to drive inflammatory Th2 in melanoma (51). In contrast, 1,25 OH-vitamin D3 substantially reduces OX40L expression while increasing expression of TGF-β in human blood CD11c+ DCs, and it ameliorates allergic bronchopulmonary aspergillosis in cystic fibrosis patients (52). A murine study showed that the regulation of vitamin D3 on OX40L expression is mediated through alteration of NF-kB p50 binding in the OX40L promoter (53).

Although CD1c+ and CD141+ DCs have different patterns of TLR expression, they both express TLR3 and secrete a high level of IL-12p70 upon activation with poly I:C (16, 54). Recent data revealed that CD1c+ DCs actually have a superior capacity to mimic by poly I:C stimulation is the main makeup for the first line of defense against microbial invasion.


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**Disclosures**

The authors have no financial conflicts of interest.

**References**


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Corrections


The sixth author’s name was published incorrectly. The correct name is Toshiyuki Hori.

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