SHORT COMMUNICATION

Expression of blood dendritic cell antigens (BDCAs) by CD1a⁺ human pulmonary cells

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KEYWORDS
Blood Dendritic Cell Antigen (BDCA); Dendritic cells; Langerhan’s cells; Lung; Human

Summary
Background: Myeloid and plasmacytoid dendritic cell (DC) subsets have been recently identified in the human lung based on their differential expression of Blood DC Antigens 1–3 (BDCAs). We investigated the expression of these antigens by isolated human pulmonary CD1a⁺ DCs, namely Langerhan’s cells.
Methods: Using an in vitro cell culture system we successfully isolated a population of relatively pure (>70%) CD1a⁺ cells from human lung tissue (n = 5 subject samples) and stained these with antibodies against the myeloid DC markers BDCA1 (CD1c) and BDCA3 (CD303), the plasmacytoid DC marker BDCA2 (CD141), the Langerhan’s cell marker Langerin and the maturation marker CD83.
Results: Among different subject samples, the isolated CD1a⁺ cells showed variable expression of Langerin, BDCAs and CD83. Interestingly, in two subject samples, which contained >50% CD83⁺ mature CD1a⁺ cells, >50% of the cells were positive for all of the BDCAs.
Conclusions: We conclude that isolated pulmonary CD1a⁺ DCs in vitro have the capacity to express both myeloid and plasmacytoid BDCA markers and that rather than subset restriction in pulmonary DCs, a significant degree of flexibility/plasticity can be induced, albeit experimentally.

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Introduction

CD1a⁺ cells, namely Langerhan cells (LCs), were the first dendritic cells (DCs) to be identified in the lung. Relatively recently, flow cytometry was used to identify three human lung DC subsets expressing the blood DC antigen 1 (BDCA1)
CD1c or the BDCA2 CD141 or the BDCA3 CD303, i.e. myeloid (m) DCs type 1, plasmacytoid (p) DCs and mDCs type 2, respectively. However, the microanatomical location of these DC subsets within lung tissue is uncertain. Furthermore, it is not clear whether pulmonary LCs comprise a fourth distinct DC subset or not. Flow cytometry of cells from lung tissue digests and bronchoalveolar lavage fluid suggest that a subpopulation of CD1a⁺ DCs expresses the BDCA1 CD1c, and could therefore be classified as pulmonary mDCs type 1. Studies aimed at classifying pulmonary DCs are challenging because these cells are relatively infrequent. Using a method previously described by us that produces a CD1a⁺-enriched population from human lung tissue digests, we aimed to characterize human lung CD1a⁺ cells based on their expression of BDCA1–3.

**Materials and methods**

**Isolation of lung DCs**

DCs were isolated from lung of grossly normal appearance after resection for lung carcinoma, approved by the Royal Brompton and Harefield Ethical Committee (n = 8 subjects). DCs were isolated at an intermediate stage of alveolar epithelial cell isolation. Lung tissue was perfused extensively (>10 times, 20 ml/5 cm² tissue) with normal saline to remove alveolar macrophages. The 8th to 10th perfusate was examined for the presence of alveolar macrophages and other leukocytes. If they were >1 × 10⁴/ml, further perfusion was performed to ensure that there were no alveolar macrophages left. The tissue was then inflated with trypsin (T8003, Sigma), incubated, 45 min at 37°C, then minced with scissors in the presence of newborn calf serum (NCS, Invitrogen, Paisley, UK). Use of trypsin, for no longer than 45 min, ensured that the epithelium was stripped from the underlying interstitial tissue, without significant collagen and elastin degradation, thus liberating only the epithelial cells and any associated epithelial DCs. The mince was incubated with 0.25% DNase (Sigma), filtered (40 μm mesh), the filtrate containing liberated cells was centrifuged (300 g) and the cell pellet re-suspended in DCCM-1 media (React Scientific, Truro, UK). These cells were plated onto culture plastic. The non-adherent, epithelial cell-enriched cells were removed. Adherent cells were immunostained for DC specific markers and for CD68 (the last to detect contaminating macrophages).

**Immunocytochemistry**

The Dako Envision kit (Dako, Ely, UK) was used to detect human BDCA2 (1:25, overnight; Miltenyi Biotech, Bergisch Gladbach), BDCA3 (1:25, overnight; Miltenyi Biotech, Guildford, UK), BDCA1(CD1c) (1:200, 3 h; Serotec, Kidlington, Oxford, UK), CD1a (1:25, overnight; Vector, Burlingame, CA, USA), CD83 (1:25, overnight; Vector), Langerin (1:50, 3 h; Beckman Coulter [Immunotech], High Wycombe, UK).

**Quantification**

At least three fields and more than 300 cells were counted per marker for each subject.

Transmission electron microscopy

Adherent cells were washed in PBS, incubated with 0.25% trypsin-EDTA (Sigma) for 3 min and 10% NCS added to inactivate the trypsin. The dislodged cells were collected, centrifuged and the cell pellet washed with PBS before fixing with 2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer.

**Results**

In three out of eight subject samples processed there was an insufficient number of CD1a⁺ DCs and they were of low purity (<70% purity). Thus these samples were not immunostained against DC antigens. A CD1a⁺ DC population of high purity (CD1a⁺ cells >70%; CD68⁺ cells 0%) was isolated from five subject samples (Table 1). Langerin was highly expressed (>65% Langerin⁺ cells) in three of these samples (#2, 3, 4). Internalisation of Langerin induces the formation of Birbeck granules which can be visualised by electron microscopy; however, none were detected.

Among the subject samples, four showed high expression of the maturation marker CD83 (>70% CD83⁺ cells) (#1, 3, 4, 5). These subject samples also contained high percentages of BDCA3⁺ cells and in two of these (#3, 4) there was also high expression of BDCA1 and BDCA2. Subject sample #2, which weakly expressed the maturation marker CD83, showed low BDCA1–3 expression. Fig. 1 illustrates differences for staining of BDCA1–3 between subject sample #3 and subject sample #2.

The antibodies did not crossreact with other antigens, being negative when applied to alveolar macrophages and human epithelial cells from the same subjects (except for weak macrophage positivity for BDCA3, data not shown).

**Discussion**

Based on the expression of BDCA1, three distinct pulmonary DC subsets have been identified previously in the lung, but it is not known whether any of these antigens can be expressed by pulmonary CD1a⁺ DCs. We separated CD1a⁺ DCs at an intermediate stage of alveolar epithelial cell isolation. Most of the isolated cells contained predominately (>70%) mature CD1a⁺ DCs, illustrated by their CD83⁺ staining. As pulmonary DCs in vivo have a predominately immature phenotype, we consider that DC maturation was induced during processing and/or adherence of DC in vitro.

Like CD1a, Langerin is a LC marker. However, there was a low frequency of Langerin expressing cells in two CD1a⁺-enriched samples, suggesting that CD1a and Langerin are not necessarily co-expressed. Accordingly, while Langerin⁺ cells reside both in the epithelium and subepithelium, CD1a⁺ cells reside almost exclusively in the epithelium. Lack of ultrastructural identification of Birbeck granules in these DCs may reflect the depletion of these granules during DC maturation.

Four samples contained significant numbers of cells expressing the mDC type 2 marker BDCA3, whereas two of these samples also contained a high percentage of cells expressing the mDC type 1 marker BDCA1 and the pDC marker BDCA2, suggesting that some DCs in the lung express all BDCA1–3. This was unexpected and in contrast to previous studies of bronchoalveolar fluid and tissue by us.
and others, in which pulmonary DCs clearly did not co-express the BDCAs 1–3. However, in these earlier studies the identified DCs had not been cultured. Thus, we suggest that our method of isolation and culture of human lung DCs is associated with maturation/activation of DCs and induced co-expression of BDCAs, particularly since the only sample containing a low percentage (5%) of mature CD83+ DCs showed low expression of all the BDCAs 1–3.

Interestingly, pulmonary mDCs have also been shown to express another pDC marker, i.e. the CD123.

**Figure 1** CD1a, CD1c (BDCA1), Langerin, BDCA2, BDCA3 and CD83 immunostaining of lung tissue-derived, CD1a+-enriched DCs. DCs from patient 3 were highly activated, as shown by strong CD83 expression and a high percentage of the cells were positive for all the DC markers. DCs from patient 2 showed low levels or no staining for CD1c (BDCA1), BDCA2, BDCA3 and CD83.

**Table 1** Subject characteristics and percentages of positively stained cells for each marker. Data are provided for each subject in the chronological order of receipt of sample. Figures in brackets denote the minimum percentage of cells calculated to be double positive for the marker studied and for CD1a (% double positive cells = % marker positive cells - % CD1a negative cells).

**Subject Characteristics**

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<tr>
<th>Subject Number</th>
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<th>Pack. Years</th>
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<td>EX-SM</td>
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<td>Primary (adenoc)</td>
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<tr>
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**Immunocytochemistry data**

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<td>6 (0)</td>
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</table>

**Abbreviations:** M: male, F: female, EX-SM: ex-smoker, C-SM: current smoker, adeno: adenocarcinoma.

**Technique limitations**

We acknowledge that: (i) DCs isolated from tissue using magnetic beads or fluorescence activated cell sorting may not be induced to co-express these markers. However, these techniques require comparatively greater numbers of DCs and are comparatively more expensive. (ii) Flow cytometric analysis would be an alternative approach to phenotyping the isolated CD1a+ DCs and identifying BDCA co-expression. However, this is...
not always feasible when low numbers of cells are isolated. We have previously found that the adherence method to isolate human lung CD1a+ DCs works well but we consider it important to report that the method likely induces maturation and co-expression of myeloid and plasmacytoid markers. Importantly, the capacity of environmental influence, albeit experimental, to induce pulmonary DCs to co-express these markers does highlight their potential plasticity.

Conclusion

DCs were isolated by adherence from resected human lung following trypsinisation. In 5 of 8 subject subjects a high percentage of the cells were CD1a+ Langerhans phenotype. Interestingly, most of these CD1a+ cells also had been induced experimentally to express both myeloid and plasmacytoid BDCA markers. The concept that factors in the cellular environment, eg viral infection,9 can induce alternative differentiation pathways has already been described for bone marrow DCs and we suggest that the adherence step in vitro induced maturation of our isolated pulmonary DCs and that there is the potential for a significant degree of flexibility/plasticity in pulmonary DCs. Investigators should be aware and consider this possibility in future studies.

Conflict of interest

None declared.

References