Clinical characterization of allergic sensitization patterns and the role of mucosal dendritic cells

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Citation for published version (APA):
Chapter 2

Dendritic cells in nasal mucosa of subjects with different allergic sensitizations

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J Allergy Clin Immunol 2011;128(4):887-90
To the Editor

The extent of the clinical response to a nasal allergen provocation in allergic rhinitis patients can depend on existing co-sensitizations. Previously we showed that a pollen provocation in patients mono-sensitized for pollen induced more symptoms than in patients with a concomitant house dust mite (HDM) sensitization (1). This effect only occurred in mono-pollen allergic individuals and not in mono-HDM allergic individuals. Levels of allergen-specific IgE, IgG4, or the biological activity of IgE did not differ between groups. Dendritic cells (DC) are not only essential in the pathogenesis of allergy, but also for expression of clinical symptoms (2). Multiple DC subtypes exist and these may functionally differ, with myeloid DC (mDC) thought to enhance and plasmacytoid DC (pDC) to inhibit immune responses (3). We hypothesized that differences between DCs could explain the differences in clinical symptoms. Outside of the Dutch pollen season, biopsies were taken at baseline and 24 hours after nasal provocation and stained for specific DC-markers CD1c (BDCA-1), CD303 (BDCA-2), CD141 (BDCA-3), CD304 (BDCA-4), and Langerhans (LH) cell-markers CD1a, and CD207 (langerin). Because DC markers can also be expressed on other cell types we have used a common approach (4;5) of also considering the morphology of DCs. Sixty-six eligible subjects (42 female, median age 23.8 yrs; range 18-62 yrs) were included in this study. Demographic characteristics were comparable for all groups; Monosens-GP (n=14); Monosens-HDM (n=9); Polysens (n=29); Controls (n=14).

Baseline nasal mucosal biopsies revealed all subtypes of dendritic cells, both in the epithelium and in the lamina propria (Figure 1 and Table I). CD141 and CD304 antibodies also stained vascular structures and to avoid misidentification we only enumerated these markers in the epithelium. All DC subtypes were detected, albeit at different levels, with the highest levels for CD207 positive cells and the lowest levels for pDC markers CD303 and CD304. Collectively, the numbers did not differ between allergic and healthy individuals. At baseline, the mDC marker CD1c in the lamina propria tended (p = 0.055) to be higher in mono-pollen sensitized subjects (median 3.8; range 0.4 - 8.9 cells/mm) than

Table I. Number of dendritic cell subtypes, characterized by immunohistochemical staining, in healthy and allergic rhinitis subjects.

<table>
<thead>
<tr>
<th></th>
<th>Healthy subjects</th>
<th>Allergic subjects</th>
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<tbody>
<tr>
<td></td>
<td>Epithelium*</td>
<td>Lamina Propria**</td>
</tr>
<tr>
<td><strong>mDC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD1c</td>
<td>0.1 (0-1.7)</td>
<td>1.5 (0-15.0)</td>
</tr>
<tr>
<td>CD141</td>
<td>0.4 (0-11.2)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>pDC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD303</td>
<td>0.0 (0-1.2)</td>
<td>0.4 (0-5.0)</td>
</tr>
<tr>
<td>CD304</td>
<td>0.0 (0-2.7)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>LH</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD1a</td>
<td>0.9 (0-5.3)</td>
<td>1.3 (0-16.2)</td>
</tr>
<tr>
<td>CD207</td>
<td>6.3 (0-20.0)</td>
<td>5.8 (0-18.9)</td>
</tr>
</tbody>
</table>

* Number of cells/mm BM, presented as median (range). ** Number of cells/mm2, presented as median (range).
poly-sensitized subjects (median 1.6; range 0 - 8.1 cells/mm). Interestingly, numbers of the pDC marker CD303 were higher (p=0.039) in epithelial layer of mono-HDM (median 0.2, range 0 – 1.1) than in poly-sensitized subjects (median 0, range 0 – 0.5). These differences were not observed for CD141 or CD304.

Next we evaluated the effect of a nasal provocation (NP) on DC subtypes, after showing a significant eosinophilia in allergic individuals only, with no differences between the different allergic sensitizations. After NP, epithelial CD1c was significantly higher (p=0.010) in the HDM mono-sensitized group (median 2.3; range 0 – 5.2) than in healthy controls.
(median 0; range 0 – 2.8), and in poly-sensitized subjects challenged with HDM (median 0; range 0 – 3.1 cells/mm BM; p = 0.032). This effect was not seen for CD1c in the lamina propria, nor for CD141. For the plasmacytoid DC marker CD303 numbers remained low in the poly-sensitized subjects (median 0, range 0 – 1.0), while increasing in mono-sensitized HDM subjects (median 0.3; range 0 – 1.6; p = 0.049) and tending to be higher in mono-pollen sensitized subjects (0.3; 0 – 2.2 cells/mm BM; p = 0.057). No significant changes were seen for CD304. We concluded that pDCs in poly-sensitized subjects do not respond to a single dose allergen provocation. After NP, CD1a in the lamina propria was higher (p = 0.039) in mono-sensitized HDM subjects (median 8.0; range 0 – 54.8) than in poly-sensitized subjects (median 0; range 0 – 70.0). Also langerin in mono-sensitized HDM subjects increased (p = 0.015) from median 3.9 (range 0.2 – 9.8) to 6.9 (range 0.3 – 11.7). This number tended to decrease in the poly-sensitized subjects (median 5.0; range 0 – 11.6 cells/mm² at baseline versus 3.4; 0 – 8.6 cells/mm² after NP; p = 0.087).

Given the opposite functions of mDCs and pDCs we assessed the immune status through the median ratio of CD1c positive (mDC) over CD303 positive (pDC) cells (Table II). This ratio dropped in the epithelium of healthy subjects after NP, and was significantly (p=0.006) different from allergic subjects. In the lamina propria the ratio in healthy subjects dropped as well, but this was mirrored in allergic subjects. Interestingly, the ratio was different in the epithelium between mono-sensitized and polysensitized subjects. In the mono-sensitized subjects there was a marked increase in the ratio after allergen challenge, whereas polysensitized subjects had a higher ratio at baseline, which slightly decreased after NP, but that still was significantly higher than in the healthy controls.

For the first time, we demonstrate the presence of four previously unidentified DC subsets in human nasal mucosa: myeloid DCs characterized by CD1c and CD141, and plasmacytoid DCs characterized by CD303 and CD304. Moreover we found that numbers of DC-subsets differ between subjects with different allergic sensitizations. However, we must consider possible overlap of markers. Most CD303 positive cells in blood also express CD304 (6), while in lung most CD1a positive DCs also express CD1c (5). We should note that the markers we have used to identify DCs in the nose may overlap within distinct DC subset so that the different stainings do not indicate more than the three distinct DC subtypes (pDC, mDC, and LH). Although the extent of this overlap and specificity cannot be fully established with our IHC protocols and could be further explored using FACS analysis of single cell preparations, the main conclusions on the presence of the different DC subtypes and the mDC/pDC should not be affected. The dynamics of several DC subsets after nasal allergen provocation were largest for HDM-monosensitized allergic subjects, while overall the dynamics were very limited for the polysensitized individuals. Elevated numbers of DCs in the nasal mucosa of allergic rhinitis subjects, as well as the response to nasal allergen challenge have been reported, with variable outcomes (7-9).
differences we found between subjects with different sensitizations hint both to allergen-specific differences in the immune response, and also to an interaction between different allergen sensitizations.

High baseline levels of mDCs in mono-pollen allergic subjects could reflect ongoing minimal persistent inflammation outside of the pollen season \(^{10}\), showing that pollen allergic individuals are not normal even when free of symptoms. Whether mDCs are responsible for maintaining allergic inflammation outside of the pollen season or for the increased response to nasal allergen provocation remains to be explored. It is not clear whether these differences are caused by the type of allergen, the number of sensitizations, or the duration of exposure. As we have not determined the activation state for different subclasses of DC by evaluating the expression levels of activation markers, we solely depend on changes in cell numbers to evaluate the status of the immune system.

The mDC/pDC ratio is equal for allergic and healthy subjects, however after NP the ratio in healthy subjects decreases, while remaining the same in allergic subjects. This suggests a lack of immunosuppression in the allergic subjects. Furthermore, in the mono-sensitized subjects the mDC/pDC ratio increased after NP compared to controls, whereas in poly-sensitized subjects this ratio decreased only slightly. This may reflect the molecular mechanism of the less severe clinical response to grass pollen in polysensitized subjects.

**Reference List**


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