

Letter to the Editor

Dendritic cells in nasal mucosa of subjects with different allergic sensitizations

To the Editor:

The extent of the clinical response to a nasal allergen provocation in patients with allergic rhinitis can depend on

existing cosensitizations. Previously, we showed that a pollen provocation in patients monosensitized to pollen induced more symptoms than in patients with a concomitant house dust mite (HDM) sensitization.¹ This effect only occurred in patients with only pollen allergy and not in patients with only HDM allergy. Levels of allergen-specific IgE or IgG₄

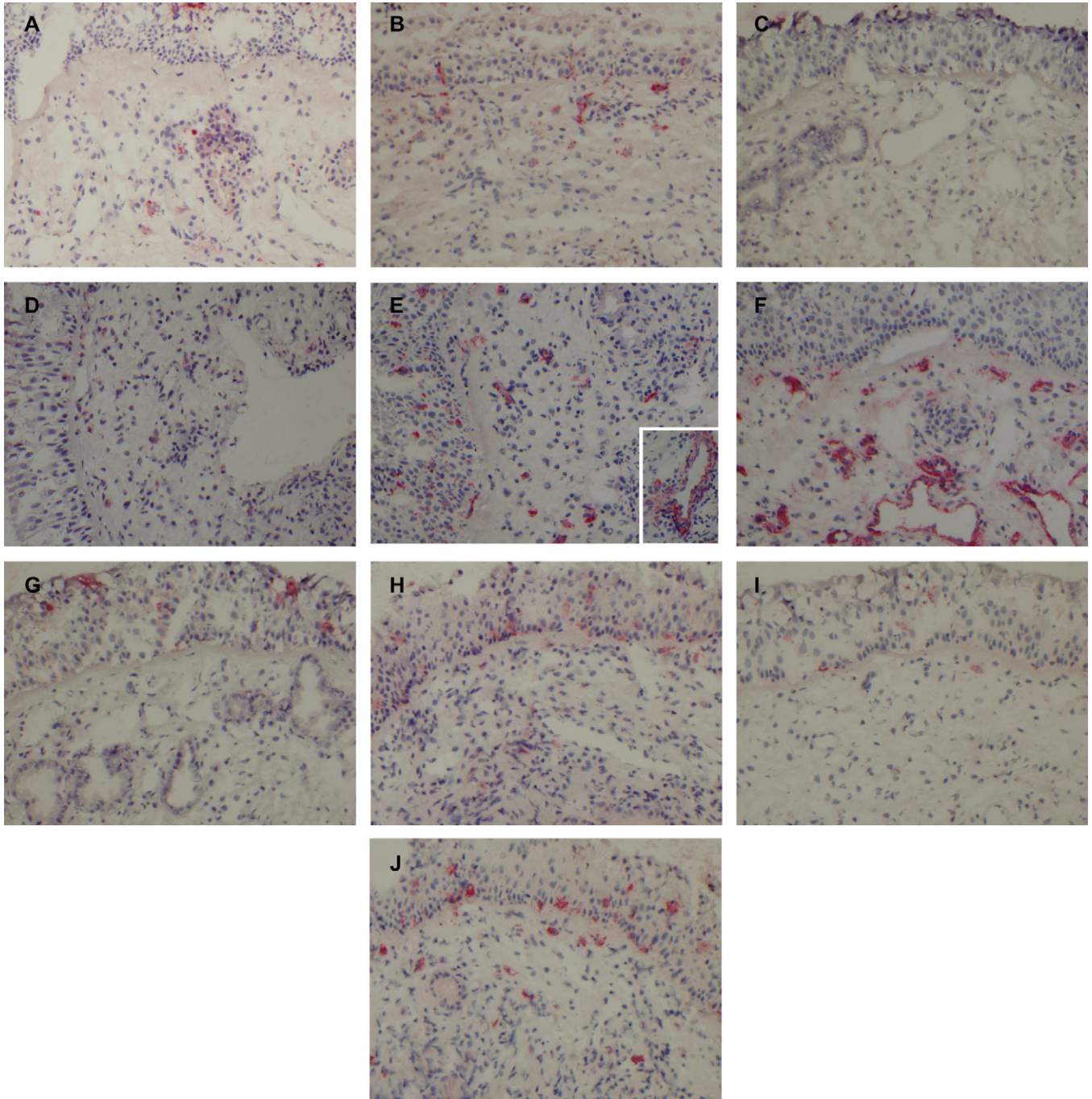


FIG 1. Immunohistochemical staining with the specific DC markers CD1c before (A) and 24 hours after (B) nasal challenge, CD303 before (C) and 24 hours after (D) nasal challenge, CD141 (E), CD304 (F), CD1a before (G) and 24 hours after (H) nasal challenge, and CD207 before (I) and 24 hours after (J) nasal challenge in nasal mucosal biopsy sections obtained from patients with allergic rhinitis.

TABLE I. Number of DC subtypes characterized by immunohistochemical staining in healthy subjects and subjects with allergic rhinitis

	Healthy subjects		Allergic subjects	
	Epithelium*	Lamina propria†	Epithelium*	Lamina propria†
mDC				
CD1c	0.1 (0-1.7)	1.5 (0-15.0)	0.4 (0-7.9)	2.4 (0-17.4)
CD141	0.4 (0-11.2)	ND	0.5 (0-4.6)	ND
pDC				
CD303	0.0 (0-1.2)	0.4 (0-5.0)	0.0 (0-2.1)	0.8 (0-9.0)
CD304	0.0 (0-2.7)	ND	0.0 (0-7.2)	ND
LH				
CD1a	0.9 (0-5.3)	1.3 (0-16.2)	1.1 (0-17.0)	2.6 (0-52.2)
CD207	6.3 (0-20.0)	5.8 (0-18.9)	8.6 (0-20.7)	4.2 (0-16.1)

ND, Not determined.

*Number of cells per millimeter of BM is presented as medians (ranges).

†Number of cells per square millimeter is presented as medians (ranges).

or the biological activity of IgE did not differ between groups.

Dendritic cells (DCs) are not only essential in the pathogenesis of allergy but also for expression of clinical symptoms.² Multiple DC subtypes exist, and these might functionally differ, with myeloid DCs (mDCs) thought to enhance and plasmacytoid DC (pDCs) to inhibit immune responses.³ We hypothesized that differences between DCs could explain the differences in clinical symptoms.

Outside of the Dutch pollen season, biopsy specimens were taken at baseline and 24 hours after nasal provocation (NP) and stained for the specific DC markers CD1c (blood dendritic cell antigen [BDCA] 1), CD303 (BDCA-2), CD141 (BDCA-3), and CD304 (BDCA-4) and the Langerhans cell (LH) 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 subjects; median age, 23.8 years; range, 18-62 years) were included in this study. Demographic characteristics were comparable for all groups: subjects with monosensitization to grass pollen allergen and relevant symptoms ($n = 14$), subjects with monosensitization to HDM allergen and relevant symptoms ($n = 9$), subjects with polysensitization and relevant symptoms ($n = 29$), and control subjects ($n = 14$).

Baseline nasal mucosal biopsy specimens revealed all subtypes of DCs, both in the epithelium and in the lamina propria (Fig 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⁺ cells and the lowest levels for the pDC markers CD303 and CD304. Collectively, cell numbers did not differ between allergic and healthy subjects. At baseline, cell numbers of the mDC marker CD1c in the lamina propria tended ($P = .055$) to be higher in pollen-monosensitized subjects (median, 3.8 cells/mm²; range, 0.4-8.9 cells/mm²) than in polysensitized subjects (median, 1.6 cells/mm²; range, 0-8.1 cells/mm²). Interestingly, cell numbers of the pDC marker CD303 were higher ($P = .039$) in the epithelial layer of HDM-monosensitized subjects (median, 0.2 cells/mm basal membrane [BM]; range, 0-1.1 cells/mm BM) than in polysensitized subjects (median, 0 cells/mm BM; range, 0-0.5 cells/mm BM). These differences were not observed for CD141 or CD304.

Next we evaluated the effect of an NP on DC subtypes after showing significant eosinophilia in allergic subjects only, with no differences between subjects with different allergic sensitizations. After NP, epithelial CD1c cell numbers were significantly higher ($P = .010$) in the HDM-monosensitized group (median, 2.3 cells/mm BM; range, 0-5.2 cells/mm BM) than in healthy control subjects (median, 0 cells/mm BM; range, 0-2.8 cells/mm BM) and in polysensitized subjects challenged with HDM (median, 0 cells/mm BM; range, 0-3.1 cells/mm BM; $P = .032$). This effect was not seen for CD1c in the lamina propria or for CD141. For the pDC marker CD303, cell numbers remained low in the polysensitized subjects (median, 0 cells/mm BM; range, 0-1.0 cells/mm BM) while increasing in HDM-monosensitized subjects (median, 0.3 cells/mm BM; range, 0-1.6 cells/mm BM; $P = .049$) and tending to be higher in pollen monosensitized subjects (median, 0.3 cells/mm BM; range, 0-2.2 cells/mm BM; $P = .057$). No significant changes were seen for CD304. We concluded that pDCs in polysensitized subjects do not respond to a single-dose allergen provocation. After NP, CD1a cell numbers in the lamina propria were higher ($P = .039$) in HDM-monosensitized subjects (median, 8.0 cells/mm²; range, 0-54.8 cells/mm²) than in polysensitized subjects (median, 0 cells/mm²; range, 0-70.0 cells/mm²). Also, langerin cell numbers in HDM-monosensitized subjects increased ($P = .015$) from a median of 3.9 cells/mm² (range, 0.2-9.8 cells/mm²) to 6.9 (range, 0.3-11.7 cells/mm²). This number tended to decrease in the polysensitized subjects (median of 5.0 cells/mm² and range of 0-11.6 cells/mm² at baseline vs median of 3.4 cells/mm² and range of 0 to 8.6 cells/mm² after NP, $P = .087$).

Given the opposite functions of mDCs and pDCs, we assessed immune status through the median ratio of CD1c⁺ (mDC) over CD303⁺ (pDC) cells (Table II). This ratio decreased in the epithelia of healthy subjects after NP and was significantly ($P = .006$) different from that seen in allergic subjects. In the lamina propria the ratio in healthy subjects decreased as well, but this was mirrored in allergic subjects. Interestingly, the ratio was different in the epithelium between monosensitized and polysensitized subjects. In the monosensitized 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 was significantly higher than in the healthy control subjects.

For the first time, we demonstrate the presence of 4 previously unidentified DC subsets in human nasal mucosa: mDCs characterized by CD1c and CD141 and pDCs 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⁺ cells in blood also express CD304,⁶ whereas in the lung most CD1a⁺ DCs also express CD1c.⁵ We should note that the markers we have used to identify DCs in the nose might overlap within distinct DC subsets so that the different stainings do not indicate more than the 3 distinct DC subtypes (pDCs, mDCs, and LHs). Although the extent of this overlap and specificity cannot be fully established with our immunohistochemistry protocols and could be further explored with fluorescence-activated cell-sorting analysis of single-cell preparations, the main conclusions on the presence of the different DC subtypes and the mDCs/pDCs should not be affected. The dynamics of several DC subsets after nasal allergen provocation were largest for HDM-monosensitized allergic subjects, whereas overall, the

TABLE II. Median ratio of CD1C/CD303⁺ cells

	Control subjects	All allergic subjects	Mono-HDM	Mono-GP	Poly-prov HDM	Poly-prov GP
Epithelium						
Baseline	1.00	1.01	1.00	1.03	1.80	1.30
24 h NP	0.51	1.00	5.96	1.52	1.00	1.00
<i>P</i> value (compared with control subjects)		.006	.002	.013	.014	.015
LP						
Baseline	3.55	2.98	3.96	3.97	3.48	1.51
24 h NP	2.17	1.92	3.13	2.07	1.45	2.36
<i>P</i> value (compared with control subjects)		NS	NS	NS	NS	NS

GP, Grass pollen; LP, lamina propria; *Mono-GP*, subjects with monosensitization to grass pollen allergen and relevant symptoms; *Mono-HDM*, Subjects with monosensitization to HDM allergen and relevant symptoms; *NS*, not significant; *Poly-prov GP*, Subjects with polysensitization and relevant symptoms, who underwent allergen provocation with grass pollen allergen; *Poly-prov HDM*, Subjects with polysensitization and relevant symptoms, who underwent allergen provocation with house dust mite allergen.

dynamics were very limited for the polysensitized subjects. Increased numbers of DCs in the nasal mucosa of subjects with allergic rhinitis, as well as the response to nasal allergen challenge, have been reported with variable outcomes.⁷⁻⁹ The differences we found between subjects with different sensitizations hint both at allergen-specific differences in the immune response and also at an interaction between different allergen sensitizations.

High baseline levels of mDCs in pollen monoallergic subjects could reflect ongoing minimal persistent inflammation outside of the pollen season,¹⁰ showing that subjects with pollen allergy 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. Because we have not determined the activation state for different subclasses of DCs 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 allergic subjects. Furthermore, in the monosensitized subjects the mDC/pDC ratio increased after NP compared with that seen in control subjects, whereas in polysensitized subjects this ratio decreased only slightly. This might reflect the molecular mechanism of the less severe clinical response to grass pollen in polysensitized subjects.

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