Asthmatic bronchial epithelium activated by the proteolytic allergen Der p 1 increases selective dendritic cell recruitment

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Background: Airway dendritic cells (DCs) are crucial for allergen-induced sensitization and inflammation in allergic asthma. After allergen challenge, an increased number of DCs is observed in airway epithelium from patients with allergy.

Objective: Because Der p 1, a cysteine protease allergen from Dermatophagoides pteronyssinus, induces chemokine production by bronchial epithelial cells (BECs), the purpose of this investigation was to evaluate the capacity of BEC exposed to Der p 1 to recruit DCs.

Methods: Chemotactic activity of BEAS-2B, a bronchial epithelial cell line, and BECs from nonatopic controls and patients with allergic asthma was evaluated on the migration of precursors, immature and mature monocyte-derived DCs (MDDCs), and CD34+–derived Langerhans cells (LCs).

Results: C-C chemokine ligand (CCL)–2, CCL5, and C-X-C chemokine ligand 10 production by BEAS-2B and BEC was increased after Der p 1 exposure, whereas the proenzyme proDer p 1 devoid of enzymatic activity had no effect. Der p 1 stimulation of BEAS-2B and BEC from both groups increased significantly the recruitment of MDDC precursors, depending on CCL2, CCL5, and C-X-C chemokine ligand 10 production. In a reconstituted polarized epithelium, apical application of Der p 1 enhanced MDDC precursor migration into the epithelial layer. Moreover, Der p 1 stimulation of BEC from patients with asthma but not from controls increased the migration of LC precursors, mainly dependent on CCL20 secretion. No migration of immature and mature DCs was observed.

Conclusion: These data confirmed that BECs participate in the homeostasis of the DC network present within the bronchial epithelium through the secretion of chemokines. In allergic asthma, upregulation of CCL20 production induced LC recruitment, the role of which remains to be determined.

Key words: Epithelial cells, dendritic cells, chemokines, allergy, lung

Dendritic cells (DCs) play a major role in the surveillance of peripheral tissue sites for incoming antigen. In airway mucosa, myeloid DCs form a dense network including different populations: interstitial DCs and Langerhans cells (LCs) close to epithelial cells. At steady state, because of the environment, immature DCs or their precursors are continuously recruited to the airway mucosa where inhaled antigens are sampled. After antigen processing, maturing DCs leave their resident sites toward the thoracic lymph nodes, where they efficiently prime antigen-specific T cells.

Whereas many studies reported how DCs achieve their migratory functions to draining lymph nodes, only few studies focused on DC migration toward antigen exposure sites. Recent attention has focused on the chemokine network, a multiparticle superfamily of chemoattractant cytokines that induce the directed migration of leukocytes and other cells. Importantly, each DC population displays a unique spectrum of chemokine responsiveness, attesting that particular profiles of chemokines are involved in the mobilization of the different subsets of DCs. Mainly, monocyte-derived DCs (MDDCs), related to interstitial myeloid DCs, respond to C-C chemokine ligand (CCL)–3/macrophage inflammatory protein (MIP)–1α and CCL5/RANTES via CCR1 and CCR5 or to CCL2/monocyte chemotactant protein (MCP)–1 via CCR2. CCL20/MIP-3α is a unique ligand for the chemokine receptor CCR6. This receptor is selectively expressed on LC precursors, a subpopulation of myeloid DCs that reside at mucosal surfaces. CCL20 has been demonstrated to be expressed in inflamed intestinal epithelial cells and keratinocytes. In bronchial epithelial cells (BECs), CCL20 secretion is strongly upregulated by exposure to ambient particulate matter or inflammatory stimuli such as IL-1β and TNF-α, whose levels are elevated and important in diseases such as asthma.

Allergic asthma is characterized by hypersensitivity against aeroallergens and the development of an allergen-specific Th2 response. In animal models, a critical role for DC is now fairly demonstrated in allergen sensitization, established airway inflammation, and alteration of...
pulmonary functions. At steady state, increased numbers of CD1a+ DCs are observed in the airway mucosa of patients with allergic asthma (AAs) compared with nonatopic donors (NAs), and the density of this network is increased after allergen exposure. In addition, BECs from NAs and AAs interact with BECs and other cells located in airway mucosa. To identify these mechanisms, we compared the capacity of BECs from NAs and AAs to produce chemokines and to induce myeloid DC recruitment after exposure to Der p 1, the major allergen of Dermatophagoides pteronyssinus. This allergen, characterized by its cysteine protease activity, can activate BEC through protease-activated receptor (PAR)–2 cleavage and can also inactivate PAR-1. In this context, BECs from NAs and AAs secreted different profiles of chemokines. In AAs, BEC activation by Der p 1 leads to the recruitment of both MDDC and LC precursors, whereas only MDDC precursors were attracted in NAs.

METHODS
Patients and bronchial sampling procedure

Main clinical characteristics of these patients are summarized in Table I. All procedures were reviewed and approved by the Hospital Institutional Review Board, and written informed consent was obtained from all subjects included in the study.

Branchial epithelial cells. Human bronchial epithelial biopsies were obtained by fiber optic bronchoscopy from 25 NAs who were being investigated for bronchopulmonary carcinoma and 8 AAs.

TABLE I. Clinical characteristics of NAs and AAs

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Age, y (mean ± SEM)</th>
<th>Sex (M/F)</th>
<th>Global Initiative for Asthma score</th>
<th>Prick test, dust</th>
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<tr>
<td>BEC NA</td>
<td>25</td>
<td>51.7 ± 11.4</td>
<td>18/7</td>
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</tr>
<tr>
<td>AA</td>
<td>8</td>
<td>36.5 ± 13.1</td>
<td>5/3</td>
<td>4 1 3 0</td>
<td>Positive</td>
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<tr>
<td>NA</td>
<td>6</td>
<td>40 ± 7</td>
<td>5/1</td>
<td>None</td>
<td>Negative</td>
</tr>
<tr>
<td>BL</td>
<td>15</td>
<td>40.5 ± 8.4</td>
<td>6/9</td>
<td>6 1 8 0</td>
<td>Positive</td>
</tr>
</tbody>
</table>

BEAS-2B and primary cultures of BEC were obtained and cultured as previously described. Confuent epithelial cells were activated with endotoxin-free Der p 1 allergen (100 and 500 ng/mL, corresponding with an evaluation of the level obtained in bronchial spaces; generous gift from G. A. Stewart, Perth, Australia), proDer p 1, the non enzymatic precursor of Der p 1 (500 ng/mL; generous gift from A. Jacquet, Gosselies, Belgium), or E64, a cysteine protease inhibitor (5 μmol/L; Sigma, St Louis, Mo). Supernatants were collected after 24-hour incubation.

Bronchial lavage. In 6 NAs and 15 AAs, bronchial lavages (BLs) were performed in a segmental bronchus of the right middle lobe by slow infusion of two 15-mL aliquots of sterile 0.9% saline solution. After centrifugation, BL fluids were collected and frozen until chemokine assays.

MDDCs
Peripheral blood from NAs was obtained according to institutional guidelines. Monocytes were isolated from mononuclear cells by using anti-CD14 mAb conjugated to microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) and cultured with IL-4 (10 ng/mL; R&D Systems, Abingdon, United Kingdom) and GM-CSF (25 ng/mL; Preprotech, Rocky Hill, NJ) for 7 days, as previously described. Mature MDDCs were obtained after 24-hour incubation with LPS (1 μg/mL). Maturity was checked by the increased expression of CD80, CD86, CD83 (all antibodies from Becton Dickinson, San Jose, Calif), and CCR7 and the downregulation of CCR2, CCR5, and C-X-C chemokine receptor (CXCR)–3 (R&D Systems).

CD34+–derived LCs
Umbilical cord blood was obtained according to institutional guidelines. CD34+ cells were isolated from mononuclear fractions by using anti-CD34 mAb associated to microbeads (Miltenyi Biotec). CD34+ cells were cultured with GM-CSF (200 U/mL), FLT3 ligand (50 ng/mL; Serotec, Oxford, United Kingdom), and TNF-α (50 U/mL; R&D Systems), as previously described. CD34+ cells were seeded for expansion in 25 to 75 cm² flasks (Corning, Acton, Mass) at 2 × 10^5 cells/mL and maintained by splitting these cultures at day 4 with medium containing fresh GM-CSF and TNF-α. At day 10, cells were resuspended in fresh cytokine-conditioned medium and further cultured until day 14.

Chemokine measurements
The concentrations of chemokines in BEC supernatants and BL were determined by sandwich enzyme immunoassay as described by the manufacturers (R&D Systems) for the determination of human CCL2/MCP-1, CCL5/RANTES, CCL7/MCP-3, CCL20/MIP-3α, CXCL8/IL-8, and CXCL10/IFN-γ–induced protein-10.

Boyden-type microchamber chemotaxis assays
Bronchial epithelial cell supernatants (1/20 in RPMI 1640 medium; 0.1% FCS) and CCL5 (positive control; 200 ng/mL; Preprotech), proDer p 1, and E64, a cysteine protease inhibitor (5 μmol/L; Sigma, St Louis, Mo). Supernatants were collected after 24-hour incubation.

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Boyden-type microchamber chemotaxis assays
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R&D Systems) were added to the lower wells of the chemotaxis chamber (48-well Boyden microchamber; Neuroprobe, Pleasanton, Calif). Migration of MDDCs collected at days 1, 3, 5, and 7 and after 24-hour LPS stimulation (5 × 10⁶ cells/well in 50 μL RPMI 1640 medium; 0.1% FCS) was performed through a standard 5-μm pore filter (Costar, Cambridge, Mass) by incubation at 37°C for 1 hour, 30 minutes. Migrated cells on the lower side of the filter were stained with May-Grünwald Giemsa (Labonord, Templemars, France) and counted in 3 randomly selected high power fields (hpf; magnification, ×40). Each assay was performed in quadruplicate. Results are expressed as the difference between mean number of cells/hpf minus the negative control (medium alone).

**Transwell insert chemotaxis assays**

CD34⁺ derived LC precursors (at day 6, CD14⁺CD1a⁻ cells) and mature LCs (at day 14, CD1a⁺E-cadherin⁺ cells) were washed and resuspended in migration buffer (HBSS), BEC supernatants (1/10), buffer alone, or CCL20/CCL19 (positive controls, respectively; 200 ng/mL; R&D Systems) was added to the lower compartment and cells (3 × 10⁵ cells) to the upper compartment, separated by transwells (5-μm pore size; Costar) for 2 hours at 37°C. Transmigrated cells were numbered by flow cytometry (Becton Dickinson). Remaining cells were double stained with fluorescein isothiocyanate anti-CD1a and phycoerythrin anti-CD14 (Becton Dickinson) and counted in 3 randomly selected high power fields (hpf; magnification, ×40). Each assay was performed in quadruplicate. Results are expressed as the difference between mean number of cells/hpf minus the negative control (medium alone).

**Identification of BEC-derived mediators implicated in DC precursor recruitment**

Chemokine depletion of BEC supernatants was performed with protein G sepharose affinity columns (Amersham Biosciences, Orsay, France) preincubated with anti-CCL5 (Pharmingen), anti-CCL20 (R&D Systems), and anti-CXCL10 antibody (Becton Dickinson) or rabbit IgG as control (1 μg/μL protein G sepharose). Solutions of recombinant human (rh) CCL5, rhCCL20, and rhCXCL10 (200 ng/mL; R&D Systems) were similarly depleted to control the efficacy of the depletion (78%, 82%, and 65% respectively; not shown).

For neutralization experiments, BEC supernatants were incubated with neutralizing anti-CCL2 (R&D Systems) and anti-CCL7 antibody (Becton Dickinson; 10 μg/mL) or with rabbit IgG for 1 hour at 37°C. Efficacy of neutralization was checked with rhCCL2 or rhCCL7 solutions (200 ng/mL; R&D Systems): 85% and 80% of neutralization, respectively (data not shown).

**Coculture model**

Polarized BEAS-2B were cultured on the lower side of transwells (5-μm pore size; Costar) coated with collagen G matrix (type I and III collagen; Biochrom KG, Berlin, Germany). After confluence, BEAS-2B were starved overnight in 50% airway epithelial cell basal medium (Promocell, Heidelberg, Germany), 50% RPMI 1640, 0.1% FCS, and transepithelial resistance was controlled (data not shown). Der p 1 (500 ng/mL) was added in the lower chamber and day 3 MDDC precursors (1 × 10⁶ cells) in the upper chamber for 24 hours in RPMI, 0.1% FCS.

After trypsin digestion, cells from the epithelial layer were stained with antigen-presenting cell–labeled anti-CD11c mAb or with the isotype control and analyzed by flow cytometry. Results are expressed as the percentage of CD11c⁺ cells (intraepithelial DC) on gated cells, because the number of CD11c⁺ cells (BEAS-2B) collected on each transwell did not differ.

**Statistical analysis**

Results are expressed as means ± SEMs, or median and interquartile range. Statistical analysis was performed by using the Wilcoxon test for paired data or the Mann-Whitney U test for unpaired data.

**RESULTS**

**Der p 1 induces the production of chemokines by BEC**

Effects of Der p 1 on chemokine production by BEAS-2B were analyzed. After Der p 1 exposure, CXCL8, CXCL10, CCL2, CCL5, and CCL20 levels were increased in BEAS-2B supernatants (Fig 1), whereas CCL7 production was not modified (data not shown). In contrast, exposure to proDer p 1 (500 ng/mL), the nonenzymatic precursor of Der p 1, did not affect chemokine production (Fig 1). Moreover, E64, an inhibitor of cysteine protease, significantly reduced CCL2, CCL5, CCL20, and CXCL10 production induced by Der p 1 (500 ng/mL; 71%, 92%, 65%, and 72% inhibition). Chemokine production was next investigated with BEC primary cultures: at baseline, the levels of CXCL8, CCL2, and CCL20 were significantly higher in supernatants of BEC from AAs compared with NAs, whereas CXCL10 level was higher in NAs. As expected, CXCL8 production by BEC from both NAs and AAs was increased by Der p 1. This allergen increased the production of CCL2, CCL5, and CXCL10 by BEC from NAs, as well as by BEC from AAs (P < .05), except for CCL5 (P = NS). Moreover, CCL20 secretion by BEC from AAs was significantly upregulated, whereas no difference was obtained with BEC from NAs. ProDer p 1 had no effect on chemokine production by primary culture of BEC (data not shown).

**Der p 1 triggers the recruitment of MDDC precursors by BEC**

We next tested the effects of BEC supernatants on MDDC precursors (days 1, 3, 5) and immature (day 7) and mature MDDC (after 24-hour LPS stimulation). All of these cells are responsive to CCL5, with maximal activity at day 3 (data not shown). Whereas supernatants from unstimulated cells had no effect, Der p 1 increased significantly the chemotactic activity of BEAS-2B on MDDC precursors taken at day 3, and at a lower level at day 5 (P < .05 compared with unstimulated cells; Fig 2, A). In contrast, Der p 1 did not modulate the migration of day 1 precursors or immature (Fig 2, A) or mature DCs (data not shown).

Chemotactic assays were also performed with supernatants from primary cultures of BEC. Unstimulated BEC from NAs and AAs had an important chemotactic activity on day 3 precursors compared with day 5 precursors (Fig 2, B), but not at days 1 and 7 (data not shown). After Der p 1 exposure, the number of recruited day 3 and 5 precursors was significantly increased. Nevertheless, unstimulated and Der p 1–exposed BECs from AAs recruited more precursors than NAs.
CCL2, CCL5, and CXCL10 are involved in MDDC precursor recruitment

Immunodepletion of stimulated BEAS-2B supernatants in CXCL10, CCL2, and CCL5 reduced by 52%, 83%, and 74%, respectively, the number of recruited day 3 MDDC precursors (Fig 2, C). The number of recruited day 5 precursors was reduced by approximately 50% by each antibody (data not shown). Moreover, neutralization of CCL7 or CCL20 had no effect on chemotactic activity of BEAS-2B (data not shown), showing that both chemokines are not involved in BEC-induced MDDC migration.

The implication of CXCL10, CCL2, and CCL5 in the chemotactic activity of BEC from NAs was next investigated. CCL2 was mainly involved in precursor recruitment (65% and 70% inhibition at days 3 and 5, respectively; data not shown at day 5). Nevertheless, CCL5 (45% inhibition at days 3 and 5) and CXCL10 (20% and 30% inhibition at days 3 and 5, respectively) were also implicated at a lower level. Similar results were obtained with Der p 1–stimulated BECs from AAs (Fig 2, C). These data suggest that CCL2, CCL5, and CXCL10 have a coordinate activity on DC precursor recruitment. Indeed, the 3 recombinant chemokines (10 ng/mL) have an additive effect on the migration of day 3 MDDC precursors (see Fig E1 in the Journal’s Online Repository at www.mosby.com/jaci).

Der p 1 increases DC precursor migration into bronchial epithelial layer

To confirm that Der p 1 modulated MDDC precursor migration, we developed a polarized model of coculture on transwell devices using BEAS-2B and day 3 MDDC precursors, as described in Methods. At baseline, approximately 25% of the cell population was MDDC in the epithelial layer. Exposure to Der p 1 significantly increased the number of CD11c+ precursors (35%) present within the epithelium (Fig 3).

Der p 1 triggers the recruitment of LC precursors by BECs from AAs

The migration of LC precursors (CD14−CD1a+ cells) and mature LCs (CD1a+E-cadherin+ cells) generated from CD34+ hematopoietic progenitors was tested in response to BEC supernatants. LC precursors were recruited by unstimulated BEAS-2B, and Der p 1 exposure increased this migration (Fig 4, A). Unstimulated BECs from both NAs and AAs induced a low recruitment of LC precursors, whereas BEC activation by Der p 1 increased their chemotactic activity only in AAs (P < .05). No migration of mature LCs was obtained whatever the BEC supernatants used (see Fig E2 in the Online Repository at www.mosby.com/jaci).

CCL20 is involved in LC precursor recruitment

Langerhans cell precursors selectively express CCR6, the receptor for CCL20.9,10 Neutralizing anti-CCL20 antibody reduced the number of LC precursors by 48% in BEAS-2B supernatants and by 87% with supernatants of BECs from AAs (Fig 4, B). Immunodepletion with anti-CCL2, anti-CCL5, and anti-CXCL10 antibody of BEC supernatants did not affect LC recruitment (4%, 6%, and 3% inhibition, respectively).

CCL20 levels are enhanced in BLs from AAs compared with NAs

Levels of CCL20 were evaluated in BL from 6 NAs and 15 AAs (Fig 5). CCL20 concentrations were significantly
increased in BL from AAs (59.5 pg/mL) compared with NAs (27.5 pg/mL).

**DISCUSSION**

Because the traffic of DCs is in part controlled by selective chemokine/receptor interactions, our study focused on the role of chemokines released by allergen-stimulated BECs on myeloid DC migration. We have demonstrated that human BECs from NAs and AAs, exposed to a very common allergen found in house dust, are able to induce the recruitment of MDDC precursors. In addition, allergen-stimulated BECs from AAs induced LC precursor migration. These profiles of migration are linked to different patterns of chemokine production by BECs.

We first focused on chemokine production by allergen-stimulated BECs. Der p 1 allergen induced an upregulation of CCL2, CCL5, CCL20, and CXCL10 mRNA expression (data not shown) and secretion by BEAS-2B and BEC primary cultures. These results extended the data showing that Der p 1 directly stimulates BEC to produce cytokines.24 In contrast with Der p 1, proDer p 1, the nonenzymatic precursor, did not modulate chemokine secretion. In addition, the protease inhibitor E64 blocks the effect of Der p 1 on chemokine production, indicating that its action is linked to its protease activity, as previously suggested.20 Proteolytic activity is an important factor for overcoming airway tolerance and induction of allergic reaction.25 Moreover, the increased expression of PAR-2 in bronchial biopsies from AAs compared with
NAs could explain the increased responsiveness of BECs from AAs to Der p 1.

The lining airway epithelium contains a network of DCs composed of 400 to 800 DCs/mm² of epithelial surface. Those DCs are continuously renewed even in the absence of stimuli. Our data confirm that steady-state DC recruitment presumably relies on constitutive chemokine production by the epithelium, but also by macrophages or DCs. DC number is increased at steady state in bronchial mucosa of AAs, and antigen inhalation accelerates the continuous renewal of DCs, underscoring the potential importance of these cells in local immune defense. Here, we demonstrate that CCL20 levels were significantly higher in the BL obtained from AAs compared with NAs, and this was previously shown for CCL2. In vitro, the increased secretion of chemokines by unstimulated BECs from AAs induced a higher MDDC recruitment. Moreover, Der p 1 exposure of BECs, even in the polarized model of coculture, resulted in an increased recruitment of MDDCs, related to interstitial DCs, lack CCR6 but display an additive effect on the recruitment of DC precursors. Increased secretion of chemokines by BECs is probably involved in the accelerated turnover of interstitial DCs observed at baseline and after allergen challenge in AAs. The preferential recruitment of day 3 and 5 precursors by DCs is related to their profile of chemokine receptors (CCR1, CCR2, CCR5, and CXCR3) and consequently can home to the airway epithelium, where they perpetuate the inflammatory response.

In contrast with NAs, BECs from AAs produced CCL20 and induced LC recruitment after allergen exposure. Therefore, allergic status is associated with a modified response of BECs to Der p 1 and a dysregulation of CCL20 secretion. The LCs follow only 1 tissue-derived constitutive and modulated signal—CCL20—and are remarkably ignorant to inflammation-related chemokine stimuli in the skin. CCL20 expression suggests its role in LC homing, in the epidermis, and in the tonsils, but also in the lung. By using CCR6-deficient mice, Lukacs et al demonstrated that CCL20 and CCR6 are involved in allergic pulmonary responses through the impaired migration of CCR6 DCs in CCR6 mice. Because DC precursors are recruited toward bronchial epithelium, we hypothesized that these precursors differentiate into mature DCs within the epithelial layer. We previously evaluated this hypothesis in the context of BEC exposure to a pathogen-associated molecular pattern derived from Klebsiella pneumoniae. Our data showed that intraepithelial DCs are phenotypically more mature than DC precursors cultured without BEC (M. Pichavant, unpublished data, May 2004). Similar experiments will be conducted within the context of allergen exposure. In human allergic asthma, the precise role of DCs is difficult to define. In vitro experiments and in vivo approaches in the humanized severe combined immunodeficiency mice show that MDDCs from AAs have a propensity to induce a Th2 response and to develop airway inflammation. The role of LCs in allergen sensitization by airway has not been studied. Interestingly, Allam et al showed that DCs from the oral mucosa are composed mainly of LCs with a particular phenotype characterized by a high FceRI expression. FceRI-activated monocytes and LC precursors acquired the ability to suppress T-cell proliferation through the production of indoleamine 2,3-dioxygenase. It is now suspected that this mechanism is important for the maintenance of tolerance within the airway mucosa. Another mechanism susceptible to regulate allergen sensitization could be linked to the ability of Der p 1 to degrade tight junctions, a situation frequently

**FIG 4.** Allergen stimulation of BECs from AAs increased LC precursor recruitment through CCL20 secretion. A, Migration of CD11c⁺CD14⁺ LC precursors (n = 7) was evaluated with supernatants of BEAS-2B and BEC from NAs and AAs. Results are expressed as the percentage of migrated cells compared with the positive control CCL20. B, CCL20 depletion of supernatants from BEAS-2B and AA BECs reduced LC precursor migration (n = 3). *P < .05; **P < .01. Unstim, Unstimulated.

**FIG 5.** CCL20 levels are increased in BLs from AAs. CCL20 levels were evaluated by ELISA in BL from NAs (n = 6) and AAs (n = 15). Results are represented as medians with interquartile ranges. *P < .05 in comparison with NAs.
observed in many subjects adopting a modern lifestyle. Therefore, this protease activity may not only facilitate the access of inhaled allergens to DC but also enhance their recruitment. For Der p 1, the coordinate action of the protease site and of the allergen epitope associated with an increased susceptibility of BEC might break down local tolerance and allow hypersensitivity development. Concerning allergen without protease function, data obtained in a mouse model of airway sensitization showed that induction of an allergic reaction requires a cofactor such as LPS, in contrast with allergen with protease activity. In summary, BEC activation by protease or molecule derived from pathogen is probably an important step during allergen sensitization.

These data demonstrated a role for BEC in the control of myeloid DC homeostasis at steady state and in the context of Der p 1 exposure, suggesting a key role for BEC in allergen hypersensitization. The process of DC migration is quantitatively and qualitatively altered in AAs. As a consequence, the respective role of MDDCs and LCs in the control of allergen sensitization in AAs remains to be determined. Control of DC subset trafficking within airway mucosa may also represent one way to limit airway inflammation or to deviate the allergen specific immune response.

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REFERENCES

29. Stumbles PA, Strickland DH, Pimm CL, Proksch SF, Marsh AM, McWilliam AS, et al. Regulation of dendritic cell recruitment into resting and inflamed airway epithelium: use of alternative chemokine...