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.
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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.[1,2] In airway mucosa, myeloid DCs form a dense network including different populations: interstitial DCs and Langerhans cells (LCs) close to epithelial cells.[3] 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.[4]

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 multipartite superfamily of chemoattractant cytokines that induce the directed migration of leukocytes and other cells.[5] 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.[6,7] Mainly, monocyte-derived DCs (MDDCs), related to interstitial myeloid DCs,[8] respond to C-C chemokine ligand (CCL)–3/macrophage inflammatory protein (MIP)–1α and CCL5/RANTES via CCR1 and CCR5 or to CCL2/monocyte chemoattractant 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,[9] a subpopulation of myeloid DCs that reside at mucosal surfaces. CCL20 has been demonstrated to be expressed in inflamed intestinal epithelial cells and keratinocytes.[10-13] 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,[14] whose levels are elevated and important in diseases such as asthma.[15]

Allergic asthma is characterized by hypersensitivity against aeroallergens and the development of an allergen-specific T_H2 response.[16] In animal models, a critical role for DC is now fairly demonstrated in allergen sensitization, established airway inflammation, and alteration of

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Abbreviations used
AA: Patient with allergic asthma
BEC: Bronchial epithelial cell
BL: Bronchial lavage
CCL: C-C chemokine ligand
CXCL: C-X-C chemokine ligand
CXCR: C-X-C chemokine receptor
DC: Dendritic cell
hpf: High power field
LC: Langerhans cell
MCP: Monocyte chemoattractant protein
MDDC: Monocyte-derived dendritic cell
MIP: Macrophage inflammatory protein
NA: Nonatopic donor
PAR: Protease-activated receptor
rh: Recombinant human

pulmonary functions.[17] 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.[18] In addition, BECs play an active role in allergic inflammation by the secretion of proinflammatory mediators. Indeed, BECs from AAs produced higher levels of cytokines such as GM-CSF, IL-6, or C-X-C chemokine ligand (CXCL)–8 and expressed more CD54/intercellular adhesion molecule 1 compared with NAs.[19]

Inhalation of antigen does not normally lead to immediate hypersensitivity, a situation linked to a particular tissue environment. Airway resident cells or their products may affect the outcome of antigen exposure and induce, or not, the development of immune response. It is therefore important to elucidate how potential allergens 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[20] 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.

Bronchial 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

Groups		n	Age, y (mean ± SEM)	Sex (M/F)	Global Initiative for Asthma score 1 2 3 4	Prick test, dust
BEC	NA	25	51.7 ± 11.4	18/7	None	Negative
	AA	8	36.5 ± 13.1	5/3	4 1 3 0	Positive
BL	NA	6	40 ± 7	5/1	None	Negative
	AA	15	40.5 ± 8.4	6/9	6 1 8 0	Positive

BEAS-2B and primary cultures of BEC were obtained and cultured as previously described.[21] Confluent 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.[22] 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.[23] 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.[9] $CD34^+$ cells were seeded for expansion in 25 to 75 cm^2 flasks (Corning, Acton, Mass) at 2×10^4 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;