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*J Immunol* 2006; 177:5912-5919; doi: 10.4049/jimmunol.177.9.5912

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Impact of Bronchial Epithelium on Dendritic Cell Migration and Function: Modulation by the Bacterial Motif KpOmpA

Muriel Pichavant,* Solenne Taront,* Pascale Jeannin,† Laëtitia Breuilh,* Anne-Sophie Charbonnier,‡ Corentin Spriet,§ Catherine Fourneau,* Nathalie Corvaia,¶ Laurent Hélot,§ Anne Brichet,* André-Bernard Tongel,*# Yves Deleneste,† and Philippe Gosset2*# 1

Mucosal immune response depends on the surveillance network established by dendritic cells (DC), APC localized within the epithelium. Bronchial epithelial cells (BEC) play a pivotal role both in the host defense and in the pathogenesis of inflammatory airway disorders. We previously showed that the outer membrane protein A from Klebsiella pneumoniae (KpOmpA), a pathogen-associated molecular pattern (PAMP) derived from Klebsiella pneumoniae, activates BEC. In this study, we evaluated the consequences of this activation on DC traffic and functions. KpOmpA significantly increased the production of CCL2, CCL5, CXCL10, and CCL20 by BEC. Stimulation of BEC increased their chemotactic activity for monocyte-derived DC (MDDC) precursors, through CCL5 and CXCL10 secretion. BEC/MDDC precursor coculture leads to an ICAM-1-dependent accelerated differentiation and enhanced maturation of MDDC. BEC/DC interactions did not affect the capacity of DC to induce T cell proliferation. However, DC preincubated with BEC increased significantly the IL-10 production by autologous T cells. Basolateral and intraepithelial DC differently enhance IL-4 and/or IL-10 synthesis according to the condition of stimulation. In vivo, intranasal injections of KpOmpA into BALB/c mice induced the recruitment of CD11c+ and I-A^n+ myeloid DC associated with bronchial epithelium activation as evidenced by CCL20 expression. These data show that KpOmpA-exposed BEC participate in the homeostasis of myeloid DC network, and regulate the induction of local immune response. The Journal of Immunology, 2006, 177: 5912–5919.

The effective host defense against bacterial infection is primarily dependent upon the innate immune system through the rapid clearance of bacteria by neutrophils and macrophages (1). In addition to a nonspecific antibacterial activity, leukocytes release inflammatory mediators that activate ACPs and facilitate the initiation of an adaptive immune response. Dendritic cells (DC) play a major role in the surveillance of peripheral tissues for incoming Ags (2, 3). In airway mucosa, immature myeloid DC constitute a dense network including interstitial DC and Langerhans cells (LC), closely located within airway epithelium (4), that act as sentinels by discarding foreign Ags and pathogens in the surrounding tissue.

Migration is an integral part of DC functions (5). Precursor cells first migrate from the bone marrow to resident sites in tissues such as lung. At steady state, due to the environment, immature DC and/or their precursors are continuously recruited to the airway mucosa where inhaled Ags are sampled (6). Upon activation by inflammatory mediators or microbial components, they undergo a complex process of maturation allowing their migration toward the regional lymph nodes (7) through a modification of adhesion molecule and chemokine receptor expression (8). Moreover, DC maturation is associated with high surface expression of MHC and costimulatory molecules, as well as secretion of immunomodulatory cytokines mainly to IL-10 and IL-12. These factors drive the differentiation and the polarization by DC of naive T cells into Th1, Th2, or alternatively into regulatory T cells. Recent data suggest that DC behavior is locally controlled by lung environment and particularly by bronchial epithelial cells (BEC) (9–11). In addition to GM-CSF, these BEC produce several cytokines and chemokines and express adhesion molecules potentially involved in DC traffic, as shown after exposure to diesel exhausted particles or allergens (6, 11). In both allergic patients and nonatopic subjects, allergen-exposed BEC trigger the recruitment of monocyte-derived DC (MDDC) precursors through the secretion of CCL2, CCL5, and CXCL10. In contrast, the migration of CD34+-derived LC precursors, depending on CCL20 secretion, is only observed with BEC from allergic patients. The recruitment of DC precursors by BEC suggests that local DC differentiation/maturation could be regulated by a confined crosstalk with BEC (12).

In this context, we demonstrated that BEC bind and are directly activated by outer membrane protein A (OmpA) from Klebsiella pneumoniae (KpOmpA), a mechanism implicated in neutrophil migration and the initiation of the innate immune response (13). OmpA has been shown to bind to and activate macrophages and immature MDDC in a TLR-2-dependent manner (14, 15). Moreover, KpOmpA is a carrier molecule suitable to generate a CD4+ and CD8+ T cell responses in different models of immunization (16–20). This model of exposure to a pathogen-associated molecular pattern (PAMP) derived from a pathogen with a lung tropism appears to us particularly relevant to evaluate the role of BEC in linking innate and adaptive immunity within airway mucosa.
To define this role, we studied in vitro the crosstalk between BEC and DC after exposure to KpOmpA, and particularly the regulation of DC traffic and functions by BEC. For this purpose, we analyzed the capacity of human BEC upon exposure to KpOmpA, to produce chemokines, to control the recruitment and the differentiation/maturation process of DC in an in vitro model of reconstituted bronchial epithelium. The in vivo relevance of this mechanism was also defined. The results showed that bronchial epithelium regulates the functions of DC and that this process might affect the development of the T cell response.

Materials and Methods

Bronchial epithelial cells

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.

Human bronchial biopsies were obtained by fiberoptic bronchoscopy from 25 nonatopic patients who were being investigated for idiopathic chronic cough (n = 5) or for bronchopulmonary carcinoma (n = 20). For 9 patients, the diagnosis of cancer was not confirmed. For the patients without tumor, there is no obvious abnormality in the airways. There are 12 smokers among these patients with a smoking history of 11 ± 3 packs/yr (mean ± SEM). These patients did not receive any anti-inflammatory or immunomodulatory medication. In patients with cancer, biopsies were collected far from the carcinoma.

The human bronchial epithelial cell line BEAS-2B and primary BEC were cultured as previously described (13). BEC were exposed to different concentrations of KpOmpA. This protein was produced according to pharmaceutical quality standards, intended for clinical trials (human). Supernatants of BEC were collected after 24 h of incubation.

Preparation of monocyte-derived dendritic cells and naïve T cells

Blood monocytes were purified by positive selection over a MACS column using anti-CD14-conjugated microbeads (Miltenyi Biotec) and were differentiated into DC by standard procedures (21). Briefly, immature DC (high expression of CD1a and CD11c) were obtained after culture for 6 days in RPMI 1640 medium supplemented with 10% heat-inactivated FCS (Invitrogen Life Technologies) and containing 10 mg/ml IL-4 and 25 mg/ml GM-CSF (R&D Systems). Mature MDDC were obtained after 24 h of incubation with 1 µg/ml LPS (from 055B5 Escherichia coli strain; Sigma-Aldrich).

Naïve T cells were isolated from the CD4+ fraction by negative selection using the CD4+ T cell isolation kit associated with CD45RO microbeads (Miltenyi Biotec). After magnetic selection of memory T cells, the negative fraction contained >95% of CD4+ CD45RA+ cells, as determined by flow cytometry (data not shown).

Chemokine and cytokine measurements

The concentrations of CCL2/MCP-1, CCL5/RANTES, CCL20/MIP-3α, and CXCL10/IP-10 chemokines were determined in BEC supernatants by ELISA (all obtained from R&D Systems). Murine CCL20 was measured in lung extracts and bronchoalveolar lavage (BAL) fluids (R&D Systems). IL-10, IL-12p70 (Diaclone), IL-6, CCL17/TARC, and CCL22/MDC (R&D Systems) were measured in BEC/DC coculture supernatants, and IL-4, IFN-γ, and IL-10 (Diaclone) in DC/T cell coculture supernatants, by sandwich enzyme immunoassay, as described by the manufacturer.

Boyden-type microchamber chemotaxis assays

Each BEC supernatants (1/20 in RPMI 1640 medium containing 0.1% FCS) and CCL5, used as positive control (200 ng/ml; R&D Systems), were added to the lower wells of the chemotaxis chamber (48-well Boyden microchamber; Neuroprobe). MDDC migration (5 × 104 cells/well in 50 µl of RPMI 1640 medium, 0.1% FCS) was performed through a standard 5-µm pore filter (Neuroprobe), at 37°C for 1 h and 30 min. Migrated cells were counted after staining on the lower side of the filter in three randomly selected high power fields (magnification ×40). Each assay was performed in quadruplicate. Results are expressed as the difference between mean numbers of cells per high power fields 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) preincubated with anti-CCL2 (BD Pharmingen) and/or anti-CXCL10 Abs (BD Pharmingen) or rabbit IgG as control (1 µg/µl protein G-Sepharose). Solutions of rhCCL5 and rhCXCL10 (200 ng/ml; R&D Systems) were similarly depleted to control the efficacy of the depletion (78 and 75%, respectively; data not shown).

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

BEC/DC precursors coculture model

BEC were cultured on the lower side of a 5-µm pore filter coated with collagen G matrix (type I and III collagen; Biochrom KG). After confluence, BEC were starved overnight in 50% RPMI 1640 medium, 0.1% FCS, and transepithelial resistance was controlled (data not shown). Biotinylated KpOmpA (20 µg/ml) was added in the lower chamber containing BEC, and day 3 MDDC precursors (1 × 105 cells) in the upper chamber for 24 h in RPMI 1640 medium containing 0.1% FCS. To evaluate the role of ICAM-1, BEC were preincubated with blocking anti-ICAM-1 Ab or an isotype control (5 µg/ml; R&D Systems) for 2 h, and washed before coculture with MDDC precursors.

Confocal microscopy and flow cytometry

The epithelial layer containing migrated MDDC precursors was assessed by confocal microscopy. MDDC precursors were stained with FITC-labeled anti-CD11c mAb, and BEC with PE-labeled anti-CD49c mAb or the corresponding isotype controls (BD Biosciences), and biotinylated KpOmpA was revealed with allophycocyanin-labeled streptavidin. The epithelial layer containing migrated MDDC precursors was assessed by confocal microscopy ( Imaging Core Facility of Calmette Campus). Imaging was done using a DM-IRE2 inverted microscope with SP2-AOBS scanhead (Leica). Acquisitions were performed using a 100×, 1.4 NA objective. The laser excitation wavelengths were selected with an AOBS, and
their power was modulated by an AOTF (AOBS and AOBF are two specific acousto-optical systems). Excitation power was between 100 and 400 μW. Three-dimensional reconstruction results of the epithelial layer were obtained using Imaris software (Bitplane). KpOmpA capture was also quantified by flow cytometry in comparison with a control, a biotinylated-mouse IgG1 isotype control (n = 3).

Day 3 MDDC precursors, and DC from the DC/BEC cell coculture model were assessed for phenotypic analysis. Epithelial layer containing migrated MDDC precursors was dissociated using trypsin solution (Invitrogen Life Technologies). Cells were stained with aliphycocyanin-labeled anti-CD11c, FITC-labeled anti-CD86, anti-HLA-DR, and anti-CD1a, vitrogen Life Technologies). Cells were stained with allophycocyanin-labeled anti-CD3 plus anti-CD28 mAbs (2 μg/ml) and analyzed by flow cytometry (FACSCalibur; BD Biosciences) with specific Ab minus the isotype control MFI (ΔMFI).

Evaluation of DC ability to induce T cell proliferation and polarization

MDDC were collected after 24 h coculture of DC precursor (day 3) with BEC, by vigorous washing of the upper chamber of the Transwell and the epithelial layer. In this case, we positively selected the DC with CD11c microparticles to remove the BEC. Importantly this treatment does not modify the DC function. The purity of this preparation was up to 92%. In a first set of experiments, basalateral DC were incubated with unlabeled autologous naïve T cells (DC7 lymphocyte ratio 1:20) in 1 ml of RPMI 1640 medium supplemented with 10% FCS during 5 days. Moreover, we evaluated the capacity of the different DC to induce the proliferation and the cytokine production of heterologous CFSE-labeled T cells. For both experiments, T cells were collected after a 5-day incubation and resuspended at 2 × 10^6 cells/ml in fresh medium. Restimulation was performed by the addition of anti-CD3 plus anti-CD28 mAbs (2 μg/ml) (BD Biosciences). Cytokine production was first analyzed by ELISA in 24-h supernatants for unlabeled T cells. Cytokine synthesis was also measured after a 6-h incubation by intracellular flow cytometry with allophycocyanin-labeled anti-IL-10, and anti-IFN-γ mAb for CFSE-labeled heterologous T cells identified with PE-Cy5 anti-CD4 mAb (all obtained from BD Biosciences).

**KpOmpA intranasal injection in mice**

Female BALB/c mice, 6–10 wk old (IFFA Credo), were anesthetized by i.p. injection of ketamine/xylazine (Sigma-Aldrich) (2.5% v/v in PBS); 30 μl of BSA (100 μg) or KpOmpA solution (100 μg) was administrated intranasally on days 0, 1, and 2. Analyses were performed 24 h later. Experiments were approved by the animal ethical committee.

**Cell infiltrate and inflammation in mouse lung tissue**

BAL was first collected; then, right lungs were collected in PBS and mechanically dissociated to extract proteins from lung tissue. With the left lobe, total cells were isolated and then stained with aliphycocyanin-labeled anti-CD11c and FITC-labeled anti-I-A^d^ mAbs, or with the isotype control (BD Biosciences). In this case, we positively selected the DC with CD11c microparticles to remove the BEC, by vigorous washing of the upper chamber of the Transwell and the epithelial layer. In this case, we positively selected the DC with CD11c microparticles to remove the BEC. Importantly this treatment does not modify the DC function. The purity of this preparation was up to 92%. In a first set of experiments, basalateral DC were incubated with unlabeled autologous naïve T cells (DC7 lymphocyte ratio 1:20) in 1 ml of RPMI 1640 medium supplemented with 10% FCS during 5 days. Moreover, we evaluated the capacity of the different DC to induce the proliferation and the cytokine production of heterologous CFSE-labeled T cells. For both experiments, T cells were collected after a 5-day incubation and resuspended at 2 × 10^6 cells/ml in fresh medium. Restimulation was performed by the addition of anti-CD3 plus anti-CD28 mAbs (2 μg/ml) (BD Biosciences). Cytokine production was first analyzed by ELISA in 24-h supernatants for unlabeled T cells. Cytokine synthesis was also measured after a 6-h incubation by intracellular flow cytometry with allophycocyanin-labeled anti-IL-10, and anti-IFN-γ mAb for CFSE-labeled heterologous T cells identified with PE-Cy5 anti-CD4 mAb (all obtained from BD Biosciences).

**KpOmpA induces chemokine secretion by BEC**

We previously described that KpOmpA induced the production of CXCL1 and CXCL8 by BEC, two chemokines involved in neutrophil recruitment (13). KpOmpA also induced a dose-dependent
production of CCL2, CCL5, CCL20, and CXCL10 but not CCL7 by BEAS-2B cells after 24 h of stimulation (with a maximal activity at 20 μg/ml) associated with specific mRNA expression at 6 h (data not shown). Similar results were obtained with BEC primary cultures. Indeed, 20 μg/ml KpOmpA significantly increased the production of CCL2, CCL5, CCL20, and CXCL10 compared with unstimulated cells after 24 h (Fig. 1A). In our hands, LPS was a poor inducer of these chemokines in BEC compared with unstimulated cells (CCL2: 68 ± 12 vs 57 ± 20; CXCL10: 68 ± 48 vs 66 ± 33; CCL20: 1295 ± 110 vs 1117 ± 240, respectively). Taken together, these data show that KpOmpA triggers the production by BEC of chemokines potentially involved in DC migration.

*Kp*OmpA triggers the recruitment of DC precursors by BEC

We next tested the effects of supernatants from BEC primary culture on the migration of MDDC precursors (days 1, 3, 5), immature (day 6), and mature MDDC obtained after 24 h of stimulation with LPS. The precursors and the immature DC were responsive to CCL5 with a maximal activity at day 3 and mature DC to CCL19 (data not shown). Whereas supernatants from unstimulated cells had no marked effect, KpOmpA-treated BEC supernatants have a strong chemotactic activity on MDDC precursors taken at day 3, and at a lower level at day 5 (p < 0.05 vs unstimulated BEC) (Fig. 1B). In contrast, supernatants of KpOmpA-activated BEC did not modulate the migration of immature (day 7) (Fig. 1B), day 1 precursors, or mature DC (data not shown). No migration of MDDC precursors was observed in response to CCL20 or KpOmpA alone (data not shown). As previously demonstrated (12), immunodepletion of KpOmpA-stimulated BEC supernatants with anti-CXCL10 and anti-CCL5 Abs reduced the number of recruited day 3 precursors (32 and 46% inhibition, respectively; Fig. 1C). Moreover, there is an additive effect of CXCL10 and CCL5 depletion on cell migration (76% inhibition). The neutralizing anti-CCL2 Ab had a weak effect on day 3 precursor migration (Fig. 1C) and neutralizing anti-CCL7 or -CCL20 Abs had no effect on chemotactic activity of BEC (data not shown).

**MDDC precursors migrate into bronchial epithelial layer and capture KpOmpA**

To confirm that KpOmpA modulated MDDC precursor migration, we used a polarized model of coculture on Transwell devices, using BEC and day 3 MDDC precursors. At baseline, approximately 25% of the cells present in the epithelial layer were MDDC. Exposure to KpOmpA significantly increased the number of CD11c+ precursors (41 ± 5%) present within the epithelium (p < 0.05, Fig. 2A). In addition, intraepithelial MDDC (CD11c+ cells) captured KpOmpA as illustrated after three-dimensional image reconstruction, by the association between CD11c labeling and allophycocyanin-conjugated KpOmpA (Fig. 2B). Flow cytometry analysis revealed that ~25% of intraepithelial DC (Fig. 2D) captured KpOmpA. Basolateral DC were also labeled but with a lower intensity (Fig. 2C).

**BEC favor DC precursor differentiation and maturation: involvement of ICAM-1**

We next analyzed the differentiation of day 3 MDDC precursors, recruited by BEC within the epithelial layer (intraepithelial), present on the other side of the Transwell (basolateral) or cultured without BEC (Fig. 3). The expression of the co-stimulatory molecules CD80 and CD86 were significantly up-regulated on MDDC precursors in contact with the basolateral side of unstimulated BEC, compared with MDDC precursors cultured without BEC (Figs. 3 and 4). This effect was more pronounced on intraepithelial DC (p < 0.05) (Fig. 3). The same profile was obtained for HLA-DR, ICAM-1, and the maturation markers, namely CD83 and CCR7 (p < 0.05). In contrast, BEC did not significantly modulate the expression of CD1a as well as the level of CD11c expression by DC (not shown). Addition of KpOmpA to MDDC precursors cultured without BEC increased the expression of CD80, CD86, HLA-DR, ICAM-1, CD83, and CCR7 (p < 0.05), but not CD1a. KpOmpA exposure has an additive effect with BEC on the modulation of DC phenotype, particularly on the expression of CD80 and HLA-DR (p < 0.05) and, in a lower manner, of CD83, CD86, and CCR7. As previously demonstrated, KpOmpA increased ICAM-1 expression on BEC or MDDC precursors alone (13) and the coculture still enhanced ICAM-1 expression on both types of cells (data not shown). Since β2 integrin-ICAM-1 interactions result in cell activation, we evaluated the potential implication of this adhesion molecule in the crosstalk between DC and BEC. Pretreatment of BEC with neutralizing anti-ICAM-1 Ab significantly reduced the expression of CD80, CD86, and HLA-DR (46, 68, and 43% inhibition, respectively) whereas this treatment did not block the effect of KpOmpA on DC alone (Fig. 4). In our hands, supernatants of unstimulated and KpOmpA-activated BEC had a weak effect on DC phenotype compared with the coculture model (data not shown).

In addition, IL-6, IL-10, IL-12, CXL10, CCL17, and CCL22 levels were evaluated in the supernatants of DC precursor/BEC cocultures. IL-10 and IL-12 were nearly undetectable in the coculture even after activation with KpOmpA, whereas this PAMP induced their secretion by MDDC alone (data not shown). In unstimulated cocultures, IL-6 levels were not modulated compared...
with the cells alone. KpOmpA increased IL-6 production in all the conditions; however, this effect was significantly lower in the stimulated cocultures compared with MDDC alone ($p < 0.05$) (Fig. 5).

CXCL10 production was not modulated by the coculture compared with cells alone (data not shown). In contrast, the secretion of CCL17 and CCL22 were increased at baseline and after KpOmpA exposure in the cocultures compared with day 3 MDDC precursors and BEC alone (Fig. 5). In cocultures, pretreatment of BEC with anti-ICAM-1 Ab significantly decreased the amounts of CCL17 (95 and 55% inhibition, without and with KpOmpA, respectively) and CCL22 (94 and 50% inhibition, respectively), whereas levels of CXCL10 and IL-6 were not affected (Fig. 5). No effect of anti-ICAM-1 Ab was observed on the activation of DC precursors or BEC alone (data not shown).

Coculture of DC with BEC modify their capacity to induce cytokine secretion but not proliferation of naive T cells. First, the capacity of basolateral MDDC cocultured with BEC to stimulate T cells was evaluated by incubating these cells with autologous CD4+/CD45RA+ T lymphocytes for 5 days. The MDDC cocultured with BEC did not modify the production of IL-4 and IFN-γ, but increased IL-10 production by T cells after restimulation with anti-CD3 plus anti-CD28 mAbs. KpOmpA activation of MDDC alone or in cocultures did not significantly modulate the capacity of DC to regulate IL-4 and IFN-γ secretion whereas there is a trend to increase the levels of IFN-γ (Fig. 6A). In contrast, treatment with KpOmpA did not affect IL-10 production in coculture with BEC whereas it increased its production with day 3 MDDC precursors.

In a second model, we analyzed T cell proliferation and cytokine production with both basolateral and intraepithelial DC in MLR. In all cases, pre-exposure to KpOmpA increased the capacity of DC alone to induce T cell proliferation (20–25% of proliferating T cells) compared with unstimulated DC (15%) or to T cells alone (5%) (data not shown and Fig. 6B). Both basolateral and intraepithelial DC induced heterologous T cell proliferation similar to that obtained with DC alone (data not shown). IFN-γ production was also increased by KpOmpA exposure; however, it does not differ with the different kinds of DC (data not shown). IL-4 and IL-10 production was similar in the presence of unstimulated basolateral DC and DC alone whereas KpOmpA induced a strong increase of both positive IL-4 and IL-10 T cell percentage only with basolateral DC. In contrast, intraepithelial DC in unstimulated conditions have an enhanced activity to induce T cell cytokine production whereas they do not respond to KpOmpA stimulation. The production of IFN-γ was not markedly affected by the coculture with BEC (data not shown).
Intranasal injection of KpOmpA induces the recruitment of CD11c<sup>+</sup> cells and I-A<sup>+</sup> cells into the airways

In a previous study, we demonstrated that one intratracheal injection of KpOmpA triggered neutrophil influx to bronchial epithelium and into the bronchial lumen (13) whereas no significant migration of myeloid DC was observed in this situation (data not shown). However, after three injections of KpOmpA, no more influx of neutrophils was observed in the BAL (3.3% at day 3 compared with 1.9% with BSA and 44% at day 1 with KpOmpA) or in the bronchial wall (data not shown). In contrast, the number of CD11c<sup>+</sup> I-A<sup>+</sup> cells in lung homogenates was significantly increased after KpOmpA exposure (Fig. 7A). Moreover, important infiltrates of CD11c<sup>+</sup> and I-A<sup>+</sup> cells were present mainly in bronchial mucosa (Fig. 7, B and C). In contrast, only a few infiltrating leukocytes were observed in lung sections of mice primed with BSA (Fig. 7C).

To demonstrate the implication of BEC in this process, we measured CCL20 production by ELISA in lung extracts and BAL and analyzed its expression by immunohistochemistry on lung sections. As shown in Fig. 7D, CCL20 production was significantly increased in lung homogenates and BAL after three intranasal injections of KpOmpA but not after only one injection. Moreover, analysis of lung sections revealed that treatment with KpOmpA increased CCL20 expression in BEC and also in subepithelial myofibroblasts compared with mice receiving BSA (Fig. 7E). An enhanced expression of ICAM-1 was also detected (data not shown) as previously reported at day 1 (13). No staining was detected with an isotype control (Fig. 7E). These data show that intranasal administration of KpOmpA induces peribronchial DC recruitment associated with an increase of CCL20 expression in BEC.

Discussion

The primary function of innate immunity is to limit the development of infections. It provides the first line of cellular defense against invading pathogens by the mobilization of neutrophils, macrophages, and DC. Both types of APC build a network of signals that instructs the adaptive immune system to mount a specific response (22). We previously reported that BEC activation by KpOmpA participates in the development of innate immune response, leading to in vivo neutrophil recruitment toward the lung (13). In the present study, we demonstrated that activation by KpOmpA of human BEC affected DC precursor migration and their subsequent differentiation/maturation, resulting in a modified T cell response.

We first focused on the production of chemokines involved in DC migration, and showed that KpOmpA increased the secretion of CCL2, CCL5, CCL20, and CXCL10 by BEC. These results extended previous data showing that KpOmpA directly stimulates BEC to produce cytokines and chemokines, a process probably related, at least, to NF-κB activation (13). Moreover, KpOmpA exposure of BEC led to an increased recruitment of MDDC precursors, through the coordinate activity of CXCL10 and CCL5. Surprisingly, CCL2 secretion is not involved in the effect of KpOmpA-stimulated BEC, in contrast to the data obtained with allergen-exposed BEC (12). Compared with Der p1-allergen, KpOmpA is a stronger inducer of CXCL10 production by BEC, this effect compensating the lack of CCL2 activity in KpOmpA-derived MDDC precursor recruitment. In addition, as previously demonstrated in the skin, we suspect that KpOmpA is as a migration-promoting stimulus for LC (19), a cell only responding to CCL20 (23) in bronchial mucosa. CCL20 expression, but not that of inflammation-related chemokines, determines LC homing in the epidermis (23), the tonsils and probably also in the lung (24). CCL20 secretion by BEC was previously reported after exposure to allergen, but only in allergic asthmatic patients and to diesel exhaust particles (11, 12). Therefore, CCL20 secretion is only induced by strong signals in BEC such as pathogen exposure or by proinflammatory mediators present within the lung environment. In summary, KpOmpA represents a strong inducer of myeloid DC migration within the bronchial epithelium.

After pathogen exposure, the lung vascular compartment is enriched in a population of mononuclear cells (25), which are able to differentiate into MHC class II<sup>+</sup> DC when exposed to the appropriate growth factors, including GM-CSF, a cytokine produced by BEC (26). This enriched population of DC precursors provides a readily available source to replenish pulmonary DC, both as part of the normal turnover of these cells and during inflammatory reactions (25, 27). In this study, we showed that airway epithelium controls the mobilization of these DC precursors through chemokine secretion. These results were confirmed in an in vitro model.
three intranasal injections of BSA (100 μg) (white columns) or KpOmpA (100 μg) (black columns). Lungs were collected 24 h after the last injection. A, Right lung was dissociated and stained for CD11c and I-A^d cells to evaluate DC recruitment into the lungs. Results are expressed as the percentage of CD11c^- I-A^d^- cells in the lung. B, Lung sections were stained for CD11c and I-A^d. Results are expressed as the number of positive cells per millimeter of epithelium or per square millimeter for the alveolar spaces (mean ± SEM). C, A representative picture of CD11c and I-A^d staining on lung sections are reported, showing a peribronchial infiltrate in KpOmpA-treated mice. D, Measurement by ELISA of CCL20 production in lung extracts and BAL fluids of mice treated with one or three daily intranasal injections of BSA or KpOmpA. *p < 0.05 compared with mice receiving BSA (n = 12). E, Lung sections were stained for murine CCL20 and with an isotype control. BEC (arrow) and some subepithelial fibroblasts (arrowhead) were positive for CCL20 in KpOmpA-treated mice whereas a weak staining was observed after BSA exposure. No staining was detected in BEC and fibroblasts with the control.

of polarized epithelium where DC precursors invaded the epithelial layer after KpOmpA exposure. Randolph et al. (28) reported that monocytes transmigrating through endothelium differentiated into DC after a complex set of signals. Whereas endothelium delivers the first signal for DC differentiation/maturation process, we hypothesized that airway epithelium may generate the second one (29). The importance of this crosstalk has been suggested in intestinal mucosa where transepithelial dendrite formation appears dependent on tight junction protein expression and on the chemokine CX3CL1 (30, 31). In our model of airway epithelium, we showed that BEC control the recruitment of MDDC precursors, the capture of KpOmpA, and the differentiation/maturation process. After 24 h of incubation with BEC, MDDC expressed higher levels of costimulatory molecules and produced higher amounts of chemokines than DC differentiated without BEC. Increased expression of CD83 and CCR7 confirmed DC maturation, and suggested that these DC could migrate to the draining lymph nodes, in response to CCR7 ligands. Moreover, ICAM-1 neutralization on BEC strongly reduced the effect of bronchial epithelium on CCL17 and CCL22 secretion and on the phenotype of recruited MDDC. This suggests that ICAM-1/LFA-1 interactions between BEC and DC precursors are involved in DC activation as well as in the subsequent recruitment of T cells and eosinophils (32). Local administration of KpOmpA in the experimental model also shows that this PAMP induces the recruitment of myeloid DC and the production of CCL20 by BEC in vivo. We have also reported that KpOmpA-exposed animals have an increased expression of ICAM-1 (13) suggesting that both CCL20 and ICAM-1 are involved in the in vivo DC/BEC crosstalk. GM-CSF production by BEC is also important in the interactions between DC and BEC, because addition of neutralizing anti-GM-CSF Ab during the coculture inhibited the secretion of CCL17 and CCL22 whereas this treatment did not affect the expression of membrane DC markers (data not shown). Other adhesion molecules or tight junction proteins may be also involved in the DC/BEC interactions. Because some DC function such as cytokine production (particularly IL-10 and IL-12) are down-regulated by BEC, additional experiments are required to evaluate the potential implication of BEC-derived inhibitors in this process (such as arachidonic acid metabolites).

An intriguing question concerns the consequence of DC recruitment and maturation into airways from healthy donors. In mice, airway exposure to an Ag, such as OVA, induces DC-dependent development of a specific tolerance through ICOS-L expression and IL-10 production (33). In contrast, association of OVA with TLR2 or TLR4 ligands triggers airway sensitization and the development of an allergic reaction, after subsequent OVA instillation (34). Concerning the polarization of Th cell, murine resting airway DC preferentially skew the T cell response toward a Th2 profile suggesting that lung environment favors this type of response (35, 36). Thus, we evaluated the capacity of MDDC precursors cocultured with BEC to activate and polarize T cells in the context or not of an exposure to a PAMP. At steady state, BEC promoted an increased production of IL-10 by T cells, suggesting that BEC may be involved in tolerance induction. In the absence of proinflammatory signals, partially mature DC probably induce an abortive proliferative response of unfit T cells. Airway exposure to a protein Ag in mice leads to the development of Ag-specific tolerance associated with induction of regulatory T cells, as shown by Akbari et al. (33). In this situation, tolerogenic lung DCs have a phenotype of mature DC expressing IL-10 and ICOS-L. We have also analyzed the T cell response in a situation with stronger T cell activation: a MLR. In this case, coculture with BEC did not affect T cell proliferation whereas their ability to induce IL-4 and IL-10 production was not markedly affected by the coculture with BEC. The fact that intraepithelial DC appear more mature than the basolateral cells (Fig. 3) could explain the difference in cytokine production by T cells. However, these differences are not sufficient to modify the T cell proliferation. These
Thus, bronchial epithelium targeting represents a novel strategy in the control of airway tolerance and, in the context of a PAMP interaction with DC. Moreover, the BEC/DC crosstalk may be implicated in inflammatory processes (37) but also control the local immune response by its action on DC traffic and functions.

In addition to its role in the innate immune response, bronchial epithelium may link innate and acquired immunity through its dialog with DC. Moreover, the BEC/DC crosstalk may be implicated in the control of airway tolerance and, in the context of a PAMP exposure, may favor the development of the immune response. Thus, bronchial epithelium targeting represents a novel strategy in vaccination process via airway mucosa.

Acknowledgments

We thank Philippe Marquillies for helpful technical assistance and Dr. Catherine Duez for the critical reading of the manuscript.

Disclosures

The authors have no financial conflict of interest.

References