

Review article

Interactions between epithelial cells and dendritic cells in airway immune responses: lessons from allergic airway disease

Micro-organisms constantly invade the human body and may form a threat to our health. Traditionally, concepts of defence mechanisms have included a protective outer layer of epithelia and a vigilant immune system searching for areas where the integrity of the outer layer may be compromised. Instead of considering these elements as two independent mechanisms, we should be treating them as a single integrated system. This review will present and discuss the role of local immune-competent cells and local epithelia in the recognition of potential pathogens and how the interaction between the two components may affect the initiation of the airway immune response. A concept emerges where airway mucosal dendritic cells act as integrators of both immunostimulatory and immunosuppressive signals that act within actively-involved mucosal tissue.

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Key words: airway epithelial cells; allergy; dendritic cells; immune response.

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Accepted for publication 15 April 2008

We are constantly exposed to environmental factors, viruses, and bacteria and some micro-organisms may constitute a threat to our health. Traditionally, concepts of defence mechanisms for these threats have included a protective outer layer of epithelia and a vigilant immune system searching for areas where the integrity of the outer layer may have been compromised. There is a growing awareness that our views of these two defence mechanisms need refining. Instead of considering them as two relatively independent mechanisms, we should be treating them as a single integrated system.

This review presents and discusses the role of local immune-competent cells and local epithelia in the recognition of potential pathogens and how the interaction between both components may affect the initiation of the immune response. As these mucosal epithelia are the first to interact with, and respond to, many different environmental factors, these signals could affect the functional characteristics of local immune competent cells and could have consequences for the subsequent immune response. To clarify the complexity of the initiation of immune responses in the context of airway mucosa, we provide an extensive description of the nature of the two main local components: dendritic cells (DCs) and epithelial cells.

The regulation events in the local mucosa cannot be viewed in isolation. This review therefore opens with an introduction to the involved T-helper cells that defines the

different types of basal immune responses. We will discuss the heterogeneity of human DCs as well as the prevalence and potential functional specializations of local DCs in human and animal airways. Ultimately, the functional characteristics of airway epithelial cells and their direct and indirect interactions with mucosal DCs will result in a model of the mucosal immune response. In this model, DCs need to integrate signals from many different sources so that the correct immune response will be initiated.

The terminology describing the processes that act on DC can be highly confusing. Different authors may use identical terms to mean different things or different terms to describe the same phenomenon. To avoid any confusion about the terminology used here, a brief description is warranted of the context we have in mind. When we refer to the *regulation* of the immune response, we describe the outcome of signals acting on the DCs. This includes the stimulation, inhibition, or modulation of the immune response. The *activation* of DCs in the mucosa includes a multitude of processes that will allow immune responses to initiate. This process includes the maturation of DCs (i.e. up-regulation of co-stimulatory molecules) and the ability of the DCs to reach the lymphatic vessel in the local tissue (i.e. up-regulation of chemokine receptors) to take the locally integrated signal away from the tissue to the draining lymph node. Differential expression levels of adhesion molecules and receptors for lymphatic

chemo-attractants contribute to the activation process of DCs in the local tissue and will have consequences for the overall outcome of the immune response. We will use the term *modulation* to describe how factors produced by these other cells influence the activation processes of DCs.

The above implies a concept in which mucosal DCs act as integrators of both immunostimulatory and immunosuppressive signals originating directly from the environment and from actively-involved mucosal tissue, acting collectively to regulate the local immune response.

The different T lymphocyte helper cells in the basal immune response

One of the critically important cells in the immune system to contact any micro-organism is the DC. After the uptake of the micro-organism, a DC leaves the local tissue and migrates to a draining lymph node where it induces the proliferation of antigen-specific T lymphocytes resulting in either a T-helper 1 (Th1) cell, T-helper 2 (Th2) cell, Regulatory T-cell (Treg) or T-helper 17 (Th17) cell response dependent on the modulation and activation status of the DC.

Th1 cells evolve when the peripheral DC has been exposed to intracellular pathogens (virus, bacteria, and some protozoa) and are characterized by the production of Interferon (IFN) γ . Th2 cells develop when the DC has been exposed to an extra cellular parasite and are characterized by the production of interleukin (IL)-4, IL-5 and IL-13. Recently, another population of Th cells has been described. Th17 cells are characterized by the production of IL-17, resulting in the recruitment of neutrophils into local tissues. This feature suggests a role in the immune response against extra cellular bacteria (1).

Another type of Th cells is the Treg cell. Both Th1 and Th2 responses can be suppressed by adaptive Treg cells through contact-dependent mechanisms and/or the production of IL-10 and TGF- β (2, 3). Although this cell type is very important in controlling the activity of both Th1 and Th2 responses, we focus in this review on the role of epithelial cells in the initiation and modulation of a Th1 or Th2 response.

Although there is considerable knowledge about the signals expressed by DCs that guide the development of

Th1 responses, less is known about the development of Th2 cells from naïve T cells. Moreover, it is still unclear which signals are responsible for the initial instruction of the DC when it still resides in a local epithelium, and is surrounded by a multitude of micro-organisms (whether pathogenic or not) or other antigenic triggers of the immune system as in, for instance, the mucosa of the gut or the upper and lower airways. This raises the interesting question of how the immune system deals with a barrage of potentially confusing signals and how it not only makes sure that the response is appropriately Th1 or Th2, but even makes sure that such a response is required at all. In auto-immune or allergic diseases, this process breaks down. An inappropriate Th1 response against a host factor contributes to the pathogenic mechanism in auto-immune diseases, whereas an inappropriate Th2 response against an otherwise innocent environmental factor contributes to the pathogenic mechanism in allergic diseases.

Dendritic cell heterogeneity

Dendritic cells constitute a heterogeneous class of specialized antigen presenting cells. There are several reasons for the complexity of the characterization of DC subtypes based on the expression of cell/cell-surface molecules. The expression of cellular molecules may depend: (i) on the myeloid or lymphoid origin of the DC, (ii) on their Th1-, Th2-, Th17- or regulatory T-cell-inducing potential after activation, and (iii) on the tissue localization within the organism. The characterization of different DC subtypes is further complicated by the fact that some molecules are also found on cell types other than DCs, and by the use of different/unique subdivisions of DCs based on author's own preferred molecules.

In human peripheral blood, we can discern at least four cell types of myeloid origin (Table 1). These mDCs develop from bone marrow-derived monocytic precursors, express common myeloid surface markers (CD11c, CD13) and require granulocyte-macrophage colony-stimulating factor (GM-CSF) *in vitro* for their survival (4). Two myeloid DC subtypes can be distinguished depending on whether they express BDCA-1/CD1c or BDCA-3, markers that are initially found in a search for specific Blood Dendritic Cell Antigens.

Table 1. Characteristics of human dendritic cell (DC) subsets in nasal mucosa

	BDCA-1 ⁺ mDC	BDCA-3 ⁺ mDC	Langerhans cell (LC)	Plasmacytoid DC (pDC)
Origin	CD34 ⁺ myeloid progenitor	CD34 ⁺ myeloid progenitor	CD34 ⁺ myeloid progenitor	Lymphoid
Markers	CD11c ⁺ BDCA-1 ⁺ (CD1c) CD33 ⁺ CD13 ⁺	CD11c ⁺ BDCA-3 ⁺	CD1a ⁺ CD1c ⁺ E-cadherin Langerin Birbeck Granules	CD123 ⁺ BDCA-2 ⁺ BDCA-4 ⁺
Growth and survival dependent <i>in vitro</i>	GM-CSF	GM-CSF	TGF- β	IL-3

A third type DC of myeloid origin can be present in certain tissues. The Langerhans cells (LCs) are a distinct population of DCs that are located in epithelial tissues such as the airway mucosa and the epidermis of the skin. LCs are characterized by the expression of CD1a, E-cadherin, Langerin, and the presence of Birbeck granules (5). The origin of LCs is not fully understood. Reports indicate that they can originate from both a CD14⁻ and a CD14⁺ myeloid precursor (4–6). In addition to GM-CSF, there is a crucial role for transforming growth factor (TGF)- β in the differentiation process of LCs. The fourth type of DC is the CD34⁺ DC, which will not be discussed in this review.

The cellular origin of plasmacytoid DCs (pDCs) differs from that of mDCs. pDCs, as their name suggests, bear a morphological resemblance to plasma cells and are related to the lymphoid lineage (7). The growth and survival of pDC depend *in vitro* on IL-3, which also explains the high level of expression of CD123 (IL-3 receptor α -chain) on these cells.

In addition to their differences in terms of identifying lineage markers, DC subsets are distinguished on the basis of different expression profiles for pathogen/pattern recognition receptors, co-stimulatory molecules, or differentiation/activation molecules. Putting aside their evolutionary origin, the expression of these molecules affects DC function, the distinctive responsiveness of DCs to pathogens and the selective production of T-cell polarizing molecules that drive the development of Th1, Th2 or regulatory T cells (8–13).

Distinct subtypes of DCs may have different functional properties. This functional specialization is, at least partly, controlled by Toll-like receptors (TLRs), a group of transmembrane proteins that play a critical role as pattern-recognition receptors (PRRs). PRRs include soluble and transmembrane molecules that bind micro-organism-derived molecules and include scavengers such as C-reactive protein, scavenger receptors, Lectins, nucleotide-binding oligomerization domain (NOD) molecules and TLRs. Toll-like receptors are under intensive investigation. They recognize pathogen-associated molecular patterns (PAMP), the molecular signatures of microbial pathogens like bacteria and viruses (13, 14).

Classical blood-derived myeloid DCs (CD11c⁺) express TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, and TLR7 (13, 14). Myeloid DC can therefore recognize several bacterial components via TLR1 as a heterodimer with TLR2 (PAM3CSK4 [Lipopeptide]), TLR2 (e.g. Lipoteichoic acid, Peptidoglycan), TLR4 (lipopolysaccharide [LPS]), TLR5 (Flagellin) and TLR6, as a heterodimer with TLR2 (MALP-2, lipoprotein), viral double-stranded (ds) RNA via TLR3 and viral single-stranded (ss) RNA via TLR7 (15).

Unlike mDCs, human pDCs express only TLR6, TLR7, TLR8, and TLR9. Through these receptors, pDCs selectively recognize DNA-containing CpG motifs via TLR9 and ssRNA via TLR7 and TLR8 (15) and appear

to be of particular importance in innate immunity against several viral pathogens (10) producing IFN- α , a type I IFN (16). It has also been shown that pDCs are able to induce Th2 responses after CD40L stimulation (16, 17), but the exact role of pDCs in the induction of specific immune responses is not fully clear yet.

To our knowledge, there are no data about the TLR-expression of mucosal LCs. Van der Aar et al. showed by using RT-PCR that epidermal LCs express TLR1, TLR3, TLR6, and TLR7 and that the expression of TLR2, TLR4, TLR5, and TLR8 is weak or even absent. Furthermore, skin derived LCs do not express TLR9 and TLR10 (18). In contrast to this report, a recent study showed, by using mAbs, that LCs from human skin express TLR1, -2, -5, -6, and -9, the cognate receptors for detection of specific bacteria-derived molecules (19). Both studies show convincing data on TLR stimulation. Because of this, discrepancies between studies could be searched in the preparation methods of LCs and the surgical specimens used.

The general opinion is that TLRs function mainly as inducers of inflammation through the production of IL-6 and tumor necrosis factor (TNF)- α , and direct innate and adaptive immunity. Most TLR ligands stimulate APCs to produce Th1-inducing cytokines such as IL-12 and IL-18 and support Th1-skewed immune responses. TLR2 and TLR5 agonist provoke Th2 immune responses under certain conditions (20). In addition, TLR2 ligation has been shown to induce Tregs (12). Low doses of LPS acting through TLR4 have been shown to induce Th2 responses (21, 22).

The molecular basis for Th1/Th2 polarization can be unexpectedly diverse as shown by the group of Kapsenberg and others. They found that both mDCs and pDCs are capable of inducing Th1- or Th2-cell responses depending on the interaction of the TLRs and other PRRs with different microbial ligands (23). The role of PAMPs, TLRs, and other Th-cell polarizing factors in the induction of Th1-cell responses seems well defined, but factors that promote Th2- or regulatory T-cell responses are less well understood.

Whether these specific effects seen *in vitro* also occur *in vivo* and whether potential Th-cell polarizing factors produced by epithelial cells may also affect DC function remains under explored. Sporri and Reis e Sousa (24) investigated whether inflammatory mediators produced *in vivo* can substitute for direct PAMP recognition, resulting in DC activation and priming. By comparing direct and indirect activation induced by microbial stimuli of TLR-sufficient and TLR-deficient DCs in an experimental animal model, it was shown that direct recognition was crucial for priming of an appropriate T cell response. Indirectly activated DCs supported CD4⁺ T cell expansion, but failed to induce Th cell differentiation. In line with this observation, Blander and Medzhitov (25) showed that the generation of peptide-MHC complexes is controlled by TLRs in a strictly phagosome-

autonomous manner, although others have contradicted this finding. These studies show that TLR mediated activation and TLR mediated MHC-peptide presentation are critically important for the development of an appropriate T cell response.

Both the indirect and direct activation route can be found in the airway mucosa. The result of this double activation loop is still unexplored. It is also unknown whether antigen presentation is regulated differently in the various DC subtypes.

Dendritic cells in allergic airway disease in animals

Animal studies using transgenic and inducible knock out mouse models suggest that mDCs and pDCs play a differential role in the pathogenesis of allergic disease. Lambrecht's group explored the role of mDCs in the allergic cellular immune response. The injection of OVA-pulsed mDCs in the airways of naïve mice resulted in the typical characteristics of asthma. mDCs were shown to migrate to the regional draining lymph nodes of the lung and induce Ag-specific T-cell activation resulting in sensitization to inhaled allergen (26, 27). Once sensitization has been established, mDCs play a critical role in the maintenance of eosinophilic airway inflammation, as was shown using a thymidine kinase (TK)-transgenic-based DC-depleting mouse model (28). The depletion of mDCs during allergen challenge in sensitized mice stops Th2 cytokine production, IgE synthesis, and the infiltration of eosinophils. The same group provided additional evidence for the role of mDCs in the late phase allergic immune response. Relevant allergen challenge in OVA-sensitized mice caused an almost 100-fold increase in of myeloid DCs in the airways. This was accompanied by an increase in the bone marrow of early myeloid progenitors, which may be related to a rise in serum eotaxin (29).

Whether these observations are specific for mDC is unclear, as a similar set of experiments have not been conducted for pDC. Animal studies did show that pDCs were important for the maintenance of tolerance to inhaled harmless antigen (30). By depleting pDCs using a pDC-specific antibody, inhalation to otherwise inert OVA led to the development of the typical histological features of asthma; goblet cell hyperplasia, airway hypereosinophilia, Th2 cytokine secretion, and IgE production. When allergen-pulsed pDCs were transferred prior to sensitization, it was no longer possible to induce these responses.

Dendritic cells in human airway mucosa

Animal studies provide us with important data about the function of DCs in allergic airway inflammation; however, for obvious reasons these animal studies cannot be repeated in humans. The exact function of the different

human DC subsets in the sensitization and maintenance of allergic airway disease is therefore less clear. Instead of functional analysis, most human studies have focused on the dynamics of DC subsets. Immunohistochemical stainings of human lung parenchyma and nasal mucosa by multiple authors have identified both mDCs (CD11c⁺ HLA-DR⁺), LCs (CD1a⁺), and pDCs (CD123⁺) (31–36). It is not fully clear whether these DC subsets reside in the human airway or enter after stimulation.

Recently, three lung DC subsets that are also present in the peripheral blood were described together with some of their functional characteristics (33, 37–39). Single-cell suspensions obtained from surgical resection specimens of normal human lung parenchyma identified the two myeloid DC subsets (CD11c⁺/BDCA-1⁺ and CD11c⁺/BDCA-3⁺), and one plasmacytoid DC subset (CD123⁺BDCA-2⁺) (33). The authors also showed that a majority of CD1c⁺ DC also express CD1a and that there are small populations of CD1a⁺ CD1c[−] DCs and CD1a[−] CD1c⁺ DCs. Unfortunately, no data were presented on additional LC markers such as E-cadherin, Langerin, or the presence of Birbeck granules and hence the identity of these cells could not be firmly established. This is relevant as it has been shown in human skin that CD1a and CD1c are both present on one homogenous population of LCs (40).

Experiments with purified DC populations from surgical specimens have shown that both pulmonary myeloid DCs express mRNA TLR transcripts (TLR1, TLR2, TLR3, TLR4, TLR6, and TLR8) and that both cell types release pro inflammatory cytokines (TNF- α , IL-1 β , IL-6, and IL-8) in response to TLR2 and TLR4 ligands. No expression of IL-10 or IL-12 has been detected. Although both cell types also express TLR3, specific ligands induce cytokine release in BDCA-1⁺ pulmonary DCs, but not in BDCA-3⁺ pulmonary DCs. In contrast, pDC express only TLR7 and TLR9 and these receptors seem functional. Isolated pDCs release pro inflammatory cytokines in response to imiquimod (TLR7) and IFN- α in response to CpG oligonucleotides (TLR9). In a mixed lymphocyte reaction pulmonary BDCA-1⁺ DCs were shown to be strong inducers of T cell proliferation, while pDCs hardly induce any T cell proliferation. BDCA-3⁺ pulmonary DCs have an intermediate T cell-stimulatory capacity (39). These results reveal potential divergent roles for the different human lung DC subsets.

Recently, KleinJan et al. showed that in symptomatic patients with perennial allergic rhinitis (PAR), the number of myeloid DCs (CD11c⁺/MHCII⁺) is higher in the epithelium and lamina propria of the nasal mucosa than in healthy control subjects (41). Moreover, these DCs had a more activated (CD86⁺) phenotype and were found in close proximity to T lymphocytes, although the implications of this last observation are not clear. The authors ascribe the differences in DC numbers between PAR patients and healthy controls to constant exposure to the relevant antigen, which is thought to induce a persistent

inflammation. A similar picture emerges for BDCA-1⁺ myeloid DC (BDCA-1/CD1c⁺ HLA-DR⁺) in the lungs of asthmatic patients. Compared to baseline in asthmatic subjects, a rapid increase (within 4–5 h) in the number of BDCA-1⁺ myeloid DC was observed in the lamina propria after allergen challenge (42). The rapid accumulation of these cells strongly suggests that they are directly recruited from peripheral blood and not formed locally from a resident precursor cell. There is also a dynamic influx of myeloid Langerhans cells after allergen exposure. Increased numbers of CD1a⁺ cells were found in the mucosa of symptomatic allergic patients with seasonal allergic rhinitis (SAR) after nasal allergen challenge (43, 44) and in patients with perennial rhinitis (41).

As well as myeloid DCs, plasmacytoid DCs also exhibit a dynamic behaviour in allergic disease, although some inconsistencies have been observed for this cell type. Jahnsen et al. succeeded in showing changes in pDCs, whereas KleinJan et al. did not. Patients with SAR were challenged topically out of season with relevant allergen daily for 7 days (34). Biopsy specimens were obtained before and during such provocation and pDCs were identified in biopsy specimens through their high levels of CD123 expression. pDCs were present in low and variable numbers in normal nasal mucosa from unchallenged allergic, and non-allergic, individuals, but cell numbers increased dramatically only after daily provocations for 7 days in allergic subjects and not after shorter exposures as compared to non-allergic subjects. These observations contradict a study by the same authors of the lower airways, where no significant recruitment of pDCs was observed in the lung just 4–5 h after allergen challenge (42). Nor was any increase in the number of CD123⁺ cells detected at baseline in comparison with nasal biopsies from PAR patients and healthy individuals (41). Unfortunately, this study provided no data about the effect of allergen provocation, but the author's assumption was that all subjects received maximum exposure to HDM as biopsies were taken during the winter when the house dust mite is most prevalent. It remains to be seen whether this assumption is correct as, in a house dust mite provocation model, nasal provocation can induce a clear exacerbation of nasal complaints (45). Other reasons for the discrepancies between the studies could be the different types (seasonal versus perennial) of allergic rhinitis patients. Perennial allergic rhinitis results in chronic nasal inflammation, which may result in a different inflammatory microenvironment from that found in acutely-induced inflammation seen in SAR patients. The second explanation may be the different time points at which the biopsies were taken by the different groups, as Jahnsen has shown that induction may need several days of constant (high) allergen exposure.

In summary, the above observations from animal studies complemented with data from human studies, suggest that myeloid and plasmacytoid DCs play a

specific role in the allergic sensitization and the maintenance of allergic inflammation, both in asthma and in allergic rhinitis. Less is known about the specific function of LCs in the human airway mucosa, but future functional research should not be restricted to the role of LCs in allergic airway disease, as many open questions remain to be answered with respect to the roles of mDCs and pDCs.

The airway epithelium

Although airway DCs are believed to be the conductor of immunological response, there is a growing awareness that these cells are activated in the context of a mucosal environment where their function is likely to be influenced by the presence of local immunomodulatory signals. A substantial proportion of these signals originate in the airway epithelium. The outer lining of the mucosa not only plays a passive role as a physical barrier between the internal and external environment, it should also be seen as an active component of the mucosal defense system.

We are constantly exposed to air pollutants, airborne allergens, and (non)-pathogenic micro-organisms (46). A majority of these environmental factors are mechanically removed by the mucociliary system, which consists of mucus containing glycoproteins, lysozymes and ciliated epithelial cells. Even when particles are not removed, they are not free to enter the local mucosal tissue as the mucosal epithelium forms a second physical barrier because of the presence of apically-located tight junctions (TJs) between adjacent epithelial cells. In addition to their passive role as a barrier, the epithelial cells are capable of responding to the environmental factors by expressing specific receptors, resulting in the release of pro- and anti-inflammatory cytokines and chemokines.

Airway epithelial cells express PRRs that recognize components of micro-organisms (TLRs) or protease activated receptors (PAR) that can interact with specific components of allergens (47–50). Airway epithelial cells express mRNA for the TLRs1–6 (47) and can respond to various stimuli such as viruses, bacteria and cytokines, resulting in the expression of different pro- and anti-inflammatory cytokines and chemokines and the modulation of the immune response. Stimulation of TLR3 by Poly (I:C) resulted in the increased secretion of IL-6, IL-8, TNF- α , GM-CSF, GRO- α , TARC, MCP-1, MIP-3 α , RANTES, IFN- β and IP-10 (47).

It is not known at present to what degree different expression levels and triggering of PRRs influence the nature of the induction of an airway immune response. As mentioned earlier, TLR-mediated activation and TLR-mediated MHC-peptide seems crucial for the induction of an antigen specific T-cell response (24, 25). This suggests that epithelial cells have a modulatory function in the airway immune response. Another interesting

subject is the regulation of the expression of these receptors in epithelial cells and whether they are expressed differently in healthy and allergic subjects.

Epithelial cells also respond to stress conditions – for example, environmental pollutants such as Diesel Exhaust Particles (DEP), particulate matter (PM), and cigarette smoke – by releasing a variety of cytokines (51, 52). Studies have shown that epithelial cells stimulated with DEPs release cytokines such as IL-6, IL-8, GM-CSF, and sICAM (51). The exposure of epithelial cells from allergic subjects to DEPs and tobacco contributed to a greater constitutive and pollutant-induced release of pro-inflammatory chemokines (53). Epithelial cells may therefore play an important role in mediating the effects of air pollutants on the inflammatory response in the airways. The precise mechanism of activation, however, has not been fully elucidated.

Airway epithelium therefore not only functions as a mechanical barrier, but it can also respond to viral and bacterial pathogens or stress conditions by releasing chemokines and cytokines, and therefore playing an active role in the airway immune response.

Pathways of antigen uptake in the respiratory tract

Even though it is clear that the concept of the epithelium as a physical barrier will have consequences for the entry of antigens, only a few studies have tried to address the question of allergen uptake by tissue-resident DCs. As a consequence, the exact mechanism of antigen uptake remains poorly understood. The classical concept was that the epithelial barrier needed to be disrupted for antigen to reach DCs. This would seem to hold true for the skin. However, in the case of mucosal surface, it was discovered that DCs can sample the environment without disrupting the epithelial barrier (54). This characteristic of DCs could reflect the daily exposition to numerous antigens and pathogens, which pass the mucosal surface without the presence of a mucosal inflammation.

Several mechanisms have been proposed for antigens or allergens to reach DCs. Der p1, one of the major components of house dust mite (HDM) was shown to cleave the tight junction adhesion protein occludin by means of the allergen's own proteolytic activity. TJ breakdown increases epithelial permeability nonspecifically, allowing Der p1 to cross the epithelial barrier. The transepithelial movement of Der p1 to DCs via the paracellular pathway may therefore be advanced by the allergen's own proteolytic activity (55). Takano et al. demonstrated a different way for DCs to sample allergen without disrupting the TJs. In biopsies taken from human allergic subjects, HLA-DR⁺ and CD11c⁺ DCs expressed claudin-1 and penetrated beyond occludin in the epithelium of the nasal mucosa (53). Interestingly, this process was only seen in allergic individuals, and not in healthy ones. This observation was also made in an

experimental model. Using a gut epithelial cell line-based *in vitro* co-culture system, DCs were shown to extend their dendrites across the epithelial barrier by means of the inducible expression of the tight junction proteins occluding, claudin 1, and zonula occludens 1 (54). Moreover, in mice, an epithelial CD103 (alphaE)-beta7 integrin-positive DC population expressing high levels of the LC-marker Langerin and the tight junction proteins Claudin-1, Claudin-7, and ZO-2 was described (56). And finally, using a three dimensional reconstruction of immunofluorescence staining of the airway mucosa of rats, 1–5% of all intra-epithelial DCs were shown to project cellular extensions between epithelial cells to the apical surface (57). These *in vitro* and *ex vivo* data imply a strong physical connection between epithelial cell and DC; tight junctions are formed to maintain the integrity of the epithelial barrier, while DCs are allowed to sample antigen at the mucosal surface by extending dendrites to the apical lumen. It is unclear whether this sampling process occurs spontaneously, but there is some evidence that it requires the expression of the fractalkine receptor CX3CR1 (58). This sampling of the exterior environment without disrupting epithelial integrity opens up a possible role for DCs in preparing for potential invasive pathogens prior to entry.

Airway DCs are therefore able to open the TJ between epithelial cells by extending their dendrites like a periscope to the lumen to sample the environment. These mechanisms proposed for the airway and gastro-intestinal tract suggest an active role for epithelial cells in the uptake of antigen. It still remains to be determined whether the DC-epithelial cell interactions in the uptake of antigen across the mucosal surface in the gastro-intestinal tract also hold true for the respiratory tract. Whether, and to what extent, airway epithelial cells are actively involved in the antigen uptake by DCs in the airway are issues requiring further investigation, as they may yield important information about the development of an airway immune response.

Epithelial cells and DCs: direct interactions

The discussion above looked at the formation of tight junctions as one example of what is believed to be mainly a structural epithelial cell-DC interaction. However, DCs also interact with epithelial cells by expressing of adhesion molecules that not only anchor them strongly in the epithelium, but also have a possible effect on their function.

Intercellular adhesion molecule (ICAM) is expressed by epithelial cells and this molecule can bind leukocyte function associated molecule-1 (LFA-1) expressed by immature DCs (59). Epithelial cells in patients with allergic rhinitis and asthma express increased levels of ICAM (60). The expression of ICAM by epithelial cells is regulated indirectly by the inflammatory cytokines

TNF- α and IL-1 (61) or directly by interaction with bacteria and viruses (62). The same interaction between ICAM-1/LFA-1 is known to support adhesion between DC and T cells, where it is involved in the induction of Th1 responses (63, 64). It is not known whether the ICAM-1/LFA-1 interaction between epithelial cells and DCs has a similar functional effect. Another adhesion molecule, which could be important in DC-epithelial cell contact, is E-cadherin. E-cadherin is expressed by bronchial epithelial cells and immature DCs (65). Importantly, E-cadherin needs to be down regulated for DCs to leave the epithelium to transfer to the lymph node (66).

Immature DCs expressing both LFA-1 and E-cadherin are therefore able to bind epithelial cells at the site of allergen entrance. No proof has been found to date that this binding takes place in airway mucosa. Nor is it known whether these interactions are purely structural or whether they have functional consequences for DC function. In addition to adhesion, ICAM/LFA-1 interaction could play an important role in the activation of tissue resident DCs by epithelial cells and contribution to the airway immune response.

Epithelial cells and DCs: DC recruitment by the production of epithelial chemokines

The exposure of epithelial cells to environmental factors triggers the expression of chemokines involved in recruitment of inflammatory cells including DCs (67–69). For instance, stimulation of epithelial cells with different allergen extracts results in the production of the chemokines IL-8/CXCL8, RANTES/CCL5 (Regulated upon Activation T-cell derived and Secreted), and TARC/CCL17 (Thymus and Activation-Regulated Chemokine). All these factors are critical in the chemotaxis of neutrophils, eosinophils, mast cells, and lymphocytes (67–69).

When we focus on DCs, there is increasing evidence that the production of chemokines by epithelial cells is also responsible for the recruitment of DCs from the peripheral circulation into the airway mucosa. The study of Reibman et al. (70) shows that primary bronchial epithelial cell cultures secrete MIP3 α /CCL20 after stimulation with pro-inflammatory cytokines and particulate matter (PM). As CCL20 is the unique ligand for CCR6 expressed by immature Langerhans' cells (LC), this study hypothesized that airway epithelial cells are involved in the recruitment of immature DCs to the mucosal tissue. In addition to CCL20, epithelial cells produce other chemokines involved in DC recruitment. Pichavant et al. showed allergen-induced expression of CCL-2 (MCP-1), CCL5 (RANTES), and C-X-C chemokine ligand 10 (IP-10) by BEAS-2B (human bronchial epithelial cell line) and primary bronchial epithelial cells. The activity of these chemokines was reflected in the increased recruitment of monocyte-derived DC precursors in the mucosa

of both healthy and allergic individuals. This effect was also seen *in vitro*, where apical application of Der p1 to reconstituted polarized epithelium enhanced monocyte-derived DC precursor migration into the epithelial layer. Interestingly, the relative contribution of epithelium to DC recruitment depended on the DC subtype being investigated. Derp1 stimulation of bronchial epithelial cells from patients with asthma, but not from controls, increased the migration of LC precursors, which is mainly dependent on CCL20 secretion (71). Other factors produced by epithelium involved in the recruitment of DCs could be CCL13/ MCP-4 or eotaxin but also neuro-peptides and complement factors (29, 72–75).

In summary, these data demonstrate the role of lung epithelial cells in the recruitment of immature DCs and specifically the role of epithelial cells of allergic individuals, through the production and release of chemotactic chemokines. Research is required into the regulation and expression patterns of the specific receptors on DCs in the airway mucosa involved in this process.

Epithelial cells and DC: epithelial factors modulating DC function

Cytokines are key regulators of the differentiation or activity of immuno-competent cells and as such they play an important role in normal and pathological immune responses. Epithelial cells express at baseline or after exposure to environmental factors known to modulate the function of DCs. Over the years, a substantial part of the research in this field has focused on the classical factors GM-CSF, TNF- α , IL-1, prostaglandin E2 (PGE₂), and TGF- β . The combined exposure of LCs to TNF- α and IL-1 is an essential step in LC activation and LC migration out of the tissue toward the draining lymph nodes (76), while GM-CSF and TGF- β are required for the differentiation of DCs from their precursors (6). PGE₂ is not involved in the activation of DCs, but rather in the modulation of their function by down-regulating the production of pro-inflammatory cytokines, such as IL-8, IL-12, MCP-1, and GM-CSF (77–79). Recently, TSLP has been put in the spotlight, as this factor is considered to be responsible for the Th2-skewing of immune responses and the pathogenesis of allergic disease. TSLP will be discussed in more detail further on.

Bronchial alveolar and nasal lavages from patients with asthma and allergic rhinitis (80) and primary bronchial epithelial cell cultures from asthmatics show increased levels of IL-1, GM-CSF, and TNF- α compared to healthy subjects. Through the expression of these cytokines, epithelial cells are able to modulate DC function. The increased expression of these cytokines appears to be directly related to allergen exposure. Airway epithelium can interact directly with allergen as shown by the release of IL-8, eotaxin, and GM-CSF after the stimulation of the proteinase-activated receptor

(PAR)-2 by the house dust mite allergens Der p1, Der p3, and Der p9 (48–50). The increased PAR-2 receptor expression in bronchial epithelium from asthmatics and the increased epithelial expression upon allergen exposure possibly accounts for the higher mediator levels seen in bronchial or nasal lavages (81–83). The consequences of this epithelial cell activation for DC function remain to be determined.

A cytokine that has been spotlighted in the allergic immune response is TSLP. TSLP is a novel IL-7 like cytokine, which is produced by epithelial cells, keratinocytes, stromal cells, and mast cells (84). The interest in TSLP was triggered by its ability to activate human immature myeloid DCs, resulting in the induction of CD4⁺ T cell responses with a proallergic phenotype (85). High levels of TSLP are expressed by human keratinocytes of atopic dermatitis patients, but not in other non-allergic types of skin inflammation (84). *In situ* hybridization techniques have been used to show that higher levels of TSLP are expressed in the bronchial mucosa/sub mucosa of asthmatic patients compared with healthy controls (86). In Th1-mediated pathology in inflammatory bowel diseases such as Crohn disease TSLP mRNA was undetectable, whereas epithelial cells isolated from human healthy colon expressed TSLP constitutively (87).

These data suggest a role for TSLP in the induction of an allergic immune response via the stimulation of myeloid DCs. Even though TSLP has a broader spectrum of action in mice in which it stimulates the growth and differentiation of pre-B-cells, peripheral CD4⁺ T-cells, and myeloid DCs, the studies in mice confirm the human data (88). In a murine, lung-specific model, over-expression of TSLP induced allergic airway inflammation that was characterized by infiltration of eosinophils and Th2

cells (89). In addition, it was demonstrated that TSLP induced pro-allergic Th2 response from naïve precursors, whilst inhibiting Th1-associated (IFN- γ) or Treg-associated (IL-10) cytokine production. Furthermore, an *in vivo* allergic inflammatory model revealed that TSLPR-KO mice demonstrated a profound reduction of inflammation in the lungs after OVA-sensitization (90).

Rescigno et al. suggested that the working spectrum of TSLP could be dose-dependent. By using MoDCs and medium from bacteria-treated epithelial cells, they showed that a narrow window of TSLP concentrations (0.07–0.15 ng/ml) inhibits IL-12 secretion (i.e. stimulate a Th2 response). When the concentrations were outside the window, DCs were able to promote IFN- γ producing T-cells (i.e. stimulate a Th1 response) (89).

Because expression of TSLP appears to be very important in the induction of a Th2 allergic immune response, the regulation of TSLP expression has been investigated recently. A role for retinoic acid signalling in the expression of TSLP was shown in epidermal keratinocytes of the mouse (91). In human airways, the expression of TSLP is triggered by activation of TLR3 by dsRNA, by infection with rhinovirus and by stimulation with Th2 cytokines (92). Regulation of TSLP expression involves NF- κ B and IFN regulatory factor 3 (IRF-3) signalling via TLR3 (93, 94). IL-4 and dsRNA have a synergistic effect on the TSLP production and suggest an amplification of Th2 inflammation via the induction of TSLP in the asthmatic airway. This is a rather surprising observation as PolyI:C stimulation and rhinovirus infection result in the induction of a Th1-type immune response.

Whether TSLP is a crucial epithelial factor in the development of allergic airway diseases like asthma and

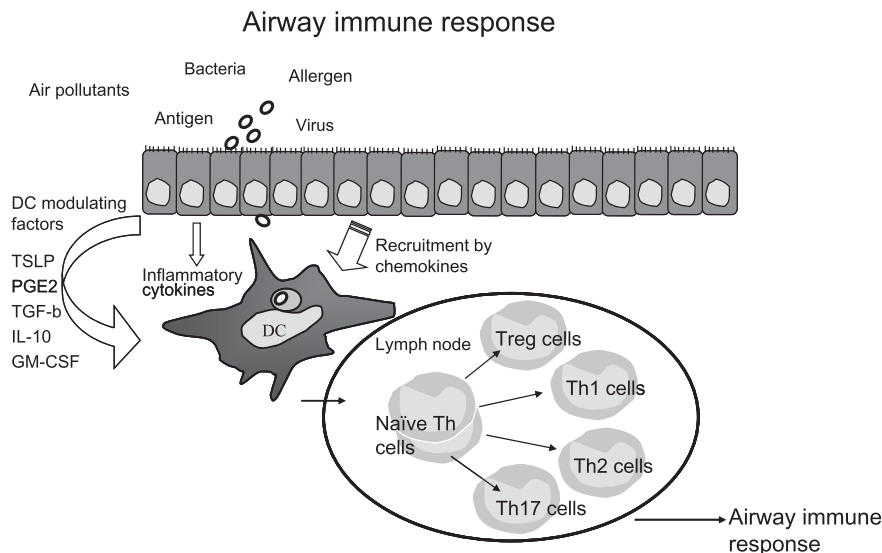


Figure 1. Activation of airway epithelial cells with different antigens/allergens not only induces the expression of chemokines resulting in recruitment of immature dendritic cells (DCs) but also creates an immunomodulatory micro-environment in which immature DCs take up antigen. A concept emerges where mucosal DCs act as integrators of both immunostimulatory and immunosuppressive signals that act within an actively involved mucosal tissue.

allergic rhinitis is an issue requiring further investigation. The immunohistochemical staining of biopsies from healthy and allergic subjects will give us an initial idea of whether expression levels of TSLP are related to allergic disease. It also will give us the opportunity to confirm the correlation observed between TSLP expression and disease severity in asthmatics.

In summary, these data show that airway epithelial factors have an important modulating role in DC function, differentiation, and maturation. An area of study that has been poorly explored is the role of epithelial cells in the inhibition of DC function, even though it has been reported that epithelial cells produce the immuno-repressive cytokines IL-10 and TGF- β . This role is suggested by the observation that a co-culture of monocyte-derived DC with Caco-2 intestinal epithelial monolayers resulted in DC with reduced expression of MHC class II, CD86, and CD80, and poor T cell stimulatory capacity. Cytokine profiles showed reduced levels of inflammatory cytokine production, and co-cultured DCs were less sensitive to stimulation via TLRs (TLR2, TLR4, and TLR6) as a result of increased levels of autocrine TGF- β production (95). Using a similar approach, commensal bacteria were also shown to mediate a tolerizing effect on the gut mucosal immune response (87, 96). Future studies should aim to identify epithelial cell-derived tolerizing factors and investigate the regulation of these factors both in healthy and allergic individuals.

Conclusion

Over the last years, it has become clear that epithelial cells play an important role in the modulation of immune response in the airways (Fig. 1). DCs can bind the

epithelial cells through the expression of adhesion molecules and tight junction molecules, which allow them to sample the mucosal surface without disrupting the mucosal integrity. The exact mechanism of antigen uptake by mucosal DCs across the epithelial barrier is not fully understood, but an active role for epithelial cells is proposed.

The activation of airway epithelial cells with different antigens/allergens not only induces the expression of chemokines resulting in the recruitment of immature DCs, but also creates an immunomodulatory micro-environment in which immature DCs exert their function. In the last decade, the characterization of the different DC subsets in human nasal and lung mucosa has taught us more about the presence and kinetics of DC subpopulations. With the help of new specific blood DC (BDC) markers and others, we will be able to characterize these specific subsets of DCs in further detail. To prevent difficulties in comparing studies, future research should aim to study both healthy and allergic subjects (seasonal or perennial) before and after the relevant allergen provocation. This will give us a detailed picture of the presence and function of potential different subtypes of DCs in the way that previous experiments have done for peripheral blood.

The cytokine/chemokine milieu created by the airway epithelium should be considered as a co-factor in the pathogenesis of allergic airway disease and as part of the normal airway immune responses. The interaction between airway epithelial cells and DCs deserves further attention. It is therefore crucial to study the contribution of epithelial cells and their role in the sensitization and maintenance of allergic airway disease is crucial. Emphasis should be placed on the use of primary tissue culture systems from healthy and allergic individuals to mimic the *in vivo* situation.

References

1. Ye P, Rodriguez FH, Kanaly S, Stocking KL, Schurr J, Schwarzenberger P et al. Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense. *J Exp Med* 2001;**194**:519–527.
2. Weiner HL. Induction and mechanism of action of transforming growth factor-beta-secreting Th3 regulatory cells. *Immunol Rev* 2001;**182**:207–214.
3. Roncarolo MG, Bacchetta R, Bordignon C, Narula S, Levings MK. Type 1 T regulatory cells. *Immunol Rev* 2001;**182**:68–79.
4. Shortman K, Liu YJ. Mouse and human dendritic cell subtypes. *Nat Rev Immunol* 2002;**2**:151–161.
5. Liu YJ. Dendritic cell subsets and lineages, and their functions in innate and adaptive immunity. *Cell* 2001;**106**:259–262.
6. del Hoyo GM, Martin P, Vargas HH, Ruiz S, Arias CF, Ardavin C. Characterization of a common precursor population for dendritic cells. *Nature* 2002;**415**:1043–1047.
7. Spits H, Couwenberg F, Bakker AQ, Weijer K, Uittenbogaart CH. Id2 and Id3 inhibit development of CD34(+) stem cells into predendritic cell (pre-DC)2 but not into pre-DC1. Evidence for a lymphoid origin of pre-DC2. *J Exp Med* 2000;**192**:1775–1784.
8. Kalinski P, Hilkens CM, Wierenga EA, Kapsenberg ML. T-cell priming by type-1 and type-2 polarized dendritic cells: the concept of a third signal. *Immunol Today* 1999;**20**:561–567.
9. Jarrossay D, Napolitani G, Colonna M, Sallusto F, Lanzavecchia A. Specialization and complementarity in microbial molecule recognition by human myeloid and plasmacytoid dendritic cells. *Eur J Immunol* 2001;**31**:3388–3393.
10. Kadowaki N, Antonenko S, Lau JY, Liu YJ. Natural interferon α / β -producing cells link innate and adaptive immunity. *J Exp Med* 2000;**192**:219–226.

11. Maldonado-Lopez R, Maliszewski C, Urbain J, Moser M. Cytokines regulate the capacity of CD8 α^+ and CD8 α^- dendritic cells to prime TH1/TH2 cells in vivo. *J Immunol* 2001;**167**:4345–4350.
12. van der Kleij D, Latz E, Brouwers JF, Kruize YC, Schmitz M, Kurt-Jones EA et al. A novel host-parasite lipid cross-talk. Schistosomal lyso-phosphatidylserine activates toll-like receptor 2 and affects immune polarization. *J Biol Chem* 2002;**277**:48122–48129.
13. Kadowaki N, Ho S, Antonenko S, Malefyt RW, Kastelein RA, Bazan F et al. Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J Exp Med* 2001;**194**:863–869.
14. Kaisho T, Akira S. Regulation of dendritic cell function through toll-like receptors. *Curr Mol Med* 2003;**3**:759–771.
15. Heil F, Hemmi H, Hochrein H, Ampenberger F, Kirschning C, Akira S et al. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. *Science* 2004;**303**:1526–1529.
16. Cella M, Jarrossay D, Facchetti F, Alebardi O, Nakajima H, Lanzavecchia A et al. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nat Med* 1999;**5**:919–923.
17. Gilliet M, Liu YJ. Generation of human CD8 T regulatory cells by CD40 ligand-activated plasmacytoid dendritic cells. *J Exp Med* 2002;**195**:695–704.
18. van der Aar AM, Sylva-Steenland RM, Bos JD, Kapsenberg ML, de Jong EC, Teunissen MB. Loss of TLR2, TLR4, and TLR5 on Langerhans cells abolishes bacterial recognition. *J Immunol* 2007;**178**:1986–1990.
19. Peiser M, Koeck J, Kirschning CJ, Wittig B, Wanner R. Human Langerhans cells selectively activated via Toll-like receptor 2 agonists acquire migratory and CD4 $^+$ T cell stimulatory capacity. *J Leukoc Biol* 2008;**83**:1118–1127.
20. Agrawal S, Agrawal A, Doughty B, Gerwitz A, Blenis J, Van Dyke T et al. Cutting edge: different Toll-like receptor agonists instruct dendritic cells to induce distinct Th responses via differential modulation of extracellular signal-regulated kinase-mitogen-activated protein kinase and c-Fos. *J Immunol* 2003;**171**:4984–4989.
21. Didierlaurent A, Ferrero I, Otten LA, Dubois B, Reinhardt M, Carlsen H et al. Flagellin promotes myeloid differentiation factor 88-dependent development of Th2-type response. *J Immunol* 2004;**172**:6922–6930.
22. Eisenbarth SC, Piggott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K. Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med* 2002;**196**:1645–1651.
23. Kapsenberg ML. Dendritic-cell control of pathogen-driven T-cell polarization. *Nat Rev Immunol* 2003;**3**:984–993.
24. Sporri R, Reis e Sousa C. Inflammatory mediators are insufficient for full dendritic cell activation and promote expansion of CD4 $^+$ T cell populations lacking helper function. *Nat Immunol* 2005;**6**:163–170.
25. Blander JM, Medzhitov R. Toll-dependent selection of microbial antigens for presentation by dendritic cells. *Nature* 2006;**440**:808–812.
26. Lambrecht BN, Pauwels RA, Fazekas De St Groth B. Induction of rapid T cell activation, division, and recirculation by intratracheal injection of dendritic cells in a TCR transgenic model. *J Immunol* 2000;**164**:2937–2946.
27. Lambrecht BN, De Veerman M, Coyle AJ, Gutierrez-Ramos JC, Thielemans K, Pauwels RA. Myeloid dendritic cells induce Th2 responses to inhaled antigen, leading to eosinophilic airway inflammation. *J Clin Invest* 2000;**106**:551–559.
28. Lambrecht BN, Salomon B, Klatzmann D, Pauwels RA. Dendritic cells are required for the development of chronic eosinophilic airway inflammation in response to inhaled antigen in sensitized mice. *J Immunol* 1998;**160**:4090–4097.
29. van Rijt LS, Prins JB, Leenen PJ, Thielemans K, de Vries VC, Hoogsteden HC et al. Allergen-induced accumulation of airway dendritic cells is supported by an increase in CD31(hi)Ly-6C(neg) bone marrow precursors in a mouse model of asthma. *Blood* 2002;**100**:3663–3671.
30. de Heer HJ, Hammad H, Soullie T, Hijdra D, Vos N, Willart MA et al. Essential role of lung plasmacytoid dendritic cells in preventing asthmatic reactions to harmless inhaled antigen. *J Exp Med* 2004;**200**:89–98.
31. Sertl K, Takemura T, Tschachler E, Ferrans VJ, Kaliner MA, Shevach EM. Dendritic cells with antigen-presenting capability reside in airway epithelium, lung parenchyma, and visceral pleura. *J Exp Med* 1986;**163**:436–451.
32. Fokkens WJ, Vroom TM, Rijntjes E, Mulder PG. CD-1 (T6), HLA-DR-expressing cells, presumably Langerhans cells, in nasal mucosa. *Allergy* 1989;**44**:167–172.
33. Demedts IK, Brusselle GG, Vermaelen KY, Pauwels RA. Identification and characterization of human pulmonary dendritic cells. *Am J Respir Cell Mol Biol* 2005;**32**:177–184.
34. Jahnsen FL, Lund-Johansen F, Dunne JF, Farkas L, Haye R, Brandtzaeg P. Experimentally induced recruitment of plasmacytoid (CD123high) dendritic cells in human nasal allergy. *J Immunol* 2000;**165**:4062–4068.
35. Bellini A, Vittori E, Marini M, Ackerman V, Mattoli S. Intraepithelial dendritic cells and selective activation of Th2-like lymphocytes in patients with atopic asthma. *Chest* 1993;**103**:997–1005.
36. Valladeau J, Duvert-Frances V, Pin JJ, Dezutter-Dambuyant C, Vincent C, Massacrier C et al. The monoclonal antibody DCGM4 recognizes Langerin, a protein specific of Langerhans cells, and is rapidly internalized from the cell surface. *Eur J Immunol* 1999;**29**:2695–2704.
37. Dzionek A, Fuchs A, Schmidt P, Cremer S, Zysk M, Miltenyi S et al. BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. *J Immunol* 2000;**165**:6037–6046.
38. MacDonald KP, Munster DJ, Clark GJ, Dzionek A, Schmitz J, Hart DN. Characterization of human blood dendritic cell subsets. *Blood* 2002;**100**:4512–4520.
39. Demedts IK, Bracke KR, Maes T, Joos GF, Brusselle GG. Different roles for human lung dendritic cell subsets in pulmonary immune defense mechanisms. *Am J Respir Cell Mol Biol* 2006;**35**:387–393.
40. Peiser M, Grützkau A, Wanner R, Kolde G. CD1a and CD1c cell sorting yields a homogeneous population of immature human Langerhans cells. *J Immunol Methods* 2003;**279**:41–53.
41. KleinJan A, Willart M, van Rijt LS, Braunstahl GJ, Leman K, Jung S et al. An essential role for dendritic cells in human and experimental allergic rhinitis. *J Allergy Clin Immunol* 2006;**118**:1117–1125.
42. Jahnsen FL, Moloney ED, Hogan T, Upham JW, Burke CM, Holt PG. Rapid dendritic cell recruitment to the bronchial mucosa of patients with atopic asthma in response to local allergen challenge. *Thorax* 2001;**56**:823–826.

43. Fokkens WJ, Broekhuis-Fluitsma DM, Rijntjes E, Vroom TM, Hoefsmit EC. Langerhans cells in nasal mucosa of patients with grass pollen allergy. *Immunobiology* 1991;**182**:135–142.
44. Godthelp T, Fokkens WJ, Kleinjan A, Holm AF, Mulder PG, Prens EP et al. Antigen presenting cells in the nasal mucosa of patients with allergic rhinitis during allergen provocation. *Clin Exp Allergy* 1996;**26**:677–688.
45. Oldenbeuving NB, KleinJan A, Mulder PG, Lumley P, de Groot EJ, van Drunen CM et al. Evaluation of an intranasal house dust mite provocation model as a tool in clinical research. *Allergy* 2005;**60**:751–759.
46. Folkerts G, Nijkamp FP. Airway epithelium: more than just a barrier! *Trends Pharmacol Sci* 1998;**19**:334–341.
47. Ritter M, Mennerich D, Weith A, Seither P. Characterization of Toll-like receptors in primary lung epithelial cells: strong impact of the TLR3 ligand poly(I:C) on the regulation of Toll-like receptors, adaptor proteins and inflammatory response. *J Inflamm (Lond)* 2005;**2**:16.
48. Vliagoftis H, Befus AD, Hollenberg MD, Moqbel R. Airway epithelial cells release eosinophil survival-promoting factors (GM-CSF) after stimulation of proteinase-activated receptor 2. *J Allergy Clin Immunol* 2001;**107**:679–685.
49. Asokanathan N, Graham PT, Stewart DJ, Bakker AJ, Eidne KA, Thompson PJ et al. House dust mite allergens induce proinflammatory cytokines from respiratory epithelial cells: the cysteine protease allergen, Der p 1, activates protease-activated receptor (PAR)-2 and inactivates PAR-1. *J Immunol* 2002;**169**:4572–4578.
50. Sun G, Stacey MA, Schmidt M, Mori L, Mattoli S. Interaction of mite allergens Der p3 and Der p9 with protease-activated receptor-2 expressed by lung epithelial cells. *J Immunol* 2001;**167**:1014–1021.
51. Terada N, Hamano N, Maesako KI, Hiruma K, Hohki G, Suzuki K et al. Diesel exhaust particulates upregulate histamine receptor mRNA and increase histamine-induced IL-8 and GM-CSF production in nasal epithelial cells and endothelial cells. *Clin Exp Allergy* 1999;**29**:52–59.
52. Roberts JW, Dickey P. Exposure of children to pollutants in house dust and indoor air. *Rev Environ Contam Toxicol* 1995;**143**:59–78.
53. Takano K, Kojima T, Go M, Murata M, Ichimiya S, Himi T et al. HLA-DR- and CD11c-positive dendritic cells penetrate beyond well-developed epithelial tight junctions in human nasal mucosa of allergic rhinitis. *J Histochem Cytochem* 2005;**53**:611–619.
54. Rescigno M, Urbano M, Valzasina B, Francolini M, Rotta G, Bonasio R et al. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol* 2001;**2**:361–367.
55. Wan H, Winton HL, Soeller C, Tovey ER, Gruenert DC, Thompson PJ et al. Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. *J Clin Invest* 1999;**104**:123–133.
56. Sung SS, Fu SM, Rose CE Jr, Gaskin F, Ju ST, Beaty SR. A major lung CD103 (alphaE)-beta7 integrin-positive epithelial dendritic cell population expressing Langerin and tight junction proteins. *J Immunol* 2006;**176**:2161–2172.
57. Jahnsen FL, Strickland DH, Thomas JA, Tobagus IT, Napoli S, Zosky GR et al. Accelerated antigen sampling and transport by airway mucosal dendritic cells following inhalation of a bacterial stimulus. *J Immunol* 2006;**177**:5861–5867.
58. Niess JH, Brand S, Gu X, Landsman L, Jung S, McCormick BA et al. CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science* 2005;**307**:254–258.
59. Schneeberger EE, Vu Q, LeBlanc BW, Doerschuk CM. The accumulation of dendritic cells in the lung is impaired in CD18^{-/-} but not in ICAM-1^{-/-} mutant mice. *J Immunol* 2000;**164**:2472–2478.
60. Vignola AM, Campbell AM, Chanez P, Bousquet J, Paul-Lacoste P, Michel FB et al. HLA-DR and ICAM-1 expression on bronchial epithelial cells in asthma and chronic bronchitis. *Am Rev Respir Dis* 1993;**148**:689–694.
61. Tosi MF, Stark JM, Smith CW, Hamedani A, Gruenert DC, Infeld MD. Induction of ICAM-1 expression on human airway epithelial cells by inflammatory cytokines: effects on neutrophil-epithelial cell adhesion. *Am J Respir Cell Mol Biol* 1992;**7**:214–221.
62. Frick AG, Joseph TD, Pang L, Rabe AM, St Geme JW 3rd, Look DC. *Haemophilus influenzae* stimulates ICAM-1 expression on respiratory epithelial cells. *J Immunol* 2000;**164**:4185–4196.
63. Smits HH, de Jong EC, Schuitemaker JH, Geijtenbeek TB, van Kooyk Y, Kapsenberg ML et al. Inter cellular adhesion molecule-1/LFA-1 ligation favors human Th1 development. *J Immunol* 2002;**168**:1710–1716.
64. Salomon B, Bluestone JA. LFA-1 interaction with ICAM-1 and ICAM-2 regulates Th2 cytokine production. *J Immunol* 1998;**161**:5138–5142.
65. Carayol N, Campbell A, Vachier I, Mainprice B, Bousquet J, Godard P et al. Modulation of cadherin and catenins expression by tumor necrosis factor-alpha and dexamethasone in human bronchial epithelial cells. *Am J Respir Cell Mol Biol* 2002;**26**:341–347.
66. Jakob T, Udey MC. Regulation of E-cadherin-mediated adhesion in Langerhans cell-like dendritic cells by inflammatory mediators that mobilize Langerhans cells in vivo. *J Immunol* 1998;**160**:4067–4073.
67. Terada N, Maesako K, Hamano N, Ikeda T, Sai M, Yamashita T et al. RANTES production in nasal epithelial cells and endothelial cells. *J Allergy Clin Immunol* 1996;**98**:S230–S237.
68. Sekiya T, Miyamasu M, Imanishi M, Yamada H, Nakajima T, Yamaguchi M et al. Inducible expression of a Th2-type CC chemokine thymus- and activation-regulated chemokine by human bronchial epithelial cells. *J Immunol* 2000;**165**:2205–2213.
69. Tomee JF, van Weissenbruch R, de Monchy JG, Kauffman HF. Interactions between inhalant allergen extracts and airway epithelial cells: effect on cytokine production and cell detachment. *J Allergy Clin Immunol* 1998;**102**:75–85.
70. Reibman J, Hsu Y, Chen LC, Bleck B, Gordon T. Airway epithelial cells release MIP-3alpha/CCL20 in response to cytokines and ambient particulate matter. *Am J Respir Cell Mol Biol* 2003;**28**:648–654.
71. Pichavant M, Charbonnier AS, Taront S, Brichet A, Wallaert B, Pestel J et al. Asthmatic bronchial epithelium activated by the proteolytic allergen Der p 1 increases selective dendritic cell recruitment. *J Allergy Clin Immunol* 2005;**115**:771–778.
72. Vanbervliet B, Homey B, Durand I, Massacrier C, Ait-Yahia S, de Bouteiller O et al. Sequential involvement of CCR2 and CCR6 ligands for immature dendritic cell recruitment: possible role at inflamed epithelial surfaces. *Eur J Immunol* 2002;**32**:231–242.

73. Hosoi J, Murphy GF, Egan CL, Lerner EA, Grabbe S, Asahina A et al. Regulation of Langerhans cell function by nerves containing calcitonin gene-related peptide. *Nature* 1993;**363**:159–163.
74. Kradin R, MacLean J, Duckett S, Schneeberger EE, Waeber C, Pinto C. Pulmonary response to inhaled antigen: neuroimmune interactions promote the recruitment of dendritic cells to the lung and the cellular immune response to inhaled antigen. *Am J Pathol* 1997;**150**:1735–1743.
75. Humbles AA, Lu B, Nilsson CA, Lilly C, Israel E, Fujiwara Y et al. A role for the C3a anaphylatoxin receptor in the effector phase of asthma. *Nature* 2000;**406**:998–1001.
76. Roake JA, Rao AS, Morris PJ, Larsen CP, Hankins DF, Austyn JM. Dendritic cell loss from nonlymphoid tissues after systemic administration of lipopolysaccharide, tumor necrosis factor, and interleukin 1. *J Exp Med* 1995;**181**:2237–2247.
77. van der Pouw Kraan TC, Boeijs LC, Smeenk RJ, Wijdenes J, Aarden LA. Prostaglandin-E2 is a potent inhibitor of human interleukin 12 production. *J Exp Med* 1995;**181**:775–779.
78. Standiford TJ, Kunkel SL, Rolfe MW, Evanoff HL, Allen RM, Strieter RM. Regulation of human alveolar macrophage- and blood monocyte-derived interleukin-8 by prostaglandin E2 and dexamethasone. *Am J Respir Cell Mol Biol* 1992;**6**:75–81.
79. Rolfe MW, Kunkel SL, Standiford TJ, Orringer MB, Phan SH, Evanoff HL et al. Expression and regulation of human pulmonary fibroblast-derived monocyte chemotactic peptide-1. *Am J Physiol* 1992;**263**:L536–L545.
80. Mattoli S, Marini M, Fasoli A. Expression of the potent inflammatory cytokines, GM-CSF, IL6, and IL8, in bronchial epithelial cells of asthmatic patients. *Chest* 1992;**101**:27S–29S.
81. Marini M, Vittori E, Hollemborg J, Mattoli S. Expression of the potent inflammatory cytokines, granulocyte-macrophage-colony-stimulating factor and interleukin-6 and interleukin-8, in bronchial epithelial cells of patients with asthma. *J Allergy Clin Immunol* 1992;**89**:1001–1009.
82. Knight DA, Lim S, Scaffidi AK, Roche N, Chung KF, Stewart GA et al. Protease-activated receptors in human airways: upregulation of PAR-2 in respiratory epithelium from patients with asthma. *J Allergy Clin Immunol* 2001;**108**:797–803.
83. Lordan JL, Bucchieri F, Richter A, Konstantinidis A, Holloway JW, Thornber M et al. Cooperative effects of Th2 cytokines and allergen on normal and asthmatic bronchial epithelial cells. *J Immunol* 2002;**169**:407–414.
84. Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002;**3**:673–680.
85. Gilliet M, Soumelis V, Watanabe N, Hanabuchi S, Antonenko S, de Waal-Malefyt R et al. Human dendritic cells activated by TSLP and CD40L induce proallergic cytotoxic T cells. *J Exp Med* 2003;**197**:1059–1063.
86. Ying S, O'Connor B, Ratoff J, Meng Q, Mallett K, Cousins D et al. Thymic stromal lymphopoietin expression is increased in asthmatic airways and correlates with expression of Th2-attracting chemokines and disease severity. *J Immunol* 2005;**174**:8183–8190.
87. Rimoldi M, Chieppa M, Salucci V, Avogadri F, Sonzogni A, Sampietro GM et al. Intestinal immune homeostasis is regulated by the crosstalk between epithelial cells and dendritic cells. *Nat Immunol* 2005;**6**:507–514.
88. Ziegler SF, Liu YJ. Thymic stromal lymphopoietin in normal and pathogenic T cell development and function. *Nat Immunol* 2006;**7**:709–714.
89. Zhou B, Comeau MR, De Smedt T, Liggitt HD, Dahl ME, Lewis DB et al. Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. *Nat Immunol* 2005;**6**:1047–1053.
90. Al-Shami A, Spolski R, Kelly J, Keane-Myers A, Leonard WJ. A role for TSLP in the development of inflammation in an asthma model. *J Exp Med* 2005;**202**:829–839.
91. Li M, Hener P, Zhang Z, Kato S, Metzger D, Chambon P. Topical vitamin D3 and low-calcemic analogs induce thymic stromal lymphopoietin in mouse keratinocytes and trigger an atopic dermatitis. *Proc Natl Acad Sci USA* 2006;**103**:11736–11741.
92. Kato A, Favoreto S Jr, Avila PC, Schleimer RP. TLR3- and Th2 cytokine-dependent production of thymic stromal lymphopoietin in human airway epithelial cells. *J Immunol* 2007;**179**:1080–1087.
93. Allakhverdi Z, Comeau MR, Jessup HK, Yoon BR, Brewer A, Chartier S et al. Thymic stromal lymphopoietin is released by human epithelial cells in response to microbes, trauma, or inflammation and potently activates mast cells. *J Exp Med* 2007;**204**:253–258.
94. Lee HC, Ziegler SF. Inducible expression of the proallergic cytokine thymic stromal lymphopoietin in airway epithelial cells is controlled by NFκB. *Proc Natl Acad Sci USA* 2007;**104**:914–919.
95. Butler M, Ng CY, van Heel DA, Lombardi G, Lechler R, Playford RJ et al. Modulation of dendritic cell phenotype and function in an in vitro model of the intestinal epithelium. *Eur J Immunol* 2006;**36**:864–874.
96. Rimoldi M, Chieppa M, Larghi P, Vulcano M, Allavena P, Rescigno M. Monocyte-derived dendritic cells activated by bacteria or by bacteria-stimulated epithelial cells are functionally different. *Blood* 2005;**106**:2818–2826.