



Interactions between dendritic cells and epithelial cells in allergic disease

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Abstract

Dendritic cells (DCs) play a crucial role in the sensitisation process. Upon encounter with an allergen, DCs require interactions with other cells and factors for triggering a primary or secondary immune response. Epithelial cells (ECs) express features of accessory cells, such as expression of HLA-DR, co-stimulatory molecules, functional FcγR, molecules of the antigen-processing machinery, and display an ability to internalise antigen. These features may authorize them to function as immunomodulators (e.g. amplification of memory T cells during secondary immune responses). ECs may increase chemokine (e.g. CCL20) secretion thereby attracting DCs. Epithelial human TSLP activates DC, which allow them to prime naive T cells for the production of pro-inflammatory cytokines, while down-regulating IFN-γ and IL-10. ECs may also influence the local polarization of types 1 and 2 antigen-presenting cells via PGE₂ by impairing the ability of maturing DC to produce bioactive IL-12 p70. PGE₂ is synergistic with IL-1β and TNF-α in the induction of functional and phenotypic maturation of DC and induce IL12 p40 production. Sensitisation via the respiratory route may be Th₂ skewed, possibly because the antigen recognition by DC occurs in an environment rich of airway EC-product such as PGE₂.

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1. Dendritic cells

1.1. Antigen presenting cells with many faces

In the following sections, antigen-presenting cells will be referred to as DCs, specifically Langerhans cells (LCs) or airway DCs. It should be appreciated though that DCs represent a heterogenous class of antigen-presenting cells. DCs can be differentiated in at least three DC lineages undergoing sequential differentiation,

resulting in intermediate blood precursors and immature DCs in peripheral tissues and organs, each with specific phenotypes and characteristics (Table 1). After migration into the tissue, the functionality of DCs is affected by the micro-environment of the tissue in which they reside. Thus, DCs from different tissues favour either cellular versus humoral immune responses by influencing T-cell cytokine production (Everson et al., 1996; Hasséus et al., 1998). Finally, DCs change phenotype and function during a maturation process. Even though allergen stimulation in many cases does not induce DC maturation as such (Lindstedt et al., 2005), it is generally accepted that in vivo DC maturation is initiated by antigen challenge if a favourable micro-environment is present. Granulocyte-

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Table 1
Characteristics of human DC subsets

DC lineage	Markers
CD34 ⁺ progenitor cells	CLA ⁺ , E-cadherin, langerin, birbeck granules
Plasmacytoid DCs	CD1a ⁺ , CLA ⁺ , co-agglutination factor XIII CD8α ⁺ , CD45RA ⁺ , CD123 ⁺ , CD1a ⁺ , CD11c ⁺ , CD13 ⁺ , CD33 ⁺ , GM-CSF receptor ⁺
Myeloid DCs	CD8α ⁺ , CD1a ⁺ , CD11c ⁺ , CD13 ⁺ , CD33 ⁺ , GM-CSF receptor ⁺
Mature DCs	Upregulation of MHC I/II, B7, ICAM-1, LFA-3 and CD40L

Ni and O'Neil (1997), Banchereau et al. (2000), and Upham (2003).

macrophage colony-stimulating factor (GM-CSF) has been shown to be essential for b6th DC differentiation from precursors and maturation, while interleukin (IL)-1 and IL-4 synergize with GM-CSF to further enhance the T-cell stimulating capacity of DCs. During the process of maturation, DCs express increased levels of cell surface antigens important for T-cell activation, e.g. major histocompatibility complex (MHC) class I/II, B7, intercellular adhesion molecule (ICAM)-1, leukocyte function-associated antigen (LFA)-3 and CD40L (Ni and O'Neil, 1997).

1.2. Dendritic cells in allergic diseases

The direct effect of DCs in sensitisation and allergy pathogenesis was demonstrated (Lambrecht et al., 1998; Lambrecht and Hammad, 2003a,b). Transfer experiments revealed that allergen-loaded DCs induce Th₂ polarisation upon aero-allergen challenge (Graffi et al., 2002; Hammad et al., 2002), and allergen-specific IgE in naive syngenic mice in absence of antigen challenge (Chambers et al., 2004). Upregulation of the expression of B7-2 (CD86) on DCs was shown to be essential for the development of IL-4 producing T cells (Ranger et al., 1996).

It is recognised that LCs and BDCA1⁺ myeloid DCs play an important role in the allergic immune response in skin and lung (Kimber et al., 2000; Lambrecht and Hammad, 2002), while the role of CD123⁺ plasmacytoid DCs (PDCs) remains unclear (Foster et al., 2003). Adoptive transfer of PDCs before sensitization was shown to prevent asthmatic reactions, suggesting a suppressive effect on the generation of effector T cells (De Heer et al., 2004). Nonetheless, the observed increase in PDC numbers in response to repeated allergen provocation (Jahnsen et al., 2000), and in atopic patients (Matsuda et al., 2002), indicate that PDCs also play an

essential role in human allergic reactions. Therefore, the recruitment of PDCs after allergen challenge may be due to an attempt by the immune system to down-regulate antigen-specific T-cell responses (Novak et al., 2004).

2. Epithelial cells and dendritic cells share the same micro-environment in vivo

2.1. Normal tissue

The juxtaposition of epithelial cells (ECs), DCs and nerve cells in skin and mucosal layers suggest that these cells interact with each other. In normal skin, the viable stratified epidermis is composed of several layers of keratinising epithelial cells interconnected by desmosomes, containing among others LCs, the main DCs in the skin (Stingl et al., 1993). Normal mucosal epithelium is a single layer of epithelial cells sealed at the apical site by tight junctions. Underneath these tight junctions is a complex network of interconnected channels where the airway DCs are found (Sertl et al., 1986). Recently, Rescigno et al. (2001) showed that in gut epithelial monolayers DCs constitutively express tight junction proteins. Although mainly derived from an in vitro co-culture system using Caco-2 cells, this might well be applicable to human airways. If this in vitro observation can be extrapolated to in vivo epithelium, DCs could connect directly with ECs by extending dendrites into the upper layers of the epithelium while the integrity of the epithelial barrier is preserved.

2.2. Tissue challenge with allergen

There is substantial evidence that DCs upon allergen challenge either induce a local response by remaining in the tissue (Constant et al., 2002) or rapidly travel away from the epithelial layers to the regional lymph nodes for initiation or amplification of the allergic immune response (Hammad et al., 2000; Kimber et al., 2000; Upham, 2003). In atopic individuals, recruitment of DCs from the circulation upon local rechallenge with allergen was demonstrated. Following local allergen challenge, the number of myeloid DCs rapidly increase in bronchial mucosal in these individuals (Jahnsen et al., 2001). In parallel, blood myeloid DC numbers decrease following allergen exposure (Upham et al., 2002). In a model of experimental asthma, DCs were shown to populate the respiratory tract submucosa within 2 h after OVA aerosol challenge (Huh et al., 2003).

3. Epithelial cells: evidence supporting integration in the immune system

There is now growing evidence suggesting that ECs in the skin and the mucosal linings of the mouth and nose, and the respiratory, gastro-intestinal and genitor-urinary tract play a critical role in homeostatis and host defence (McKenzie and Sauder, 1990; Lambrecht and Hammad, 2003b). Once the passive lines of defence represented by the skin and the mucosal membranes are breached, ECs and DCs present at the site of entry are the first cells to encounter the antigen. The fate of DCs following allergen challenge has been extensively reviewed elsewhere (Kimber et al., 2000; Lambrecht and Hammad, 2003a). Yet, there is also a wealth of direct and indirect evidence supporting the theory that ECs, by virtue of their production of cytokines, growth factors and membrane proteins, are integrated in the immune system.

Table 2 shows a selection of the information currently available. This list does not try to acknowledge all the work in the field, but to give a comprehensive overview of the immunological markers expressed by ECs and known to be involved in maintenance of immuno-homeostasis and/or induction of immune responses. Supposing that the available in vitro data can be extrapolated to in vivo, ECs could be able to affect

Table 3

Epithelial cells: possible impact on immune cells

Impact	Epithelial products
Activation	IL-2, IL-10, IL-12, MIP-2, PGE2, TSLP
Adhesion/homing	CD44, ICAM-1, VCAM-1
Chemotaxis/migration	MCP-1, MIP-3 α , IL-8, IL-16, IP-10, RANTES, SCF, TGF- β , TNF- α
Differentiation	G-CSF, GM-CSF, M-CSF, IL-6
Proliferation	CD40, IL-1 α , IL-1 β , IL-2

a variety of mechanisms involved in various levels of the immune system. As can be deduced from Table 3, these mechanisms involve activation, adhesion/homing, chemotaxis, differentiation and proliferation. However, the presented data should be considered as indicative and not as conclusive for several reasons.

First, these studies were performed on cells from various sources, on biopsies, primary epithelial cells and various cell lines, in submerged and airlifted cell or reconstituted human tissue assay systems. Furthermore, the panel of responses studied by the respective investigators was subjective.

Second, it was not assessed to what extend the cells in the respective test systems were representative for the in vivo counterparts. Thus, the reported observa-

Table 2

Overview of proteins involved in immunomodulation and expressed by skin and lung epithelial cells

Tissue	Methodology	Challenge	Affected parameters	References
Skin	Biopsies	Allergens	IL-1 α , IP-10, MIP-2; IL-12	Enk and Katz (1992)
		None	ICAM-1, IL1- β , MCP-1, TNF- α , IL-1RA, IL1-RII, G-CSF, M-CSF	Bernard et al. (2002) and McKenzie and Sauder (1990)
		Injury	PDGF; PDGF-receptor; IL-1 β	McKenzie and Sauder (1990) and Antoniadis et al. (1991)
	Keratinocytes	Allergens	IL-8, IL-1 α	Coquette et al. (2003)
		Viscum Album L	IL-1 α , IL-2, IL-6, IL-8, IL-10, TNF- α	Gorter et al. (2003)
Mucosa	BAL-fluid	Bronchiectatic	TSLP	Soumelis et al. (2002)
		Micro-organisms and microbial products (including parasites)	MMP-8	Prikk et al. (2001)
	Cells (lines)		G-CSF, GM-CSF, ERK12, IL-6, IL-8, SCF, MCP-1, TNF- α , G α , RANTES, IP-10	Jung et al. (1995), Rasmussen et al. (1997), Schroth et al. (1999), Krishnamoorthy et al. (2000), Pompen et al. (2000), Saba et al. (2002), Schulz et al. (2002), Hurley and McCormick (2003), Young et al. (2003), and Spurrell et al. (2005)
		TNF- α + IL-1 β	Eotaxin-2, eotaxin-3, RANTES, IL-1 α , IL-1 β , TNF- α , IL-16, MCP-1, TGF- β , LTB4, RANTES, GM-CSF, IL-6, IL-8, VCAM-1, MIP3 α /CCL20, M-CSF	McKenzie and Sauder (1990), Jung et al. (1995), Koyama et al. (1999), Propst et al. (2000), Cheng et al. (2001), Leir et al. (2003), Reibman et al. (2003) and Heiman et al., 2005
		Air particles	IL-8, IL-6	Hetland et al. (2004)
		Allergens	IL-6, IL-8, G-CSF, RANTES, GM-CSF, M-CSF, MCP-1	De Wever and Roggen (2001)
		IL-4, IL-5, IL-10, IL-13	ICAM-1	Bianco et al. (1998)

Table 4
Characteristics of frequently used human lung epithelial cells

Human lung cell line	Characteristics
16HBE14o [−]	Polarised; ciliated; tight-junctions; no mucin production
BEAS-2B	No tight-junctions, poorly attaching to the extracellular matrix
Calu-3	Polarised; ciliated; tight-junctions; mucin production; metabolically active; active transport pumps
A549	Polarised; no tight-junctions; no mucin production; metabolically active

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tions may not reflect properly what happens in vivo. The importance of this concern cannot be underestimated. Comparison of the gene expression profiles in human keratinocyte monolayer cultures (HKC), reconstructed epidermis (RE) and normal human skin biopsies revealed significant differences between the HKC and the RE, and the skin biopsies (Bernard et al., 2002). Similar observations were made for cancerous alveolar type II cells, such as the frequently used human A-549 cell line, compared to non-cancerous human type II alveolar and bronchial cells (Kobayashi et al., 2004). In addition, phenotypic analysis of various frequently used human lung epithelial cells revealed defects that could bias the interpretation of the obtained data (Table 4). Finally, analysis of the A-549 cell line by RT-PCR seems to indicate that the expression of membrane markers (e.g. Tol-like receptors) and cytokines, as well as the responsiveness of the cells to a panel of respiratory allergens, is strongly influenced by the medium in which the cells are grown (Roggen, unpublished data).

Third, the quality of the compounds used for stimulation of the cells was not always controlled. As shown in Table 2, microbial products, e.g. lipopolysaccharide (LPS), stimulate ECs to release a variety of cytokines and chemokines. Thus, contaminated allergen samples are likely to produce a mixed or diverted cellular response leading to misinterpretation of the data related to the allergen under investigation.

4. Signaling of NF- κ B signal transduction pathways in epithelial cells may lead to DC recruitment and maturation

Various forms of cutaneous and mucosal trauma call up a cascade of cellular and molecular events trying to make repairs or to combat intruders. The main pathway initiating this process is the nuclear factor (NF)- κ B signalling pathway. Activation of the tran-

scriptional factor NF- κ B causes enhanced expression of genes encoding inflammatory cytokines, acute phase proteins, immunoreceptors and chemokines important in the recruitment of neutrophils and eosinophils. For example, eotaxin, IL-6, IL-8, MIP-2, GM-CSF, IP-10 and ICAM-1 all have NF- κ B binding sequences in their promoter regions (Hayden and Ghosh, 2004).

4.1. Signal transduction pathways emanating from allergen-induced trauma—learnings from the lung

Der p 1, a cysteine protease and a major allergen from the house dust mite *Dermatophagoides pteronyssinus*, was demonstrated to produce functional changes in the barrier function of the epithelium, probably by disrupting the epithelial tight junctions (Wan et al., 1999). Preliminary studies using enzymatically active serine protease variants seem to support a role for cell detachment in respiratory sensitisation. Indeed, a correlation was observed between the capacity of protease variants to disintegrate in vitro airlifted human alveolar type II-like cell (A-549) monolayers, and in vivo production of protease-specific IgE antibodies in mice (Kristensen and Roggen, unpublished data). Stacey et al. (1997) found that Der p 1 acts by promoting the activation of NF- κ B by interference with the function of the cytoplasmic inhibitor I κ B- α .

Signalling to NF- κ B causes the upregulation of IL-8 gene expression and release in human bronchial and alveolar ECs (Shibata et al., 1996; Hayden and Ghosh, 2004). In vivo, this increase would result in the recruitment of neutrophils to the site of injury and release in the environment a variety of molecules, including defensins, TNF- α and serine proteases (Sibille and Reynolds, 1990). Human neutrophil peptide (HNP)-1 (α -defensin), but not human β -defensin (HBD)-2, was shown to increase IL-8 and IL-1 β mRNA expression in a dose-dependent manner, as well as IL-8 protein secretion and NF- κ B DNA binding activity, in human bronchial ECs (Sakamoto et al., 2005). These observations suggest an allergen-induced self-amplifying system resulting in increasing amounts of IL-1 β and defensins, as well as the NF- κ B driven proteins, e.g. IL-6, IL-8, GM-CSF, ICAM-1. Thus, a micro-environment is created favouring recruitment of immature DCs. Indeed, defensins are suggested to be involved in chemotaxis of human immature DCs (Territo et al., 1989). However, there is evidence suggesting that this effect is indirect and acts through defensin-mediated IL-1 β release from ECs. Reibman et al. (2003) demonstrated that human bronchial ECs exposed in vitro to IL-1 β were triggered to induce the expression and release of MIP-3 α (CCL20), a chemokine selectively inducing migration of immature

DCs (iDCs) (Fig. 1A). Upon arrival at the site of insult, iDCs probably interact physically with ECs. One of the consequences of the activation of the NF- κ B signalling pathway is the upregulation of the expression of adhesion molecules, e.g. ICAM-1. It is likely that epithelial ICAM-1 (Stacey et al., 1997; Schneeberger et al., 2000; Poynter et al., 2002) and E-cadherin (Carayol et al., 2002) bind to LFA-1 and E-cadherin, both expressed by iDCs. Thus, the iDCs are concentrated at the site of entrance of the allergen, thereby facilitating antigen-uptake by the iDCs. In addition, antigen-uptake occurs in a micro-environment with increasing levels of epithelial GM-CSF, IL-1 β and prostaglandin (PG)E₂ (Vancheri et al., 2004) and neutrophilic TNF- α . These molecules are known to be essential for DC maturation and migration (Fig. 1B).

4.2. Signal transduction pathways triggered by TNF- α

TNF- α is stored in granules and is known to be released in large amounts primarily from mast cells and macrophages as a result of immunological stimulation involving IgE-dependent mechanisms. Other cell types that are a significant source of TNF- α include epithelial cells, eosinophils and neutrophils. TNF- α delivers its signal to the target cells through the species unspecific membrane TNF-receptor (TNFR)-1 and the species specific TNFR-2. Interaction between TNF- α and the TNFRs elicits a general inflammatory response, involving NF- κ B activation, mainly through enhanced release of pro-inflammatory/chemotactic mediators and upregulation of adhesion molecules (Russo and Polosa, 2005).

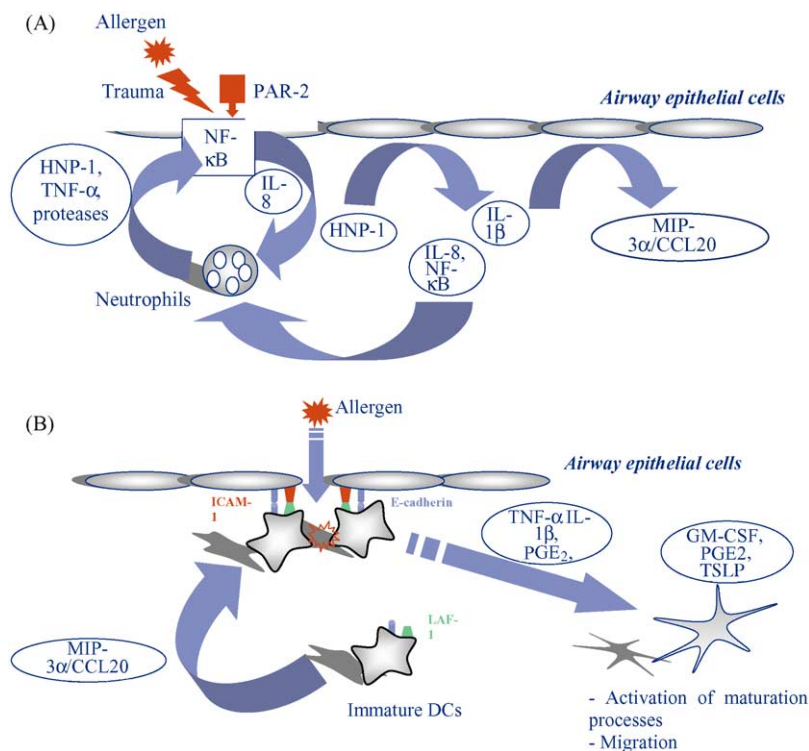


Fig. 1. Allergen-induced modification of the micro-environment (A) and recruitment of immature DCs (B). (A) Stimulation of the NF- κ B signalling pathway in ECs is triggered by any form of allergen-induced trauma or by activation of PAR-2. This stimulation will result in the release of IL-8 and recruitment of neutrophils. Neutrophils will release a number of factors, including defensins, TNF- α and serine proteases; Defensin HNP-1 will further enhance epithelial IL-8 production by enhancing binding of NF- κ B to DNA. In addition, other neutrophilic defensins and serine proteases will result in tissue injury and additional NF- κ B activation. Thus, a self-amplifying process is initiated. HNP-1 will also increase epithelial IL-1 β expression and release, resulting in the excretion by ECs of MIP-3 α (CCL20) known to attract immature DCs. (B) Immature DCs are attracted to the site of insult by MIP-3 α . Activation of the NF- κ B pathway will also result in upregulation of adhesion molecules, e.g. ICAM-1 and E-cadherin. These adhesion molecules will be engaged to establish direct EC–DC contact, thereby facilitating antigen-uptake. Sequentially, immature DCs will be mobilised by TNF- α , IL-1 β and PGE₂ to move to the lymphoid tissue, while GM-CSF, PGE₂ and TSLP will further support DC maturation towards DC facilitating the development of Th₂ immunity.

4.2.1. Learnings from the skin

The mechanisms involved in skin LC mobilisation after contact with allergens and irritants have been extensively reviewed elsewhere (Kimber et al., 2000).

Topical exposure of mice to contact allergens and skin irritants is known to stimulate the upregulated expression of various epidermal cytokines, including TNF- α , IL-1 β , IL-6, IL-10, GM-CSF and MIP2. In mice and humans, TNF- α and IL-1 β are known to provide mandatory signals for the mobilisation of LCs.

TNF- α is an inducible product of keratinocytes. The mobilization and migration of LCs in response to skin sensitisation or skin irritation are dependent upon TNF- α acting directly on epidermal LCs via TNF-R2 receptors. The consequence of this interaction is activation of the NF- κ B signalling pathway (Section 4.1). While TNF- α is a mandatory signal for LC migration, it is not alone sufficient for the successful mobilization and movement of these cells. There is a requirement also for IL-1 β which in mouse epidermis is a constitutive and inducible product of LCs themselves.

The observation that PGE₂ is produced substantially in skin exposed to antigen, suggests that this molecule plays a role in skin immune responses. Kabashima et al. (2003) demonstrated that PGE₂ promotes the migration and maturation of LCs through the PGE₂-EP4 signaling pathway.

One of the likely consequence of the signals provided by TNF- α and IL-1 β is the altered expression of integrins and adhesion molecules (e.g. E-cadherin, ICAM-1, CD44 and α 6 integrin) that collectively facilitate the movement of LCs from the skin to draining lymph nodes. The modulation of E-cadherin was demonstrated to be affected among others by TNF- α . Migration to the lymphoid tissue demands that LCs cross the basement membrane at the dermal–epidermal junction before their onward movement through the dermal matrix. Passage across the basement membrane will require the possession by LCs of very late antigen (VLA)-6 as well as proteolytic activity. Epidermal LCs possess matrix metalloproteinase (MMP)-9 which is induced or upregulated following skin sensitisation, possibly in response to TNF- α and/or IL-1 β (Fig. 2A).

Another product of keratinocytes that is upregulated during skin sensitisation is IL-10. In contrast to TNF- α and IL-1 β , IL-10 seems to inhibit the functional activity of LCs and DCs primarily by operating at the pro-inflammatory cytokine synthesis (Fig. 2A).

4.2.2. What is known for the lung

In contrast to keratinocytes, lung epithelial cells do not seem to upregulate the expression of TNF- α upon

contact with respiratory allergens (Roggen, unpublished data). However, in vitro studies have demonstrated that a cocktail of TNF- α and IL-1 β stimulates the upregulation of the expression of cytokines, chemokines and adhesion molecules, including TNF- α (Table 2). In vitro studies suggest that exogenous TNF- α triggers normal human bronchial ECs to enhance ICAM-1 expression, probably through interaction with TNF-R1 (Krunosky et al., 2000). The mechanism by which endogenous TNF- α triggers DCs to excrete cytokines and chemokines (e.g. IL-1 β) is not clear, as the presence of TNF-R2, responsible for LC activation in the skin, has not been identified yet on lung DCs.

In vivo, TNF- α and IL-1 β probably originate from IgE-loaded cells, e.g. mast cells, by allergen-mediated cross-linking of the IgE-receptors on these cells. These observations are consistent with Poynter et al. (2002) demonstrating that mice sensitised with ovalbumin (OVA) develop in lung tissue a rapid and protracted activation of the inhibitor of κ B kinase (IKK) following a single challenge with nebulised (OVA). IKK activation was followed by nuclear localisation of NF- κ B within the lung epithelium. Thus, a secondary stimulus may induce the expression of TNF- α by airway ECs, leading to the cascade of events observed in the skin (Section 4.2.1) (Fig. 2B).

4.3. Signal transduction pathways triggered by CD40, a member of the TNFR family

Keratinocytes and airway epithelial cells do not appear to express either CD80 or CD86, ligands for the T cell co-stimulatory pathway mediated through CD28. However, it was shown that these cells do express CD40, a member of the TNFR family playing an important role in the regulation of humoral and cell-mediated immunity. The ligand for this receptor is CD40L, type II transmembrane protein classified as a member of the TNF family. It is expressed on activated CD4⁺ and CD8⁺ T cells, eosinophils, mast cells, basophils, NK cells and activated DCs (Grewal and Flavell, 1998).

In atopic individuals and a mouse model for asthma, DCs are recruited from the circulation and populate the respiratory tract submucosa upon local rechallenger with allergen (Jahnsen et al., 2001; Huh et al., 2003). It is likely that in this process activated DCs (CD40L⁺) interact with ECs expressing CD40. It has been shown that engagement of CD40 induces in airway ECs a significant increase in expression of the chemokines RANTES, monocyte chemoattractant protein (MCP)-1 and IL-8, and ICAM-1, which are involved in T-cell recruitment, and iDC recruitment and maturation (Sec-

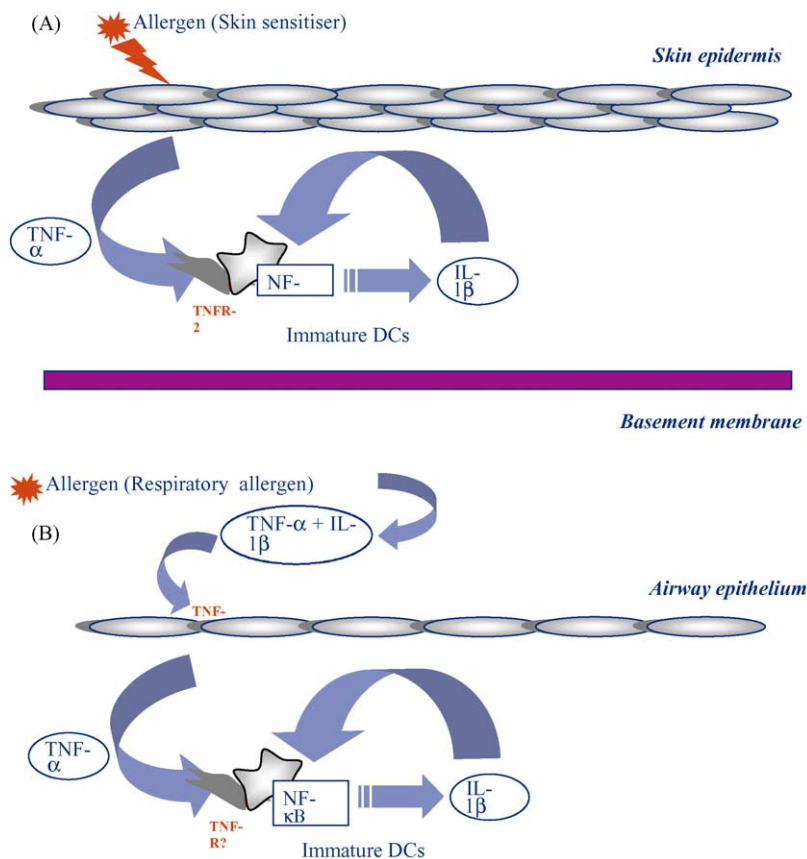


Fig. 2. Allergen-induced mobilisation of LCs (A) and airway DCs (B). (A) Trauma will stimulate keratinocytes in the skin to upregulate the expression and excretion of $\text{TNF-}\alpha$ and PGE_2 . Sequentially, $\text{TNF-}\alpha$ will bind to the TNF-R2 receptor on LCCs, resulting in upregulation of $\text{IL-1}\beta$ release by LCs by a process involving the $\text{NF-}\kappa\text{B}$ signalling pathway. $\text{IL-1}\beta$ will enhance its own excretion by stimulating LCs. (B) Lung epithelial cells apparently required exogenous $\text{TNF-}\alpha$ and $\text{IL-1}\beta$ as a trigger to upregulate expression and excretion of endogenous $\text{TNF-}\alpha$. Indirect evidence suggest that exogenous $\text{TNF-}\alpha$ acts through TNF-R1 . Sequentially, mobilisation and migration of DCs might be as described for LCs in the skin. In contrast to the skin, the TNF-R involved in lung DC stimulation has not been identified.

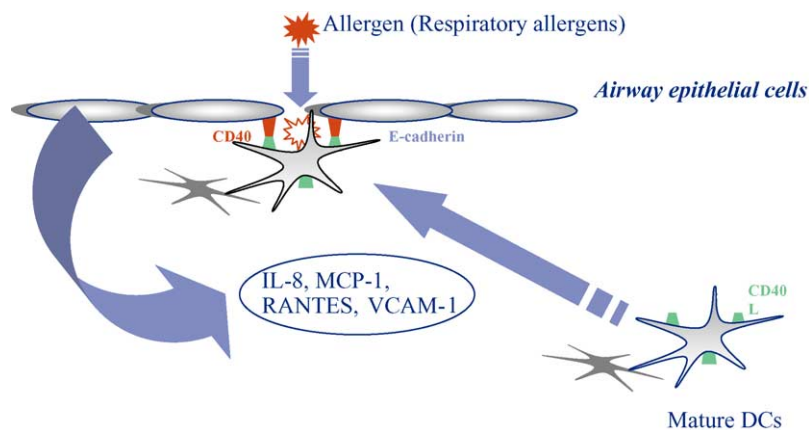


Fig. 3. CD40-CD40L mediated recruitment of mature DCs. Upon rechallenge of mucosal epithelium with allergen, CD40^+ DCs move from the circulation to the lung to populate the lung mucosal. Upon arrival, DC-CD40L is likely to engage in binding to CD40 , expressed by lung ECs. This interaction will result in upregulation of the expression and excretion of cytokines and chemokines involved in recruitment of immature DCs, and DC maturation and migration.

tion 4.1). Importantly, CD40 ligation seems to enhance the sensitivity of airway ECs to the effects of TNF- α and IL-1 β on the expression of RANTES, MCP-1, IL-8 and VCAM-1 (Section 4.2.2). This observation seems to suggest that activated DCs promote an immune response to a previously encountered allergen by signalling information to the ECs (Propst et al., 2000) (Fig. 3).

Bianco et al. (1998) demonstrated that Th₂ cytokines (IL-4, IL-5, IL-10 and IL-13) significantly enhanced ICAM-1 expression by lung ECs. In contrast, Th₂-derived cytokines IL-4 and IL-13 were found to attenuated the positive effects of CD40 cross-linking on ICAM-1 expression (Propst et al., 2000). From these observations, it can be hypothesised that the interaction between activated DCs and ECs is impaired, resulting in a less efficient immune response to a recall allergen.

4.4. Induction of inflammatory reactions by activation of the PAR-2 receptor

In the respiratory system, the protease-activated-receptor (PAR-2) appears to play a dual role, being both pro-inflammatory and anti-inflammatory. There is clinical evidence that PAR-2 is upregulated in respiratory epithelium from patients with asthma (Knight et al., 2001). PAR-2 belongs to the family of G-coupled receptors which are in vivo activated by serine proteases to induce inflammatory, mitogenic and chemotactic functions. The signalling pathways following activation of PAR-2 have yet to be investigated in detail, but seem to involve the NF- κ B signalling pathway. The formation of inositol trisphosphate and diacylglycerol, followed by Ca²⁺ mobilisation and activation of protein kinase C, seems to suggest phospholipase C activation. Certain actions of PAR-2 agonists are partially sensitive to the general tyrosine kinase inhibitor genestein, indicating the likely involvement of tyrosine kinases other than c-Src (Kawabata, 2002).

In vivo, PAR-2 activation is the result of proteolytic modification by serine proteases released by neutrophils, e.g. after recruitment by ECs (Section 4.1). Interestingly, house dust mite allergens Der p 1 (cysteine protease), and Der p 3 and Der p 9 (serine proteases) seem to exert their allergenic potency through the PAR-2 signaling pathway of lung ECs (Sun et al., 2001; Asokanathan et al., 2002). It was shown that activation of PAR-2 on airway ECs triggers the release of GM-CSF (Vliagoftis et al., 2001), and of PGE₂, a Th₂ promoting factor, and IL-6, IL-8 and matrix metalloproteinase (MMP)-9, factors having the potential to induce or prolong inflammation (Thompson et al., 1999).

Alternatively, PAR-2 activation may affect immune responses by processes involving cytokine/chemokine degradation. Indeed, PAR-2 activation of mast cells was found to result in the release of serine proteases reported to be involved in TNF-degradation (Alshurafa et al., 2004). In vitro studies using a variety of serine proteases demonstrated proteolytic susceptibility of IL-6, IL-8, MCP-1, G-CSF and GM-CSF. In contrast, M-CSF was found stable within the settings of the experiments. To what extent proteolytic modification of the micro-environment resulting from EC stimulation has in vivo relevance remains to be established (Kristensen and Roggen, unpublished results).

5. The third signal concept

The concept of a third signal, separated from MHC/TCR and CD28 ligation, provided by DCs that influence Th₂ differentiation was proposed. However, there is still no DC-induced strictly Th₂-inducing factor identified. Nonetheless, several costimulatory molecules have been implicated in Th₂ responses, such as ICOSL (Eisenbarth et al., 2003), OX40L (de Jong et al., 2002; Salek-Ardakani et al., 2003) and sCD30 (Bengtsson et al., 1997). The fact that ICOS-ICOSL ligand costimulation between T cells and DCs is critical for Th₂-driven inflammation (Gonzalo et al., 2001) and T_{reg} cell-mediated tolerance (Akbari et al., 2002), as pointed out above, is intriguing and suggest that these processes are related. Moreover, the cell surface Notch ligand family members delta and jagged on DCs were recently shown to influence Th₁ and Th₂ differentiation, respectively (Amsen et al., 2004). These ligands were differentially expressed by DCs stimulated with either cholera toxin, PGE₂ or LPS. Also, ectopic expression of jagged and delta polarized naive T cells into Th₂ or Th₁ cells, respectively.

Recently, the direct role of ECs in modulating DC function in allergic inflammation was demonstrated. Human thymic stromal lymphopoietin (TSLP) is expressed by epithelial cells of inflamed tonsils and by skin keratinocytes in atopic dermatitis (Soumelis et al., 2002). TSLP-activated DCs prime naive T cells to produce pro-allergic cytokines (IL-4, IL-5, IL-13 and TNF- α), while downregulating IFN- γ and IL-10. TSLP may also have an indirect effect on CD40 engagement of ECs and on the expression of cytokines and chemokines involved in iDC recruitment and maturation (Section 4.3).

PGE₂ is another factor that promote Th₂ cytokine production, by impairing the ability of maturing DCs to produce bioactive IL-12 p70 (Kalinski et al., 1997). PGE₂

synergizes with IL-1 β and TNF- α in the induction of functional and phenotypical maturation of DCs (Kalinski et al., 1998), and induces IL12 p40 production, which as a monomer or homodimer function as an IL-12 antagonist (Kalinski et al., 2001). PGE₂ is produced by airway epithelial cells (Vancheri et al., 2004, Section 4.4), which may influence the local balance of Th₁/Th₂ polarizing agents. The route of antigen entry also seems to be of importance for the generation of T helper cells. Sensitization to inhaled antigen via the respiratory route appears to be Th₂ skewed (Constant et al., 2000), possibly because the antigen recognition by DCs occurs in an environment rich of airway epithelial cell-product such as PGE₂.

6. DCs possibly regulate ECs

As described in Section 4.3, activated DCs may inform ECs when a trauma is caused by a recall allergen through mechanisms involving CD40–CD40L cross-linking.

DCs seem to be responsible also for pathogenic events at EC level, such as goblet cell hyperplasia (GCH) and/or metaplasia (GCM). Both GCH and GCM are thought to be induced directly by the Th₂ cytokines IL-9 and IL-13. IL-13 has the potential to induce STAT-6 phosphorylation in ECs, and this alone is sufficient to induce GCM and GCH. It was shown that signalling through the epidermal growth factor receptor (EGFR, c-erbB) on goblet cells through ligands expressed by ECs is critical in the induction of GCM and airway remodelling. The Th₂ cytokines IL-4 and IL-13 in conjunction with Der p 1 allergen induce the release of TGF- α , a known ligand for the EGFR. Although Th₂ cells and inflammatory cells are the most likely source of IL-4, IL-9 and IL-13, it has been shown that DCs produce IL-13, opening the possibility that they might be directly involved in inducing GCH (Lambrecht and Hammad, 2003b).

7. Summary

Overall, allergens seem to activate in ECs the NF- κ B signaling pathway through mechanisms that are not fully clear yet. The consequence of this stimulation is the upregulation of the expression of TNF- α and IL-1 β , both known to be essential for mobilisation and migration of DCs to the lymphoid tissue. Stimulated ECs also release MIP-3 α /CCL20, a chemokine that is chemotactic specifically for iDCs, and upregulate the expression of among others GM-CSF and adhesion molecules, such as ICAM-1. Thus, ECs are likely to promote antigen-uptake and provide a micro-environment supporting the initiation of processes leading to the maturation of iDCs.

Upon contact with an allergen, ECs release molecules (e.g. TSLP, PGE₂) suspected to deviate immune responses towards Th₂ mediated responses. Upon re-encounter with the allergen, Th₂ cytokines seem to impair the interaction between ECs and activated DCs, normally leading to a prompt immune response to a recall allergen. This interaction is probably driven by the activated DCs through mechanisms involving CD40–CD40L cross-linking.

In conclusion, the cytokine/chemokine micro-environment, produced by local ECs, seems to influence inflammatory processes, recruitment and maturation of specific subsets of immature as well as recruitment of mature DCs. However, our understanding of the mechanisms mediated by ECs and how they correlate with sensitisation and pathogenesis of allergy is not complete.

Well controlled in vivo and in vitro experiments should be performed to map in detail the series of events activated by an allergen when contacting skin or mucosal cell layers.

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