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Lung dendritic cells and host immunity to infection

B.N. Lambrecht, J-B. Prins, H.C. Hoogsteden

Lung dendritic cells and host immunity to infection. B.N. Lambrecht, J.-B. Prins, H.C. Hoogsteden. ©ERS Journals Ltd 2001.

ABSTRACT: The lung is a portal of entry for numerous microbial pathogens, against which evolution has created an adequate innate and adaptive immune response. Dendritic cells (DCs) are central to the integration of innate and specific immunity. These cells are located within the epithelium and interstitium of the lung where they are influenced by the innate immune system.

Upon recognition and internalization of microbial antigens, DCs migrate to the draining lymph nodes of the lung to initiate the specific cellular and humoral immune response. By their capacity to integrate stimuli derived from the pathogen, the host and the environment, they are specialized to induce a protective immune response while at the same time avoiding damage to the host.

It is becoming increasingly clear that dendritic cells are involved in the induction of immunity to viruses, bacteria, mycobacteria and fungi. Some pathogens subvert the function of dendritic cells to escape immune recognition. Not surprisingly, if dendritic cell function fails, the consequence for the host is immunodeficiency.
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Dept of Pulmonary and Critical Care Medicine, Erasmus Medical Centre Rotterdam, Rotterdam, the Netherlands.

Correspondence: B.N. Lambrecht, Dept of Pulmonary and Critical Care Medicine, Erasmus University Rotterdam (Room Ee2263), Dr. Molewaterplein 50, 3015 GE Rotterdam, The Netherlands.
Fax: 31 104089453

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The lung is continuously exposed to the outside world and is a portal of entry for viral, bacterial, and fungal infection. Throughout evolution, an extensive defence mechanism has been developed to protect humans from these potentially lethal assaults. In its most primitive form, present also in invertebrates, the defence system consists mainly of nonspecific mechanisms, such as antibacterial peptides (α- and β-defensins), mannose binding protein, lysozyme, lactoperoxidase, opsonizing collectins (*e.g.* surfactant), activation of complement, and interferons. Later, the innate nonspecific cellular defence system evolved. This important aspect of pulmonary immunity consists of phagocytic cells (alveolar macrophages (AMs), neutrophils, eosinophils) and natural killer (NK) cells. They have the capacity to recognize and neutralize bacterial antigen or virus-infected cells. These cells are endowed with so-called pattern recognition receptors, which are encoded in the germline deoxyribonucleic acid (DNA) of the species and have been selected through evolution to recognize conserved bacterial products (cell wall constituents, bacterial DNA motifs) or viral motifs (double-stranded ribonucleic acid (RNA)) [1]. Finally, the adaptive specific cellular immune response, which first appeared in higher vertebrates, provided humans with T- and B-lymphocytes and the exceptional capacity to recognize a plethora of foreign- and self-antigen by the process of clonal rearrangement and somatic mutation of their respective T-cell receptor (TCR) and immunoglobulin genes. The function of adaptive immunity is to strengthen and regulate the innate defence mechanisms and to build immunological memory so that subsequent challenges are efficiently overcome.

The evolution of the immune system and the environmental pressures upon it have created an extraordinarily complex regulatory system, in which molecules and cells of the nonspecific line of defence reciprocally influence the lymphocytes of the adaptive immune response to induce an optimally protective immune response, while at the same time avoiding tissue-damaging autoimmunity [2]. Dendritic cells (DCs) are a particular group of cells of the innate defence system that are central to the integration of nonspecific and specific immunity [2, 3]. These professional antigen-presenting cells (APC) are located at sites of the body where maximal microbial encounter occurs, such as the skin, gut and lung. In contrast to T- and B-lymphocytes, DCs have retained many of the pattern recognition receptors of the ancient immune system and have the unique capacity to sense stimuli, such as tissue damage, necrosis, bacterial and viral infection. This review describes the role of lung DCs in the initiation and control of pulmonary immunity to infection.

Previous articles in this series: No. 1: Pilette C, Ouadrhiri Y, Godding V, Vaerman J-P, Sibille Y. Lung mucosal immunity: immunoglobulin-A revisited. *Eur Respir J* 2001; 18: 571–588.

T-lymphocyte activation and the need for antigen-presenting dendritic cells

Naive T-lymphocytes of the adaptive immune response need DCs to become fully activated. Circulating naive T-lymphocytes have a limited capacity to leave the blood stream and migrate into peripheral tissues. Instead, they extravasate through specialized high endothelial venules in the T-cell area of the central lymphoid structures before re-entering the bloodstream *via* the efferent lymphatics. By this migratory behaviour, they are spatially separated from the antigen at the portal of entry of infection (*e.g.* skin or mucous membranes for most naturally occurring infections) [4]. Therefore, an important requirement that precedes the induction of adaptive immunity is the transport of antigen from the site of initial exposure to the T-cell area of the draining lymph nodes. Although some microbes can directly gain access to these nodes, the transportation of antigen *via* the afferent lymphatics is a specialized function of DCs [5, 6].

The TCR on T-lymphocytes can only recognize antigen in the context of major histocompatibility complex (MHC) molecules. A primary function of the APC is to recognize, internalize and efficiently process the antigen into immunogenic peptides for presentation on MHC class I and class II molecules (fig. 1) [7]. Immature DCs express various receptors, such as calcium-type lectin receptors (mannose receptor, DEC-205, langerin, dectin), immunoglobulin receptors and complement receptors, which can be used for receptor-mediated endocytosis, macropinocytosis or phagocytosis of exogenous antigens [8]. Entry *via* these receptors targets antigen to a specialized MHC class II containing endocytic compartment (MIIC) where exogenous antigen is loaded onto MHC class II molecules and subsequently targeted to the cell surface, especially when the antigen is delivered in an inflammatory context [8]. The capacity to take up antigen is a feature of immature DCs residing in peripheral tissues, and is largely lost during the migration of DCs into the draining lymph nodes. This way, immature DCs effectively make a "snapshot" of the antigens present in a peripheral inflammatory site. Following migration into the draining lymph nodes, the mature DC becomes a reporter of its earlier environment and displays the immunogenic peptides picked up in the periphery in the cleft of MHC class I and MHC class II molecules to the TCR on the responding T-cell [4]. Because of this function, DCs have been aptly called "the sentinels of the immune system". Compared with B-cells and macrophages, DCs are extremely efficient in rapidly generating surface peptide-MHC ligands after exposure to exogenous antigen [9].

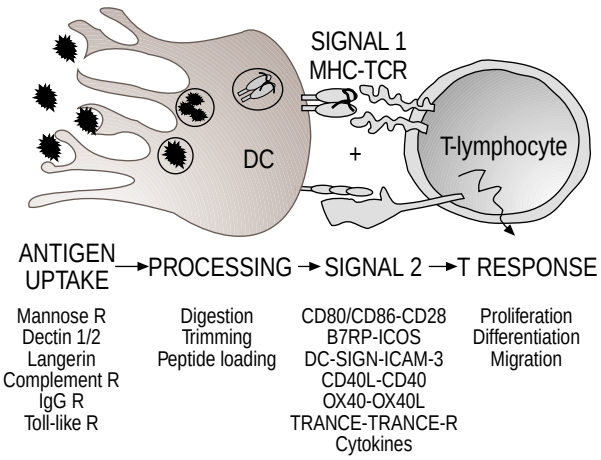

Fig. 1. – The interaction of dendritic cells (DCs) with naive T-cells. DCs capture foreign antigen using a variety of pattern recognition receptors and process the antigen for delivery onto major histocompatibility complex (MHC) antigens. Foreign antigens are loaded onto MHC class II in an MHC class II rich compartment (MIIC). T-lymphocytes need two signals to become activated. The first signal is the recognition of the antigen-MHC by the T-cell receptor (TCR). The second signal is a costimulatory signal provided by interaction of ligands on the DC to counter-receptors on the T-cell. Recognition of signals 1 and 2 occurs in the draining lymph nodes and leads to T-cell proliferation, differentiation and migration to the peripheral tissues. DC-SIGN: DC-specific ICAM-3 grabbing nonintegrin; ICAM: intercellular cell adhesion molecule; TRANCE: tumour necrosis factor related activation-induced cytokine; IgG: immunoglobulin-G.

In addition to TCR ligation by peptide-MHC (signal 1), T-lymphocytes need a so-called costimulatory signal 2, which is provided by the concerted action of costimulatory molecules expressed on the surface of the mature DC interacting with reciprocal receptors on the naive T-cell (fig. 1) [3, 10]. These interactions take place in the draining lymph nodes and are important for clonal expansion, differentiation, and avoidance of anergy in T-cells [7]. Upon initial encounter of a DC with a T-cell, adhesion molecules, such as DC-specific ICAM-3 grabbing nonintegrin (DC-SIGN) and CD54 (intercellular adhesion molecule (ICAM)-1), interact with leukocyte functional associated antigen (LFA)-3 and CD11a/CD18 (LFA-1) to retain the naive T-cell and to approximate the two cell types. This nonspecific interaction is of sufficient strength to allow the initial screening of the low-affinity TCR for recognition of its specific peptide-MHC on the DC [11]. In very close proximity to the peptide-MHC, DCs express CD80 and CD86, which gives an activating signal to CD28 on the naive T-cell [7]. Upon TCR recognition and CD28 stimulation, T-cells produce interleukin (IL)-2 to proliferate and upregulate the expression of CD40L. The latter molecule is a member of the tumour necrosis factor (TNF) receptor family, and signals to CD40 on DCs to increase the production of cytokines (*e.g.* IL-12) and the expression of CD80 and CD86, further intensifying the interaction [12]. Further downstream of the cascade, membrane interactions involve other members of the TNF-receptor family (OX40L/OX40, tumour necrosis factor related activation-induced cytokine (TRANCE)-R/TRANCE, 4-1BBL/4-1BB), which induce mutual activation, differentiation and survival of DCs and T-cells [13, 14]. Soluble products, such as IL-1β, IL-6, TNF-α and substance P, are released in what has been aptly called the immunological synapse forming between the APC and the naive T-cells, efficiently contributing to costimulation [15].

Dendritic cells determine the outcome of T-cell priming

T-lymphocyte responses are operationally divided on the basis of the cytokines produced and the functional effects exerted after encounter of antigen-specific T-cells with antigen [16]. CD4+ T-helper (Th)1 lymphocytes are effector cells that predominantly secrete IL-2, interferon (IFN)-γ and TNF-β to activate macrophages and cytotoxic T-cells. CD4+ Th2 lymphocytes secrete IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13 to induce a predominantly humoral immune response, sometimes dominated by the formation of immunoglobulin (Ig)-E. Additionally, it is thought that Th2 cells are important for mounting an eosinophilic response and for the expulsion of gastrointestinal parasites [17]. As DCs carry antigen from the periphery to the draining lymph node for presentation, it is not surprising that they are crucial in instructing naive precursor Th0 cells to become either Th1 or Th2 cells [18, 19] (fig. 2). The most critical factors for determining Th differentiation during infection are the cytokine milieu at the site of infection, the type and dose of infecting organism, the natural route of exposure, and the genetic background, age and prior infection history of the host. IL-12, IFN-γ and IL-18 are critical for the development of polarized Th1 responses, as illustrated by reduced Th1 responses in mice in which these genes were deleted [17, 20]. Conversely, IL-4 acting in concert with IL-6 is crucial for Th2 development [21]. However, DCs have not been shown to produce IL-4, so the early sources of this cytokine are probably the naive T-cell, or cells of the innate immune system, such as NK1.1 T-cells or mast cells [17]. The development of Th2 responses by naive T-cells could be the default pathway in the absence of IL-12 production by DCs [22].

In addition to the production of polarizing cytokines by DCs, it has been suggested that costimulatory molecules expressed on the surface of the DC are also essential for determining Th differentiation, although considerable controversy surrounds this issue. In this context, ICAM-1 and CD40 favour Th1 development, whereas OX40L, T1/ST2L, and perhaps CD86

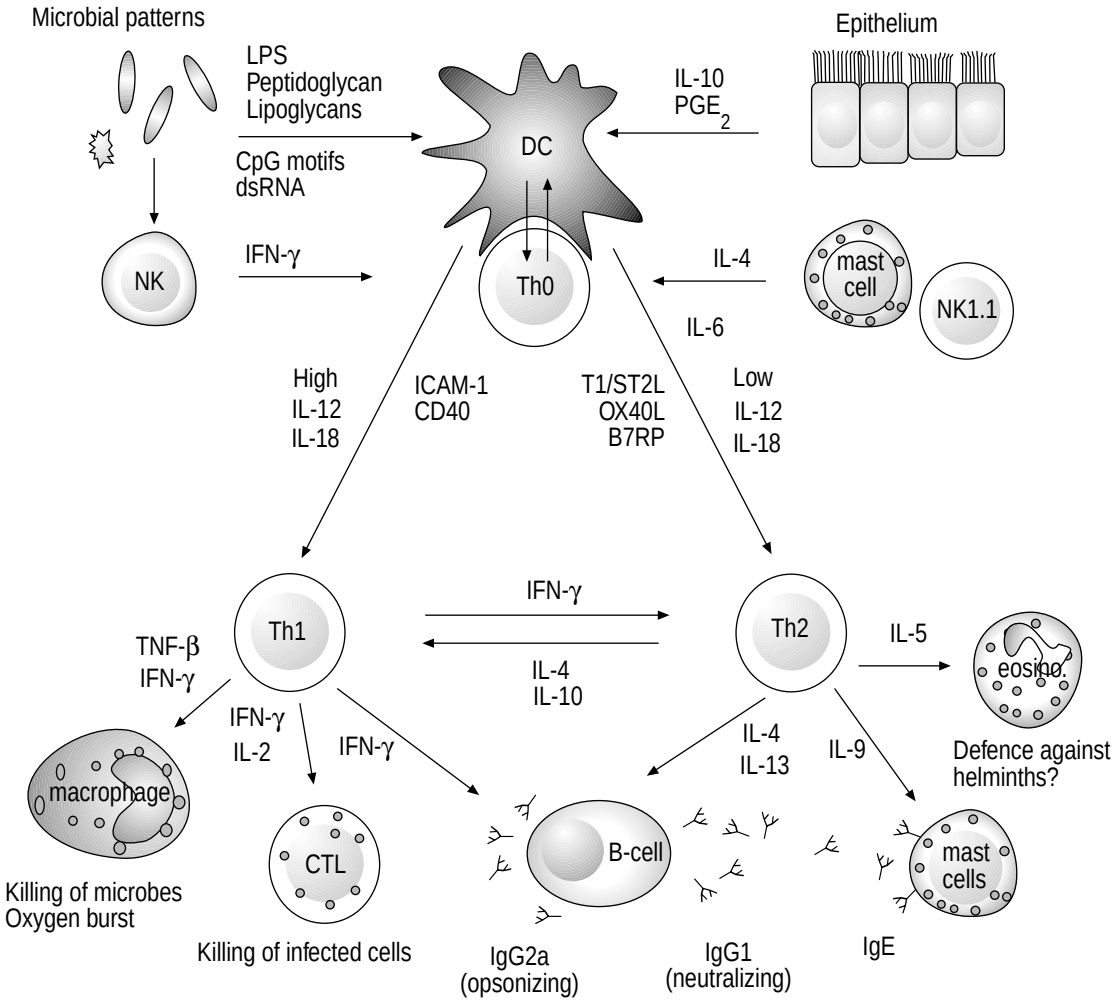

Fig. 2. – Differentiation of T-helper (Th) responses by dendritic cells (DCs). B7RP: B7-related protein; CTL: cytotoxic lymphocyte; dsRNA: double-stranded ribonucleic acid; LPS: lipopolysaccharide; NK: natural killer cell; PGE_2: prostaglandin E_2; IFN: interferon; IL: interleukin; TNF: tumour necrosis factor; Ig: immunoglobulin; ICAM: intercellular cell adhesion molecule; CpG: Cytosine-p-Guanine; eosino.: eosinophil.

promote Th2 development [19, 22]. To complicate things further, there seem to be different developmental lineages of DCs, each inducing different Th responses, hence the termination DC1 and DC2. In the mouse, spleen-derived DC1s are of lymphoid origin, express CD8a+ and produce large amounts of IL-12, leading to Th1 responses. Conversely, DC2s are myeloid-derived, express CD11b and produce very little IL-12 [23]. This is the complete opposite in humans. DC1s are monocyte-derived cells and, therefore, of myeloid origin, whereas DC2s express T-cell markers and are probably of lymphoid origin [24]. The lineage difference of DCs cannot, however, be the only explanation for the Th1/Th2 decision. It has indeed been shown that rodent myeloid DCs (*i.e.* DC2s) produce IL-12 upon proper stimulation and become strong stimulators of Th1 cells [25, 26].

Dendritic cells indirectly and directly stimulate B-cell responses

Although naive B-cells can recognize antigen through their B-cell receptor, they often need cognate CD4+ Th cell help before they can become fully activated and perform Ig isotype switching. In the mouse, Ig switching towards production of opsonizing antibodies IgG2a requires Th1 CD4+ cells, whereas switching towards neutralizing IgG1 or anaphylactic IgE requires Th2 CD4+ cells. Therefore, DCs have critical roles: they induce migration and activation of different subsets of CD4+ T-cells, activate B-cell activation and promote Ig switching [26, 27]. Moreover, recent studies have shown that DCs can carry unprocessed antigen from the periphery to the B-cell area of lymph nodes for direct presentation to recirculating naive B-cells and for inducing the survival of plasmablasts [28]. Germinal centres contain a particular subset of germinal centre DCs that can influence germinal centre T-cells during memory B-cell generation [29].

The airway dendritic cell network under baseline conditions

Studies in rodents and humans have shown that an extensive network of bonemarrow-derived DCs reside within the mucosa of the nose and the large conducting airways (fig. 3), the alveolar lumen and septum, and the connective tissues surrounding blood vessels and pleura [30–33]. Considerable phenotypic and functional heterogeneity exist in DCs within these compartments. Mucosal DCs are equipped with phagocytic receptors and have a rapid turnover rate, reflecting the continuous sampling for antigen and prompt migration of these cells to the draining lymph nodes [5, 34]. Some intraepithelial DCs in humans show characteristic Birbeck granules and have been called Langerhans′ cells, by analogy with the skin DCs. In contrast, alveolar wall DCs have a slower turnover time and have not (yet) been shown to migrate into the draining lymph nodes of the lung.

As for all DCs that reside in the periphery, lung

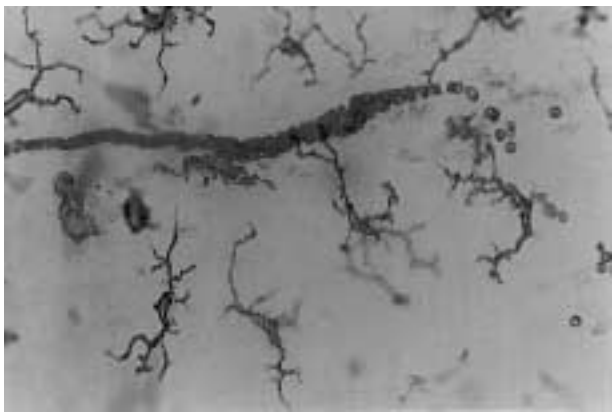

Fig. 3.–The dendritic cell (DC) network in the conducting airways. Mouse tracheal whole mounts were stained with moAb M5/114 for major histocompatibility complex class II molecules (I–E) (original magnification ×400). Numerous DCs with long delicate processes can be seen throughout the conducting airways.

DCs have an immature phenotype, specialized for antigen uptake and recognition, but not yet capable of stimulating naive T-cells, because they lack costimulatory molecules [26, 30, 31, 35]. Although the precise regulatory mechanisms that keep DCs in an immature state are largely unknown, it is proposed that: 1) as airway DCs reside in the lateral intercellular space formed by the basal layer of epithelial cells, it is very likely that mediators (prostaglandin (PG)E_2, IL-10 or nitric oxide (NO)) or membrane ligands (epithelial cadherin) expressed by epithelial cells are critical for downregulating airway DC function [26, 36]; and 2) an equally critical, but largely unexplored mechanism could be the network of unmyelinated nerve endings that also resides within the lateral intercellular spaces and interacts with airway DCs [37]. This network contains vital neuromediators such as calcitonin-gene related peptide (CGRP) and substance P, which are important for the crosstalk between nervous and immune systems [15]. Indeed, it has been shown that the baseline function of Langerhans′ cells in the skin is suppressed by CGRP-containing nerve endings [38].

Separate studies by HOLT and coworkers [31, 39] have focused on the AM as playing a critical role in regulating the function of alveolar wall DCs, by secreting a number of suppressive molecules, such as NO, transforming growth factor (TGF)-β, 1α,25-dihydroxyvitamin D3, PGE_2 and IL-1 receptor antagonist [31, 39]. Moreover, AMs also directly inhibit the proliferation of T-cells, while allowing partial activation [40]. The strongest arguments for a suppressive role come from studies in rodents where AMs were depleted *in vivo* by inhalation of toxic liposomes. In these animals, the response to innocuous protein antigens was greatly enhanced and accompanied by tissue damage to the alveolus [39, 41]. It is thought that the suppressive properties of AMs serve to protect the delicate gas exchange mechanism of the alveolus from being damaged by overtly vigorous inflammatory reactions to inhaled nonpathogenic antigen.

In the absence of infection or inflammation, there seems to be a continuous migration of DCs from the

airways into the draining lymph nodes [6, 34]. One explanation could be that DCs continuously sample the environment for incoming antigens, even under baseline conditions. The localization of immature airway DCs, extending cell projections to the upper layers of the airway epithelium (fig. 3), and the capture of harmless inhaled proteins is consistent with this function [31]. Alternatively, by analogy with the gut and internal organs, an explanation for the continuous migration could be that immature DCs capture bronchial epithelial cells that have become apoptotic as part of their normal turnover. The transportation of apoptotic bodies and the subsequent presentation by DCs in the absence of infection might induce peripheral tolerance to self-antigens derived from bronchial epithelial cells. This would protect the immune system from mounting an immune response to self-antigen when bronchial epithelial cells are rendered apoptotic or even necrotic by infection with respiratory viruses or bacteria [2, 42]. Whether the induction of tolerance would be a function of the migrating DC or occurs only after transfer of self-antigen from migrating lung DCs to resident lymphoid DCs remains to be solved. These nonmigratory lymphoid DCs have been implicated in the regulation of central (*i.e.* thymic) and peripheral tolerance to self-antigen within the CD4 and CD8 pool [2].

The lung dendritic cell network under inflammatory conditions: induction of immunity

Under inflammatory conditions, the function of the lung DC network changes dramatically (fig. 4). In rodents, it has been shown that exposure to the mycobacterium bacillus Calmette-Guérin (BCG) [43], *Moraxella catarrhalis* [44], *Bordetella pertussis* [45], heat-killed *Listeria monocytogenes*, bacterial lipopolysaccharide (LPS) [30], *Mycoplasma* spp. (unpublished data), Sendai virus [46], influenza virus [47], and allergen [48] occassionally induces marked increases in the numbers and activation status of airway DCs. The mechanisms by which immature DCs are recruited into the lung are largely unknown. Based upon *in vitro* and animal studies, it is very likely that monocytic precursors are recruited from the bloodstream and acquire an immature DC phenotype after transendothelial migration into the tissues and exposure to DC-differentiating factors [49].

The signals that attract these immature DCs are largely produced by the epithelium and stromal cells (myofibroblasts) of the airways and potential pathways could include the following. 1) Lung inflammation and tissue damage induced by microbial invasion or lipopolysaccharide (LPS) can be accompanied by expression of inflammatory chemokines, such as macrophage inflammatory protein (MIP)-1α and -β, monocyte chemotactic protein 1–4, and regulated upon activation, normal T-cell expressed and secreted (RANTES), which have all been shown to be chemotactic for immature DCs [45, 50, 51]. However, the chemokine MIP-3α, which is expressed in airway mucosal cells, is probably the most critical factor in attracting immature lung DCs *via* a chemokine receptor (CCR)6-dependent mechanism [51]. 2) Mediators of the innate immune system can similarly and logically attract DCs into the mucosa. The family of antimicrobial epithelial β-defensins is expressed in the bronchial epithelial cells of nearly all vertebrates, where they can be released in high concentrations upon microbial invasion or upregulated by stimulation with LPS and TNF-α. Recently, it was shown that human β-defensin-2 (HBD2) has strong chemotactic activity on immature DCs *via* a CCR6-dependent mechanism, linking direct antibacterial effects with induction of adaptive immunity [52]. Alternatively, bacterial activation of the alternative complement pathway generates C5a, which is chemotactic for immature DCs and a number of inflammatory cells [45, 50]. 3) Finally, microbial invasion also induces the release of cytokines and DC growth factors from resident cell types. TNF-α acts as an important mediator of DC influx, possibly by inducing chemokines, HBD2, platelet activating factor and by increasing the expression of cell adhesion molecules on endothelial cells. An important role for epithelial granulocyte macrophage colony stimulating factor (GM-CSF) is suggested by the finding that adenoviral delivery of this cytokine to the airways induces dramatic changes in the number and immunostimulatory capacity of airway and interstitial DCs ([53] and the authors′ unpublished data) and the fact that GM-CSF accelerates the differentiation of monocytes into immunostimulatory DCs in the lung vascular bed [49].

The induction of inflammation by microbes in the lung accompanies the induction of immunity because: 1) immature DCs that have recognized antigen are induced to migrate *via* the afferent lymphatics into the draining lymph nodes of the lung; and 2) DCs are activated to express the necessary costimulatory molecules (CD80, CD86, see earlier) for stimulating naive T-cells. The molecular mechanisms regulating this maturation process are slowly being elucidated. The pattern recognition receptors expressed on immature DCs can sense microbial danger and tissue damage and signal *via* the conserved Toll-like receptor (TLR) signal transduction pathway, effectively leading to activation of the nuclear factor (NF)-κB transcription factors, and cellular maturation [1, 54]. The Toll protein was originally described in the fruit fly *Drosophila melanogaster*, where it leads to activation of NF-κB-like kinases, leading to the production of antifungal peptides. Similar proteins that confer disease resistance have been found in plants. Thus, Toll proteins represent a host defence mechanism that has been conserved over hundreds of millions of years of evolution. One such pattern-recognition receptor expressed by DCs is the LPS receptor, which consists of (soluble) CD14 that binds Gram-negative LPS complexed to LPS binding protein and signals *via* the transmembrane TLR-4 receptor. A similar mechanism applies to bacterial peptidoglycan from Gram-positive cell walls, which binds to CD14 and signals *via* the TLR-2 [55]. Other factors, such as unmethylated Cytosine-p-Guanine (CpG) motifs in bacterial DNA or double-stranded viral RNA, are strong stimulators of innate immunity and maturation factors for DCs

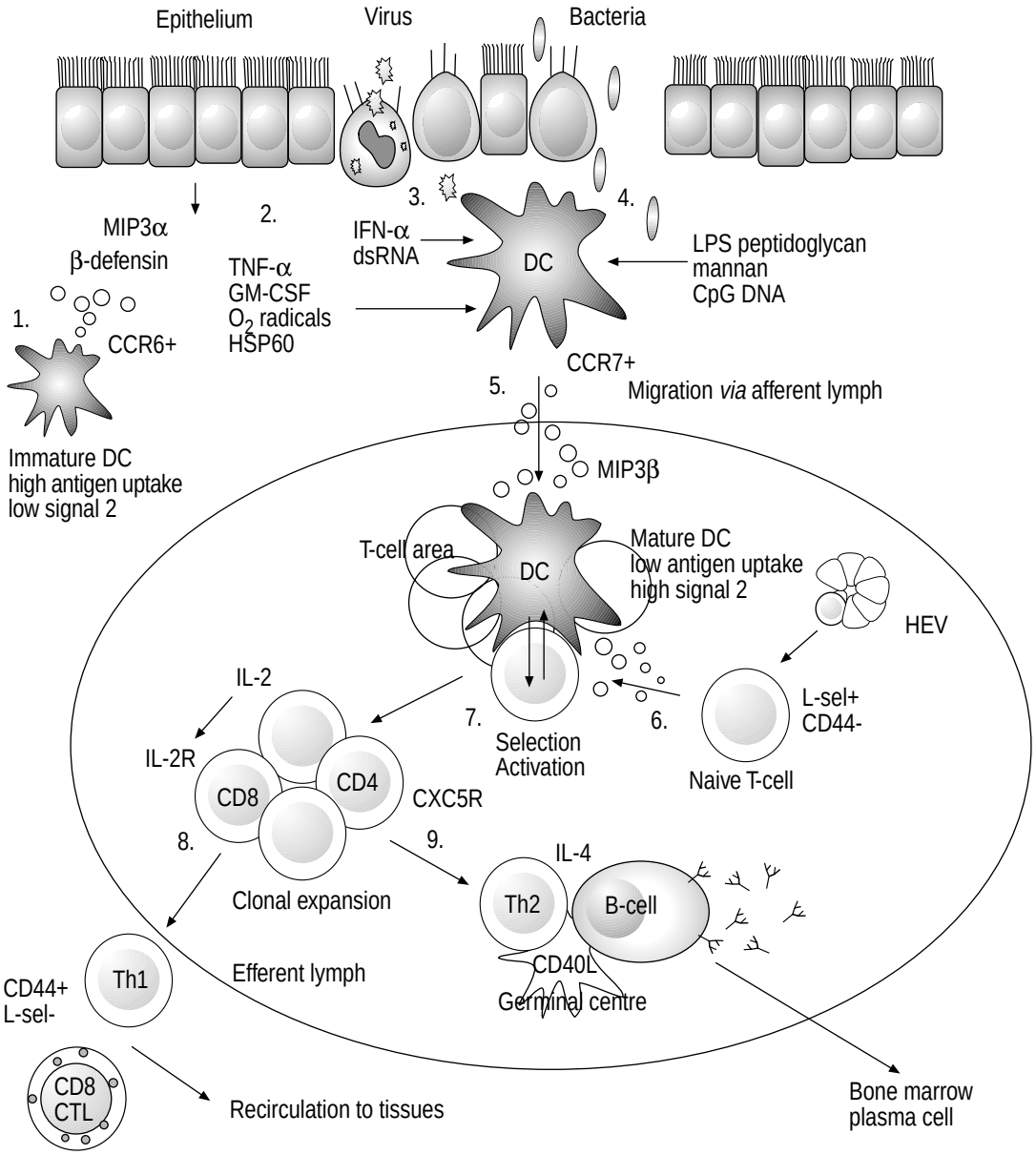

Fig. 4.–Induction of the antimicrobial immune response. 1. Upon exposure to microbial stimuli, epithelia produce macrophage inflammatory protein (MIP)3α and β-defensin to attract chemokine receptor (CCR)6+ immature dendritic cells (DCs). 2. Damaged resident cell types produce inflammatory mediators (tumour necrosis factor (TNF)-α, heat shock protein-60 (HSP60)) and growth factors that attract and activate the DC. 3. During viral infections, DCs phagocytose apoptotic virus-infected cells or are direct targets for viral invasion. The local release of interferon (IFN)-γ or virus-derived motifs (double-stranded ribonucleic acid (dsRNA)) further activates the DC. 4. Alternatively, DCs can phagocytose bacteria. Certain bacterial patterns (lipopolysaccharide (LPS), Cytosine-p-Guanine deoxyribonucleic acid (CpG DNA)) further activate the DCs. 5. The recognition of infection and tissue damage by DCs upregulates the CCR7; DCs migrate to the T-cell area of draining lymph nodes where the ligand MIP3β is constitutively expressed. 6. In the T-cell area, DCs produce chemokines to attract naive T-cells that continuously leave the bloodstream *via* the high endothelial venules (HEV). 7. Naive T-cells are first arrested and then selected for specificity for microbial antigens. The latter event induces their activation. 8. The activation of T-cells leads to autocrine production of interleukin (IL)-2 and to clonal expansion of antigen-specific CD4+ and CD8+ T-cells. These cells differentiate into effector cells that leave the lymph node *via* the efferent lymphatic. Effector cells have the capacity to kill infected cells or to activate macrophages. 9. Upon contact with DCs, some antigen-specific CD4+ T-cells upregulate CXCR5 receptor and migrate to the B-cell follicles of the draining lymph node. Here, they further interact with germinal centre DCs to induce CD40L-dependent B-cell immunoglobulin (Ig) switching and affinity maturation (germinal centre reaction). Most high-affinity B-cells go to the bone marrow to become long-lived Ig-producing plasma cells. Th: T-helper; CTL: cytotoxic lymphocyte; GM-CSF: granulocyte macrophage colony stimulating factor.

[56, 57]. Maturation can also be induced by tissue damage and its accompanying release of oxygen radicals, heat shock proteins (HSP) and changes in the balance between suppressive (IL-10, PGE_2, NO) and inflammatory mediators (TNF-α, IFN-α, GM-CSF, IL-1) [3, 22, 36, 54]. In this respect, it is interesting to note that HSP60, endogenously released by tissue damage, also signals *via* CD14 and TLR-4 [54].

It is critical that DCs that have recognized and taken up foreign antigen migrate to the draining

lymph nodes. This process is exceptional in that maturing DCs have to migrate against chemotactic gradients that attract immature DCs into the inflammatory site. To achieve this, maturing DCs produce inflammatory chemokines leading to ligand-induced downregulation of CCR1, 5 and 6 receptors. However, the expression of CCR7 is increased [51, 58]. A ligand for CCR7 is secondary lymphoid tissue chemokine (SLC; 6Ckine), which is expressed at afferent lymphatic endothelium, efficiently guiding DCs into the afferent lymphatics. Another ligand for CCR7 is MIP-3β (Epstein-Barr virus-induced molecule 1 ligand chemokine), which is constitutively expressed in T-cell zones of lymph nodes, possibly attracting maturing DCs and naive T-cells into these areas [59]. The importance of directed migration of DCs into the lymph nodes is underscored in CCR7 knock-out mice or SLC mutant mice who fail to mount a primary immune response [59].

The migration of airway DCs in response to an immunogenic stimulus is rapid; within 12 h, lung-derived DCs can be traced in the T-cell area of draining mediastinal lymph nodes of the lung [5, 6, 19, 60]. DCs reaching the draining lymph nodes are fully mature and specialized to stimulate naive T-cells. Moreover, they produce high levels of constitutive chemokines, such as DC chemokine and MIP-3β, which attract naive T-cells [3, 59]. Not surprisingly, when antigen is delivered by DCs in the airways, the initial activation and first cycle of division in naive T-cells occur in the draining mediastinal lymph nodes [6]. When a new protein antigen is presented by DCs in the airways, activation followed by proliferation occurs almost exclusively in antigen-specific T-cells. Under these conditions, there is minimal induction of bystander activation in nonantigen specific cells [6]. This process is very rapid as some antigen-specific T-cells have already undergone two cell divisions 48 h after introducing antigen into the lung. After 3–4 days, effector CD4+ and CD8+ lymphocytes are generated in the draining lymph nodes, which then leave the lymph node *via* the efferent lymphatics and recirculate throughout the body [6, 47]. In contrast to most naive L-selectin+ T-cells, these activated effector cells are predisposed to migrate to inflammatory tissues by their strong expression of inflammatory chemokine receptors (CCR1, CCR2, CCR5, CXC1), integrins, CD44 hyaluronate receptor, and by down-regulation of L-selectin [61]. Other effector CD4+ T-cells are stimulated by OX40L on DCs to upregulate the CXCR5 and to migrate into lymph node germinal centres where the ligand B-cell attracting chemokine is expressed [59]. These CD4 effectors produce IL-4, stimulate B cell Ig production, and induce the germinal centre reaction, critical for the generation of high-affinity antibodies [62].

Effector and memory CD4+ and CD8+ lymphocytes are poised to migrate to sites of inflammation or virus replication within the lung, and, upon recognition of their cognate ligand, will regulate the lung defence mechanisms to clear the pathogen (see later). There is now further evidence that the lung DC is the most important APC in stimulating effector CD4+ cells, as was recently shown for the response to inhaled soluble antigen in rodents [32, 37]. This interaction is likely to occur within the inflamed airways and lung parenchyma and does not require a migratory DC. The nonmigratory DCs situated around lung venules and within the alveolar wall, are, therefore, ideal candidates for presentation and amplification of effector T-cell reactions during the effector and/or memory response, especially when resident AMs have become immunostimulatory, *e.g.* under the influence of locally released GM-CSF and TNF-α [37, 39].

Dendritic cells and immunity to viruses

Innate immunity against viral infections depends upon type I IFNs (IFN-α/β), which interfere with viral replication (hence their name) by induction of a number of IFN-stimulated genes. Additionally, IFN-α/β increases MHC class I expression on virus-infected cells and activates NK cells. Although type I IFNs are produced by virus-infected epithelial cells and fibroblasts, another important early source of large amounts of IFN-α/β is the circulating pool of enigmatic natural IFN-producing cells (IPC), which are rapidly attracted to sites of viral replication and inflammatory lymph nodes [63]. There is now considerable evidence that these IPCs are identical to the plasmacytoid monocytes or CD4+CD11c- blood precursors of type 2 lymphoid DCs (pre-DC2) [24, 64]. Interestingly, viral infection of IPCs in the absence of exogenous cytokines induces their differentiation into lymphoid DC2s that produce extremely high levels of antiviral IFN-α/β [63]. Moreover, virus-infected DC2 cells have the capacity to stimulate naive T-cells to become IFN-γ and IL-10 producing effectors. The release of IFN-α by IPCs and virus-infected cells will further promote the maturation and migration of tissue-resident myeloid DCs, also contributing to the generation of antiviral effector T-cells. Therefore, pre-DC2s/IPCs that are attracted into sites of viral infection perform the two master functions of the innate immune system: 1) they kill viruses; and 2) they initiate and dictate adaptive immune responses [1]. The involvement of these cells in viral lung infections has not been studied but clearly deserves much attention.

NK cells are another important aspect of innate immunity to viruses, not least by their capacity to perform antibody-dependent cellular cytotoxicity, their potential to induce apoptosis in target cells and their secretion of IFN-γ. Murine studies have shown that DCs can directly activate the cytotoxic activity of NK cells through membrane interactions (CD80, CD40L) and by production of IFN-α, IL-12, IL-15 and IL-18 [65]. A population of oligoclonal T-cells, called NKT-cells, respond to their natural microbial ligand α-galactosyloceramide, presented on the non-classical MHC class I molecule CD1d and expressed on DCs [3]. Although the latter event strongly induces IFN-γ production in responding NKT-cells, it is also possible that IL-4 is produced under certain conditions (*e.g.* presentation by DC2) [3].

The adaptive immune response to viral infection

involves a population of CD8+ cytotoxic lymphocytes (CTLs), which have the capacity to kill virus-infected cells and which constitute immunological memory to infection. The TCR on CD8+ CTLs recognizes cytosol-derived viral peptides presented on MHC class I molecules. All of the body's cells express MHC class I, but only professional APCs have the necessary costimulatory molecules to stimulate naive CD8+ CTL cells. Therefore, induction of immunity in the CD8+ pool requires that: 1) DCs are infected with virus (endogenous pathway of MHC class I loading); or 2) that they acquire exogenous viral antigen from other infected cells (exogenous pathway of MHC class I loading). A number of viruses (influenza virus, respiratory syncytial virus, measles virus (MV), herpes virus, cytomegalovirus, and dengue virus) can directly infect myeloid and lymphoid DCs *in vitro* [63, 66]. For example, infection with influenza virus occurs in almost all DCs exposed to the virus, as seen in their expression of haemagglutinin and nonstructural protein 1 [67]. The infection must not, however, lead to rapid cell death, as this would eliminate induction of immunity. To avoid destruction, DCs produce IFN-α, which induces the MxA gene to protect the cell from the cytopathic effects of viral infection [56]. Active viral replication is not a requirement, as inactivated influenza virus presented by DCs induces strong CTL activity *in vitro* [67]. When infection or active replication do not occur within DCs, an efficient, alternative pathway to generate MHC class I viral peptides is the phagocytosis of virus particles or virus-infected epithelial cells that have become apoptotic, a process called cross-presentation [68].

As the respiratory tract is often the site of primary viral replication of many of the previously mentioned viruses, it is evident that the airway DC is the most relevant APC for viral infections *in vivo*, as suggested in a number of viral models of influenza and Sendai virus [46, 47]. Airway DCs express: 1) specific receptors that are used by viruses to enter the cell, *e.g.* the ICAM-1 receptor for rhinovirus, and the CD46 receptor for measles virus; but also 2) the mannose receptor that is used to recognize sugar moieties on the capsular and envelope glycoproteins on a variety of viruses [35, 69]. The cross-presentation pathway is also particularly relevant for loading lung DCs with exogenous virus antigen, as apoptosis is the dominant mechanism by which many respiratory viruses induce damage to bronchial and alveolar epithelial cells.

It is controversial whether the induction of full cytotoxic activity in CD8+ cells by DCs requires CD4+ Th1 cell help. Like T-cell-dependent antibody production by B-cells, many CD8+ cytotoxic cell responses are dependent on CD4+ Th1 cells, providing IL-2 for proliferation and IFN-γ for activation (fig. 4). In the "licence to kill" theory, originally proposed by LANZAVECCHIA [70], it is thought that CD40L expressed on virus-specific CD4+ T-cells activates the DC to prime CD8+ CTL activity. The interaction between CD4+ Th1 cells and CD8+ CTLs is therefore indirect and does not occur simultaneously, with the DC acting as a temporal bridge. It is, however, possible that direct viral infection of DCs as well as microbial stimuli (LPS, microbial DNA) can bypass the need for CD4+ Th1 help [71].

One final aspect of long-lived adaptive immunity to infections within the respiratory tract is the presence of secretory IgA in epithelial lining fluid, inhibiting viral (and bacterial) adherence. The production of IgA antibodies is T-cell-independent, but requires cytokines from nonB-cells. Dendritic cells: 1) induce surface IgA expression on CD40-activated naive B-cells; and 2) through their release of IL-10, TGF-β and an unknown factor induce secretion of both IgA1 and IgA2 subclasses by plasma cells [72]. It has been suggested that direct interactions between B-cells, DCs and epithelial cells (providing IL-5) occur locally in the airway epithelium, leading to the extralymphoid production of IgA [54].

Dendritic cells and immunity to bacteria, fungi and parasites

Innate immunity to bacterial and fungal pathogens consists mainly of β-defensins, bacteriostatic enzymes, alternative activation of complement, production of C-reactive protein and most importantly, uptake followed by phagocytic cell killing (respiratory burst). Phagocytic neutrophils and monocytes predominate in acute pyogenic infections, whereas macrophages are more prevalent in chronic or granulomatous infections. Another important function attributed to NK cells is recognizing conserved bacterial structures and effectively killing cells infected with intracellular bacteria (*e.g. L. monocytogenes*). In contrast to most T-lymphocytes that express a unique αβ TCR, a subclass of intraepithelial CD4-CD8- T-cells express a common γδ TCR. This TCR reacts with glycolipid antigens in a CD1-restricted manner and could be important for recognizing conserved motifs of intracellular bacteria.

Adaptive immunity to bacteria is both humoral and cellular. Neutralizing immunoglobulins and epithelial IgA protect against extracellular organisms through a variety of mechanisms: neutralization of toxins, complement lysis, and interference with adherence to cell surfaces [17]. Opsonizing antibodies are important for complement fixation and for enhancing the efficiency of macrophage killing. T-lymphocytes mediate a variety of reactions, including recruitment and activation of macrophages, induction of delayed hypersensitivity (granuloma formation) and the provision of help for Ig production by B-cells. The central role of lymphocytes in protecting against infection is illustrated in acquired immune deficiency syndrome (AIDS), where a progressive loss of CD4+ cells leads to a dramatic susceptibility to bacterial, fungal and viral pathogens. Over the last 15 yrs, it has become clear that it is not the induction of an immune response *per se* that determines the outcome of a pulmonary bacterial infection, but rather the development of a response that is optimally tuned to clear the pathogen. Inappropriate responses to infection can cause severe pathology. Central to the understanding of adaptive immunity to bacterial infections was the discovery of the Th1/Th2 concept by MOSSMANN *et al.*

[16]. By the nature of their antagonistic effects on the opposite subset (*e.g.* IL-4 suppressing IFN-γ in Th1 cells), the relative frequencies of the two Th subsets can determine whether a given immune response is protective or pathological [17]. In the murine model of *Leismania major* infection, Th1 cells are associated with protection, whereas Th2 cells are clearly associated with susceptibility, leading to a fatal disease [17]. For bacteria, the adaptive immune response has evolved to induce a strongly polarized Th1 response to clear the infection. This is especially critical for intracellular bacteria that depend on IFN-γ: 1) to activate macrophages to either contain (*e.g.* in the case of tuberculosis) or eradicate the infection; and 2) to switch Ig production towards opsonizing IgG2a. Th2 cells are inadequate in both respects.

In the absence of bacterial stimuli, resting lung myeloid DCs induce weak Th2 responses to inhaled harmless antigens [19, 26]. This may be due to the pulmonary environment, which is rich in IL-10 or PGE_2, mediators known to downregulate IL-12 production in DCs [22, 36]. This response can be dramatically changed during bacterial infection. According to KALINSKI *et al.* [22], the characteristics of the pathogen and the microenvironmental tissue damage that it induces are instrumental in directing the type of Th response that will ensue, to ensure an optimal immune response. Several pathogen-associated molecules (LPS, lipoteichoic acid, peptidoglycan, bacterial DNA) have been shown to induce IL-12 and/or IL-18 production from DCs, and to induce their migration into T-cell areas, effectively inducing IFN-γ-producing Th1 cells [20, 26, 57]. While it is clear that the majority of "danger" signals, such as LPS, activate DCs to promote a Th1 response, the nature of the pathogen-associated molecules involved in initiating a Th2 response are poorly understood. These molecules could be used by the pathogen to subvert the cellular immune response. As an example, a filarial nematode-secreted glycoprotein called ES-62 was shown to influence Th2 development by DCs even in the presence of IL-12 [25]. For this parasite, Th2 responses are associated with decreased clearance from the tissues.

Mycobacterial infection deserves separate attention. Immunity to mycobacteria is mediated *via* macrophages, whose activation depends upon IFN-γ-producing CD4+ Th1 cells, and by CD8+ CTLs that lyse infected macrophages harbouring cytoplasmic mycobacteria. Recently, it was shown that γδ T-cells and CD8+ T-cells reacting to mycobacterial glycolipids on CD1 molecules are also important in mediating control of the disease [73]. Although macrophages have been shown to be potent inducers of effector T-cells *in vitro*, studies in mice have shown that DCs can also efficiently phagocytose the attenuated BCG strain and migrate to the draining lymph nodes of the lung to induce protective immunity against *Mycobacterium tuberculosis* challenge *in vivo* [18]. In humans, the presence of the primary Ghon complex is a clear illustration of the movement of mycobacteria from the site of inoculation to the draining lymph nodes. It is likely that DCs are the vehicles of this transport. Moreover, it has been shown that human DCs phagocytose *M. tuberculosis* and efficiently prime mycobacterium-specific CD4+ and CD8+ T-cells *in vitro*, with at least some mycobacterial antigens being presented on CD1 [74].

Granuloma formation is a consistent finding in infections caused by mycobacteria, reflecting the need to contain the organism when it is not efficiently cleared by macrophages. It was recently shown that early granulomata induced by BCG in rats are characterized by large numbers of DCs expressing the rat marker OX62 [75]. These DCs were seen to interact with T-cells in the lymphoid collar surrounding the area of epithelioid cells and Langhans' giant cells. As the granulomata matured, increasing numbers of DCs were seen to surround the lesion. Similarly, patients with the less aggressive tuberculoid leprosy (*M. leprae*) have multiple CD1+ DCs surrounding the granulomata, whereas those with progressive lepromatous lepra lack infiltrating DCs [76]. The latter findings suggest that DC infiltration into the granuloma is critical in containing infection. Thus, as DCs have the capacity to activate effector T-cells, it is unclear why these granulomata do not resolve. One possible explanation could be the production of large amounts of the Th2 cytokine IL-10 within granulomata, known to reduce costimulatory activity and IL-12 production in DCs and to convert the phenotype of mycobacterium-infected DCs into bactericidal macrophage-like cells [3, 36].

Dendritic cells and immunodeficiency

As DCs are central to the integration of innate and adaptive immunity, it is highly likely that disruption of DC function leads to increased susceptibility to infection. Indeed, DCs have been implicated in the pathogenesis of a variety of acquired immunodeficiency states. Measles infection causes a profound immune suppression, which leads to an increased susceptibility to secondary infections, a major cause of childhood mortality in developing countries. Respiratory tract DCs are a primary target for MV infection and dissemination to lymph nodes. Infection of DCs with MV leads to the formation of giant multinucleated reticuloendothelial cells (Whartin-Finkeldey cells) that are thought to represent syncytia of DCs and activated T-cells, and are sites of vigorous viral replication [77]. Infected DCs actively suppress "bystander" T-cell proliferation, and when they interact with activated T-cells, both cell types are eliminated by apoptosis [77, 78]. Moreover, MV infection also causes defective activation and suppression of IL-12 production by DCs. Not surprisingly, interaction of MV with DCs leads to a profound defect in cell-mediated immunity, as exemplified by the loss of the delayed type hypersensitivity reaction to recall antigens [66]. Similarly, the interaction of mucosal DCs and human immunodeficiency virus (HIV) leads to transmission of the virus to the lymph nodes [79]. Surface receptors, such as CD4, the costimulatory molecule DC-SIGN and the chemokine receptor CCR5, are used by the virus to attach to the DC surface, or to enter the cell [11]. Upon interaction

with virus-carrying DCs, naive CD4+ T-cells become infected with the virus, ultimately causing their destruction [80]. Aside from their role in dissemination of HIV, it is thought that DCs can function as an important reservoir of HIV during the latent phase of the disease. When patients with HIV infection reach the AIDS stage, there is a striking reduction in the natural IFN-producing cells, recently identified as pre-DC2s (see earlier), although its implication in the progression of immunodeficiency is not established [64].

The use of systemic corticosteroids is associated with a reduction in cell-mediated immunity, leading to enhanced susceptibility to a number of pathogens. Corticosteroids reduce the antigen-uptaking capacity of DCs and influence the expression of costimulatory molecules and the level IL-12 production [81, 82]. Moreover, inhaled steroids have been shown to reduce the numbers of airway DCs by inducing apoptosis, although it is unclear whether this alone can lead to immunosuppression [83].

Dendritic cells as cellular vaccines for infectious diseases?

The fact that DCs are crucial inducers of adaptive immunity has led to their exploitation as potential cellular vaccines, inducing long-lasting immunity that may mimic that of natural infection. During the last 2 yrs, numerous clinical trials using DCs to induce strong CTL activity against tumours have been initiated, highlighting the feasibility of such an approach [3]. Similarly, the possible use of DCs to protect against viral or bacterial infection is actively investigated in a number of laboratories. Dendritic cells pulsed with a lymphocytic choriomeningitis virus peptide were able to induce strong CTL activity and protect against subsequent live virus infection in mice [84]. Similarly, human DCs pulsed with influenza peptide were able to elicit long-lasting CTL activity *in vivo*, although protection against subsequent infection was not tested [85]. In mouse studies, it was also recently shown that pulsing DCs *in vitro* with *Chlamydia trachomatis* antigen, *Pseudomonas aeruginosa* antigen and attenuated *Mycobacterium* spp. was sufficient to protect against subsequent challenge of the lungs with live organisms [18, 86, 87]. Although it is not feasible to vaccinate large numbers of patients with (autologous) DCs in the prevention of infections, alternative strategies could be aimed at targeting a vaccine to the resident DCs of the body. In this context, it is clear that the antigens encoded by DNA vaccines are expressed and presented predominantly by DCs in the lymph nodes draining the site of injection [88]. Alternatively, the administration of attenuated bacteria (*e.g.* apathogenic *Salmonella typhimurium* subspecies) that are genetically modified to express novel antigens, might be a future strategy for loading DCs *in situ*, as these strains are actively phagocytosed by immature DCs [89].

Concluding remarks

The defence system of the host lung against microbes relies on the communication between cells of the innate and adaptive immune system. Dendritic cells use the receptors of the innate immune system to recognize and internalize pathogens, and carry these antigens to the draining lymph nodes. Over the past few years, it has become clear that these cells perform essential and pivotal functions in the induction and regulation of adaptive cellular and humoral immunity. The knowledge of dendritic cell biology is rapidly expanding; in the future, it will undoubtedly be possible to see strategies that will utilize the unique properties of these cells for the prevention and therapy of infectious diseases.

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