Eur Respir J, 1993, 6, 1213–1220
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European Respiratory Journal
ISSN 0903 - 1936

SERIES ' PULMONARY IMMUNE CELLS'
Edited by U. Costabel and C. Kroegel

Pulmonary immune cells in health and disease: Dendritic cells and Langerhans' cells

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Dendritic cells and Langerhans' cells. A.J. Hance. ©ERS Journals Ltd 1993.
ABSTRACT: The activation of T-lymphocytes recognizing specific antigens is a crucial and early event in the development of an immune response, but T-lymphocytes cannot respond to antigens without help of a second cell type called accessory cells or antigen-presenting cells. Studies from several groups have indicated that pulmonary dendritic cells and Langerhans' cells, like their counterparts in other tissues, are potent accessory cells, and suggest that these cells may play an extremely important role in initiating lung immune responses. The purpose of this review is to summarize current information concerning pulmonary dendritic cells and Langerhans' cells, including their origin, distribution in the lung and functional capabilities. The possible role of these cells in certain lung diseases of immune origin will also be discussed.
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Keywords: Dendritic cells
Langerhans' cells

The activation of T-lymphocytes, recognizing specific antigenic epitopes on foreign proteins, is a crucial and early event in the development of an immune response. T-lymphocytes cannot respond to antigens without the help of a second cell type, called the accessory cell or antigen-presenting cell [1, 2]. The accessory cell performs several distinct roles in the activation of the T-lymphocyte. Firstly, the accessory cell must degrade the foreign antigen, and then express the partially degraded antigen on its surface in association with major histocompatability complex (MHC) molecules (human leucocyte antigen (HLA) molecules in man). Secondly, the accessory cell, expressing the processed antigen must encounter a T-lymphocyte capable of recognizing the antigen/MHC complex through its T-cell antigen receptor. Finally, in the course of the resulting interaction between the accessory cell and T-lymphocyte, the accessory cell must deliver activation signals to the T-lymphocyte, which stimulate the proliferation of lymphocytes and induce activities necessary for lymphocyte effector functions (*e.g.* cytotoxicity, secretion of cytokines). Most cell types cannot perform all of these accessory cell functions, and "professional" accessory cells are usually required to initiate immune reactions [3].

For many years, the alveolar macrophage was considered to be the most important accessory cell present in the lung. Although alveolar macrophages can clearly serve as accessory cells, most studies have found that alveolar macrophages in the normal human lung are, in fact, relatively weak accessory cells (reviewed in [3]). In addition, these cells secrete a variety of mediators, which can inhibit the activation and function of T-lymphocytes. Strikingly, when experimental animals are depleted of alveolar macrophages *in vivo*, the ability of these animals to mount an immune response against inhaled antigens is actually improved [4]. These findings have called into question the importance of alveolar macrophages in initiating immune responses, and have given impetus to the search for other lung cell populations which could efficiently play the role of accessory cells in the lung.

Recently, dendritic cells (DC) and Langerhans' cells (LC) have been identified in the normal lung. Studies from several groups have indicated that pulmonary DC and LC, like their counterparts in other tissues, are potent accessory cells, and suggest that these cells may play an extremely important role in initiating lung immune responses. The purpose of this review is to summarize current information concerning pulmonary DC/LC, including their origin, distribution in the lung, and functional capabilities. In addition, their possible role in certain lung diseases of immune origin will be discussed.

Pulmonary dendritic cells and Langerhans' cells

Unlike alveolar macrophages, which represent up to 10–15% of all cells in the alveolar interstitium, DC and LC are present in relatively small numbers. As their name suggests, DC have an elongated form, with multiple long cytoplasmic extensions, which can be observed to retract and extend when cells are maintained in culture [5, 6]. The nucleus of DC is irregular and highly convoluted. The cells contain the usual cytoplasmic organelles in abundance, including rough endoplasmic reticulum, a

well-developed Golgi apparatus, mitochondria and acidic lysosomes [7–11]. Consistent with an apparent lack of phagocytic activity, the cells do not contain phagolysosomes.

LC, thought to be derived from DC, are also present in the human lung. In most respects, the morphology of LC is similar to that of DC, but LC are distinguished from dendritic cells by the presence of characteristic pentalaminar plate-like cytoplasmic organelles, called Birbeck granules, visible only by electronmicroscopy [6, 7, 12]. In the normal human lung, LC are present only within the airway epithelium (see below). It remains unclear whether all cells of DC lineage within the airway epithelium are LC. Studies in our laboratory indicate that the majority of cells present within the airway epithelium of humans which have other morphological criteria typical of DC also contain Birbeck granules, but the number of granules is usually low, and serial sections must often be evaluated to demonstrate their presence [8]. In contrast, Birbeck granules have not been identified in DC present in the airways of rodents, and these cells are commonly referred to as "intraepithelial DC" [9, 10].

DC are widely distributed in the normal lung, including the pleura, alveolar septal interstitium, pulmonary capillaries, peribronchiolar connective tissue and bronchus associated lymphoid tissue (BALT) [8, 10, 13–16]. As indicated above, LC (humans) or intraepithelial DC (rodents) are also present between the basement membrane and the lumen of airways, interspersed between the airway epithelial cells. When airway epithelium is cut parallel to the lumen and stained for DC, the cells are found to form a highly-developed intraepithelial network, resulting from the interdigitation of the long cytoplasmic processes of DC between the epithelial cells [14, 17], and highly reminiscent of the network formed by LC in the skin. The density of airway DC is highest in the trachea, and decreases progressively in smaller airways, although LC/DC can be identified in respiratory bronchioles [8, 17].

Origin

DC/LC, first identified in the skin, are now known to be present in human tissues. DC are derived from bone marrow stem cells, presumably the same cells that give rise to other haematopoeitic cells [5, 6]. The differentiation pathway for DC has not been established, but progenitor cells with morphological and phenotypic properties typical of DC and distinct from those of monocytes, have been found in the bone marrow [18]. It is thought that DC are released from the bone marrow into the blood. DC are present in peripheral blood, but represent <0.5% of mononuclear cells [19]. Tissue LC and DC may also re-enter the blood after stimulation. It is unclear what proportion of circulating DC have arrived directly from the bone marrow, and whether the functional properties of circulating cells, which have or have not previously resided in tissues, are similar.

DC are thought to arrive in the lung and other tissues *via* the capillary bed (fig. 1), and are present in the lung before birth [9]. The cells are mobile, and can migrate along channels present between cells and connective tissue components of the interstitial tissues [8]. LC are probably derived from DC which have migrated into the bronchial epithelium. As in other tissues, the differentiation of DC into LC in the lung appears to occur only in the presence of epithelial cells, but not all epithelial cells are equally effective in inducing the recruitment and differentiation of DC. Thus, in the normal lung, LC are found within the airway epithelium, but not the alveolar epithelium [8]. LC can be found in additional sites in pathological circumstances, but again are associated with epithelial cells. Firstly, LC accumulate at sites of alveolar epithelial hyperplasia, where they are found infiltrating the hyperplastic type II pneumocytes [8, 20, 21]. Secondly, some, but not all, pulmonary carcinomas are infiltrated by LC, occasionally with extremely large numbers of cells [21]. Tazi *et al.* [21] have recently demonstrated a close correlation between the infiltration of epithelial cells by LC and the production of the cytokine, granulocyte macrophage colony stimulating factor, (GM-CSF) by the epithelial cells. These findings support the conclusion that the GM-CSF may play an important role in the recruitment and/or differentiation of airway LC. Intratracheal instillation of lipopolysaccharide and administration of interferon-γ to animals also increases the number of intraepithelial DC, but the mechanisms of action have not been defined [16, 17]. DC/LC are not present in the bronchial lumen, and few if any DC and LC are recovered by bronchoalveolar lavage from normals. Up to 5% LC can be recovered by lavage from smokers or patients with diseases producing epithelial hyperplasia [7, 22].

Surface phenotype

(MHC) molecules. As expected for potent accessory cells, lung DC and LC strongly express class I MHC molecules (HLA-A,-B and -C in man), and all forms of class II MHC molecules (HLA-DR,-DP and -DQ in man) [17, 23–25]. Interstitial dendritic cells from rats have been reported to express class II MHC molecules more strongly than airway dendritic cells [26].

Cluster designation 1(CD1) molecules. DC in parenchymal tissue of humans express CD1c molecules, whereas intraepithelial LC are $CD1a^+$ [8]. Although $CD1a^-$ LC have not been described, CD1a and CD1c molecules cannot be used to unequivocally identify LC and DC, respectively. Firstly, varying proportions of LC can co-express CD1a and CD1c molecules [8, 21], and the LC accumulating in granulomas of histiocytosis X (see below) may be essentially all $CD1c^+/CD1a^+$ (Tazi, unpublished). Furthermore, cells with the $CD1c^+/CD1a^+$ phenotype, but without detectable Birbeck granules (so-called "indeterminate cells") are present in the lung and other tissues [8]. Some evidence suggests that CD1 molecules may be involved in the internalization of antigen [11, 27]. In addition, T-lymphocytes which recognize antigens expressed in association with CD1 molecules have been described, indicating that these molecules can be involved in antigen presentation [28]. Further studies are needed to

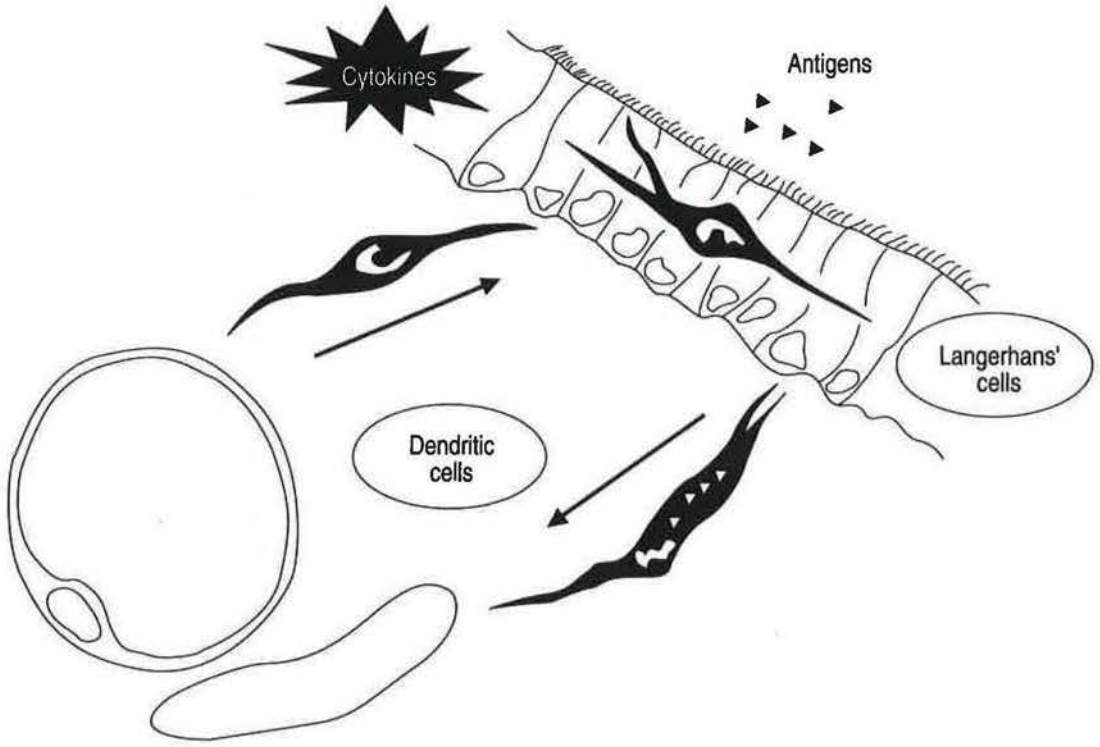

Fig. 1. – Langerhan's cell (LC)/dendritic cell (DC) in the lung. DC are thought to arrive in the lung parenchyma *via* the pulmonary capillaries. Some DC can then migrate into the bronchial epithelium and differentiate into LC. Intraepithelial LC are ideally positioned to capture antigens (small triangles) penetrating into the airways, and these cells can internalize the antigens and carry them to regional lymphatic tissues *via* the pulmonary lymphatics. The distribution and function of DC/LC is under the control of cytokines present in the extracellular space. Lung DC/LC also appear to have considerable ability to activate T-lymphocytes *in situ*, and this lymphostimulatory activity increases at sites of immune/inflammatory reactions.

define the functional capacities of LC/DC expressing different isoforms of CD1. Antibodies recognizing CD1 molecules on murine DC are not available.

Receptors and adhesion molecules. LC/DC in other tissues may express receptors for immunoglobulins, including FcγRII, FcεRI and FcεRII [5, 10, 29, 30]. These molecules could be involved in uptake of antigens recognized by immunoglobulins. Fc receptors for immunoglobulin G (IgG) are much more abundant on airway DC than interstitial DC [25, 26]. Receptors for the complement fragment C3bi are also present in low density on lung DC [10, 23, 25]. As discussed below, numerous molecules belonging to the integrin and adhesin families are expressed by lung DC [17, 23, 24, 26], and probably play a role both in the interaction of DC with other cells and the migration of DC. These molecules can be differentially expressed on subpopulations of DC, and the expression of some of these molecules can change substantially as a result of inflammatory stimuli, or in the course of purification of DC [17, 26]. A variety of other receptor-like molecules have been identified on DC/LC in the lung, including CD4 molecules, interleukin-2 (IL-2) receptors, and the CD45 leucocyte common antigen [17, 24, 25]. DC/LC are also positive for the S-100 protein, but this marker is also expressed in other cells types.

Isolation of pulmonary DC

The study of the functional capacities of lung DC/LC requires the isolation of these cells. Procedures have been developed by several groups for the isolation of DC from human and rodent lungs [13, 14, 16, 26, 31, 32]. Because they are present in only small numbers in the lung, multistep procedures are required to obtain purified populations. In general, cell suspensions are obtained by proteolytic digestion of lung tissue, and DC are purified on the basis of low buoyant density and their tendency to be loosely adherent cells, which pass through nylon wool columns [13, 14], and adhere only transiently to petri dishes [16, 31]. To obtain highly purified populations, additional steps are required, either based on the positive selection of DC (*e.g.* isolation of cells strongly expressing class II MHC molecules by panning) [26], or the elimination of contaminating populations. For example, both the removal of cells expressing Fc receptors for IgG and the elimination of autofluorescent cells using the cell sorter are useful in eliminating macrophages [13, 32]. A technique for selective recovery of DC present in larger airways has recently been described [26]. It is likely, however, that cells recovered from "lung parenchyma" include both DC present within the smaller airways and those found within the interstitial space.

Only a fraction of DC originally present in the tissues are recovered by these techniques. In addition, the isolation procedures themselves may both eliminate subpopulations of DC (*e.g.* removal of DC expressing Fc receptors) [13, 25, 26] and result in modifications of the surface phenotype of the DC which are recovered [26]. Finally, cytokines released in the course of cell isolation, or those added to culture media in order to improve the survival of DC *in vitro*, may modify the functional capacities of the cells [19, 33, 34]. Thus, it remains uncertain to what extent the functional activities of DC studied *in vitro* reflect that of cells *in situ*. Despite this caveat, the analysis of purified DC *in vitro* has provided considerable insight into the function of lung DC.

Function of pulmonary DC and LC

The signals required to stimulate T-lymphocytes vary considerably as a function of their state of activation. In general, "naive" T-lymphocytes (those which have never been previously activated) require signals which are no longer required by "memory" T-lymphocytes (those which have been previously activated, but have returned to a resting state) or recently activated cells [3]. DC/LC appear to be particularly effective in stimulating "naive" T-lymphocytes. In this respect, several studies have indicated that antigen-pulsed DC/LC have a strong capacity to initiate immune responses against previously unencountered antigens. Because other types of accessory cells (*e.g.* B-lymphocytes and macrophages) are ineffective in these systems, DC have been referred to as "nature's adjuvant" [35]. Similarly, DC have a strong capacity to stimulate $CD8^+$ cytotoxic T-lymphocyte precursors in the absence of help from $CD4^+$ T-cells [36, 37]. Once T-lymphocytes have been initially activated by DC, the cells can subsequently respond to antigen presented by a variety of different accessory cells [38].

To serve as accessory cells, DC and LC must perform several distinct functions. The ability of lung DC and LC to perform these different accessory cell functions has been evaluated in a number of studies.

Presentation of exogenous antigens. Foreign antigens present in the extracellular milieu are generally recognized by $CD4^+$ T-lymphocytes, which interact with antigens presented in association with class II MHC molecules [3]. Thus, these exogenous antigens must be internalized by the accessory cell, partially degraded, and subsequently re-expressed on the cell surface in association with class II MHC molecules. The pathways involved in antigen processing by DC and LC are incompletely understood. Birbeck granules are thought to be formed by cell processes folding back onto the cell membrane, and have been implicated in the internalization of antigens [11, 27]. CD1a molecules are present on the cell surface and in the lumen of the "granules", where they communicate with the cell surface, and may play a role in the formation of Birbeck granules. Some DC, which lack Birbeck granules, can also internalize antigens [5]. The folding back of dendritic processes onto the cell membrane of DC also occurs, and the cell membranes at these sites are rich in CD1c molecules, suggesting that similar mechanisms of internalization may be involved [8]. In addition, skin LC possess acidic endocytic vesicles, thought to be necessary for antigen degradation [11], and synthesize de novo class II MHC molecules [39]; the loss of these activities is associated with impaired ability to present external antigens [11, 39]. Lung DC from rodents and humans have been shown to present exogenous antigens [13–16, 26, 40].

Presentation of endogenous antigens. Antigens encoded by intracellular parasites, such as viruses, typically follow a distinct processing pathway, and are expressed on the cell surface in association with class I MHC molecules, permitting their recognition by $CD8^+$ T-lymphocytes [41]. Splenic DC can be infected with a number of viruses, including influenza and human immunodeficiency virus (HIV), and these cells stimulate virus-specific $CD8^+$ cytotoxic lymphocytes [5, 37, 42, 43]. Nonacs *et al.* [37] recently demonstrated that productive infection of DC with virus, not just the presence of viral antigens, was necessary for the generation of $CD8^+$ cytotoxic cells, and the responses were greatly increased by the presence of $CD4^+$ lymphocytes responding to exogenous antigens. Infection of DC/LC by HIV has been suggested to play a role in the pathogenesis of acquired immune deficiency syndrome (AIDS) [42, 43]. Viral infection of lung DC/LC has not been directly demonstrated, and the role of these cells in stimulating $CD8^+$ cytotoxic lymphocytes in the lung has not been defined.

Migration of DC and LC. Essentially all T-lymphocytes in the normal lung are "memory cells", recognizing antigens with which the immune system has had previous contact [3, 44]. Thus, the stimulation of a primary immune response to inhaled antigens requires that the accessory cell transport the newly arrived antigen to regional or central lymphoid tissues, where lymphocytes capable of recognizing previously unseen antigens are present. Studies in other tissues have shown that DC and LC do transport antigens, and that lymphatics must be intact for migration to regional lymph nodes [5, 45, 46]. Lung DC and LC may also leave the lung and migrate to regional lymphoid tissues, presumably *via* the afferent lymphatics; these cells could also reach the spleen *via* the peripheral circulation. Lung DC are known to express a variety of surface molecules, such as adhesins and $\beta 1$ and $\beta 2$ integrins, which are thought to be involved in the attachment to endothelial cells and connective tissue components, and which could participate in the migration of DC [23–26]. Further studies are necessary, however, to better characterize the pathways followed by lung DC and LC in antigen transport, and to define the factors which regulate this important process.

Lymphostimulatory activities. The lymphostimulatory activity of DC has most frequently been assessed by measuring the ability of purified cells to stimulate the proliferation of syngeneic T-cells, allogeneic T-cells, or periodate treated T-cells in the absence of added antigen.

Lung DC, like DC from many sources [5, 47], have the remarkable ability to stimulate lymphocyte proliferation in these assays, and, on a cell-for-cell basis, lung DC are generally 10–100 fold superior to blood monocytes or alveolar macrophages in inducing lymphocyte proliferation [13, 15, 16, 25, 26, 31, 48–50]. Alveolar macrophages actually suppress the lymphocyte proliferation induced by DC [13, 14, 31, 40, 50].

LC and DC can produce a variety of cytokines, including IL-1, IL-6, tumour necrosis factor-α (TNF-α) and macrophage inflammatory proteins (MIP) [51–53]. Nevertheless, other cell types that function poorly as accessory cells also produce these factors [23, 53]. Furthermore, the addition of neutralizing antibodies to these cytokines do not inhibit DC-induced lymphocyte proliferation, and factors produced in the course of DC/lymphocyte interactions do not stimulate the proliferation of bystander lymphocytes [5]. These findings suggest that the release of soluble mediators does not explain the lympho-stimulatory activity of DC. Of more importance appears to be the expression by DC of a variety of surface molecules, which interact with specific receptors on the surface of T-lymphocytes. These molecules appear to serve two purposes. Firstly, they reinforce the low affinity interactions resulting from contact between the antigen receptor on the T-lymphocyte and the antigen/MHC molecules present on the DC. In addition, these co-stimulatory molecules deliver signals necessary for the activation of the T-lymphocyte. Numerous molecules have been described on DC and LC, including those present on DC/LC in the lung, which can interact with T-lymphocytes (table 1). Although antibodies against a single molecule inhibit lymphocyte proliferation only partially, the use of combinations of antibodies can essentially eliminate lymphostimulatory activity [23, 54, 55]. Nicod and El Habre [23] have demonstrated that antibodies to lymphocyte function associated antigen-3 (LFA-3), CD18 and CD29 cause moderate to marked inhibition of the proliferation of allogeneic T-cells induced by lung DC.

Table 1. – Surface molecules expressed by DC/LC participating in accessory-cell/lymphocyte interactions*

Surface molecule on dendritic cell	Ligand on T-lymphocyte	Present on lung DC/LC
Antigen + MHC	T-cell receptor	+
Class I MHC	CD8	+
Class II MHC	CD4	+
ICAM-1 (CD54)	LFA-1 (CD11a)	+
LFA-3 (CD58)	CD2	+
B7/BB1	CD28	?
CD4	Class II MHC	+
LFA-1 (CD11a)	ICAM-1 (CD54)	+

*CD: cluster designation. Some molecules may be expressed on only a subpopulation of lung DC/LC. MHC: major histocompatability complex; ICAM: intercellular adhesion molecule; LFA: lymphocyte function associated antigen; DC: dendritic cell; LC: Langerhans' cell.

Heterogeneity

Although DC and LC represent cells of a single lineage, it is increasingly clear that the cells are heterogeneous, both in terms of the types of molecules expressed on their surface and in their ability to perform the different functions required of accessory cells [25, 26]. Furthermore, not only the distribution, but also the phenotype and functional activities of DC/LC can be influenced strongly by mediators present in the local milieu.

Although DC/LC can perform all of the functions required of accessory cells, it is important to recognize that a given cell may not express all of these functional activities simultaneously. Rather, DC may express these activities sequentially, over time. This has been most clearly demonstrated for skin LC from rodents. Fresh skin LC efficiently process and present antigens, but have little lymphostimulatory activity [33, 56, 57]. Following culture *in vitro*, the cells lose their ability to process antigens, but develop strong lymphostimulatory properties. This has led to the idea that, under some circumstances, LC serve primarily as "sentinels" that transport potential antigens to regional lymph nodes, without generating immune reactions at the site of antigen deposition [56]. In this regard, Gong *et al.* [26] have shown that airway dendritic cells were more effective in stimulating antigen-induced lymphocyte proliferation than were DC isolated from parenchymal tissues. In contrast, DC from parenchymal tissues were superior to airway DC in stimulating the proliferation of allogeneic T-cells. The differences between airway and parenchymal DC were less dramatic than those observed comparing fresh and cultured skin LC, and both airway and parenchymal DC had considerable activity in both assays.

A number of studies suggest that the functional capabilities of DC and LC can be modified by cytokines. For example, both IL-1 and GM-CSF can dramatically improve the lympho-stimulatory activity of skin LC, and TNF-α and GM-CSF improve the survival of LC/DC *in vitro* [19, 33, 34]. Similarly, changes in the surface phenotype of lung DC present at sites of inflammatory reactions have been reported [17, 26], and the incubation of lung DC in the presence of supernatants from activated T-cells improves their ability to stimulate lymphocyte proliferation [15]. Thus, cells which possess only modest ability to stimulate lymphocyte proliferation in the normal lung may develop potent lymphostimulatory activity in the course of pulmonary immune responses, or under other pathological conditions.

Role in lung diseases

Because pulmonary DC/LC are potent accessory cells, it is probable that they play an important role in lung diseases resulting from abnormal immune responses. Evidence supporting this idea has been presented for several diseases; three examples are briefly considered here.

Pulmonary histiocytosis X. Histiocytosis X (HX), also called Langerhans' cell granulomatosis, is defined pathologically

by the presence of destructive granulomatous lesions containing LC [7, 58]. In children, pulmonary involvement usually occurs in the context of a systemic disease involving multiple tissues. In adult patients, isolated pulmonary involvement is common, and occurs almost exclusively in cigarette smokers [7, 58]. Pulmonary lesions are centred on bronchioles and destroy the airway involved. The evolution of the pathological lesions supports the idea that the disease results from an immune response initiated by LC. Thus, in early lesions, LC and lymphocytes are the only cells present in large numbers. As the lesions evolve, inflammatory cells become more prominent and the number of LC decreases. The lesions heal by scarring and few, if any, LC can be identified. LC are known to accumulate at sites of pulmonary epithelial hyperplasia [8, 21]. Thus, bronchiolar abnormalities may predispose to pulmonary HX, thereby accounting for the strong association between HX and cigarette smoking. Bronchiolar epithelial cells involved by the process produce more GM-CSF than normal epithelial cells, and this cytokine may play a role in recruiting the large numbers of LC present in early lesions, as well as augmenting their lymphostimulatory capacities (Tazi, unpublished). The antigens involved in the process are unknown, but we have suggested that the immune response could be directed against the airway cells themselves [58].

Lung transplantation. Two major complications in lung transplantation are the development of graft rejection and the appearance of bronchiolitis obliterans. Both conditions are thought to be mediated, at least in part, by cytotoxic T-lymphocytes recognizing allogeneic MHC molecules expressed on cells in the transplanted lung [59]. Although many cell types in the transplanted lung can express class I and class II MHC antigens, most parenchymal cells are poor accessory cells, and therefore lack the ability to stimulate resting lymphocytes recognizing alloantigens. Furthermore, parenchymal cells do not migrate to lymphoid tissues, and therefore would not come in contact with naive T-lymphocytes capable of recognizing such alloantigens. Accordingly, it has been suggested repeatedly that "passenger leucocytes" present in the graft must migrate to lymphoid tissues to initiate T-lymphocyte immune responses directed against MHC molecules [60]. Because LC/DC strongly express both class I and class II MHC antigens, possess potent lymphostimulatory activity, and can migrate to regional and central lymphoid organs, the LC/DC present in the lung at the time of transplantation may be a major source of cells that migrate to lymphoid tissue and stimulate T-lymphocytes recognizing donor MHC molecules. Once activated in the lymphoid tissues, these T-lymphocytes could return to the lung to serve as effector cells.

Over time it is likely that donor LC/DC initially present in the grafted lung are replaced by LC/DC derived from the recipient's bone marrow, and such recipient LC/DC would not be a direct target for alloreactive T-lymphocytes. The time required for total replacement of lung LC/DC by recipient cells has not been investigated. The role of LC/DC (of either donor or recipient origin) in the activation of cytotoxic lymphocytes within the lung in the course of the effector phase of graft rejection or the development of bronchiolitis obliterans has not been studied. Increased numbers of DC have been observed, both within the tracheal and bronchial epithelium and within submucosal tissues of transplanted lungs showing evidence of bronchiolitis obliterans, compared to that in transplants without signs of this complication [61].

Asthma. LC are abundant in the epithelium of the upper respiratory tract and in the large conducting airways. Thus, these cells are ideally placed to process inhaled antigens, including those producing allergic reactions. The recovery of LC from bronchial biopsies has been reported to be increased when comparing patients with allergic asthma and allergic but nonasthmatic controls [62].

$CD4^+$ T-lymphocytes can be divided into subsets on the basis of the lymphokines they produce: helper T-lymphocyte (TH)1 cells (producing IL-2 and INF-γ) and TH2 cells (producing IL-3, IL-4, IL-5 and IL-10) [63]. The development of allergic reactions appears to be dependent on the activation of TH2 T-cells that release cytokines required for the stimulation of IgE production by B-lymphocytes and the recruitment and differentiation of eosinophils and mast cells [64]. LC are capable of activating previously established T-cell clones of both the TH1 and TH2 phenotype. Interestingly, however, studies in experimental animals have indicated that under some circumstances LC preferentially activate TH2 cells [65], and hapten-specific T-cell clones derived by repeated stimulation of T-cells using LC all expressed the TH2 phenotype [66]. The mechanisms by which LC favour the expansion of TH2 cells remain unclear, but may be related to the ability of LC to secrete IL-1 (a co-stimulator for TH2 cells but not TH1 cells), and the absence of INF-γ production by LC (which inhibits the development of TH2 cells) [51–53]. Taken together, these results suggest that LC may be important accessory cells in the development of allergic immune reactions in the lung.

Conclusions

Cells of DC/LC lineage are widely distributed in the lung, and work from several groups suggests that these cells may be the most potent accessory cells in this organ. DC/LC are heterogeneous, and current evidence indicates that differences in differentiation (DC *vs* LC), distribution, and exposure to cytokines present in the extracellular milieu can influence the ability of these cells to internalize antigens, express surface molecules involved in migration and cell-cell interactions, and stimulate lymphocyte proliferation. Because of their strong ability to initiate immune responses, DC/LC are likely to play an essential role in the development of normal immune responses. Further work is needed to understand the factors controlling the number, distribution and functional activities of these cells in the lung. It is likely that this information will provide important insights into the pathogenesis of lung diseases characterized by abnormal immune responses.

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