

Antigen Presentation in the Lung

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Studies from our laboratory and elsewhere have implicated populations of dendritic cells in lung and airway tissues as key regulators of both qualitative and quantitative aspects of T cell responses to local antigenic challenge. Under steady state conditions, they are specialized for uptake of antigen, and require additional maturation signals for full expression of their T cell-stimulating activity. Their functional phenotype appears to be controlled via a complex series of interactions with both bone marrow-derived, mesenchymal, and possibly neuroendocrine cells; failure(s) in one or more of these regulatory interactions may be important etiologic and/or pathogenic factors in a variety of respiratory immunoinflammatory disease.

The epithelial surfaces of the lungs and conducting airways are continuously exposed to mixtures of antigens present in ambient air. These mixtures are dominated by ubiquitous non-pathogenic antigens from a variety of plant and animal sources, in particular pollens, animal danders, and insect products, and these are interspersed with varying levels of potentially pathogenic antigens derived from viable and nonviable microorganisms. The adaptive immune system in the lung is faced with the task of accurately categorizing these stimuli, such that T cell responses that are qualitatively appropriate for neutralization of each agent are selected. Secondly, it must tightly control the intensity and duration of these responses, in order to preserve the integrity of the fragile, highly vascularized epithelial surfaces in the organ, particularly those at which gas exchange occurs.

The latter is partially achieved via aerodynamic mechanisms that result in progressive filtration of > 95% of inhaled antigens as they traverse the conducting airways, so that the overall level of antigen exposure at a given site is inversely related to its depth within the respiratory tree. The epithelial surfaces within the major conducting airways in which the bulk of inhaled antigen is deposited are protected via the scrubbing action of the overlying mucociliary escalator, and the small proportion of inhaled antigen that escapes this mechanism and penetrates into the underlying epithelial layer is then dealt with via specialized antigen-presenting cells (APCs), in particular dendritic cell (DC) populations, within and below the epithelium. The alveolar surfaces in the deep lung are policed instead by macrophage populations, again backed up by APC populations below the alveolar epithelium.

It is becoming increasingly clear that regulation of the function(s) of local APC populations, in particular DCs, is central to the maintenance of overall immunological homeostasis within the lung. This brief review focuses on a series of issues that are relevant to our understanding of these regulatory processes, in particular in relation to the etiology and pathogenesis of immunoinflammatory respiratory diseases.

DC POPULATIONS IN THE LUNG AND AIRWAYS

DCs are distributed throughout the respiratory tract, from the nasal mucosa to the lung pleura. The most prominent populations (Figure 1) are localized within the epithelium of the conducting airways, in which they form a rich network comparable to the Langerhans cells (LC) population in the epidermis (1–3), and within the lung parenchyma, in particular at the interseptal junctions between adjacent alveolar units (3–5).

Immunostaining of these cells in frozen sections reveals their prominent dendritiform morphology and constitutive high-level expression of MHC class II antigen (although a minor subpopulation of MHC class II-negative DCs may also be present [6]), together with a range of species-specific markers such as CD1 in human and NLDC-145 in mouse. Flow cytometric analysis of DCs derived via enzymatic digestion of lung tissues has identified a wide array of additional function-associated surface molecules (7–9), and moreover suggests the possibility that functional heterogeneity (developmental and/or lineage related) may be an intrinsic feature of these populations.

TURNOVER OF LUNG AND AIRWAY DCs: STEADY STATE VERSUS INFLAMMATION

We have used a rat radiation chimera model to ascertain steady state turnover times for DC populations in the conducting airway epithelium and lung parenchyma in relation to epidermal LCs in the same animals. These experiments have provided estimates of 2 and 7–10 d, respectively, for the half-lives of the airway epithelial and lung wall DC populations, compared with > 21 d for those in the skin (10).

The rapid steady state turnover of these respiratory tract DCs, in particular those in the airway epithelium, is rivaled in peripheral tissues only by those in the gut wall, hinting at the importance of resident DCs in immune surveillance at the major mucosal surfaces of the body (10).

This rapid steady state turnover of airway DCs can be further accelerated during acute inflammation, in particular in response to inhaled bacterially derived stimuli (3, 11). Recruited DCs in these models appear within 1–2 h of challenge, cycle rapidly through the inflamed tissues, and eventually migrate into regional lymph nodes (RLNs); this provides a potentially highly efficient mechanism for transfer of incoming antigen to the T cell system (11). Comparable rapid recruitment of DCs into airway tissues occurs in response to productive local viral infection (12) and aerosol challenge with inert soluble recall antigen in animals primed for either helper T cell type 1 (Th1)- or Th2-polarized immunity (13). We have also noted increased accumulation of airway epithelial DCs in animals chronically exposed to airborne irritants, in particular pine dust, which contains a range of allergens including abietic and pleiatic acids (3).

In vitro studies indicate that these DCs are rapidly responsive to a wide range of CC chemokines, as well as fMLP and complement cleavage products (13), which may explain their recruitment as the “default” response to inhalation of such a disparate range of inhaled stimuli.

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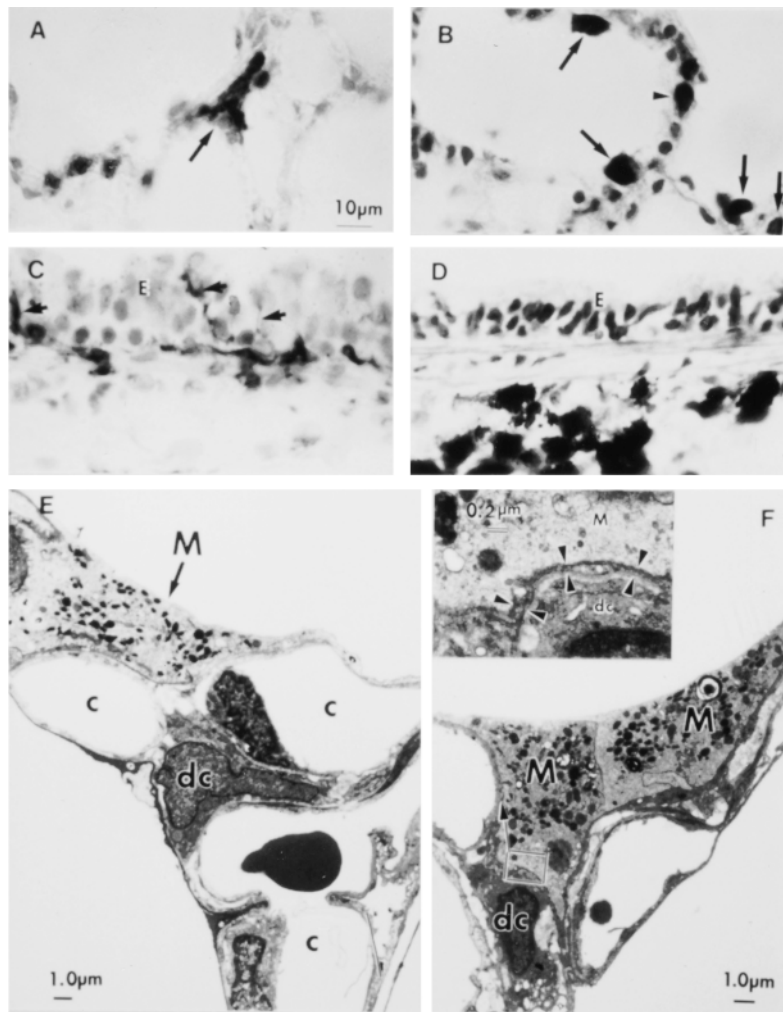


Figure 1. Lung and airway DC populations in the rat. DCs and macrophages are shown closely juxtaposed in both airway and peripheral lung tissues. (A) Interstitial Ia^+ DC (arrow) straddling an alveolar septal junction. (B) ED1 $^+$ macrophages (arrows) on the luminal surface of the alveolar space. A small interstitial macrophage (arrowhead), in contrast, is situated in a septal wall, possibly within the vasculature. (C) Ia^+ DC within the tracheal epithelium. The cell bodies lie close to the basement membrane and their processes (arrows) interdigitate between the epithelial cells. (D) Subepithelial macrophages in trachea heavily stained with the ED2 MAb. Note the lack of staining in the epithelium. (E) Electron micrograph of pulmonary alveolar macrophage spread on the Type I epithelial lining of an alveolus in the region of a septal junction, with its characteristic electron-dense cytoplasmic inclusions (lysosomes, phagosomes, and residual bodies). Note the candidate dendritic cell in the interstitial tissue with irregularly shaped indented nucleus. (F) Electron micrograph of two pulmonary alveolar macrophages at a septal junction, separation from a candidate dendritic cell by the intervening Type I alveolar epithelial cell and basal lamina (arrowhead). E, Epithelium; M, macrophage; c, capillary; dc, dendritic cell. Original magnification: (A–D) $\times 730$; (E and F) $\times 4,000$; inset, $\times 17,000$. Reproduced by permission of the Rockefeller University Press from Holt, P. G., J. Oliver, N. Bilyk, C. McMenamin, P. G. McMenamin, G. Kraal, and T. Thepen. 1993. Downregulation of the antigen presenting cell function(s) of pulmonary dendritic cells *in vivo* by resident alveolar macrophages. *J. Exp. Med.* 177:397–407.

FUNCTIONAL STUDIES OF RESPIRATORY TRACT DCs

Antigen Acquisition

The capacity of airway (4) and lung parenchymal DCs (5, 14) to accumulate antigen *in situ* has been formally demonstrated in a series of studies using both aerosol and intratracheal inoculation as methods of exposure.

It appears likely, by analogy with DCs propagated *in vitro* from bone marrow or blood-borne precursors, that lung-derived DCs employ mannose receptor-mediated endocytosis as their principal means of antigen uptake. This conclusion stems from independent studies of rat (8) and human lung DCs (9), both of which exhibit levels of endocytic activity comparable to those of “immature” *in vitro*-propagated DCs. It also appears that considerable variation exists within these populations in relation to rates of endocytosis, which is possibly related to maturational differences (8).

It has also been suggested that lung DCs may acquire processed antigen from local tissue macrophages (15). This possibility requires further detailed testing; however, it appears plausible on the basis of the close juxtaposition of macrophages and DCs throughout lung and airway tissues (Figure 1 [4, 5]).

T Cell Activation *In Vitro*

The functional capacity of lung DCs in *in vitro* T cell activation systems appears to be related to the way the cells are

treated after initial dissociation of lung tissue by enzymatic digestion. In particular, if the preparative procedure includes a step of overnight incubation, DCs harvested from these cultures exhibit relatively high levels of APC activity (7, 9, 16). In contrast, analysis of their activity after rapid separation from tissue digests via flow cytometry reveals much lower levels of APC function (4, 5).

High levels of expression of APC function by these latter lung DCs was revealed only after overnight incubation in cultures deliberately supplemented with cytokines such as interleukin 1 (IL-1) and/or granulocyte-macrophage colony-stimulating factor (GM-CSF) (8, 17, 18). This functional upregulation is accompanied by redistribution of presynthesized MHC class II to the cell surface, and upregulation of CD80/86 expression (8). It is noteworthy that major upregulation of APC activity was also achieved via overnight incubation in medium supplemented with serum alone, suggesting that the studies using prolonged incubation steps for DC preparation may be detecting changes that have occurred *ex vivo*. These may be due to activation via adherence, or possibly via cytokines present in the serum supplement (5).

Collectively, these findings are consistent with the “sentinel” role proposed generally for peripheral tissue DCs (19), that is, these cells are specialized for antigen uptake while *in situ* within the epithelial surfaces of the respiratory tract, but require additional maturation signals before the antigen can

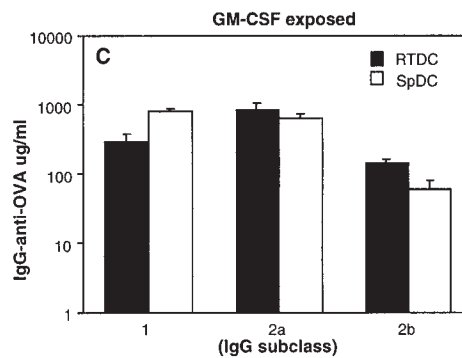
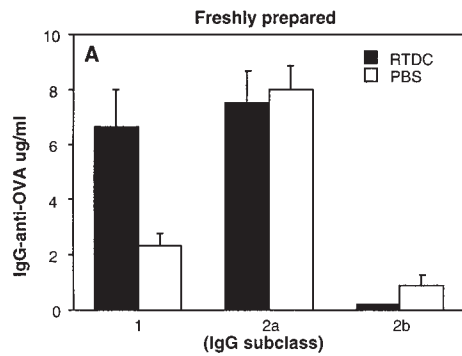


Figure 2. Helper T cell priming by ovalbumin (OVA)-pulsed respiratory tract dendritic cells. Helper T cells were primed with (left) fresh or (right) GM-CSF-exposed OVA-pulsed RTDCs, and challenged with soluble OVA. Results shown are antibody titers on day 10 postchallenge. RTDC, Respiratory tract dendritic cells; SpDC, splenic dendritic cells. (Data based on Stumbles, P. A., J. A. Thomas, C. L. Pimm, P. T. Lee, T. J. Vennail, S. Proksch, and P. G. Holt. *J. Exp. Med.* 1998;188: 2019–2031.)

be presented to T cells, usually only after migration to RLNs. As argued earlier (5), this compartmentalization of functions may be a highly effective means of protection of the delicate epithelial surfaces of the lung against potentially toxic T cell activation events.

Qualitative Aspects of T Cell Regulation: Th1/2 Switching

A study from our laboratory has examined the capacity of freshly isolated and GM-CSF-treated lung DCs to prime and restimulate Th1 and Th2-dependent IgG subclass antibody responses in rats. These experiments revealed that freshly isolated antigen-pulsed but otherwise unmanipulated lung DCs prime selectively for Th2-dependent antibody responses (Figure 2 [8]), mirroring the normal “Th2 default” that is observed in initial immune responses to aerosol exposure of animals to antigen (20). Repeated challenge with the same cells progressively boosted Th2-dependent antibody production without effects on the Th1-dependent subclass (Figure 3).

In addition, these experiments demonstrated that GM-CSF maturation of the DCs markedly increased their overall potency as APCs and led to selective upregulation of Th1-stimulatory functions, resulting in priming for high-level Th0-like immunity (Figure 2). The latter was associated with a switch from IL-10 to IL-12 production by the DCs. It was of interest to note in this context that sustained production of IL-12 required signals additional to GM-CSF, in particular tumor necrosis factor α (TNF- α) or CD40L (8).

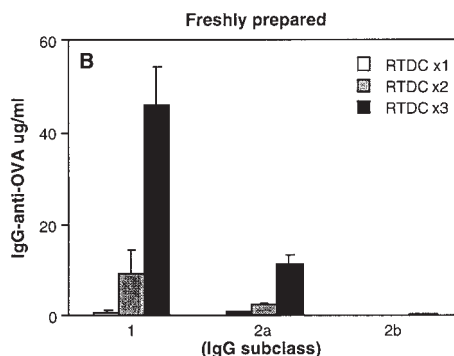


Figure 3. Repeated challenge with unmanipulated OVA-pulsed RTDCs. Animals were given one to three challenge doses of pulsed DCs; other details are as in Figure 2.

ENDOGENOUS CONTROL MECHANISMS REGULATING DC FUNCTION IN THE LUNG

Given the potential dangers to the host of uncontrolled upregulation of DC functions within lung tissues, it may be confidently predicted that efficient mechanisms would be operative locally to maintain activity levels within defined limits. One of these mechanisms appears to involve the functions of local lung tissue macrophages. Earlier studies from our group indicated that the capacity to demonstrate the APC activity of lung DCs within tissue digests was severely compromised unless endogenous tissue macrophages were first removed from the cell suspensions (4), and subsequent work implicated nitric oxide (NO) in this inhibitory mechanism (5, 21).

NO exerts a variety of effects in this context, which include inhibition of GM-CSF-mediated upregulation of APC activity by lung DCs (5, 21), as well as direct inhibitory effects on responding T cells via dephosphorylation of key signaling kinases such as Jak3/STAT5 (Janus Kinase 3/signal transducer and activator of transcription 5) (22). The macrophages may use multiple mechanisms in this context (e.g., IL-10, TGF- β , etc.), particularly in humans, where NO production by these cells is much less prominent (23).

While the precise nature of the molecular mechanisms involved remains to be determined, the results of a series of experiments involving selective *in vivo* depletion of alveolar macrophages (AMs) in rats and mice argue that these mechanisms are extremely potent *in vivo*. These include the findings that AM-deficient animals are hyperresponsive to intratracheally inoculated (24) or aerosolized antigen (25), and the additional demonstration that lung DC populations display rapid upregulation of APC activity *in vivo* within 24 h of AM depletion (5). The close *in vivo* juxtaposition of macrophages and DCs in the lung (Figure 1) would appear to be conducive to such regulatory interactions, and it is feasible (although unproven) that these interactions may also be bidirectional.

PHARMACOMODULATION OF LUNG DCs

There is growing interest within the pharmaceutical industry in lung and airway DCs as strategic targets for selective anti-inflammatory drugs, and also in their susceptibility to currently available therapeutic agents. In relation to the latter, we have shown that topical or systemic steroids are potent downregulators of DC recruitment in animals during acute inflammation (26), and these also appear to have inhibitory effects on airway DC networks in humans with atopic asthma (27, 28). There is also *in vitro* evidence suggestive of possible effects of β -agonists on cytokine production by airway DCs (29).

LUNG AND AIRWAY DCs AND MACROPHAGE POPULATIONS IN HUMAN DISEASE

Low-level chronic inflammation in smokers is associated with increased numbers of DCs on both the alveolar surface and in the bronchial mucosa (30, 31). Obliterative bronchiolitis associated with chronic lung rejection in patients receiving transplants is also associated with elevated DC numbers in the bronchial wall (32).

A growing body of evidence also suggests that upregulation of respiratory tract DC populations may be an etiologic factor in allergic respiratory disease. This includes the demonstration of upregulation of nasal mucosal DCs in rhinitis after deliberate (33) or environmental (34) allergen challenge, and findings illustrating changes in the number and surface phenotype of bronchial mucosal DCs in subjects with atopic asthma (27, 28, 35).

The significance of these latter observations is not fully understood. However, it is hypothesized that functional changes in airway DC networks that facilitate local activation of allergen-specific Th2 cells in the airway mucosa of subjects with atopic asthma may be a key factor in the chronic phase of atopic asthma, and in particular may help to "drive" the cycles of local tissue damage/repair that underlie the airway remodeling process in this disease (36).

It is feasible that one of the major stimuli for functional changes in airway DCs in this disease may be hyperproduction of GM-CSF by local mesenchymal cells, in particular airway epithelial cells (37). Several lines of evidence from animal models lend general support to this thesis (38–40).

It is, in addition, feasible that changes in local macrophage populations may play a role in enhancing T cell activation in chronic atopic asthma. Indirect evidence in support of this possibility has been provided via a range of studies involving functional analysis of human bronchoalveolar lavage (BAL) cells, and immunohistochemical analysis of bronchial biopsy samples (41, 42), and given the potent immunomodulatory role of lung macrophages in the animal models (see above), this issue warrants further detailed investigation.

ONTOGENY OF LUNG AND AIRWAY DC POPULATIONS: SIGNIFICANCE IN RELATION TO DISEASE SUSCEPTIBILITY

Immunohistochemical studies of the rat model indicate that lung DC networks are poorly developed at birth. The airway population increases slowly in number over the first few weeks of life, and does not reach adult equivalence until about 1 wk postweaning (43). This process involves numerical increases in these cells, and upregulation of MHC class II expression, which is low at birth (43). During the preweaning period the lung DCs express poor APC function and are relatively refractory to GM-CSF and interferon γ (IFN- γ) stimulation (6). In addition, inhaled bacterial stimuli, which elicit rapid influx of DCs into the airway wall of adult rats, trigger low responses in young animals (6).

It is of interest to note that in the skin of these animals, the epidermal LC population appears mature by approximately 7 d after birth, suggesting the possibility that microenvironmental influences specific to lung tissue may be responsible for this delayed postnatal maturation of local DC networks.

We have suggested that this maturational deficiency in local immune surveillance functions may be responsible for the increased susceptibility of preweaning animals to respiratory infections (6). It is conceivable that variations in the kinetics of postnatal maturation of the airway DC network may also be

an important determinant in the overall process of allergic sensitization in humans. This possibility is suggested on the basis of the following observations from our group (36, 44).

1. Priming of Th cells against inhalant allergens frequently occurs transplacentally during late fetal life when the immature immune system is intrinsically Th2 biased, resulting in initial expression of low-level Th2-polarized immunity against these allergens (45).

2. These early Th2 responses are progressively modified during infancy and early childhood via direct exposure to inhaled allergen, and in the majority of cases (nonatopics) these responses are redirected toward the Th1 phenotype via immune deviation (46–48).

3. The available evidence from model systems, in particular our studies of the rat (8), strongly suggest that Th1/2 switch regulation in responses to inhaled antigens is controlled by respiratory tract DCs.

4. The capacity of these DCs to switch toward the Th1 phenotype is dependent on their capacity to respond to GM-CSF signals (8), a capacity that is attenuated in lung DCs from immature animals (6).

Thus, protection against the development of long-term Th2-polarized immunological memory against inhalant allergens may be dependent on timely postnatal maturation of the Th1 switching functions of airway DCs, and any significant delay in this process may constitute a risk factor for subsequent atopic asthma.

FUTURE DIRECTIONS

Considerable progress has been made in understanding how antigen presentation and subsequent T cell activation are regulated in the lung environment, in particular in relation to the role of DCs. On the basis of this progress, several discrete areas of investigation would appear to merit high priority for research in the immediate future.

1. Heterogeneity within local DC populations: Issues such as DC1 versus DC2, lymphoid versus myeloid, mature versus immature, can be resolved only by careful phenotyping of cells harvested from normal versus diseased lung.

2. The functional phenotype of airway DCs in chronic asthma: This may be the key to the riddle of excessive local T cell activation in this disease, but the technical problems associated with functional studies of these cells in human biopsy samples are daunting.

3. Molecular mechanisms of Th1/2 regulation by lung DCs: The current focus on IL-10/IL-12 balance may prove to be simplistic in the long term; the role of costimulators may be crucial, including novel costimulators. It is nevertheless intriguing that the "default" Th2-skewing bias of resting airway DCs is associated with low-level constitutive CD86 expression in the absence of CD80.

4. Local microenvironmental regulation of lung and airway DC subsets: As well as the effects of NO from multiple cellular sources, it is highly likely that local fibroblastic and epithelial cells (37, 40, 49), and also neuroendocrine cells (50), will prove to play significant roles; considerable scope exists for well-controlled cell biological and whole animal studies relevant to these possibilities.

5. Postnatal maturation of lung DC functions during infancy: Potentially a key determinant of susceptibility to both respiratory infections and respiratory allergy; at the moment, these studies appear feasible only in animal models.

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