Review

ACQUISITION OF ANTIGENS BY AIRWAY DENDRITIC CELLS. DO WE KNOW ENOUGH?

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ABSTRACT

The respiratory system is endowed with a number of structural and functional barriers that protect it against harmful and innocuous material from taking advantage of its vast surface area to gain access into the organism. These barriers include; 1) the surfactant system 2) a highly efficient mucociliary escalator system 3) a population of highly phagocytic macrophages and 4) an epithelium endowed with tight junctions. However, despite these barriers, pulmonary immune responses are easily generated by introduction of antigens into the airways. These responses are thought to be mediated via dendritic cells, which are located in the basal aspect of the epithelium,and the most potent antigen presenting cells in the lung. Although there is substantial information on the nature of interaction between dendritic cell and particles from *in vitro* experiments, there is little information on how the particles breach the barrier to reach the immunocompetent cells. An understanding of how these particles pass the epithelial barrier to reach the immunocompetent cells is important in the development of mucosal vaccines. Insights into how this may happen are discussed.

Key words: Immune Cells, Respiratory Tract

INTRODUCTION

The surface epithelium of the respiratory tract is the most extensive surface that interfaces directly between man and his environment. In an average human weighing about 74 Kg, it may comprise up to 143 m^2 (Gehr *et al.,* 1978) in surface area. This surface is exposed to a large load of particulate matter that is dependent on the level of environmental pollution, inhalability of the pollutant and the breathing habit of the individual (Stahlhofen *et al.,* 1994; Roth *et al.,* 1997). It has been estimated that the human airways may be exposed to as much as 7 Kg of pollutants per year (Phalen 1984) although this may vary depending on the amount of particulate matter suspended in the inhaled air. To counter the danger posed by these particles, the lung is endowed with several structural and functional barriers that constitute its innate immunity against harmful and innocuous particles from reaching the rest of the body organs. These barriers include the following 1) the surfactant system (Schürch *et al*., 1990; Gehr *et al.,* 1990), 2) a highly efficient mucociliary escalator system (Kilburn 1968), 3) a population of highly phagocytic macrophages (Geiser *et al.,* 1990; Crystal 1991), and 4) a continuum of epithelium endowed with tight junctions (Breeze and Wheeldon 1977; Barry 1987). Nevertheless, numerous epidemiological studies have shown that particulate air pollution is associated with increased morbidity and mortality (Brunekreef 1997; Pope *et al.,*2009 Pope *et al.,* 2014). Further, immune response related diseases are common in the lung, which suggests that breach of this barrier occurs. One factor that has been said to predispose to breaching of the barrier is particle-overload (Oberdöster *et al.,* 1994). The phenomena of particle overload suggest that the lung barrier

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system can only handle up to a given maximum particulate volume beyond which the particles will inevitably get into contact with the epithelium and become interstitialised (Marrow 1988; Oberdöster *et al.,* 1994) where they may reach the dendritic cells. Furthermore, twenty years ago, Gehr and Schürch(1992) reported that upon inhalation, small particles are forcefully displaced by the surfactant lining film towards the airway epithelium forming a depression on the epithelial cells. The displacement enables the particles to interact with airway macrophages and perhaps dendritic cells (Gehr *et al.,* 1996) thereby determining their fate.

Dendritic cells are recognized as the main antigen presenting cells in the lung (Holt *et al.,* 1990; Nicod 1997) and the nature of how they acquire antigens is a prerequisite in understanding the consequences of particle-lung interaction. Of particular importance is how the antigens can reach DC, which are the most competent antigen presenting cells in the lung (Holt *et al.,* 1990; Nicod, 1997). The location of DCon the basal surface of the epithelium and lack of cells such as M cells in the airway epithelium suggests that there may be active and yet unresolved mechanisms of antigen delivery to the DC in the airways. Are the antigens phagocytosed and delivered byairway macrophages? Do they penetrate through the airway epithelial cells, or do the antigens pass through the tight junction. Is there a possibility that the DC collect the particles by pushing cytoplasmic processes beyond the tight junctions into the airway lumen (Rescigno *et al.,* 2001; Vermaelen *et al*, 2001; Takano *et al*., 2005)? How does this antigen delivery change during epithelial injury and repair, which is a common feature of inflammatory airway disease, like asthma (Gaurav and Agrawal 2013)? These questions need urgent answers.

Dendritic cells

Dendritic cells (DC) are professional antigen presenting cells that play a critical role in generating primary and secondary immune responses against specific antigens (Steinman, 1991). Priming of naive T lymphocytes, proliferation and functional differentiation of antigen-specific T cells as well as development of antigen specific T cell tolerance depends on an appropriate T cell - dendritic cell interaction (Banchereau *et al.,* 2000). The high capacity of DC to stimulate T lymphocytes is attributed to (1) a high DC level of adhesion molecules that may favour T cell receptor engagement (Steinman, 1991), (2) high expression levels of co-stimulatory molecules on the DC that facilitate T cell activation (Katayama *et al.,* 1997; Quaratino *et al.,* 2000) as well as (3) expression of high level of major histocompatibility complex molecules (Inaba *et al.,* 1994; Cella *et al.,* 1997).

DC were first isolated from murine spleen suspension and defined on morphological grounds (Steinman and Cohn, 1973). They were shown to display a distinctive dendritic morphology (Figure1). Subsequently, the DC were defined by functional criteria, notably the lack of endocytic activity *invitro* and *in vivo*, the inability to retain antigens or immune complexes on their surface and a low labelling index in vitro (1.5–2.5%) following administration of [^{3}H] thymidine (Steinman and Cohn, 1974). Nonphagocytic DC were later isolated from rat peripheral lymph (Pugh *et al.,* 1983) and human lung parenchyma (Nicod *et al.,* 1987). The lack of endocytic activity was a paradox since antigen uptake and processing is expected to precede antigen presentation (Steinman and Swanson 1995).

Life cycle of dendritic cells.

The origin and ontogeny of DC have been reviewed in detail (Hart, 1997; Granucci and Zanoni 2009). Evidence of bone marrow origin of dendritic cells was

demonstrated from irradiation reconstitution experiments following bone marrow transplantation (Katz et al., 1979)(Figure 2). Furthermore, perturbation of the respiratory tract mucosa is knownto induce mobilization of DC in the respiratory epithelium. Inhalation of viruses, bacterial and soluble protein antigens result in recruitment of DC into the respiratory mucosa followed by DC migration to the lymph node (Mc Williams*et al.,* 1996). Subsequently, the migratory immature dendriticcells undergo maturation and become professional antigen presenting cells (Banchereau and Steinman, 1998). Maturation changes DC in many ways that explain their potent antigen presenting capacity to naive T cells.These changes include: (1) a high expression of T lymphocytes co-stimulatory molecules such as B7-2 (Inaba *et al.,* 1994), (2) production of IL-12 (Cella *et al.,* 1996), (3) redistribution of MHC class II molecules from nonlysosomal vesicles to the cell surface (Pierra *et al.,* 1997, Turley *et al.,* 2000), (4) an upregulation of CCR7 that guide migration of DC into the T cell areas (Forster *et al.,* 1999), (5) expression of DC survival molecule, TRANCE-R (Wong *et al.,* 1997), (6) a high expression of adhesion molecules such as ICAM-1, ICAM-3 and LFA-3 (Banchereau and Steinman, 1998) and (7) downregulation of dextran uptake (Cochand *et al.,* 1999). In addition, human DC havebeen shown to reduce their phagocytic capacity on maturation (Kiama et al., 2001).

Dendritic cells in the epithelial tissue.

An interdigitating network of DC has been described in the intestinal and respiratory tract of rat and humans (Holt *et al.,* 1988; Pavli *et al.,* 1993). Studies from P. Holt's laboratory (Schon-Hegrad *et al.,* 1991; Holt *et al.,* 1994) and other laboratories (Nicod *et al.,* 1987; Gong *et al.,* 1992) have identified two main populations of DC in the lung that differ in location, phenotype and turnover. One population which exhibits a high turnover (2 to 3 days), and a more immature phenotype residing in the conducting airways just below the epithelium (Sertl *et al.,* 1986; Holt *et al.,* 1988) and the second population, that is fairly sparse, exhibiting a more advanced stage of differentiation, a slower turnover (7 days) located in the alveolar septa (Holt *et al.,* 1994).

Densities of DC in the rat are highest in the upper airways (600-800/mm^2) and decrease with progression down the respiratory tree, reaching 75/mm^2 in the small airways of the peripheral lung (Schon-Hegrad *et al.,* 1991). The airway mucosal DC population is capable of up regulation in response to both acute and chronic inflammation (McWilliams*et al.,* 1994). Targeting these cell population with vaccines presents high potential of inducing strong mucosal and systemic immunity. However, a good understanding of how the potential vectors reach the DC is a prerequisite in achieving desirable immunogenic responses.

Function of Dendritic cells.

DC are professional antigen presenting cells (Steinman, 1991 van Spriel and de Jong 2014). They phagocytose, processand present immunogenic epitopes in the context of MHC class I and/or class II molecules for recognition by T lymphocytes (Hart, 1997; Alberts 1998). Besides presenting the antigens, the DC are endowed with an array of costimulatory molecules whose interaction with complementary molecules on the T lymphocytes ensures optimal T cell activation (Banchereau and Steinman, 1998). In addition to their antigen presenting function, DC serve a sentinel function. DC survey epithelial surfaces such as the skin, respiratory tract and gastrointestinal tract for agents that may present a threat to health. The sentinel position of the DC stands out after a challenge of the respiratory mucosa with *Moraxella catarrhalis*. Inhalation of *M. catarrhalis* organisms is accompanied by an amplification of active DC surveillance in the airways that results in an increase in the traffic of the DC between the airway

epithelium and the regional lymph nodes (McWilliams*et al.,* 1994). Besides stimulating T lymphocytes, DC are now known to have effects on B cell growth and immunoglobulin secretion (Banchereau *et al.,* 2000). DC activate and expand T-helper cells, which in turn induce B cell growth and antibody production (Briere *et al.,* 1999). Human DC have been reported to skew isotope switching of CD40-activated naive B cells in presence of IL-10 and TGF-β towards IgA secreting cells (Fayette *et al.,* 1997). Immunoglobulin A is the major class of immunoglobulin present in the mucosa of the healthy respiratory tract and is thought to be the most important immunoglobulin for lung defence (Lamm, 1997). This suggests that DC are in control of mucosal immunity, since DC located in the airway epithelia could directly influence the isotype switch of B cells towards IgA. Other functions of the DC include induction of central and peripheral tolerance (Banchereau and Steinman, 1998), control of Th1/Th2 directed immune responses (Rissoan *et al.,* 1999) and linking of innate and adaptive immunity via production of interferon alpha (Palucka and Banchereau, 1999).

Our studies and other reports have demonstrated that DC are efficiently phagocytic for a variety of particles such as, 1) polystyrene particles (Kiama *et al,* 2001; 2) puff ball spores,3) biodegradable microspheres made of poly(lactid-co-glycolid) acid (PLGA) (Walter *et al.,* 2001), and 4) *Salmonella typhimurium* (Kiama *et al.,* 2006; Dreher *et al.,* 2001). The uptake of *Salmonella* and PLGA particles by DC is important because of their potential use in drug delivery and as vectors in delivery of DNA vaccines. This is because, *Salmonella* not only invade DC very efficiently but also induces the expression of costimulatory signals for T cell (Dreher *et al.* 2001) and hence can serve as a carrier of the vaccine. *Salmonella* was also found to induce formation of microvesicles after infection of DC (Kiama *et al.,* 2006). The significance of these microvesicles (Szakal *et al*., 1988) in amplification of immunity on salmonella based vaccine remains to be explored (Obregon *et al*., 2006). Whereas much is known on the interaction of particles with DC and with alveolar macrophages there is no sufficient data on how the antigens reach the DC (Vermaelen and Pauwels, 2005; Takano *et al*., 2005).

Surfactant barrier

The primary role of pulmonary surfactant is to reduce surface tension forces in the lung and to stabilize pulmonary alveoli (Schürch *et al.,* 1976). However, it is now generally well recognized that surfactant may also serve the role of a barrier to inhaled antigens reaching the epithelium (Pison *et al.,* 1994) and the dendritic cells. It does this through several mechanisms that include: 1) enhancing mucociliary clearance (De Sanctis *et al.,* 1994), 2) improving phagocytosis of particles by alveolar macrophages (Pison *et al.,* 1994), and 3) displacing small particles to the vicinity of macrophages and epithelial cells (Gehr *et al.,* 1990; Schürch *et al*., 1990; Geiser *et al.,* 2000). In view of the concomitant observation of surfactant deficiencies and poor airways clearance in lung diseases such as cystic fibrosis and asthma (Griese *et al.,* 1997; Meyer *et al.,* 2000), unravelling the role which surfactant may play as a barrier of particles to reach the epithelium and subsequently the dendritic cells would provide useful information on how to treat such diseases. Furthermore, emerging evidence indicate that surfactant proteins A and D modulates dendritic cell function and helper T cell polarization (Nayak et al, 2012, Schleh et al 2012)

Mucociliary barrier

The efficiency of the mucociliary barrier depends on among other factors, the morphological integrity of the cilia structure and mucus components (Werner *et al.,* 1996). Impairment of the mucociliary barrier, which is common in diseases such as cystic fibrosis and

asthma, predisposes to bacterial colonization of the airways (Werner *et al.,* 1996). Although impaired clearance is known to result in prolonged retention of deposited particles, its contribution to antigens gaining access to dendritic cells through the epithelium has not been determined. Delayed clearance of particles deposited in the airways would predispose to a breach of airway epithelial barrier highlighting the risk of particles gaining access to dendritic cells.

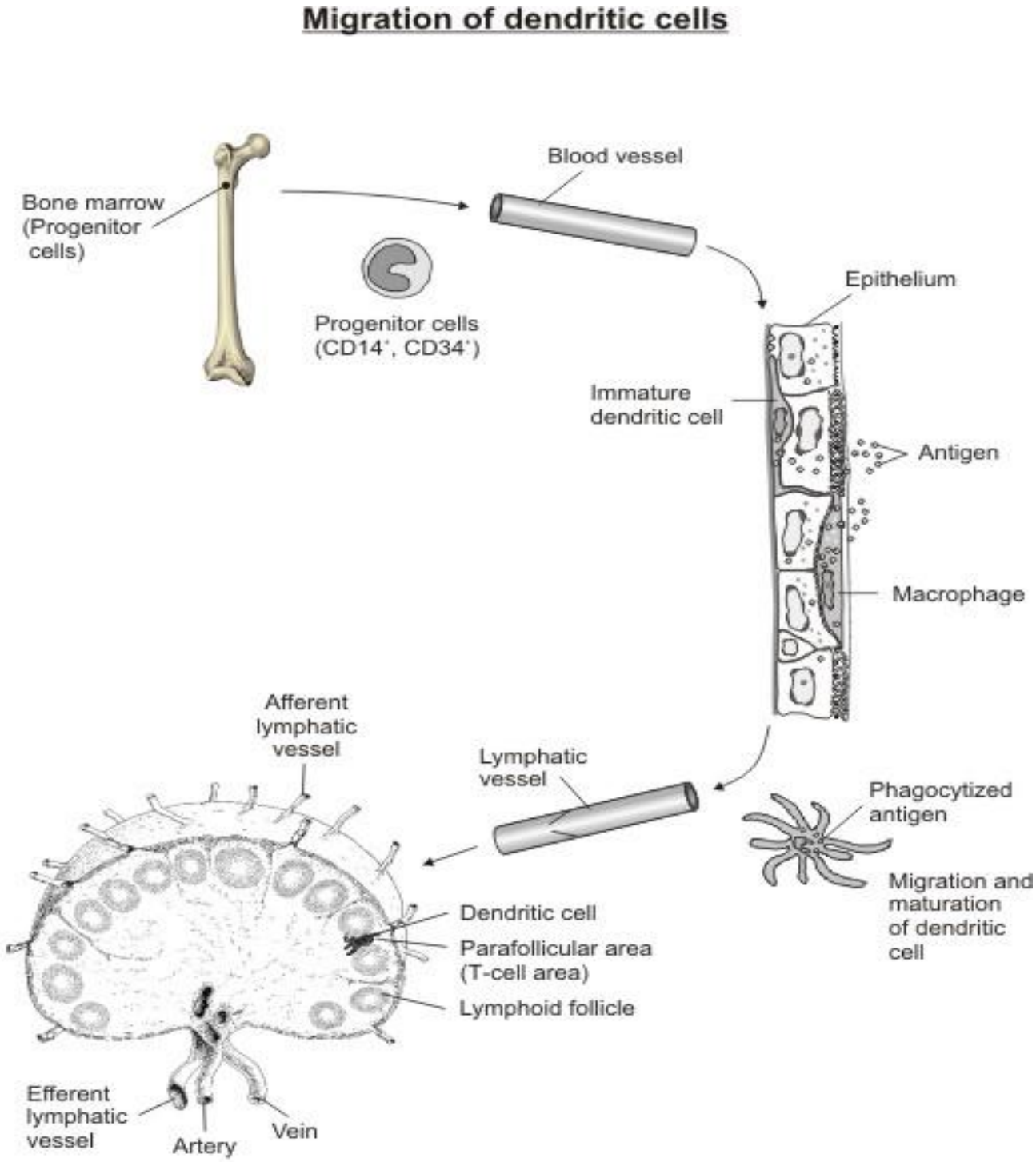

Figure 2: A schematic illustration of the life cycle of Dendritic cell. The Dc precursors migrate from the bone marrow to the epithelium where they differentiate to immature

dendritic cells. The immature dendritic cells take up antigen and migrate to the regional lymph node in the process maturing to the professional antigen presenting cells.

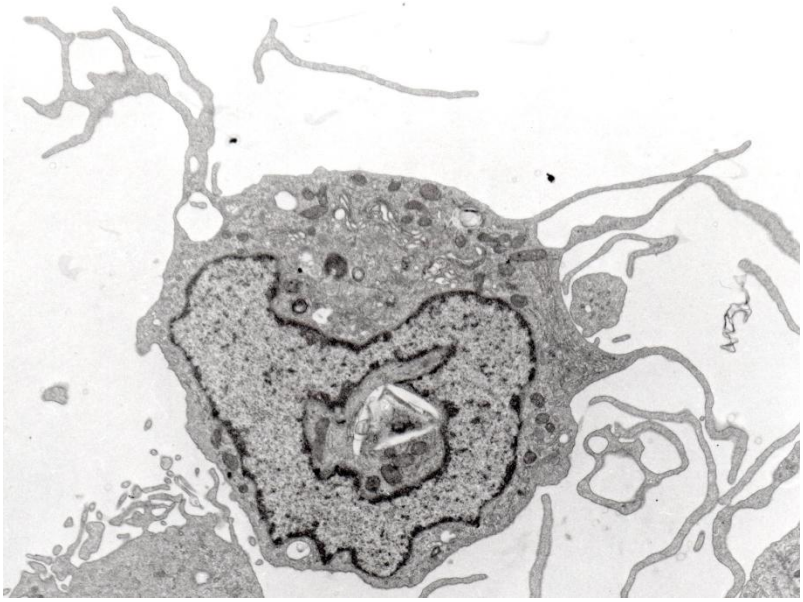

Figure 1: An Electron micrograph of a dendritic cell displaying the long dendritic processes that are typical for this cell type.

Macrophage Barrier

Airway macrophages are highly phagocytic and are rapidly recruited to the locations where particles have been deposited (Geiser *et al.*, 1994). Macrophage overloading though, may interfere with the ability of these cells to phagocytise (Oberdoster *et al.,* 1994). The phenomenon of particle-overload assumes that macrophages can only engulf up to a given maximum volume of particles (Morrow 1988; Oberdöster et al., 1994). Morrow (1988) suggested that alveolar macrophage functions begin to be impaired when on average 6 % of its volume is filled by phagocytosed particles. The capacity of pulmonary DC to serve as antigen presenting cells for heat killed Listeria after in vivo challenge is observed only when the dose of heat-killed Listeria exceeded 10^9 organisms per rat (MacLean *et al.,* 1996). Moreover, elimination by alveolar macrophages *in v*ivo is associated with increased pulmonary immune response to intra-tracheal administered heat-killed Listeria (Kradin*et al.*, 1999) implying that macrophages serve a protective role. This suggests that particle overload plays a role in the breaching of the epithelial barrier.

Epithelial barrier

A continuous layer of epithelial cells joined by tight junctions (Breeze and Wheeldon 1977) lines the pulmonary airways. The tight junctions completely prevent the diffusion of macromolecules through the intercellular spaces across the epithelium (Balda and Matter, 1998). Transport of the particles to the DC cells presupposes their passage across the epithelium, although the route they take has not been determined. Vermaelen and colleagues (2001) reported that fluorescein isothiocyanate (FITC)- conjugated macromolecules are transported to the tracheal lymph nodes by airway DC after an intratracheal instillation. However, the mechanism in which the macromolecules passed through the epithelium to reach the DC was not provided. Takano and colleagues (Takano *et al.*, 2005) showed that dendritic cells easily access antigens beyond epithelial tight junctions in human nasal mucosa, but of allergic rhinitis only.

Another *in vitro* model using mouse tracheal epithelial cells and mouse bone marrow dendritic cells showed impaired migration of metalloproteinase-9-deficient dendritic cells through tracheal epithelial tight junctions (Ichiyasu *et al*., 2004). Furthermore, there is evidence that dendritic cells play an important role in the pathogenesis of allergic asthma, and there is an increased number of dendritic cells in the airway mucosa of patients with chronic obstructive pulmonary disease (Vermaelen and Pauwels, 2005).

In conclusion, once particles have evaded thesurfactant, mucociliary, macrophages and the epithelial barrier they come into contact with the professional antigen presenting cells, the dendritic cells. One or several mechanisms may be involved in breaching each barrier which could determine the outcome of the dendritic cell-particle interaction. Thus, concerted studies ought to be carried out to unravel how each barrier is breached perhaps identify whether there is a critical barrier and thus answer the questions. Are all the barriers equally important or is there one that is more redundant?. Or even the question. Do we know enough about the barriers?

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