

EDITORIAL

Dendritic cells and lung antigen responses

Dendritic cells have taken a prominent place in immunological research over the years, largely because of their unique ability to prime naive T cells. While the low numbers of dendritic cells that can be isolated from blood or tissue impeded early experiments, this obstacle has been overcome by the elucidation of maturation pathways and the possibility of generating reasonable numbers of pure dendritic cells *in vitro*. Hopes are high that the dendritic cell will be the basis of therapeutic strategies aiming at the modification of the immune response in the development of vaccines and tumour defence (reviewed in [1]).

As mentioned above, dendritic cells are classically identified on the basis of function, the priming of naive T cells, and morphology, through their dendritic processes. Recent insight into the maturation of dendritic cells has demonstrated that this definition still allows for considerable variation. A recurrent problem in this line of research is the lack of specific dendritic cell markers, which complicates the experimental isolation processes. Essentially, dendritic cells in suspension are still identified through the exclusion of all other cell lineages rather than positive selection and their developmental stages are defined by the change of surface marker intensity over time, such as major histocompatibility complex (MHC), CD1a and the costimulatory molecules CD80 and CD86. These markers, however, are not exclusive to dendritic cells.

All dendritic cells arise from bone marrow precursors and their maturation pathways are similar in the human, murine and rat systems. Based on maturation stages and growth factors three lineages are currently defined: the myeloid dendritic cell, which differentiates via monocytic stages and localizes to a multitude of tissues, the skin Langerhans' cell, which is morphologically characterized by Birbeck granules and the lymphoid dendritic cell. Both myeloid dendritic cells and Langerhans' cells differentiate from circulating CD34⁺ progenitors in the presence of granulocyte macrophage-cell stimulating factor (GM-CSF) and tumour necrosis factor alpha (TNF α) [2,3]. The lineage commitment is indicated by the expression of cutaneous lymphocyte antigen (CLA), identifying cells that will give rise to Langerhans' cells [4,5]. CLA⁺ cells proceed through a CD14⁺ monocytic stage to either macrophages, in the presence of M-CSF or interleukin (IL) -10 [6,7], or into dendritic cells. The latter share CD1a, CD83, CD86 and factor XIIIa expression with the Langerhans' cells, but lack Birbeck granules and E-cadherin and demonstrate slightly different patterns of cytokines [8]. Numerous papers have

demonstrated that CD14⁺ blood monocytes may also be driven towards dendritic cells in the presence of GM-CSF and IL-4 resulting in a surface receptor pattern similar to interstitial and blood dendritic cells [8–11]. The development of lymphoid dendritic cells from a CD34⁺ precursor, in contrast, is GM-CSF-independent and supported by Flt3-ligand, IL-3 and TNF α [12–14]. In the mouse, where they were first identified, but not in humans, CD8 α selectively discriminates between lymphoid and myeloid dendritic cells [15]. Some reports, however, fail to differentially detect myeloid and lymphoid dendritic cells and question a clear-cut separation into these lineages, such as Masten and Lipscomb in the mouse lung [16].

Dendritic cells reside in the tissue as immature antigen-presenting cells, but with a high efficiency of antigen uptake. As they mature under the influence of cytokines, pathogens or contact with T cells, the expression of a number of costimulatory molecules and MHC increases to optimize antigen presentation and reduce their ability of antigen uptake (reviewed in [1]).

The question currently being addressed, is how dendritic cells activate the immune response, and how the cytokines produced by dendritic cells themselves and their micro-environment could promote the development of an allergic response. Two recent studies reach opposing conclusions on whether myeloid dendritic cells and lymphoid dendritic cells differ in their ability to skew T cells towards the T helper 1 (TH1) or TH2 phenotype (Table 1). Rissoan *et al.* argue that human lymphoid dendritic cells promote a TH2 response via an IL-4 independent mechanism, and myeloid dendritic cells, through the production of IL-12, promote the TH1 response [17, editorial 18]. On the other hand Pulendran *et al.* demonstrate that in mice the lymphoid dendritic cell subset is associated with TH1 cytokine production in T cells and myeloid dendritic cells skew towards the TH2 phenotype through induction of IL-4 and IL-10 [19]. It remains to be shown whether the opposing results reflect species differences or can be attributed to the varying methods to obtain the dendritic cells. In the human study myeloid dendritic cells were differentiated from monocytes *in vitro* with GM-CSF and IL-4, and lymphoid dendritic cells from CD4⁺CD11c⁺ lineage⁺ cells with IL-3 and CD40 ligand transfected L cells [17]. In the murine study, mice were injected with either polyethyleneglycol-modified GM-CSF to expand the myeloid dendritic cells and Flt3 ligand to expand both lineages, and the cells were isolated from spleens by flow cytometric sorting [19]. Both studies address the *in vitro* interaction of dendritic cell subsets

Table 1. Summary of selected studies cited and experimental strategies followed (major points only)

Study	Species	Dendritic cells	Read out
Lambrecht <i>et al.</i> [23]	Rat	Generated <i>in vitro</i> from bone marrow precursors	<i>In vivo</i> – histology, BAL, serum IgE
Xia <i>et al.</i> [24]	Rat	Isolated from lung	<i>In vitro</i> T-cell restimulation
Havenith <i>et al.</i> [25]	Rat	Isolated from spleen	<i>In vivo</i> injection of DCs, <i>in vitro</i> restimulation
Liu <i>et al.</i> [28]	Rat	Isolated from the thoracic duct	<i>In vivo</i> and <i>in vitro</i> antigen presentation, serum immunoglobulin, immunocytochemistry
Thepen <i>et al.</i> [27]	Rat	Isolated from spleen	Immunohistochemistry, plaque-forming assay
Vremec <i>et al.</i> [15]	Mouse	Isolated from lymphoid organs	Phenotyping and short-term cultures
Masten <i>et al.</i> [16]	Mouse	Isolated from lung	Phenotyping, lymphoproliferation assay
Pulendran <i>et al.</i> [19]	Mouse	Isolated from spleen	<i>In vivo</i> T-cell priming, cytokine production, serum immunoglobulin
Rissoan <i>et al.</i> [17]	Human	Generated <i>in vitro</i> from bone marrow precursors	<i>In vitro</i> coculture of DCs and T cells, cytokine ELISAs
Kalinski <i>et al.</i> [22]	Human	Generated <i>in vitro</i> from monocytes	<i>In vitro</i> coculture of DCs and T cells, cytokine ELISAs

with T cells, or the combined interaction of T cells, B cells and dendritic cells ([20] reviewed in [1,21]).

However, following the report by Kalinski *et al.* [22], it seems more likely that subset independent, dendritic cells can give rise to either a TH1 or TH2 response. They demonstrated the importance of the microenvironment during maturation: a prostaglandin E₂-rich environment has a lasting inhibitory effect on IL-12 production by the dendritic cell and promotes a TH2 response, whereas in its absence a TH0/TH1 response is favoured.

We are still far from a final conclusion on the influence of the microenvironment on dendritic cell maturation and the consequences this might have on the interaction with T cells and the development of TH1 or TH2 polarization.

Lambrecht *et al.* in this issue [23] followed the alternative strategy of an *in vivo* rat model to clarify whether antigen presentation by dendritic cells in the airways was sufficient to induce sensitization (Fig. 1). The dendritic cells and control macrophages were generated *in vitro* through the maturation of bone marrow precursors in the presence of GM-CSF, incubated with ovalbumin and intratracheally instilled into the recipient rats. After 10 days the rats were rechallenged three times with ovalbumin and the *in vivo* immune response monitored. This was represented by airway histology, differential bronchoalveolar lavage (BAL) cell counts and immunoglobulin (Ig) E measurements. Table 1 summarizes the strategies used to generate dendritic cells and the experimental readouts in the studies cited in this editorial.

Previous studies in rodents provided the background knowledge for this study (Table 1), demonstrating that

resident and intratracheally instilled antigen-laden dendritic cells home to the local lymph nodes to prime naive T cells [24,25]. There is also general consensus, that only the dendritic cell is capable of priming the naive T cells in the lung, not the B cell [16] or the alveolar macrophage [25]. In fact, it was demonstrated that the low response to alveolar macrophages not only required a 10-fold higher cell concentration but was also mediated by an alternative mechanism independent of cell viability [25]. Earlier studies illustrated that alveolar macrophages downregulate the T-cell response in the lung [26,27], a finding mirrored in the study presented [23].

As mentioned above, the recent advance in knowledge about dendritic cell maturation pathways, permits the use of *in vitro*-generated dendritic cells, an approach followed by both Lambrecht *et al.* [23] and Rissoan *et al.* [17]. The culture in GM-CSF by Lambrecht *et al.* favours myeloid dendritic cell development and the authors demonstrate that a single pulse of antigen-incubated dendritic cells is sufficient to sensitize the animals to further antigen contact (Fig. 1). They make a point of avoiding *in vitro* rechallenge experiments, which in their eyes might be misleading, and demonstrate the histological changes after *in vivo* secondary antigen exposure, which are characterized by a mononuclear cell infiltrate selective for the antigen. At that point, however, the response is not obviously polarized to either a TH1 or TH2 cytokine pattern, in the absence of significant eosinophilia, serum IgE or BAL cytokines. The absence of specific IgE is slightly unexpected since the Brown Norway rat is predisposed to eosinophilia and IgE synthesis, but is attributed to the mode of antigen delivery. Regarding the

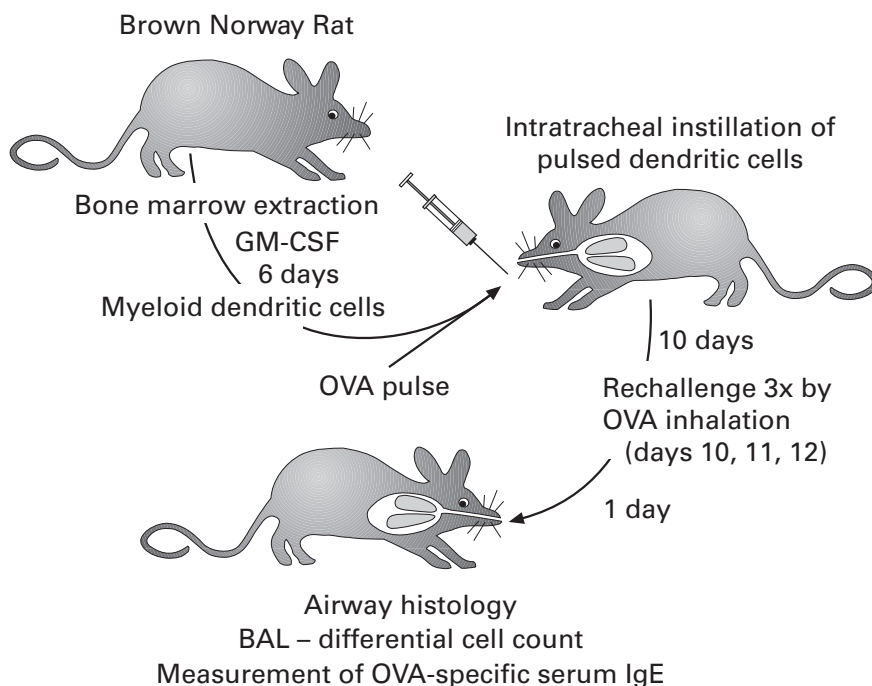


Fig. 1. Schematic representation of the protocol used by Lambrecht *et al.* [23]. Dendritic cells were generated *in vitro* from bone marrow precursors, pulsed with antigen and introduced into the trachea of recipient animals

failure to detect cytokines, it is worth mentioning that the T cells in previous studies had to be restimulated *in vitro* for detectable levels of interleukin protein production [17,19,22].

The finding that the instillation of dendritic cells induces sensitization supports the role of the dendritic cell in the elicitation of asthma, but the lack of skewing towards a particular cytokine pattern argues for an involvement of the tissue microenvironment and the need for prolonged exposure to the antigen.

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