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Dendritic cells as immune regulators: the mouse model

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Abstract

Dendritic cells (DC) are central to the immune system because of their role in antigen presentation leading to either tolerance or immunity among cells of the adaptive immune response. It is becoming increasingly evident that DC show extensive plasticity in terms of their origin and function, giving rise to a number of subsets represented differentially in all lymphoid organs. This article considers the tolerogenic capacity of murine DC and draws a distinction between DC that induce tolerance in the immature state and immunity in an inflammatory context, and those that act as regulatory cells inducing immunosuppression in the presence of inflammation.

Keywords: immunity • dendritic cells • spleen • tolerance • immunosuppression

Introduction

Cells of the immune system continually monitor a host for invading pathogens and participate in clearing those pathogens so the body remains free of infection. This active response is an immunogenic response. Under non-infectious conditions, cells of the immune system are also continually exposed to proteins of the host and any immune responses which develop are suppressed so the host maintains a state of self-tolerance. The cells that direct the outcome of pathogen exposure are the antigen-presenting cells (APC), and these include dendritic cells (DC), macrophages and some B lymphocytes. APC continually filter the molecular environment for both host and pathogen proteins. The potential for control of the uptake and presentation of environmental antigens by APC is important for immunotherapy aimed at both the tolerogenic and immunogenic capacity of APC. Achieving therapeutic control over DC could have impact on immunization for pathogens and cancers on the one hand, and immunosuppression for autoimmune diseases and organ transplant rejection on the other. To date, the difficulty has been to determine the conditions under which DC behave in an immunogenic *versus* a regulatory or suppressive manner. Spleen has been the organ of focus in many DC studies due to its unique role as a site of DC development [1–3]. It is also a site for DC exposure to blood-borne pathogens and apoptotic or dying cells [4].

The range of murine dendritic cell types

The main function of DC is to endocytose and process antigen from pathogens or apoptotic cells, and to present antigen as peptide in the context of major histocompatibility complex (MHC) Class I and II molecules for T-cell recognition and activation. The outcome of T-cell activation can be either immunity or tolerance depending on the signals that accompany T-cell receptor (TCR) recognition of antigen [5].

Cell surface markers like CD11c, CD11b, CD8α, CD4, MHC-II and CD45RA are most widely used for phenotypic characterization of DC subsets. Different subsets of DC are present across

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lymphoid organs like spleen, bone marrow (BM), lymph nodes and thymus, with different subsets distinguishable by function and marker expression. The murine thymus, for example, contains mainly $CD8\alpha^+$ conventional (c)DC that also express CD11c and MHC-II, which develop within this organ [6], while $CD8\alpha^-$ cDC resident in murine thymus are thought to be immigrants [6]. Spleen is distinct in that it contains multiple DC subsets including plasmacytoid (p) DC, and the $CD8\alpha^-$ and $CD8\alpha^+$ subsets of cDC. The $CD8\alpha^-$ cDC in murine spleen are $CD11c^+CD11b^+$MHC-II^+B220^- cells, and can be further distinguished on the basis of CD4 expression [7]. The $CD8^+$ cDC are also distinguishable as $CD11b^-CD4^-$ cells [8], while pDC can be characterized by marker expression as $CD11c^{lo}CD11b^-CD8\alpha^{-/+}$MHC-$II^{-/+}B220^+$ cells, with $CD8\alpha$ and MHC-II expression dependent on the state of maturation. pDC are also marked by secretion of interferon-α (IFN-α), which is effective in mediating viral clearance [9, 10]. Both cDC subsets are derived from precursors originating in BM, which migrate to spleen where they develop into immature, steady-state DC [8]. As well as differing in marker expression, cDC differ in their location within organs, with $CD8^+$ cDC localizing to the T-cell areas of spleen and $CD8^-$ cDC to the marginal zone [8].

Distinct monocyte-derived DC with the functional and phenotypic characteristics of cDC are also recruited into sites of inflammation. Langerhans cells (LC) also appear to be inflammatory DC and represent a distinct monocyte-derived cell type expressing Langerin (CD207) that resides in the epidermis and the intestinal epithelium [11]. LC migrate to lymph nodes in response to contact with antigen in the presence of inflammation or 'danger' signals. Inflammatory DC derive from circulating monocytes, which differentiate under the influence of inflammatory factors like granulocyte-macrophage colony stimulating factor (GM-CSF), tumour necrosis factor (TNF)-α and IL-4 [12–14]. They are distinct from the DC subsets described in spleen in the resting state, although they have phenotypic and functional similarity to $CD8\alpha^-$ cDC. The developmental relationship between cDC and inflammatory DC is not yet clear, although recent evidence for a common dendritic progenitor in BM for cDC and pDC [2, 15], now implicates inflammatory DC as a different lineage with a distinct myeloid progenitor and developmental pathway more closely aligned with monocyte development.

Recent studies have shown that by 6 weeks of age, mouse spleen contains ~2.4% DC which express MHC-II on their cell surface and are immunocompetent [16]. A primary function of DC is uptake of antigen from a site of infection with subsequent migration to lymph nodes and presentation of antigen to $CD4^+$ T cells in the context of MHC-II molecules. If DC also become activated *via* pathogen-associated danger signals, which signal through toll-like receptors (TLR) on the DC, then there is up-regulation of T-cell co-stimulatory molecules like CD86 and CD40, leading to T-cell activation and an immunogenic response [8]. All cDC subsets in spleen can endocytose antigen. However, capacity to present antigen to T cells and to initiate an immune response differs with their $CD8\alpha$ phenotype. $CD8\alpha^-$ cDC endocytose foreign antigen, and induce a $CD4^+$ T-cell response both in the presence and absence of bacterial lipopolysaccharide (LPS) as a 'danger' signal, while $CD8\alpha^+$ cDC stimulate $CD4^+$ T cells only after LPS treatment [17]. Some cDC subsets can endocytose exogenous antigen and present it to $CD8^+$ T cells in the context of MHC-I molecules in a process termed 'cross-presentation' [18], whereby foreign antigen is endocytosed and degraded within endosomes to be presented as peptide in the context of MHC-I molecules. This pathway is important for clearance of pathogens like viruses or parasites that do not infect APC, and for removal of apoptotic normal and cancerous cells [18]. In terms of cDC in spleen, $CD8\alpha^+$ cDC are able to cross-present antigen most efficiently [17, 18].

Functional studies on DC are difficult due to the problem of isolating small subsets of cells and of maintaining their functional potential. Removal of cells from the host, injection into recipient animals, or staining cells with antibody for FACS analysis, can lead to activation. It is difficult to obtain an *ex vivo* population in the resting or steady-state for use in comparative studies.

The tolerogenic function of dendritic cells

A wealth of evidence now indicates that DC maturation or activation status rather than DC lineage *per se* determines the immunogenicity of DC [19]. DC tolerogenicity is not therefore a characteristic of a specific subset, or lineage of DC, but a feature of the environmental niche surrounding the developing cell. Indeed, an important characteristic of DC is their functional plasticity and their ability to adopt different APC characteristics depending on the cytokine milieu or inflammation site in which they are located [20]. Several environmental factors are well known to support the development of tolerogenic/suppressive DC including the immunosuppressive cytokines IL-10 and transforming growth factor-β (TGF-β), as well as other factors like hepatocyte growth factor, granulocyte colony stimulating factor (G-CSF), prostaglandin E_2 and histamine [21, 22].

DC that are tolerogenic or suppressive contribute to peripheral tolerance mechanisms essential in supporting the state of central or thymic tolerance established at birth. Peripheral tolerance is maintained by multiple mechanisms including anergy or deletion of self-reactive T cells, and the induction of regulatory T cells (Tregs), which suppress the function of self-reactive T cells. It is well established that immature cDC resident in spleen are tolerogenic in the steady-state, taking up antigen from the environment as soluble molecules or apoptotic cells, and presenting these to T cells in the absence of a co-stimulatory signal, often with low MHC-II expression, so inducing to a tolerogenic outcome [23–26]. In contrast, these same cells also become highly immunogenic when exposed to inflammatory or 'danger' signals, inducing strong immunogenic responses in naïve T cells. Activating proinflammatory cytokines can include TNF-α, IFN-γ and CD40-ligand

Table 1 Subsets of murine monocytes and DC with regulatory function

Cell type (References)	Phenotype	Development	Mode of action
DCreg [20, 32, 35]	$CD11c^{lo}CD11b^{hi}CD45RB^{+}$	HSC cultured over fibroblastic splenic stroma. Also identified *in vivo*.	Produce IL-10 and induce IL-10 producing $CD4^{+}$ Treg.
diffDC [34]	$CD11c^{lo}CD11b^{hi}$MHC-II^{lo}	Mature DC cultured over endothelial splenic stroma (contact-dependent). Also identified *in vivo*.	Inhibit T-cell proliferation through NO production.
DCreg [33]	$CD11c^{lo}CD11b^{hi}$MHC-II^{lo}	HSC cultured over endothelial splenic stroma (contact-independent). Also identified *in vivo*.	Inhibit T-cell proliferation through NO production.
Myeloid suppressor cells [39–41, 43]	$CD11b^{+}Gr\text{-}1^{+}$	Numbers increase in animals with tumours and after traumatic stress. Also found in CNS of animals with EAE.	Induce T-cell anergy through ARG1 and/or NOS2 activity.

(CD40-L) [19], while danger signals can include LPS, CpG motifs and double stranded (ds) RNA. For example, $CD8\alpha^{+}$ cDC have been shown to be highly immunogenic and able to induce $CD8^{+}$ cytotoxic T cells in response to viral infection [18, 27]. DC of the same type can also function in cross-presentation of apoptotic cells to $CD8^{+}$ T cells, mediating deletion of reactive T cells, although the mechanism of deletion is unclear [28]. Much of the work on immature, tolerogenic DC has involved *in vitro* cultured cells, and the identity of the *in vivo* cell equivalent is unclear. Many studies have involved DC produced by culture of BM cells with GM-CSF and IL-4 [29], which yields a subset of DC very similar to $CD8\alpha^{-}$ cDC. Several papers now report the ability of these cells to induce both regulatory and anergic T cells [30, 31]. Tolerance induction is commonly mediated by pDC, through induction of $CD4^{+}CD25^{+}$ Tregs. Indeed, in the collagen-induced arthritis model in mice, orally induced tolerance due to collagen administration has been associated with pDC and their production of induction of 2,3 indoleamine dioxygenase (IDO) with subsequent formation of Tregs [30]. Tregs then have an inhibitory effect on T-cell proliferation and their production of inflammatory cytokines [30]. Soluble factors produced by tolerogenic DC could represent valuable immunotherapeutic agents for control of T-cell proliferation and activation in autoimmune diseases.

Furthermore, both immature and mature pDC can induce tolerogenic responses. Immature pDC induce anergy in $CD4^{+}$ T cells, and mature pDC can induce regulatory T-cell function in $CD8^{+}$ T cells [24]. While the induction of $CD4^{+}$ T-cell anergy by immature pDC is thought to occur through DC-T-cell contact in the absence of costimulatory molecules, the induction of regulatory function in $CD8^{+}$ T cells by mature pDC may be mediated by the activation of the pDC by CD40L [24].

Regulatory dendritic cells and immunosuppression

Recent evidence also implicates a role for various subsets of murine DC termed 'regulatory' DC (DC_{reg}) in the suppression of T-cell responses. These are listed in Table 1. While a number of different examples exist in the literature, this group of cells is not well defined [20, 32–34]. Several reports have described the induction of DCregs with suppressive or regulatory function for $CD4^{+}$ T cells after *in vitro* co-culture of haematopoietic progenitors, precursors or even DC above splenic stroma [33–35]. Indeed, evidence that splenic stromal cells support DC haematopoiesis is consistent with a central role for microenvironments in spleen in DC development [1, 36, 37].

Some authors describe DCregs that can induce the development of Treg cells [20, 35]. These were defined as 'immature' DC expressing CD45RB, isolated directly from BM or induced by culture of BM progenitors with stroma comprising a combination of fibroblastic and endothelial cells [32]. Others have described DCregs, which do not induce Treg formation, but are immunosuppressive due to their production of inhibitory factors. Tang *et al.* [33] reported DCregs as $CD11c^{lo}CD11b^{hi}$MHC-II^{lo} DC developing when BM-derived haematopoietic stem cells (HSC) were cultured over a spleen endothelial stroma. IL-10 released by stromal cells appears to be an important factor in their development [33]. Zhang *et al.* [34] describe a distinct DCreg subset called 'diffDC', also with suppressive function for T cells. These cells were derived *in vitro* from mature $CD11c^{hi}$MHC-II^{hi} DC induced after culture of BM cells with GM-CSF and IL-4. When cultured above a splenic stroma, they proliferated and

differentiated further dependent on stroma-produced soluble TGF-β and contact with fibronectin [34]. These two DC subsets are quite distinct in terms of lineage origin, although both are regulatory in that they directly inhibit $CD4^+$ T-cell proliferation through release of nitric oxide, which interferes in the IL-2 signalling pathways important in T-cell proliferation [33, 34]. DCregs described by Tang *et al.* [33] also activate $CD4^+$ T cells in terms of cytokine production, but are unable to induce their proliferation.

The production of DCreg in response to infection has also been described. Wong and Rodriguez [38] showed expansion of a $CD11c^{lo}CD8^-$MHC-$II^{lo}CD45RB^{hi}$ subset of DCreg in response to infection with *Plasmodium yoelii, P. berghei* and LPS treatment. The expansion of DCreg lasted until 10 days after infection and corresponded with a drop in numbers of cDC [38]. The DCreg population was shown to stimulate Tregs, which secrete IL-10 and suppress $CD4^+$ T-cell function and appear to resemble the DCreg described previously by Svensson *et al.* [32]. Indeed, descriptions and mode of action of DCreg differ, and the challenge for immunologists now is to reconcile cells identified by *in vitro* studies, with *in vivo* subsets, and to determine the *in vivo* conditions that favour one cell type over another.

The ubiquitous myeloid suppressor cells

Recently, myeloid suppressor cells (MSC) were described that have a $CD11b^+CD11c^{-/lo}Gr\text{-}1^{+/lo}$ phenotype, and so bear some phenotypic relationship with monocytes and myeloid DC. This cell type has been described in several mouse models and in association with a variety of disease states. The exact phenotype and mechanism of action of MSC appears to vary with cell location and the disease state of the host, so it is possible that different subsets of this cell type exist, although they generally reflect inflammatory monocytes.

Gallina *et al.,* [39] describe a $CD11b^+Gr\text{-}1^+$ cell type, which is expanded in tumour-bearing mice. These cells up-regulate arginase (ARG1) and nitric oxide synthase (NOS2) activity in response to IFN-γ and IL-13, leading to an environment containing reactive nitric oxide species and depleted levels of L-arginine. This results in T-cell apoptosis, impaired proliferation and failure of T cells to express functional antigen-specific TCR [39]. Other groups report cells that act through either ARG1 or NOS2 [40, 41]. MSC reported in mice that have undergone traumatic stress like surgery, are $CD11b^+Gr\text{-}1^+$ myeloid cells with up-regulated ARG1 activity, and are found in spleen in close proximity to T-cell zones [40]. The MSC described by Rossner *et al.,* [41], develop from BM cells cultured with GM-CSF and resemble $CD11b^+Ly\text{-}6C^+Ly\text{-}6G^{lo}$ monocytes. These become suppressive in the presence of IFN-γ and appear to require cell-cell contact and NOS2 activity to achieve this effect [41]. A subset of $CD11b^+Ly\text{-}6C^{hi}Ly\text{-}6G^-$ MSC that resemble monocytes have also been described by Zhu *et al.* [42]. These were observed in the neural tissue of experimental autoimmune encephalitis (EAE)-immunized mice. They can up-regulate both ARG1 and NOS2 in response to a variety of cytokines, including IFN-γ and IL-4, so suppressing T cells in a mechanism initiated by cell-cell contact [42]. Recently Weber *et al.* described type II monocytes and their suppressive effect upon adoptive transfer into mice with induced experimental autoimmune encephalomye-litis (EAE) [43]. Transferred cells ameliorated disease and reduced T-cell infiltration into the central nervous system, due to production of Tregs and T helper type 2 cells through increased IL-10 and TGF-β expression, which led to an anti-inflammatory response.

Differences between the myeloid cell types described by these groups could be explained by the different disease states of the mice from which they were isolated. MSC differ by comparison with described DCregs in that they are associated with disease states and often appear to contribute to pathology as opposed to playing a role in immunosuppression.

What are regulatory dendritic cells?

Dendritic cells are emerging as a highly versatile cell type that displays plasticity in response to environmental factors. They can induce two opposing immune states: tolerance and immunity. Several theories have been proposed regarding the subsets of cells involved in these two processes. Most of the well-described steady-state DC have tolerogenic capacity as immature cells, but can become immunogenic upon maturation/activation in the presence of a danger signal. Tolerance mediated by these cells most likely involves ubiquitous self-antigens, like those released by apoptotic cells. Other subsets of cells, including DCregs and MSC, function to turn down an immune response under inflammatory conditions. Cells like these would be important in ensuring that tolerance is maintained even when pathogens are present.

Increasing evidence now suggests that DC can emerge from a number of precursors and can acquire a range of immunosuppressive or regulatory functions under different environmental triggers. MSC may also have a developmental origin common with some DC and particularly diffDC [34], but appear to be distinct from other DCregs. Our conclusion is that DCregs represent a broad class of cells, which participate in a number of ways to turn down an immune response. Their role in homeostasis and switching off immune responses therefore reflects yet another immune capacity for DC, which now extends to immunostimulation, induction of tolerance, maintenance of peripheral tolerance and immunosuppression.

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