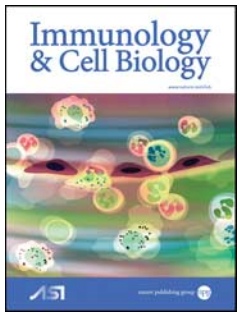

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Heterogeneity, functional specialization and differentiation of monocyte derived dendritic cells

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Abstract

Dendritic cells (DCs) are professional antigen presenting cells that consist of functionally and phenotypically heterogeneous populations. Monocyte derived dendritic cells (moDCs) are a DC subset that have been attracting increasing interest due to their potent influence on adaptive immune function and their rapid accumulation upon an inflammatory stimulus. Whilst early studies on moDCs mainly addressed infection, their emergence and function in other settings such as autoimmunity and allogeneic organ transplantation are now being increasingly appreciated. In this review, the relationship between murine monocyte subsets and the moDCs that arise from them is discussed. Their role in initiating and modulating innate and adaptive immune responses in various pathophysiological scenarios is also explored, including how they may separate their labour from conventional DCs. How these findings might relate to their human counterparts is also discussed. Overall, monocytes and moDCs exhibit complex and heterogeneous behaviours that are critical in responses against microbial invasion, autoimmunity and allograft rejection.

Introduction

Dendritic cells (DCs) are the key professional antigen-presenting cells of the immune system. They are a highly heterogeneous population, comprised of many different subsets, each with distinct morphological, developmental and functional characteristics. Unlike the well-studied lymphoid resident conventional DCs (cDCs) and plasmacytoid DCs (pDCs) that arise from defined common DC precursors, monocyte derived dendritic cells (moDCs) arise from monocyte precursors in vitro and in vivo [1, 2]. They have been implicated in the immune response to various infective and inflammatory conditions as well as in the pathogenesis of several autoimmune diseases. Most of what is known about them has been derived from mouse studies. Hence, in this review, we discuss the current understanding of the developmental and functional characteristics of murine moDCs and their monocyte precursors in the context of autoimmunity and organ transplantation. We explore the division of labour between moDCs and cDCs. We also highlight the phenotypic and functional differences of the various moDC populations reported in the literature. Whilst these observations regarding murine cells may not always directly translate to their human equivalents, they nevertheless provide important insights into the complex biology underlying this important component of the immune system.

Monocytes

Monocytes are circulating phagocytic leukocytes that have been classically considered to be precursors of macrophages in many tissues [3, 4]. They comprise approximately 10% and 4% of circulating leukocytes in human and mouse blood respectively [5]. M-CSF is critical whereas GM-CSF, Flt3L and lymphotoxin α1β2 are redundant for monocyte development [5].

Mouse blood monocytes are identified by their expression of CD115, CD11b, F4/80 and Dectin-1. They can be separated into two subsets based on the variable expression of Ly6C and CX_3CR1 [5, 6]. They share a number of typical morphological features such as an irregular cell shape, oval shaped nucleus, cytoplasmic vesicles and a high cytoplasm to nucleus ratio, but vary greatly in size and shape [5].

Monocyte subsets in inflammation and repair

There are at least two major monocyte subsets based on function and surface phenotype. The classical, more abundant monocyte (70 – 80% of circulating monocytes) that is recruited to inflamed tissues is $CCR2^{+}CX_3CR1^{low}Ly6C^{+}CD62L(L\text{-}Selectin)^{+}$. Morphologically larger and more granular, it is often referred to as the "inflammatory" monocyte [6], as it produces abundant TNF-α and IL-1 in response to infection and injury [7]. Upon infection, $Ly6C^{+}$ monocytes exit the bone marrow into the circulation via CCR2 [7]. Interestingly, adoptively transferred monocytes from $CCR2^{-/-}$ mice could still traffic to sites of infection, suggesting that CCR2 is not required for their migration into tissues [7]. This monocyte subset is also the in vivo precursor of monocyte-derived dendritic cells (moDCs) [2, 8].

The second monocyte subset, operationally defined as $CCR2^{-}CX_3CR1^{high}Ly6C^{-}$, comprises 20 – 30% of circulating monocytes [5, 6]. Smaller in size and lacking L-Selectin (CD62L) [5], these $Ly6C^{-}$ cells are often referred to as "patrolling monocytes" due to their involvement in innate surveillance of tissues such as the vascular endothelium [4]. Although they mainly remain within blood vessels, they can rapidly extravasate (within 1 – 2 hrs) into surrounding tissues in response to tissue damage (e.g. tissue irritants or aseptic wounding) or infection (eg: *Listeria monocytogenes* peritoneal infection), whereupon they display an early

but transient inflammatory response characterized by production of TNFα up-regulation of genes associated with IL-1, lysozyme, defensins, complement, TLRs, scavenger receptors, IgFc receptors and chemokines [4, 5]. This inflammatory response by these non-classical monocytes is only transient so that by 8 hours after *Listeria* infection, classical monocytes become the main producers of inflammatory cytokines [5].

Examination of transcription factors used by the two monocyte subsets suggests that extravasated Ly6C$^-$ monocytes, after the conclusion of their transient inflammatory response, initiate a typical macrophage differentiation program (characterized by up-regulation of cMaf and MatB, but not RelB and Pu.1), whereas Ly6C$^+$ monocytes initiate a DC differentiation program (characterized by up-regulation of RelB and Pu.1 but not cMaf and MafB) [4]. Ly6C$^-$ monocytes promote tissue healing after injury. They are recruited to the healing myocardium where they promote healing through myofibroblast accumulation and collagen deposition [9]. After an early accumulation of Ly6C$^+$ monocytes, induced muscle necrosis resulted in later preponderance (after 4 days) of Ly6C$^-$ monocytes, whereupon they exhibited features of anti-inflammatory macrophages [10]. Interestingly, whereas Ly6C$^+$ monocytes can facilitate cancer metastasis, Ly6C$^-$ monocytes contribute to cancer immunosurveillance [11].

In humans, the current consensus view has three monocyte subsets characterized by CD14^{high}CD16$^-$CX$_3$CR1^{low}, CD14^{hi}CD16^{int}CX$_3$CR1^{hi} and CD14^{int}CD16^{hi}CX$_3$CR1^{hi} [12]. The first is similar to the "inflammatory" monocyte and the latter two are more similar to the "patrolling" monocyte. Similarly, human monocytes can also differentiate into DCs in vitro [1]. As the focus of this review is mainly on the differentiation and function of differentiated moDCs in vivo, and given that most of what is known in this area has been generated from mouse studies, most of our discussion will focus on murine monocytes and moDCs. Whilst findings derived from animal studies may not always directly translate to the human immune

system, they can still provide important insights into relationships and associations that may also occur in the human.

Developmental relationship between monocyte subsets

The existence of monocyte subsets raises the question about their developmental relationship. After depletion with poison-loaded liposomes, the first monocytes to recover were Ly6C$^+$ monocytes (within 18 hours), whereas Ly6C$^-$ monocytes recovered over several days [13]. Analysis of bone marrow in chimeric mice 8 weeks after irradiation and reconstitution with wild-type and CCR2$^{-/-}$ cells revealed that Ly6C$^+$ monocytes were predominantly comprised of CCR2$^{-/-}$ cells (presumably their inability to exit the bone marrow resulted in their accumulation), while Ly6C$^-$ monocytes were moderately dominated by wild-type cells. Furthermore, in the blood, both Ly6C$^+$ and Ly6C$^-$ monocyte subsets were dominated by wild-type cells [14]. These results suggest that the precursors of Ly6C$^-$ monocytes require CCR2. Whilst there is a possibility that some radiation cells may have contaminated these findings, when taken together with the observations from the earlier study these results are consistent with the premise that the Ly6C$^+$ subset is the precursor to the Ly6C$^-$ population (**Figure 1**).

Monocytes as effector cells

Monocytes contribute as circulating precursors for macrophages and moDCs in tissues [8]. They also contribute directly as effector cells in the immune response against microbial pathogens. Successful clearance of *Listeria monocytogenes*, for instance, requires the activation of both innate and adaptive immune responses, the former of which includes the production of TNFα and interferon-ɣ (IFN-ɣ) by monocytes and their derivative cells [8].

Depletion of monocytes and neutrophils by the administration of an antibody that sees both Ly6C and Ly6G resulted in profound susceptibility to the infection [15]. This was especially so when the antibody was administered within the first 24 hours post infection. Similarly, antibody blockade of CD11b (which prevents monocyte and neutrophil recruitment) within 24 hours of infection with *Listeria monocytogenes* resulted in profound susceptibility [16]. In these settings, it appears that monocytes can mediate bacterial killing through phagocytosis and phagolysomal enzyme activity or through the production of reactive oxygen species [8].

Pulmonary infection with *Mycobacterium tuberculosis* results in the recruitment of various inflammatory cells including monocytes, macrophages, DCs and neutrophils into the bronchoalveolar space. The recruited monocytes are $CCR2^{+}$ (hence corresponding with the $Ly6C^{+}$ subset) and are reduced in frequency in $CCR2^{-/-}$ mice [17]. They subsequently give rise to macrophages and/or DCs. $CCR2^{-/-}$ mice are highly susceptible to *Mycobacterium tuberculosis* infection (with high mortality and up to 100-fold greater bacterial replication), especially at high infection doses, a finding which is associated with delayed T cell priming and reduced IFN-ɣ secretion by $CD4^{+}$ T cells in the lung [17]. Monocytes also appear to be important in early control of infection with *Toxoplasma gondii*. Infection in $CCR2^{-/-}$ and $MCP\text{-}1^{-/-}$ mice resulted in a lack of recruitment of $Ly6C^{+}$ monocytes and in increased mortality, even though the induction of normal Th1 responses remained intact [18].

Differentiation of monocytes into macrophages

Whilst the concept that monocytes act as precursors for various populations of macrophages in tissues had been generally accepted in the past [3], this paradigm has come under challenge in recent years. In the traditional framework, monocytes were thought to give rise to various steady state macrophages contained within specific compartments of bone

marrow, thymus, secondary lymphoid organs (including germinal centre macrophages, lymph node subcapsular macrophages, splenic marginal zone and red pulp macrophages), as well as macrophages in non-lymphoid tissues (e.g. liver Kupffer cells, lung alveolar macrophages and microglial cells in the central nervous system) [3]. According to this paradigm, during inflammatory states associated with infection, injury or malignancy, monocytes were also thought to be recruited to inflamed tissues where they were induced to differentiate into various types of inflammatory macrophages which variably participate in diverse functions such as clearance of dead cells, removal of pathogens, tissue repair and regulation of innate and adaptive responses [3].

It has become increasingly clear, however, that this concept of a definitive monocyte-macrophage axis does not provide an entirely satisfactory picture of macrophage ontology in vivo. In support of a new conceptual framework, some recent reports now contend that monocytes do not contribute significantly to many tissue macrophage populations in the steady state and even some that arise during inflammation. Instead, most of these populations may actually be derived from embryonic precursors which are seeded before birth and capable of self-renewal, even into adulthood [19]. Fate mapping using both constitutive and CX_3CR1-dependent deletion found no evidence of monocyte contribution to a number of steady state macrophage populations such as liver Kupffer cells, lung alveolar, splenic or peritoneal macrophages [14]. Instead, the authors concluded that the major macrophage populations in these organs are established prenatally during development and continued to be maintained without contribution from monocytes. Further evidence for this concept was provided by a second study in which $CD45.2^+$ and congenic $CD45.1^+$ mice were parabiotically joined for between 2 – 12 months [20]. While peripheral blood monocytes exhibited a degree of non-host chimerism (approximately 15% for $Ly6C^+$ monocytes and 40% for $Ly6C^-$ monocytes), lung, peritoneal, splenic, red pulp and bone marrow macrophages

as well as microglial cells showed negligible chimerism, consistent with a lack of monocyte contribution. Even more remarkably, a second set of parabiotic experiments between wild-type CD45.1$^+$ and CCR2$^{-/-}$ CD45.2$^+$ mice showed that, despite an even more pronounced non-host chimerism in CCR2$^{-/-}$ parabionts (approximately 70% and 90% for Ly6C$^+$ and Ly6C$^-$ monocytes respectively; due to defective bone marrow emigration by CCR2$^{-/-}$ cells), the macrophage populations continued to show negligible non-host chimerism (even after 12 months of parabiosis).

Notwithstanding the above, monocytes can give rise to certain macrophage populations under particular conditions [19]. For example, a short-lived intestinal CX$_3$CR1$^+$ macrophage population was found to be derived from Ly6C$^+$ monocytes in adoptive transfer studies [19]. Similarly, a proportion of dermal macrophages in the steady state were derived from Ly6C$^+$ monocytes [21]. Interestingly, an analysis of the origins of four distinct populations of cardiac macrophages revealed that they were primarily maintained by local proliferation at the steady state, whilst both infiltration of Ly6C$^+$ monocytes and local proliferation contributed during inflammation [22]. Overall, it seems that the origins of macrophages can vary between tissues and between inflammatory states, and hence each circumstance needs to be considered individually.

MoDCs

Monocytes can differentiate into moDCs in vitro [1] and in vivo [2, 8]. The concept that moDCs arising in inflammatory contexts could play important roles in the induction and regulation of immune responses has led to a number of studies aimed at better understanding and defining these DCs.

Generation of moDCs in vitro

Monocytes from human or mouse peripheral blood or bone marrow, when cultured with GM-CSF and IL-4, differentiated into DCs with typical dendritic morphology and high levels of MHC and co-stimulation molecules [1, 23]. A variety of methods using different combinations of cytokines and culture conditions have been since reported [24].

These systems availed the study of DC biology. While there was significant variability in reported findings depending on the particular conditions of the experiments involved, overall in vitro moDCs were found to be highly capable of antigen uptake, processing and presentation, as well as being stimulatory in mixed leukocyte reactions (MLRs) [1]. Treatment with a number of compounds, such as LPS, TNFα, IFN-ɣ or CD40L, have been found to induce in vitro moDC maturation, as defined by expression levels of MHC II and co-stimulatory molecules, or by functional changes in antigen presentation and modulation of T cell responses [24]. Interestingly however, when monocytes (as opposed to moDCs) were incubated in LPS prior to culture with GM-CSF and IL-4, their differentiation into moDCs was impaired (only 30% became DCs) compared to when they were cultured in LPS, GM-CSF and IL-4 simultaneously (approximately 75% became DCs) [25]. These observations highlight the critical importance of the conditions in which monocytes are cultured in determining their ultimate fate. Indeed it appears that TLR signalling on monocytes (prior to differentiating into moDCs) and moDCs have opposite effects, such that signalling on monocytes inhibits DC development, while signalling on moDCs induces their maturation [24].

The plasticity of monocyte-moDC development is further illustrated by the skewing of DCs toward a Langerhans' resembling phenotype (CD324, CD207, CCR6) when IL-4 was replaced by IL-15 [26]. Replacement of IL-4 by IFN-α resulted in short-lived TNF-related

apoptosis-inducing ligand (TRAIL) expressing moDCs that were more potent at stimulating T cell responses [27]. On the other hand, exposure of in vitro derived moDCs to agents such as vitamin D3, corticosteroids, rapamycin, cyclosporine, tacrolimus, aspirin or retinoic acid, results in the DCs adopting a stable semi-mature phenotype characterized by low expression of MHC II and co-stimulatory molecules but elevated expression of indoleamine dioxygenase, IL-10, TRAIL and PDL-1 [28]. These cells show tolerogenic properties as they down regulate adaptive responses, in part through the induction of regulatory T cells.

These in vitro manipulations avail the production of large numbers of tailored moDCs for potential clinical benefit. One such potential benefit is in the use of moDCs as therapeutic vaccines against malignant tumours. Tolerogenic DCs generated by culturing peripheral blood monocytes in GM-CSF, IL-4 and vitamin D3 resulted in a nearly 3-fold increase in kidney allograft survival in macaque primates [29]. Whilst these results indicate potentially significant benefits in using manipulated moDCs, DC therapy has not yet become routine clinical treatment.

Differentiation of monocytes into moDCs in vivo

Monocytes differentiating into DCs in vivo was first reported by tracking the fate of the monocytes that phagocytosed subcutaneously injected fluorescent latex microspheres [2]. While most of these $CD11b^{+}F4/80^{-}$ monocytes remained at the site of injection and eventually became macrophages, 25% of them migrated to T cell areas of the draining lymph node over 3 – 4 days, where they up-regulated MHC II, CD11c, CD80 and CD86, consistent with their differentiation into DCs. After the injection of microspheres with different fluorescent labels in adjacent but not overlapping subcutaneous sites, the resulting moDCs in the draining lymph nodes contained only one colour of microsphere and not both. This

suggested that the microspheres had actually been taken up locally by monocytes that had then trafficked to the lymph node and differentiated into moDCs en route, rather than free microspheres being transported passively in lymphatic channels before being taken up by resident DCs upon arrival in the lymph node. A later report found that the in vivo differentiation of monocytes into moDCs was blocked by the presence of bacteria or bacterial LPS [30]; this accords with the earlier in vitro findings of LPS inhibiting in vitro moDC development [25]. That inflammatory monocytes are truly the precursors of the moDCs derived in this model was shown in a subsequent report using $CX_3CR1^{gfp/+}$ mice in which monocytes and their daughter cells could be tracked by analysing GFP^+ cells [31]. After ingesting the fluorescent microspheres, moDCs that trafficked to the draining lymph node expressed Gr1 (Ly6C/Ly6G), thereby suggesting that they corresponded to the $Ly6C^+$ inflammatory monocyte subset. In contrast, $CCR2^-Gr1^-$ monocytes did not give rise to these moDCs.

Possible heterogeneity of moDC populations in vitro and in vivo

There have been recent discussions regarding the proper identification of in vitro derived moDCs generated under GM-CSF. Classically, cells within the $CD11c^+$ $MHCII^+$ fraction resulting from cultures of murine bone marrow cells with GM-CSF have been thought to be homogeneous populations of DCs. Differences within these cells were thought to reflect only variation in maturation stage. However, recent reports have contended that these differences actually result from the presence of two distinct cell types within this fraction [32]. By examination of ontogeny and gene expression, the presence of both bone fide DCs and monocyte-derived macrophages was detected within this population. If validated, these findings might have significant implications for the interpretation of numerous studies that have assumed that this population is mainly comprised only of moDCs.

Whilst the generation of moDCs in vitro has often been accomplished through stimulation with GM-CSF, in vivo moDCs can be derived in the absence of GM-CSF [33]. Instead, M-CSF has been found to be a critical cytokine required to drive the differentiation of moDCs in vivo. Nevertheless, moDCs do become more abundant in mice in which levels of GM-CSF are increased. Indeed, owing to their abundance, we used moDCs from GM-CSF transgenic mice as an in vivo source of moDCs for our functional evaluation [34]. Thus, GM-CSF can still be a critical factor influencing moDC differentiation, particular under conditions where GM-CSF levels are elevated.

Regardless of the potential differences in growth factors that regulate moDC differentiation in vitro and in vivo, there may also be significant moDC heterogeneity in vivo. Most studies examining moDCs in vivo have used similar cell surface markers to define similar populations that are likely to be analogous [8, 34, 35]. However, an additional population of quite different moDCs was reported to accumulate in the skin-draining lymph nodes of mice which had been treated with LPS 1 day earlier [36]. This DC subset is unique amongst other reported moDC populations in that they arise only in the skin-draining lymph nodes (not in other lymph nodes or the spleen) after LPS treatment (but not other TLR ligands or inflammatory stimuli). Furthermore, they are also uniquely characterized by the expression of CD206 (mannose receptor) and DC-SIGN. This DC-SIGN expressing population has phenotypical and functional differences from historically ascribed moDC populations. We confirmed that, unlike traditionally defined moDCs, this $CD206^{+}DC\text{-}SIGN^{+}$ population does not arise in the spleen following LPS administration [34]. Another recent study has also found that they do not accumulate in LPS treated $Flt3l^{-/-}$ mice despite the presence of normal monocyte numbers, with mixed bone marrow chimera experiments excluding the possibility that this is due to loss of cDC help [37]. The significance of this heterogeneity has not been

fully resolved, although the possibility that this population might be more closely related to migratory cDCs has not been excluded.

moDCs in infection and disease

moDCs are rare in steady state tissues and lymphoid organs but become abundant during inflammation [8]. They have been found to arise during viral, bacterial, fungal and protozoan infections: pulmonary influenza infection [33], genital herpes simplex virus-2 infection [38], systemic *Listeria monocytogenes* infection [8], gastrointestinal *Salmonella Typhimurium* infection [39], pulmonary *Streptococcus pneumoniae* infection [33], pulmonary *Mycobacterium tuberculosis* infection [40], pulmonary *Cryptococcus neoformans* infection [41], and pulmonary *Aspergillus fumigatus* infection [42], cutaneous *Leishmania major* infection [35], and systemic *Trypanosoma brucei* infection [43]. They have also been implicated in the pathogenesis of autoimmune and allergic diseases: experimental autoimmune encephalomyelitis (EAE) [44], rheumatoid arthritis [45], inflammatory colitis [46] and asthma [47]. Thus, moDCs are likely to play a role in any disease with substantial inflammation.

The role of moDCs in innate immune responses

moDCs have been implicated in a number of innate and adaptive immune responses under various conditions (**Table 1**) . One of the earliest reports of moDCs arising in vivo under disease conditions found that they became abundant in the spleen of mice 2 days after *Listeria monocytogenes* infection [8]. These moDCs were originally called Tip-DCs (TNFα and inducible nitric oxide synthase (iNOS) producing) and were characterized by $CD11b^{+}CD11c^{int}Mac\text{-}3^{+}$; up-regulated MHC II, CD40, B7.1 and B7.2; and CCR2 dependent recruitment. They were distinguished from macrophages, as the former neither expressed

F4/80 nor adhered to culture plates in vitro. CCR2$^{-/-}$ mice lacked Tip-DCs, had profound TNF and iNOS deficiencies, and died early upon bacterial challenge, suggesting that these moDCs were critical in facilitating bacterial clearance.

The iNOS production by moDCs during *Brucella melitensis* infection was dependent on TLR4 and TLR9 stimulation [48]. Gastrointestinal *Salmonella* infection induces rapid accumulation of Gr1^{+} monocytes [39] and within 5 days, they differentiate into moDCs (up-regulated MHC II, CD80 and CD86). As well as producing iNOS, these moDCs rapidly phagocytosed and killed bacteria. Despite this, the moDCs were found to be weak at inducing OT-II T cell proliferation after incubation with *Salmonella* expressing OVA.

With such potent effector molecules, it is not surprising that moDCs can also elicit immunopathology. During chronic *Trypanosoma brucei* infection, moDCs accumulated in the liver and lymphoid organs, contributing to tissue toxicity such as hepatic inflammation, ischaemia and necrosis [43]. This damage was exacerbated by IL-10 deficiency, which resulted in enhanced differentiation of monocytes into moDCs.

The role of moDCs in the regulation of adaptive immune responses

CD4^{+} T cell proliferation

moDCs have been found to be capable of inducing proliferation of antigen specific CD4^{+} T cells ex vivo under a number of different experimental conditions [49]. moDCs from the mediastinal lymph node or lung of house dust mite-exposed mice were found to be as efficient as CD11b^{+} migratory DCs (and more efficient than CD103^{+} migratory DCs) at inducing proliferation of naïve cognate CD4^{+} T cells in vitro [47]. These moDCs were already activated. Under steady state conditions, we found that like pDCs, moDCs are markedly less

efficient than cDCs at inducing $CD4^+$ T cell proliferation [34] (see below under Division of Labour).

The role of moDCs in the induction of $CD4^+$ T cell proliferation in vivo has been less well characterized. Nevertheless, in a model of pulmonary infection with *Aspergillus fumigatus*, $CCR2^+Ly6C^+$ inflammatory monocytes were found to accumulate in infected lungs and draining lymph nodes, where they differentiated into $CD11b^+$ moDCs [42]. Depletion of $CCR2^+$ cells (by diphtheria toxin treatment of CCR2.DTR mice) reduced the amount of *Aspergillus* transported from lungs to lymph nodes, abolished pulmonary $CD4^+$ T cell proliferation and prevented fungal clearance. In contrast, we observed a significant increase in the proliferation of adoptively transferred $CD4^+$ T cells in the spleens of diphtheria toxin treated CCR2.DTR mice [34], suggesting that moDCs can also inhibit T cell proliferation in the spleen in vivo. The differences in these observations may reflect variability in moDC function in different tissues, possibly in order to support the initiation of adaptive responses at peripheral sites of inflammation, while preventing potentially deleterious over activation of these functions in lymphoid organs.

$CD4^+$ T cell polarization

Several studies have indicated that moDCs induce Th1 and Th17 responses. After cutaneous infection with *Leishmania major*, moDCs could induce Th1 polarization [35]. Furthermore, we also recently reported that GM-CSF responsive $CCR2^+$ moDCs are critical for Th17 induction, and for the development of EAE [44]. Depletion of moDCs in CCR2.DTR mice significantly delayed the development of EAE, whereas deletion of cDCs in CD11c.DTR mice had no effect on disease progression. Examination of lymph nodes from moDC depleted mice revealed significant reduction in numbers of IL-$17A^+$ $CD4^+$ T cells and

reduced IL-17A levels in culture supernatants. Consistent with this, purified moDCs induced significantly greater production of IL-17 from OT-II $CD4^+$ T cells than cDCs ex vivo. Most interestingly, while GM-CSF has been previously found to be dispensable for in vivo moDC development [33], this study found that it was required to allow moDCs to mediate Th17 induction. Thus, only GM-CSF responsive moDCs, and not $Csf2rb^{-/-}$ moDCs (which do not respond to GM-CSF) can induce Th17 responses in vitro and in vivo. These findings confirm an in vivo role for Th17 induction by moDCs after previous reports of moDC mediated Th17 induction in vitro. They also suggest that, while GM-CSF is not required for moDC recruitment in vivo, it is required for their polarizing function.

Although most studies report that moDCs induce Th1 and Th17 responses, moDCs can foster Th2 responses under certain stimuli such as alum adjuvant or dust mite exposure. After alum injections, moDCs induced persistent Th2 responses in the draining lymph nodes [50]. After induction of asthma by house dust mite allergens, lung moDCs could induce Th2 polarization, albeit less efficaciously than conventional DCs [47].

Overall, the variability of these observations suggest that there is a significant degree of plasticity in moDC function and that the type of T cell response they induce is dependent on the particular inflammatory context.

Cross presentation

Although $CD8^+$ cDCs have been recognized as the classical DC subset responsible for cross presentation of exogenous antigen to $CD8^+$ T cells, several studies have suggested that moDCs may also be involved. Administration of measles virus nucleoprotein or 2,4-dinitrofluorobenzene onto the buccal mucosa resulted in recruitment of $Gr1^+$ monocytes and their differentiation into moDCs in a CCR6/CCL20 dependent mechanism [51]. These moDCs

were found to be critical for in vivo cross priming of antigen specific $CD8^+$ T cells, as illustrated by the loss of cross priming in CCR6 deficient mice, and its restoration with adoptive transfer of CCR6 sufficient $Gr1^+$ monocytes. In a model of HSV-1 reactivation, depletion of monocytes and moDCs by administration of clodronate-containing liposomes was found to reduce the stimulation of antigen-specific memory $CD8^+$ T cells in inflamed peripheral tissues, further suggesting a role in cross-presentation [52].

Immunoglobulin class switch recombination

moDCs present in the gut-associated lymphoid tissues have been found to be critical for the induction of IgA class switch recombination and IgA production by B cells in the gastrointestinal mucosa [53]. This was dependent on TLR, iNOS and transforming growth factor-β.

Division of labour between moDCs and cDCs

The fact that DC subsets are located differently anatomically and secrete different cytokines would suggest some division of labour. However, most previous studies of moDC function have examined their behaviour in isolation, making it difficult to gauge their contribution to immune responses relative to other DC subsets (i.e. lymphoid resident DCs or tissue resident DCs). We recently conducted side-by-side comparisons between how moDCs and cDCs affect T cell responses [34]. We found ex vivo moDCs to be 10 – 20 times less efficient than cDCs at driving $CD4^+$ T cell proliferation. moDCs were in fact capable of inhibiting cDC induced T cell proliferation through a nitric oxide dependent process. In contrast, we observed that moDCs were highly efficient at driving Th1 and Th17 responses in vitro and inhibiting Th2 responses in vivo. Overall, our observations suggest that there is

some division of labour between cDCs and moDCs, with cDCs acting as initiators predominantly of proliferation (corresponding to their lymphoid location), and moDCs acting as initiators predominantly in differentiation and as regulators (corresponding with their target location).

In the light of this division of labour between moDC and lymphoid resident DC subsets, it is interesting to speculate whether further subgroups of moDCs might also exhibit a similar division of labour, therefore allowing for the variety of functions that moDCs have been found to exhibit. It remains to be seen whether this is indeed the case.

Monocytes and moDCs in innate allo-recognition

Despite an increasingly well-developed understanding of the role of moDCs in infection and inflammation, their role in mediating allograft rejection in the setting of organ transplantation has been less well explored. Nevertheless, a number of recent studies have proposed that monocytes might be capable of innately sensing allogeneic non-self resulting in their subsequent differentiation into moDCs that can then activate T cells and initiate allograft rejection. Whilst inflammation and tissue damage-associated danger signals arising from surgical procedures are likely to contribute to moDC development in this context, these studies suggest that moDCs can also be induced directly by allogeneic stimulation independent of external danger signals.

Initial evidence of this concept was provided in experiments in which $RAG^{-/-}$ mice (which lack B cells, T cells and NK cells) were injected with allogeneic or syngeneic splenocytes into the ear pinnae [54]. Injection of allogeneic splenocytes induced significantly greater swelling and skin infiltration with myeloid cells (mainly neutrophils, monocytes and macrophages) than injection of syngeneic splenocytes, but only if hosts had been previously

primed with allogeneic cells or an allogeneic skin graft. Interestingly, it was also found that the adoptive transfer of syngeneic monocytes from mice that had previously been primed with allogeneic cells, was sufficient to prime a significant allogeneic immune response in otherwise naïve hosts. This suggested that monocytes were able to recognize allo-antigen and therefore prime an immune response.

A subsequent study comparing moDC recruitment in syngeneic and allogeneic murine models of heart and kidney transplantation, found that innate allo-recognition by monocytes resulted in their differentiation into mature moDCs, and that this process was required to initiate rejection under certain conditions [55]. Using CX3CR1$^{gfp/+}$/CD45.1 recipients, which allowed for cells of monocyte lineage to be identified, allogeneic heart transplants were found to be infiltrated with significantly more moDCs than syngeneic transplants. moDCs in allografts exhibited a more mature phenotype, had greater production of IL-12p40, and persisted for longer periods of time than those in syngeneic grafts. Transplantation of BALB/c RAG-/- or C57BL/6 RAG-/- organs into C57BL/6 RAG-/-ɣc /-CX3CR1gfp/+ hosts (which lack B cells, T cells and NK cells) showed similar results, suggesting that this innate allo-recognition was not dependent on recipient lymphoid cells. Transplantation of NK depleted BALB/c RAG-/- hearts into C57BL/6 RAG-/-ɣc-/-CX3CR1gfp/+ also yielded similar results, suggesting that donor lymphoid cells were also not required. The caveat here is that NK cells are notoriously difficult to fully deplete. Results from experiments with a bone marrow plug transplantation model comparing the response of C57BL/6 RAG-/-ɣc-/-CX3CR1gfp/+ hosts to transplantation with syngeneic C57BL/6 or allogeneic NOD grafts from wild type or RAG-/- ɣc-/- backgrounds, similarly found greater numbers of moDCs in allografts than syngeneic grafts. There were, however, significantly greater numbers of moDCs in wild type allografts than in RAG-/-ɣc-/-, suggesting that donor lymphoid cells do contribute to the host monocyte response, but that they are not required for eliciting it.

Unfortunately, while elegant and compelling, a potential contribution of surgery-induced danger signals could not be fully excluded in this study. Therefore, in order to avoid the potentially confounding impacts of surgery associated tissue damage, we recently employed a system involving the enumeration of splenic moDCs after i.v. transfer of freshly isolated allogeneic cells [56]. Using this experimental model, in which external danger signals were minimised, we found that host moDCs accumulated rapidly (within 1 day) following exposure to allo-antigen. This accumulation was mainly associated with recruitment of cells from outside the spleen rather than from differentiation or proliferation of in-situ monocytes. Using $RAG^{-/-}\gamma c^{-/-}$, scid $\gamma c^{-/-}$ and NK cell deficient $Mc11^{fl/fl}$Ncr1-Cre mice, we found that lymphoid cells and NK cells were required to elicit this phenomenon.

Monocytes as precursors of conventional DCs

Several reports have attempted to characterise the potential monocyte contribution to steady state cDCs in peripheral tissues. In one such study, in which macrophage/DC precursors (MDPs) and monocytes from CX_3CR1^{gfp}/CD45.1 mice were adoptively transferred into wildtype CD45.2 mice, $Gr1^{high}$ inflammatory monocytes in the blood, which originally derive from MDPs, were found to develop into steady state cDCs in the intestinal lamina propria and the lung, but not to splenic cDCs [57]. The latter were found to develop directly from MDPs without a monocytic intermediate. A second similar study revealed that adoptively transferred $Gr1^{high}$ as well as $Gr1^{low}$ monocytes were able to give rise to lung DCs under non-inflammatory and inflammatory conditions [58]. When combined with other studies showing distinct non-monocyte precursors that specifically generate particular lymphoid resident cDC populations, these observations suggest that monocytes give rise to steady state cDCs only in peripheral tissues and not lymphoid organs.

Under inflammatory conditions, monocytes are capable of differentiating into several populations of peripheral tissue cDCs as well as lymphoid resident cDCs. Examination of dermal and interstitial DC subsets during infection suggest significant monocyte contribution to these populations. Additionally, following ultraviolet depletion, epidermal Langerhans cells have been shown to be reconstituted by Csf1r dependent $Gr1^+$ monocytes specifically recruited to the inflamed skin [59]. Vaginal epithelial Langerhans cells, which in contrast to their epidermal counterparts are mainly repopulated by non-monocyte bone marrow derived precursors at the steady state, were also reconstituted by $Gr1^+$ monocytes during HSV-2 infection [60]. Although less convincing, there is some evidence that monocytes can contribute toward lymphoid resident cDCs during inflammation. After the adoptive transfer of CD45.2 monocytes into irradiated CD45.1 hosts, approximately 20% of the transferred donor monocytes were found to have differentiated into splenic $CD8^+$ and $CD8^-$ cDCs [23]. A subsequent study similarly using monocyte adoptive transfer during *Leishmania major* infection, also revealed evidence of monocyte differentiation into splenic $CD8^+$ and $CD8^-$ cDCs [35].

Overall, the available evidence supports the concept that, in addition to being able to differentiate into specialised inflammatory moDCs, monocytes are also capable of acting as precursors for several populations of peripheral tissue cDCs at the steady state, as well as peripheral tissue and lymphoid resident cDCs during inflammation.

Conclusion

What was once a simple paradigm used to explain the role and function of monocytes within the broader immune response (i.e. monocytes traffic in blood to peripheral tissues where they differentiate into macrophages), has now been replaced by an increasingly compartmentalized framework which attempts to recognize the complexity of the

developmental and functional relationship between monocytes, macrophages and moDCs. It has become apparent that the developmental pathways of each of these cell types are highly variable and can be influenced by intrinsic aspects of host biology and extrinsic aspects of the type of inflammatory stimuli. Furthermore, multiple studies have implicated one or more of these populations in promoting many different aspects of innate and adaptive immunity. The newly reported functional differences between moDCs and cDCs foreshadow more exploration. Whilst there remain some disputes on some of the detail, it is clear that monocytes, macrophages and moDCs are critical for the immune system's ability to effectively respond to microbial threats, allorejection and autoimmunity. An important caveat is that most of the findings discussed here have been derived from animal studies which may not always directly translate to the human immune system. Nevertheless, they provide guidance for future studies that might more directly examine the role of moDCs in the human. Such future research focused on further investigating the origins and functions of these cells in humans may allow for greater clarity in this framework and potentially avail novel therapeutic targets.

Figure Legends

Figure 1: Developmental relationship between murine monocyte subsets. Ly6C$^+$ inflammatory monocytes egress from the bone marrow into the blood via a CCR2$^+$ dependent process. They can then differentiate into Ly6C$^-$ patrolling monocytes. Under inflammatory conditions they can upregulate MHC II and CD11c and differentiate into moDCs.

References

1. Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *The Journal of experimental medicine* 1994; **179**(4)**:** 1109-18.

2. Randolph GJ, Inaba K, Robbiani DF, Steinman RM, Muller WA. Differentiation of phagocytic monocytes into lymph node dendritic cells in vivo. *Immunity* 1999; **11**(6)**:** 753-61.

3. Gordon S, Taylor PR. Monocyte and macrophage heterogeneity. *Nature reviews. Immunology* 2005; **5**(12)**:** 953-64.

4. Auffray C, Fogg D, Garfa M, Elain G, Join-Lambert O, Kayal S *et al.* Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. *Science (New York, N.Y.)* 2007; **317**(5838)**:** 666-70.

5. Auffray C, Sieweke MH, Geissmann F. Blood monocytes: development, heterogeneity, and relationship with dendritic cells. *Annu Rev Immunol* 2009; **27:** 669-92.

6. Geissmann F, Jung S, Littman DR. Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity* 2003; **19**(1)**:** 71-82.

7. Serbina NV, Pamer EG. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nature immunology* 2006; **7**(3)**:** 311-7.

8. Serbina NV, Salazar-Mather TP, Biron CA, Kuziel WA, Pamer EG. TNF/iNOS-producing dendritic cells mediate innate immune defense against bacterial infection. *Immunity.* 2003; **19**(1)**:** 59-70.

9. Nahrendorf M, Swirski FK, Aikawa E, Stangenberg L, Wurdinger T, Figueiredo JL *et al.* The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. *The Journal of experimental medicine* 2007; **204**(12)**:** 3037-47.

10. Arnold L, Henry A, Poron F, Baba-Amer Y, van Rooijen N, Plonquet A *et al.* Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. *The Journal of experimental medicine* 2007; **204**(5)**:** 1057-69.

11. Hanna RN, Cekic C, Sag D, Tacke R, Thomas GD, Nowyhed H *et al.* Patrolling monocytes control tumor metastasis to the lung. *Science (New York, N.Y.)* 2015; **350**(6263)**:** 985-90.

12. Wong KL, Yeap WH, Tai JJ, Ong SM, Dang TM, Wong SC. The three human monocyte subsets: implications for health and disease. *Immunologic research* 2012; **53**(1-3)**:** 41-57.

13. Sunderkotter C, Nikolic T, Dillon MJ, Van Rooijen N, Stehling M, Drevets DA *et al.* Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response. *Journal of immunology (Baltimore, Md. : 1950)* 2004; **172**(7)**:** 4410-7.

14. Yona S, Kim KW, Wolf Y, Mildner A, Varol D, Breker M *et al.* Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* 2013; **38**(1)**:** 79-91.

15. Czuprynski CJ, Brown JF, Maroushek N, Wagner RD, Steinberg H. Administration of anti-granulocyte mAb RB6-8C5 impairs the resistance of mice to Listeria monocytogenes infection. *Journal of immunology (Baltimore, Md. : 1950)* 1994; **152**(4)**:** 1836-46.

16. Rosen H, Gordon S, North RJ. Exacerbation of murine listeriosis by a monoclonal antibody specific for the type 3 complement receptor of myelomonocytic cells. Absence of monocytes at infective foci allows Listeria to multiply in nonphagocytic cells. *The Journal of experimental medicine* 1989; **170**(1)**:** 27-37.

17. Peters W, Cyster JG, Mack M, Schlondorff D, Wolf AJ, Ernst JD *et al.* CCR2-dependent trafficking of F4/80dim macrophages and CD11cdim/intermediate dendritic cells is crucial for T cell recruitment to lungs infected with Mycobacterium tuberculosis. *Journal of immunology (Baltimore, Md. : 1950)* 2004; **172**(12)**:** 7647-53.

18. Robben PM, LaRegina M, Kuziel WA, Sibley LD. Recruitment of Gr-1+ monocytes is essential for control of acute toxoplasmosis. *The Journal of experimental medicine* 2005; **201**(11)**:** 1761-9.

19. Ginhoux F, Jung S. Monocytes and macrophages: developmental pathways and tissue homeostasis. *Nature reviews. Immunology* 2014; **14**(6)**:** 392-404.

20. Hashimoto D, Chow A, Noizat C, Teo P, Beasley MB, Leboeuf M *et al.* Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. *Immunity* 2013; **38**(4)**:** 792-804.

21. Tamoutounour S, Guilliams M, Montanana Sanchis F, Liu H, Terhorst D, Malosse C *et al.* Origins and Functional Specialization of Macrophages and of Conventional and Monocyte-Derived Dendritic Cells in Mouse Skin. *Immunity* 2013.

22. Epelman S, Lavine KJ, Beaudin AE, Sojka DK, Carrero JA, Calderon B *et al.* Embryonic and adult-derived resident cardiac macrophages are maintained through distinct mechanisms at steady state and during inflammation. *Immunity* 2014; **40**(1)**:** 91-104.

23. Leon B, Martinez del Hoyo G, Parrillas V, Vargas HH, Sanchez-Mateos P, Longo N *et al.* Dendritic cell differentiation potential of mouse monocytes: monocytes represent immediate precursors of CD8- and CD8+ splenic dendritic cells. *Blood* 2004; **103**(7)**:** 2668-76.

24. Leon B, Lopez-Bravo M, Ardavin C. Monocyte-derived dendritic cells. *Seminars in immunology* 2005; **17**(4)**:** 313-8.

25. Palucka KA, Taquet N, Sanchez-Chapuis F, Gluckman JC. Lipopolysaccharide can block the potential of monocytes to differentiate into dendritic cells. *Journal of leukocyte biology* 1999; **65**(2)**:** 232-40.

26. Mohamadzadeh M, Berard F, Essert G, Chalouni C, Pulendran B, Davoust J *et al.* Interleukin 15 skews monocyte differentiation into dendritic cells with features of Langerhans cells. *The Journal of experimental medicine* 2001; **194**(7)**:** 1013-20.

27. Santini SM, Lapenta C, Logozzi M, Parlato S, Spada M, Di Pucchio T *et al.* Type I interferon as a powerful adjuvant for monocyte-derived dendritic cell development and activity in vitro and in Hu-PBL-SCID mice. *The Journal of experimental medicine* 2000; **191**(10)**:** 1777-88.

28. Raker VK, Domogalla MP, Steinbrink K. Tolerogenic Dendritic Cells for Regulatory T Cell Induction in Man. *Frontiers in immunology* 2015; **6:** 569.

29. Ezzelarab MB, Zahorchak AF, Lu L, Morelli AE, Chalasani G, Demetris AJ *et al.* Regulatory Dendritic Cell Infusion Prolongs Kidney Allograft Survival in Nonhuman Primates. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* 2013.

30. Rotta G, Edwards EW, Sangaletti S, Bennett C, Ronzoni S, Colombo MP *et al.* Lipopolysaccharide or whole bacteria block the conversion of inflammatory monocytes into dendritic cells in vivo. *The Journal of experimental medicine* 2003; **198**(8)**:** 1253-63.

31. Qu C, Edwards EW, Tacke F, Angeli V, Llodra J, Sanchez-Schmitz G *et al.* Role of CCR8 and other chemokine pathways in the migration of monocyte-derived dendritic cells to lymph nodes. *The Journal of experimental medicine* 2004; **200**(10)**:** 1231-41.

32. Helft J, Bottcher J, Chakravarty P, Zelenay S, Huotari J, Schraml BU *et al.* GM-CSF Mouse Bone Marrow Cultures Comprise a Heterogeneous Population of CD11c(+)MHCII(+) Macrophages and Dendritic Cells. *Immunity* 2015; **42**(6)**:** 1197-211.

33. Greter M, Helft J, Chow A, Hashimoto D, Mortha A, Agudo-Cantero J *et al.* GM-CSF controls nonlymphoid tissue dendritic cell homeostasis but is dispensable for the differentiation of inflammatory dendritic cells. *Immunity* 2012; **36**(6)**:** 1031-46.

34. Chow KV, Lew AM, Sutherland RM, Zhan Y. Monocyte-Derived Dendritic Cells Promote Th Polarization, whereas Conventional Dendritic Cells Promote Th Proliferation. *Journal of immunology (Baltimore, Md. : 1950)* 2016; **196**(2)**:** 624-36.

35. Leon B, Lopez-Bravo M, Ardavin C. Monocyte-derived dendritic cells formed at the infection site control the induction of protective T helper 1 responses against Leishmania. *Immunity* 2007; **26**(4)**:** 519-31.

36. Cheong C, Matos I, Choi JH, Dandamudi DB, Shrestha E, Longhi MP *et al*. Microbial stimulation fully differentiates monocytes to DC-SIGN/CD209(+) dendritic cells for immune T cell areas. *Cell* 2010; **143**(3)**:** 416-29.

37. Meredith MM, Liu K, Darrasse-Jeze G, Kamphorst AO, Schreiber HA, Guermonprez P *et al.* Expression of the zinc finger transcription factor zDC (Zbtb46, Btbd4) defines the classical dendritic cell lineage. *The Journal of experimental medicine* 2012; **209**(6)**:** 1153-65.

38. Iijima N, Mattei LM, Iwasaki A. Recruited inflammatory monocytes stimulate antiviral Th1 immunity in infected tissue. *Proceedings of the National Academy of Sciences of the United States of America* 2011; **108**(1)**:** 284-9.

39. Rydstrom A, Wick MJ. Monocyte recruitment, activation, and function in the gut-associated lymphoid tissue during oral Salmonella infection. *Journal of immunology (Baltimore, Md. : 1950)* 2007; **178**(9)**:** 5789-801.

40. Mayer-Barber KD, Andrade BB, Barber DL, Hieny S, Feng CG, Caspar P *et al.* Innate and adaptive interferons suppress IL-1alpha and IL-1beta production by distinct pulmonary myeloid subsets during Mycobacterium tuberculosis infection. *Immunity* 2011; **35**(6)**:** 1023-34.

41. Osterholzer JJ, Curtis JL, Polak T, Ames T, Chen GH, McDonald R *et al.* CCR2 mediates conventional dendritic cell recruitment and the formation of bronchovascular mononuclear cell infiltrates in the lungs of mice infected with Cryptococcus neoformans. *Journal of immunology (Baltimore, Md. : 1950)* 2008; **181**(1)**:** 610-20.

42. Hohl TM, Rivera A, Lipuma L, Gallegos A, Shi C, Mack M *et al.* Inflammatory monocytes facilitate adaptive CD4 T cell responses during respiratory fungal infection. *Cell Host Microbe.* 2009; **6**(5)**:** 470-81. doi: 10.1016/j.chom.2009.10.007.

43. Guilliams M, Movahedi K, Bosschaerts T, VandenDriessche T, Chuah MK, Herin M *et al.* IL-10 dampens TNF/inducible nitric oxide synthase-producing dendritic cell-mediated pathogenicity during parasitic infection. *J Immunol.* 2009; **182**(2)**:** 1107-18.

44. Ko HJ, Brady JL, Ryg-Cornejo V, Hansen DS, Vremec D, Shortman K *et al.* GM-CSF-responsive monocyte-derived dendritic cells are pivotal in Th17 pathogenesis. *J Immunol.* 2014; **192**(5)**:** 2202-9. doi: 10.4049/jimmunol.1302040. Epub 2014 Jan 31.

45. Campbell IK, van Nieuwenhuijze A, Segura E, O'Donnell K, Coghill E, Hommel M *et al.* Differentiation of inflammatory dendritic cells is mediated by NF-kappaB1-dependent GM-CSF production in CD4 T cells. *Journal of immunology (Baltimore, Md. : 1950)* 2011; **186**(9)**:** 5468-77.

46. Rivollier A, He J, Kole A, Valatas V, Kelsall BL. Inflammation switches the differentiation program of Ly6Chi monocytes from antiinflammatory macrophages to inflammatory dendritic cells in the colon. *The Journal of experimental medicine* 2012; **209**(1)**:** 139-55.

47. Plantinga M, Guilliams M, Vanheerswynghels M, Deswarte K, Branco-Madeira F, Toussaint W *et al.* Conventional and monocyte-derived CD11b(+) dendritic cells initiate and maintain T helper 2 cell-mediated immunity to house dust mite allergen. *Immunity* 2013; **38**(2)**:** 322-35.

48. Copin R, De Baetselier P, Carlier Y, Letesson JJ, Muraille E. MyD88-dependent activation of B220-CD11b+LY-6C+ dendritic cells during Brucella melitensis infection. *J Immunol.* 2007; **178**(8)**:** 5182-91.

49. Langlet C, Tamoutounour S, Henri S, Luche H, Ardouin L, Gregoire C *et al.* CD64 expression distinguishes monocyte-derived and conventional dendritic cells and reveals their distinct role during intramuscular immunization. *Journal of immunology (Baltimore, Md. : 1950)* 2012; **188**(4)**:** 1751-60.

50. Kool M, Soullie T, van Nimwegen M, Willart MA, Muskens F, Jung S *et al.* Alum adjuvant boosts adaptive immunity by inducing uric acid and activating inflammatory dendritic cells. *The Journal of experimental medicine* 2008; **205**(4)**:** 869-82.

51. Le Borgne M, Etchart N, Goubier A, Lira SA, Sirard JC, van Rooijen N *et al.* Dendritic cells rapidly recruited into epithelial tissues via CCR6/CCL20 are responsible for CD8+ T cell crosspriming in vivo. *Immunity* 2006; **24**(2)**:** 191-201.

52. Wakim LM, Waithman J, van Rooijen N, Heath WR, Carbone FR. Dendritic cell-induced memory T cell activation in nonlymphoid tissues. *Science (New York, N.Y.)* 2008; **319**(5860)**:** 198-202.

53. Tezuka H, Abe Y, Iwata M, Takeuchi H, Ishikawa H, Matsushita M *et al.* Regulation of IgA production by naturally occurring TNF/iNOS-producing dendritic cells. *Nature.* 2007; **448**(7156)**:** 929-33.

54. Zecher D, van Rooijen N, Rothstein DM, Shlomchik WD, Lakkis FG. An innate response to allogeneic nonself mediated by monocytes. *Journal of immunology (Baltimore, Md. : 1950)* 2009; **183**(12)**:** 7810-6.

55. Oberbarnscheidt MH, Zeng Q, Li Q, Dai H, Williams AL, Shlomchik WD *et al.* Non-self recognition by monocytes initiates allograft rejection. *The Journal of clinical investigation* 2014; **124**(8)**:** 3579-89.

56. Chow KV, Delconte RB, Huntington ND, Tarlinton DM, Sutherland RM, Zhan Y *et al.* Innate Allorecognition Results in Rapid Accumulation of Monocyte-Derived Dendritic Cells. *Journal of immunology (Baltimore, Md. : 1950)* 2016; **197**(5)**:** 2000-8.

57. Varol C Fau - Landsman L, Landsman L Fau - Fogg DK, Fogg Dk Fau - Greenshtein L, Greenshtein L Fau - Gildor B, Gildor B Fau - Margalit R, Margalit R Fau - Kalchenko V *et al.* Monocytes give rise to mucosal, but not splenic, conventional dendritic cells. 2007; (0022-1007 (Print)).

58. Landsman L, Varol C, Jung S. Distinct differentiation potential of blood monocyte subsets in the lung. *Journal of immunology (Baltimore, Md. : 1950)* 2007; **178**(4)**:** 2000-7.

59. Ginhoux F, Tacke F, Angeli V, Bogunovic M, Loubeau M, Dai XM *et al.* Langerhans cells arise from monocytes in vivo. *Nature immunology* 2006; **7**(3)**:** 265-73.

60. Iijima N, Linehan MM, Saeland S, Iwasaki A. Vaginal epithelial dendritic cells renew from bone marrow precursors. *Proceedings of the National Academy of Sciences of the United States of America* 2007; **104**(48)**:** 19061-6.

Table 1: The role of moDCs in innate and adaptive immune responses

	Response	*Experimental model*	*Anatomical Compartment*	*Reference*
Innate Responses	iNOS dependent bacterial killing	*Listeria monocytogenes* infection	Spleen	Serbina *et al.* [8]
	Phagocytosis of bacteria	Gastrointestinal *Salmonella* infection	Gastrointestinal tract	Rydstrom *et al.* [39]
	iNOS mediated tissue toxicity	*Trypanosoma brucei* infection	Spleen, liver, lymph nodes	Guilliams *et al.* [43]
Adaptive responses	Induction of CD4+ T cell proliferation ex vivo	Intravenous LPS administration	Skin draining lymph nodes	Cheong *et al.* [36]
		Intramuscular alum-OVA administration	Draining lymph nodes	Langlet *et al.* [49]
		Intranasal house dust mite exposure	Mediastinal lymph nodes	Plantinga *et al.* [47]
	Induction of CD4+ T cell proliferation in vivo	Pulmonary *Aspergillus fumigatus* infection	Lungs and mediastinal lymph nodes	Hohl *et al.* [42]
	Cross presentation to CD8+ T cells	Measles virus infection	Buccal mucosa	Le Borgne *et al.* [51]
		HSV-1 infection	Peripheral non lymphoid tissues (e.g. kidney)	Wakim *et al.* [52]
	Suppression of CD4+ T cell proliferation	OT-II CD4+ T cells in CCR2.DTR mice	Spleen	Chow *et al.* [34]
	Induction of Th1 polarisation	*Leishmania major* infection	Draining lymph nodes	Leon *et al.* [35]
	Induction of Th2 polarisation	Intranasal house dust mite exposure	Mediastinal lymph nodes	Plantinga *et al.* [47]
	Induction of Th 17 polarisation	Experimental autoimmune encephalomyelitis	Spleen	Ko *et al.* [44]
		OT-II CD4+ T cells in CCR2.DTR mice	Spleen	Chow et al. [34]
	iNOS and TGF-β dependent IgA production and class switch recombination	Commensal bacteria	Gastrointestinal tract lymphoid tissues	Tezuka *et al.* [53]
	Mediation of innate allorecognition and transplant rejection	Allogeneic heart transplantation	Cardiac allografts	Oberbarnscheidt *et al.* [55]