Monocyte-derived Inflammatory Dendritic Cells in the Granuloma During Mycobacterial Infection

Heidi A. Schreiber and Matyas Sandor

Abstract The monocyte-derived, inflammatory dendritic cell subset plays an important role during immune responses against infections. This review will focus on the complex, changing role of this subset during mycobacterial infection. Studies demonstrate that in addition to sustaining a systemic anti-mycobacterial response, the inflammatory dendritic cell subset present in *Mycobacterium*-induced granulomas also influences local immune regulation within the granuloma over the course of infection. This review will also survey the literature on how similar and different inflammatory dendritic cell subsets during other infections.

Keywords Mycobacteria • Dendritic cells • Granuloma • Acute and chronic infection • $CD4^+$ T cells • BCG

1 Introduction

The discovery of dendritic cells (DCs) nearly four decades ago has provided immunologist with the missing link to efficiently bridge the innate and adaptive immune response (Steinman et al. 1973, 1974a, b). DCs are comprised of heterogeneous populations, with each subset uniquely qualified to support either immunity during infection and tolerance in the face of autoimmunity. Under steady-state conditions, conventional DCs make up a very small percentage of tissue and lymphoid-resident cells. In the face of infection the immune system must respond quickly. Circulating, peripheral blood monocytes provide a powerful reservoir of immune artillery, as they can efficiently give rise to DCs under infectious conditions. This monocyte-derived DC (moDCs) lineage that arises during infection shares many functional and phenotypic similarities to classical DCs, and are therefore termed 'inflammatory DCs' (*inflam*DCs). There has been a recent surge in data that irrefutably dem-

M. Sandor (✉)
Department of Pathology and Laboratory Sciences, University of Wisconsin, 53705, Madison, WI, USA
e-mail: msandor@wisc.edu

J. D. Lambris, G. Hajishengallis (eds.), *Current Topics in Innate Immunity II,*
Advances in Experimental Medicine and Biology 946,
DOI 10.1007/978-1-4614-0106-3_16, © Springer Science+Business Media, LLC 2012

onstrates the importance of this subset during infection by a wide array of pathogens (viruses, bacteria, fungi, protozoan, etc.). This review will discuss the origin and phenotype of moDCs, and their involvement during infection by various pathogens. The newly immerging role *inflam*DCs play during *Mycobacterium* infection will be specifically highlighted in this review.

2 Development and Phenotype

DC precursors, both classical and monocyte-derived inflammatory, originate in the bone marrow from hematopoietic stem cells. Both subsets share the same myeloid precursor that gives rise to monocyte/macrophage and DC lineages, known as the macrophage-DC precursor (MDP) (Fogg et al. 2006). However, the classical DC and monocyte developmental pathway diverges henceforth (Liu et al. 2009). Although *in vitro* culture systems can generate a large number of DCs by culturing monocytes with cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF) (Inaba et al. 1992; Sallusto and Lanzavecchia 1994), and early *in vivo* data suggested the generation of a DC-like population from both murine and human monocytes (Randolph et al. 1998, 1999), recent *in vivo* data strongly supports the idea that monocytes do not give rise to classical lymphoid organ DCs during steady-state conditions. *In vivo* experiments that utilize either adoptive cell transfer models or GFP reporter mice driven by the monocyte-specific lysozyme M promoter, all demonstrate that monocytes do not have classical DC progenitor activity or are able to reconstitute classical lymphoid organ DCs during steady-state conditions (Fogg et al. 2006; Naik et al. 2006; Varol et al. 2007; Jakubzick et al. 2008; Liu et al. 2009). However, monocytes have been shown to replenish some DC populations in peripheral organs, such as the intestines, lung, and skin (Holt et al. 1994; Ginhoux et al. 2006; Varol et al. 2007). MDP exposure to fms-like tyrosine kinase 3 ligand (Flt3L) (McKenna et al. 2000; Waskow et al. 2008) or GM-CSF (Dai et al. 2002) in the bone marrow results in the divergence of conventional DC and monocyte developmental pathways, respectively. Conventional DC (cDC) development is reviewed in depth elsewhere (Liu and Nussenzweig 2010). In addition to early exposure of the growth factor GM-CSF, the monocyte lineage is further selected for at the transcriptional level. Increased expression of transcription factor PU.1 has been shown to favor monocyte lineage development (Dahl et al. 2003). Interestingly, continued expression of PU.1 further promotes monocyte differentiation into DCs by suppressing the macrophage-inducing transcription factor MafB, and vice versa (Bakri et al. 2005). Conversely, the transcription factor STAT3 sustains cDC Flt3-dependent differentiation (Laouar et al. 2003). Table 1 summarizes this and other defining characteristics between cDCs and monocyte-derived *inflam*DCs.

From the bone marrow, monocytes can be classified into two subpopulations based on high- or low-surface expression of Ly6C. Ly6C^{high} and Ly6C^{low} populations were originally and often times currently used interchangeably with Gr-1^{high} and Gr-1^{low}, respectively. However, in addition to recognizing Ly6C, the monoclo-

Table 1 Defining characteristics between cDCs and monocyte-derived *inflam*DCs. (Source: Fogg et al. 2006; Liu et al. 2009; D'Amico et al. 2003; Geissmann et al. 2010; Laouar et al. 2003; Dahl et al. 2003; Liu el al. 2007; Auffray et al. 2009; Fleming et al. 1993; Geissmann et al. 2003; Cheong et al. 2010; Kamphorst et al. 2010)

	Conventional DCs	Monocyte-derived *Inflam*DCs
BM precursor	CDP	MDP
Growth factor for development	Flt3L	M-CSF
Transcription factors	STAT3	PU.1
Precursor frequency in blood	Low (0.2%)	High (10%)
Distinguishing markers	Flt3 (CD135)	CD115, Gr-l/Ly6C, CD209a, CD14
T cell priming ability		
$CD8^+$ T cell (cross presentation)	+++	+++
$CD4^+$ T cell (MHCII)	+++	+

nal antibody Gr-1 (RB6-8C5) also recognizes Ly6G, a marker not expressed on monocytes (Fleming et al. 1993). Therefore, Ly6C expression is the more accurate description of murine monocyte subsets. The $Ly6C^{high}$ and $Ly6C^{low}$ monocyte populations in mice are believed to correspond to the human $CD14^{high}CD16-$ and $CD14^{int}CD16^+$ populations, respectively (Randolph et al. 2008). The two murine $Ly6C^{high}$ and $Ly6C^{low}$ monocyte populations can be further phenotyped by expression of different chemokine and adhesion molecules.

The conventional $Ly6C^{high}$ monocytes are also $CCR2^{pos}$, $CD62L^{pos}$, $CCR5^{neg}$, $CD11c^{neg}$ and CX_3CR1^{low}, and the $Ly6C^{low}$ population is CX_3CR1^{high}, $CCR2^{neg}$, $CD62L^{neg}$, CX_3CR1^{pos}, and $CD11c^{int}$ (Geissmann et al. 2003; Sunderkotter et al. 2004; Tacke et al. 2007). These two populations are not independently derived as the $Ly6C^{high}$ population has been shown to give rise to the $Ly6C^{low}$ population, both in bone marrow and in the periphery (Sunderkotter et al. 2004; Tacke and Randolph 2006; Arnold et al. 2007; Nahrendorf et al. 2007; Varol et al. 2007). Monocyte emigration from the bone marrow is controlled by chemokine receptors CCR2 on the $Ly6C^{high}$ population, and CX_3CR1 and CCR5 on the $Ly6C^{low}$ counterpart, but the mechanisms behind this control are not known (Combadiere et al. 2008). Once within the blood, the $Ly6C^{low}CX_3CR1^{high}$ population has been elegantly imaged crawling along vessels under steady-state conditions regardless of the direction of flow, but rapidly extravasated into tissue in the presence of infection (Auffray et al. 2007). A recent study from the Steinman group definitively demonstrated the differentiation of monocytes into DCs *in vivo* in response to microbial stimuli (Cheong et al. 2010). This study demonstrated the inflammation-induced recruitment of moDCs into the T cell areas of the affected lymph node and subsequent expression of DC-SIGN (CD209a). The moDC mobilization they observed was dependent on CCR7, TLR4, CD14, and Trif expression on the DC. In spite of the many characteristics moDCs share with cDCs, antigen presentation is not one of them. A recent, definitive report by Kamphorst et al. exhaustively compared the antigen presentation capacity of cDCs and moDCs following several mechanisms of antigen capture (Kamphorst et al. 2010). This study demonstrated that while moDCs are efficient at priming $CD8^+$ T cells through cross presentation, the cDC compartment was much

more effective at presenting antigen to MHCII-restricted CD4$^+$ T cells. While the inflamDCs may appear very similar to cDCs in terms of costimulatory molecules, MHCII expression and morphology, they may differ internally upon acquisition of antigen. A recent study by McCurley and Mellman demonstrated that the moDC subset in humans had a higher protease content, and as a result, had higher lysosomal proteolysis compared to cDCs (McCurley and Mellman 2010). This high lysosomal proteolytic activity results in quick degradation of intracellular antigen and may explain the poor ability of *inflam*DCs to present MHCII peptide.

In the face of infection the monocyte lineage provides a quick, profuse source of cells able to differentiate into the necessary populations of antigen presenting cells (APCs) necessary for quick combat. In this next section we will discuss their potential to differentiate into *inflam*DCs and the role they play during infection by bacteria, viruses, fungi and protozoa.

3 Function of Monocyte-derived Inflammatory DCs During Infection

3.1 Mycobacteria

All things considered (i.e., prevalence, new cases and deaths per year, economic burden, drug resistance, etc.) infection with *Mycobacterium* remains one of the most devastating diseases worldwide. The granuloma is the hallmark of mycobacterial infections, and is the interface for the host immune response and bacterial persistence. Functional granulomas protect an estimated two billion individuals from active disease by attenuating bacterial growth and dissemination (Ulrichs and Kaufmann 2006). One of the defining characteristics of *Mycobacterium* infection is that even in the face of an early, strong immune response, the host often fails to completely eradicate the bacteria, resulting in a long-term chronic infection. Therefore, when bearing in mind *Mycobacterium* infection it is best to view both the immunological and bacteriological events in two separate stages, acute and chronic. For example, granulomas formed during the acute stage of infection largely protect the host by limiting bacterial dissemination and inadvertent tissue damage. However, during chronic infection these well-formed granulomas provide the mycobacteria with a long-term survival niche (Volkman et al. 2004; Russell 2007). Understanding the role of DCs during *Mycobacterium* infection is still in its infancy, but the growing body of data supports the idea that they have an essential, influential role during both the acute and chronic stages. The predominating DC subset involved during both stages is monocyte-derived *inflam*DCs, largely characterized by Ly6C^{int}CD11b^{high}CD11c^{low} expression (Reljic et al. 2005; Humphreys et al. 2006; Wolf et al. 2007; Schreiber et al. 2010). However, the mechanisms governing monocyte recruitment, differentiation, infection, lymphatic antigen transport, and T cell priming are not equally understood for both the acute and chronic stages. There is more information regarding the role of *inflam*DCs during the acute phase

of infection. However, recent studies are now focusing on the chronic phase. Data from these studies suggests a changing role of monocyte-derived *inflam*DCs during chronic *Mycobacterium* infection.

Recruitment of this subset to the site of acute *Mycobacterium* infection seems to occur in both a CCR2- and CCR5-dependent manner and not CX_3CR1-dependent, as demonstrated by infection of mice with mutated expression of these chemokine receptors (Hall et al. 2009). Following *Mycobacterium tuberculosis* (MTb) infection, CCR2 deficient mice were highly susceptible to high doses and had a delayed immune response when challenged with low infectious doses (Peters et al. 2001; Scott and Flynn 2002). Conversely, mice over expressing CCR2 better controlled *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) infection by faster granuloma formation, lower bacterial burden and increased transport of bacterial antigen to the draining lymph nodes by DCs (Schreiber et al. 2008). Interestingly, humans with a single nucleotide polymorphism in the MCP-1, which resulted in increased peripheral levels of the chemokine, had increased susceptibility of MTb infection, suggesting a possible desensitization to the CCR2 ligand over time (Flores-Villanueva et al. 2005; Serbina et al. 2008). In the absence of a CCR2-expressing monocyte subset, CCR5 expression may compensate and vice versa as CCR5-deficient mice also showed no significant difference in Mtb control (Badewa et al. 2005). Studies examining the immune response in the absence of both chemokine receptors have not been done. The global requirement of $CD11c^{pos}$ DCs for the initiation of T cell immunity and bacterial control following MTb infection was definitively demonstrated by transiently depleting DCs using pCD11c-diphtheria toxin receptor transgenic mice (Tian et al. 2005). Depleting the CD11c population prior to infection resulted in a delayed $CD4^+$ T cell response to MTb and impaired control of MTb replication. In back to back reports, Wolf and colleagues demonstrated that the $CD11b^{pos}CD11c^{pos}$ DC population recruited to the lung after MTb infection is readily infected and facilitates the transport of MTb antigen to the draining lymph node (Wolf et al. 2007). Eliminating a primary chemokine network utilized by this subset en route to lymph nodes using *plt* mice, which lack CCR7 ligands CCL19 and CCL21ser, they demonstrated the necessity of mycobacteria transport by $CD11b^{pos}CD11c^{pos}$ DCs (Wolf et al. 2007) and *Mycobacterium*-specific IFNγ-producing $CD4^+$ T cells(Khader et al. 2009). Early studies that demonstrated a potent T cell stimulatory activity by lymph node DCs after BCG infection did not entirely separate lymph node-resident conventional DCs from recruited monocyte-derived *inflam*DCs (Jiao et al. 2002). However, recent studies specifically addressing the activation capacity of $CD4^+$ T cells specific for *Mycobacterium* antigen 85B (P25) during early Mtb infection found *inflam*DCs to be poor T cell stimulators and elicit much less IFNγ production compared to other DC subsets (Wolf et al. 2007). As previously described for *Listeria monocytogenes* and Salmonella, evidence for the transfer of antigen from *inflam*DCs to lymph node-resident DCs also seems to be a phenomenon occurring during acute *Mycobacterium* infection as well. Eliminating the mechanism of *inflam*DC transport to the lymph nodes by using *plt* mice, resulted in 95% fewer bacilli reaching the lymph nodes, which effectively eliminated the source of antigen (Wolf et al. 2007). In addition to CCR7-expressing

migratory DCs aiding in antigen delivery to the lymph node, it was also found that CCR7-deficient mice infected via aerosol with Mtb had decreased bacterial load in the spleen during chronic infection, demonstrating the requirement for CCR7-expressing migratory DCs for bacterial dissemination during acute Mtb infection (Kahnert et al. 2007). There are numerous studies testing the ability of DCs *ex vivo*, and bone marrow and moDCs *in vitro,* to take up mycobacteria, mature, produce cytokines and prime T cells. This is reviewed in greater detail elsewhere (Schreiber et al. 2010). Collectively, these studies yield conflicting results by demonstrating the ability of DCs to both stimulate and suppress immunity *ex vivo* and *in vitro*. Which further reaffirms the idea that DCs, particularly *inflam*DCs, play a multifaceted role during mycobacterial infection.

Recent data from our group has supported this multifunctional role for *inflam*-DCs during *Mycobacterium* infection, particularly within the granuloma. Using *in vivo* imaging, a recent study by Egen and colleagues demonstrated the intense movement of T cells within the acute granuloma (Egen et al. 2008). Their data showed that newly arrived T cells efficiently scan granuloma-resident APCs. This scanning is necessary, as newly primed T cells require a second antigen encounter at the site of inflammation in order to rapidly secrete cytokines (Mohrs et al. 2005). By phenotyping monocyte-derived *inflam*DCs in both acute and chronic BCG-induced granulomas, we found that their phenotype varied greatly from the progression of acute to chronic infection. *Inflam*DCs found in acute granulomas had high expression of MHCII, CD40, CD80 and CD86, and were able to support IFNγ production from newly arrived primed *Mycobacterium*-specific P25 $CD4^+$ T cells (Schreiber et al. 2010). However, as infection progressed the *inflam*DCs within the chronic granuloma had decreased expression of MHCII and T cell costimulatory molecules, and had a significant increase in T cell inhibitory molecules PD-L1 and PD-L2. These DCs could no longer support IFNγ production from newly arrived *Mycobacterium*-specific P25 $CD4^+$ T cells. This inability was in part dependent on PD-L1/2:PD-1 signaling, as blockade of this pathway partially restored IFNγ production. Monocyte-derived *inflam*DCs, by affecting local IFNγ availability, may contribute to granuloma maturation and bacterial persistence (Fig. 1).

Enabling bacterial persistence during mycobacterial infection has partially led to the belief that chronic granulomas are 'walled-off' structures, inaccessible to immune surveillance. One defining feature of moDCs is their profound ability to migrate. Therefore, we asked the question of whether chronic granulomas were assessable to *inflam*DCs, and if so, were they able to then leave the granuloma with antigenic cargo en route to the draining lymph nodes? Using a kidney capsule transplant model, which consists of grafting granuloma-containing tissue underneath the kidney capsule of uninfected recipients, we were able to study $CD11c^{pos}$ cellular migration into and out of both acute and chronic *Mycobacterium*-induced granulomas using CD11c enhanced yellow fluorescent protein (CD11c-EYFP) mice with ubiquitously fluorescing DCs (Schreiber et al. 2010. *Manuscript Under Review*) (Lindquist et al. 2004). This study demonstrated that *inflam*DCs had considerable access to enter and exit chronic granulomas, even more so than acute lesions, with a turnover rate of ~60% and 30%, respectively, by one week. Determining the life

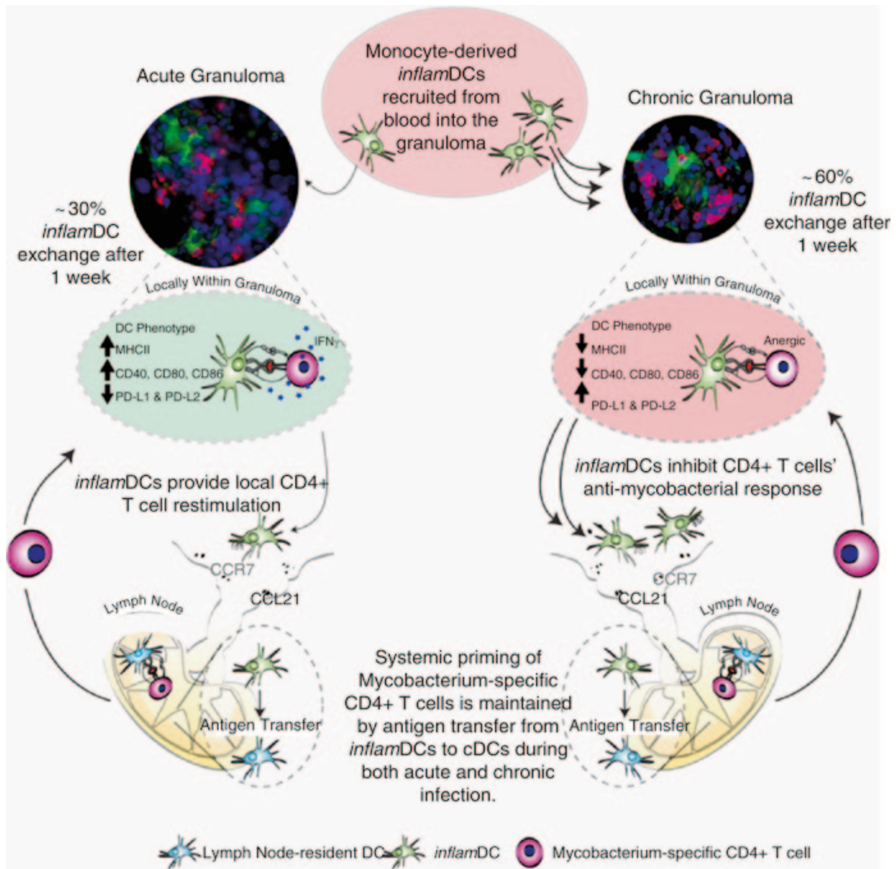

Fig. 1 Changing role of *inflam*DCs in *Mycobacterium*-induced granulomas over the course of infection

span of *inflam*DCs within the granuloma is complicated. Circulating monocytes have a relatively short half-life, as do migrating DCs; however, monocytes have also been shown to live for months after differentiation (Whitelaw 1966; Kamath et al. 2002; Liu et al. 2007; Gonzalez-Mejia and Doseff 2009). By staining acute and chronic granulomatous lesions with a marker for apoptosis and CD11c, the DCs present do not appear to be dying locally within the lesions during either acute or chronic infection (Fig. 2). It is difficult to discern whether the change in DC flux throughout the course of infection is due to a general shrinking of the lesions or potential differences in DC expansion locally due to M-CSF availability. *Inflam*-DCs leaving granulomas migrated not only to the draining lymph nodes, but also to other systemic lymphoid organs. This systemic migration supported priming of P25 $CD4^+$ T cells during both acute and chronic infection, albeit to a lesser extent in the latter. Grafting both acute and chronic granulomas into $MHCII^{-/-}$ recipients resulted in total abrogation of P25 $CD4^+$ T cell activation, demonstrating the necessity for

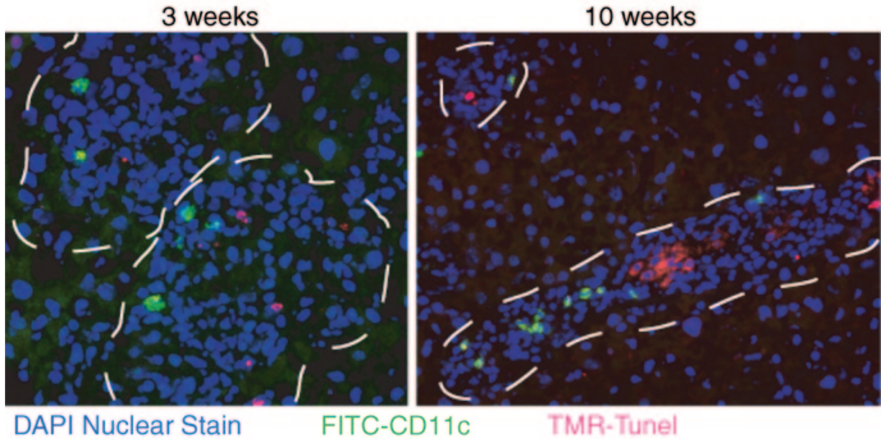

Fig. 2 Tunel staining on sections from 3- and 10-week BCG-infected mice. White dashed line indicates periphery of granuloma lesions. Staining with CD11c (green) and apoptosis indicator, Tunel (red), demonstrates that DCs are not undergoing apoptosis within the granuloma throughout chronic infection

recipient MHCII^{pos} cells. Eliminating the mechanism used by inflamDCs to migrate from the granuloma to the draining renal lymph node by transplanting granulomas from CCR7$^{-/-}$ mice, abrogated P25 CD4^{+} T cells activation during acute infection. Collectively demonstrating the role of *inflam*DCs during T cell priming as antigenic ferries (Randolph 2006).

It is evident that the changing phenotype and function of *inflam*DCs in mycobacterial granulomas plays an important role, for better or worse, in the disease course. Better understanding this cellular compartment during both acute and chronic *Mycobacterium* infection will inevitably lead to a better understanding of the disease and potential targets for therapeutic intervention.

3.2 Other Bacteria

Pioneering work on *inflam*DCs largely came from studies done by the groups of Pamer and Leenen on *Listeria monocytogenes* (Auffray et al. 2009). A Gram-positive intracellular bacterium, *L. monocytogenes* got its species name '*monocytogenes*' after the observation of increased monocytes in infected rabbits (Murray et al. 1926; Serbina et al. 2008). The early requirement for monocyte-derived Ly6C-expressing cells was further confirmed by depleting mice of this population using the RB6-8C5 monoclonal antibody within 24 h of *L. monocytogenes* infection, resulting in death within six days (Czuprynski et al. 1994). Upon infection with *L. monocytogenes*, Ly6C^{high} cells egress from the bone marrow in considerable numbers by CCR2-mediated signals largely in response to monocyte chemoattractant protein-1 (MCP-1)

(Kurihara et al. 1997; Sunderkotter et al. 2004; Serbina et al. 2006; Tsou et al. 2007). Induction of MCP-1 expression during *L. monocytogenes* infection is dependent on cytosolic recognition of the bacterium and independent of the MyD88 signaling pathway (Serbina et al. 2003). MCP-1 is also significantly induced after infection by *Toxoplasma gondii*, *Aspergillus fumigatus* and *Mycobacterium tuberculosis* (Lin et al. 1998; Blease et al. 2001; Robben et al. 2005). Mice lacking CCR2 succumb to fatal infection by *L. monocytogenes* and lack the $CD11b^{int}CD11c^{int}$ population that is morphologically similar to the splenic $CD11c^{high}$ population (i.e., pleomorphic nuclei, dendrites and numerous vacuoles) (Kurihara et al. 1997; Serbina et al. 2006). This $CD11b^{int}CD11c^{int}$ population present during *L. monocytogenes* infection also produced TNF- and iNOS (Tip), thereafter referred to as TipDCs (Serbina et al. 2003). Kang et al. recently demonstrated that the differentiation of TipDCs from the recruited $Ly6C^{high}$ monocytes was dependent on the ability of splenic $CD11c^{pos}$ cells to induce IFNγ production from NK cells (Kang et al. 2008). In addition to CCR2, Auffrey and colleagues demonstrated a role for chemokine receptor CX_3CR1 on the recruitment of $Ly6C^{pos}(GR1^{pos})CD115^{pos}$ moDC precursors to the spleen during *L. monocytogenes* infection (Auffray et al. 2009). A follow up study by this group used $Cx_3cr1^{gfp/+}$ mice to elegantly image *in vivo* monocyte recruitment during *L. monocytogenes* infection (Auffray et al. 2007). Here, the $GR1^{neg}CX_3CR1^{pos}$ monocytes observed patrolling the vasculature rapidly extravasated into inflamed tissue and initiated a macrophage-like transcriptional program by up regulating cMaf and MafB. Conversely, the $GR1^{neg}CX_3CR1^{pos}$ subset took on a DC transcriptional profile by up regulation of Pu.1 and RelB genes within 2 h post infection. Once recruited to the site of infection both differentiation of monocytes into TipDCs and production of IL-12 wash shown to be MyD88 dependent (Serbina et al. 2003; Zhan et al. 2010). CX_3CR1 is also required by moDCs recruited to the intestinal lamina propria during *Salmonella typhimurium* infection in order to extend their dendrites into the intestinal lumen and transport antigen to the lymph node (Niess et al. 2005). MoDCs are among the better migratory DC populations; however, their ability to directly prime the immune system has been called into question in infectious models across the board (Randolph et al. 2008). Using a *Salmonella typhimurium* bacterial infection model, Ravindran and colleagues demonstrated that although a recruited $Ly6C^{high}$ DC-like population was required for induction of an anti-Salmonella $CD4^+$ T cell response, the $Ly6C^{high}$ phagocytes themselves were not directly responsible for this induction (Ravindran et al. 2007). Rather, the $Ly6C^{high}$ population was necessary for transporting the antigen from the site of infection to the draining lymph nodes. Rydstrom and colleagues also demonstrated the inability of a monocyte-derived TipDC-like population to activate antigen-specific T cells following *Salmonella* infection (Rydstrom and Wick 2007).

3.3 Viruses

In addition to bacteria, studies using viral infection models have significantly broadened our understanding of *inflam*DCs. Under steady state conditions, few

monocytes are recruited to the vaginal epithelium. Although, following intravaginal infection with herpes simplex virus-2 (HSV-2), recruited GR-1^{pos} monocytes readily gave rise to vaginal epithelial DCs, including CD11c^{pos}MHCII^{pos} subsets (Iijima et al. 2007; Eidsmo et al. 2009). Similar to the granuloma kidney capsule transplant model previously discussed, Wakim and colleagues grafted HSV-infected dorsal root ganglia under the kidney capsule of recipient naïve mice, which resulted in viral reactivation (Wakim et al. 2008). They sophisticatedly demonstrated that the recruited monocyte-derived Ly6C^{high} DCs were able to restimulate memory viral-specific T cells within the graft. In addition to HSV, monocyte-derived *inflam*DCs are also readily involved in influenza infection. Upon infection with the virulent influenza A virus CD11c^{pos}CD11b^{high}Ly6C^{pos} *inflam*DCs are recruited in a CCR2-dependent manner directly into the lymph nodes, as their recruitment was not hindered in neither *plt* nor CCR7$^{-/-}$ mice (Nakano et al. 2009). When purified and stimulated *ex vivo*, these LN recruited *inflam*DCs produced IL-12(p70) and had the ability to stimulate OTII CD4^{+} T cells. A study by Aldridge et al. also found CCR2-dependent recruitment of Ly6c^{pos}CD11b^{pos} TipDCs into the lungs following influenza A infection (Aldridge et al. 2009). Interestingly, they found that these TipDCs presented antigen to cytotoxic CD8^{+} T cells in the lung and limiting the number of DCs recruited using pioglitazone, a synthetic pharmacological agent that suppressed CCL2 secretion, they could dampen inflammation-induced pathology. That indicated that too many TipDCs are not additive in protection and could even be lethal if not controlled. Viral models have also supported evidence for the dual requirement of inflamDCs migrating from the site of infection and lymph node-resident DCs in the initiation of T cell immunity. Belz and colleagues demonstrated that during both HSV and influenza infection, efficient antigen-specific T cell priming required the collaboration of both lymph node-residing DCs and DCs originating from the site of infection (Belz et al. 2004). Using an HSV skin infection model, Allan and colleagues further demonstrated that skin-originating DCs were required to shuttle antigen to the draining lymph node, where lymph node-resident CD8α^{pos} DCs were principally responsible for initiating the T cell response (Allan et al. 2006).

3.4 Fungi

The role of *inflam*DCs during fungal infection remains one of the less understood areas. *Aspergillus fumigatus* was previously shown to recruit monocytes in a CCR2-dependent mechanism in mice (Blease et al. 2000). A recent study from the Pamer group reconfirmed Ly6C-expressing monocyte recruitment using CCR2 and demonstrated that this cellular compartment was responsible for transporting *Aspergillus* antigen from the lung to the draining lymph node for CD4^{+} T cell priming (Hohl et al. 2009).A more recent study demonstrated that *A. fumigatus* infection of neutropenic hosts resulted in an even more robust recruitment of *inflam*DCs, suggestive of a novel defense mechanism to circumvent loss of neutrophils (Park et al. 2010).

Antigenic hand-off was also demonstrated in a recent study by Erlsand and colleagues, whom investigated the immune response to an attenuated vaccine strain of *Blastomyces dermatitidis* (Ersland et al. 2010). They found that monocyte-derived *inflam*DCs were quickly recruited to the subcutaneous immunization site and along with skin-migratory DCs, shuttled antigen to the lymph nodes; however, lymph node-resident DCs were responsible for priming $CD4^+$ T cells.

3.5 Protozoan

More so than other infectious models, the recruitment and differentiation of *inflam*DCs from recruited monocytes was definitively demonstrated by Leon and Ardavin using the protozoan parasite *Leishmania major* (Leon et al. 2007). Mice infected with *L. major* received an adoptive transfer of purified monocytes, traceable by the Ly5.1/5.2 congenic markers. After 72 h post transfer, two DC lineages arose from the transferred monocytes: $CD11c^{low}Ly\text{-}6C^{high}MHCII^{int}$ and $CD11c^{int}Ly\text{-}6C^{int\text{-}high}MHC\ II^{int\text{-}high}$ populations. The moDCs were also the predominate cellular compartment infected and the only cells presenting *L. major* peptide LACK in the context of MHCII, suggesting that they contribute to anti-parasitic $CD4^+$ T cell response. In a follow up study, this same group demonstrated that during *L. major* infection monocytes were recruited through inflamed dermal venules by binding of PSGL-1 with P/E-selectin and L-selectin with PNAd, and migration through high endothelial venules relied only the latter interaction (Leon and Ardavin 2008). A recent study also confirmed that the same monocyte-derived *inflam*DC ingesting the parasite *L. major* in mice was the same counter subset in humans (Zhan et al. 2010). Monocyte-derived *inflam*DCs have also been studied in the protozoan pathogen, *Toxoplasma gondii.* During infection, $GR\text{-}1^{pos}CCR2^{pos}CX_3CR1^{low}$ monocytes are recruited to inflamed tissue in a CCR2-dependent manner, demonstrated by lethality observed in CCR2 or MCP-1 deficient mice (Robben et al. 2005). Adoptive transfer of inflammatory monocytes into $CCR2^{-/-}$ *T. gondii* infected mice restored protection (Dunay et al. 2008).

4 Conclusions

The dynamic, crucial role *inflam*DCs play during infection is only beginning to be fully understood and appreciated. From our discussion here, *inflam*DCs represent a common thread that can be drawn between the various infectious models. This monocyte-derived lineage is rapidly recruited to the site of infection, be it liver, lung, spleen or skin, within hours. The TNF and iNOS-producing capacity of these cells in the face of various pathogens represents a common line of innate defense. One of the most significant commonalities of *inflam*DCs shared between the various pathogens is their role in shuttling antigen from the site of infection to the respective drain-

ing lymph nodes and transferring this precious cargo to lymph node-resident DCs, which relies on their professional migratory abilities. The mechanism(s) behind this antigenic transfer are currently not known. Based on the current literature, a recent review by Randolph et al. speculates that this transfer may be occurring by two potential mechanisms (Randolph et al. 2008). The first scenario involves the moDCs dying upon arrival to the lymph node and lymph node-resident DCs reprocessing the dead DC and its antigenic cargo. The second model suggests that moDCs transfer intact portions of their plasma membrane that contain MHC/peptide complexes to lymph node-resident DCs. Better understanding the mechanism behind antigenic exchange will increase our understanding of the initiation of adaptive immunity during infection and may potentially provide a target for immune intervention.

Our discussion on the role of *inflam*DCs during *Mycobacterium* infection demonstrates the plasticity this monocyte-derived linage can have during a single infection over time. Figure 1 illustrates this changing role. During acute infection *inflam*DCs facilitate an anti-*Mycobacterium* T cell response by reboosting newly recruited $CD4^{+}$ T cells within the granuloma to produce IFNγ, an essential cytokine to promote macrophage killing of intracellular bacteria. As infection progresses, traffic of inflamDCs into and out of chronic granulomas supports *Mycobacterium*-specific T cell priming in the lymph nodes by providing a continuous source of granuloma-residing antigen. However, once these newly primed T cells reach the granuloma, the phenotype of the local *inflam*DCs does not support IFNγ production, thereby contributing to bacterial persistence.

In closing, this review demonstrates that the monocyte-derived *inflam*DCs are often the dominant DC subset present at sites of inflammation induced by a variety of infectious agents. DCs are a necessary element of both the innate and adaptive immune response; therefore, making it important to increase our understanding of their function. At the site of infection *inflam*DCs have been shown to both induce and suppress the immune response, and can also critically effect the host-pathogen interaction. Many questions still remain regarding this DC subset. Some of them include: How does the *inflam*DC regulate components of the inflammatory response? What is their expected lifespan within an inflammatory lesion? What is the extent of their traffic to and from the inflammatory lesions? And are they able to sustain systemic immunity? In regards to *Mycobacterium*-host interactions and the anatomical structure of the granuloma, which is required for mycobacterial containment, the *inflam*DC plays a critical role in the pathogenesis. They facilitate dissemination of bacteria and bacterial antigens, which drives long-term immunity, and regulate intragranuloma processes; thereby representing an important part of the puzzle of long-term bacterial containment

References

Aldridge, J. R., Jr., C. E. Moseley, D. A. Boltz, N. J. Negovetich, C. Reynolds, J. Franks, S. A. Brown, P. C. Doherty, R. G. Webster and P. G. Thomas. (2009) TNF/iNOS-producing dendritic cells are the necessary evil of lethal influenza virus infection. *Proc Natl Acad Sci U S A*. 106, 5306-5311

Allan, R. S., J. Waithman, S. Bedoui, C. M. Jones, J. A. Villadangos, Y. Zhan, A. M. Lew, K. Shortman, W. R. Heath and F. R. Carbone. (2006) Migratory dendritic cells transfer antigen to a lymph node-resident dendritic cell population for efficient CTL priming. *Immunity*. 25, 153-162

Arnold, L., A. Henry, F. Poron, Y. Baba-Amer, N. van Rooijen, A. Plonquet, R. K. Gherardi and B. Chazaud. (2007) Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. *J Exp Med*. 204, 1057-1069

Auffray, C., D. Fogg, M. Garfa, G. Elain, O. Join-Lambert, S. Kayal, S. Sarnacki, A. Cumano, G. Lauvau and F. Geissmann. (2007) Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. *Science*. 317, 666-670

Auffray, C., D. K. Fogg, E. Narni-Mancinelli, B. Senechal, C. Trouillet, N. Saederup, J. Leemput, K. Bigot, L. Campisi, M. Abitbol, T. Molina, I. Charo, D. A. Hume, A. Cumano, G. Lauvau and F. Geissmann. (2009) CX3CR1+ CD115+ CD135+ common macrophage/DC precursors and the role of CX3CR1 in their response to inflammation. *J Exp Med*. 206, 595-606

Auffray, C., M. H. Sieweke and F. Geissmann. (2009) Blood monocytes: development, heterogeneity, and relationship with dendritic cells. *Annu Rev Immunol*. 27, 669-692

Badewa, A. P., L. J. Quinton, J. E. Shellito and C. M. Mason. (2005) Chemokine receptor 5 and its ligands in the immune response to murine tuberculosis. *Tuberculosis (Edinb)*. 85, 185-195

Bakri, Y., S. Sarrazin, U. P. Mayer, S. Tillmanns, C. Nerlov, A. Boned and M. H. Sieweke. (2005) Balance of MafB and PU.1 specifies alternative macrophage or dendritic cell fate. *Blood*. 105, 2707-2716

Belz, G. T., C. M. Smith, L. Kleinert, P. Reading, A. Brooks, K. Shortman, F. R. Carbone and W. R. Heath. (2004) Distinct migrating and nonmigrating dendritic cell populations are involved in MHC class I-restricted antigen presentation after lung infection with virus. *Proc Natl Acad Sci U S A*. 101, 8670-8675

Blease, K., B. Mehrad, N. W. Lukacs, S. L. Kunkel, T. J. Standiford and C. M. Hogaboam. (2001) Antifungal and airway remodeling roles for murine monocyte chemoattractant protein-1/CCL2 during pulmonary exposure to Asperigillus fumigatus conidia. *J Immunol*. 166, 1832-1842

Blease, K., B. Mehrad, T. J. Standiford, N. W. Lukacs, J. Gosling, L. Boring, I. F. Charo, S. L. Kunkel and C. M. Hogaboam. (2000) Enhanced pulmonary allergic responses to Aspergillus in CCR2$^{-/-}$ mice. *J Immunol*. 165, 2603-2611

Cheong, C., I. Matos, J. H. Choi, D. B. Dandamudi, E. Shrestha, M. P. Longhi, K. L. Jeffrey, R. M. Anthony, C. Kluger, G. Nchinda, H. Koh, A. Rodriguez, J. Idoyaga, M. Pack, K. Velinzon, C. G. Park and R. M. Steinman. (2010) Microbial stimulation fully differentiates monocytes to DC-SIGN/CD209(+) dendritic cells for immune T cell areas. *Cell*. 143, 416-429

Combadiere, C., S. Potteaux, M. Rodero, T. Simon, A. Pezard, B. Esposito, R. Merval, A. Proudfoot, A. Tedgui and Z. Mallat. (2008) Combined inhibition of CCL2, CX3CR1, and CCR5 abrogates Ly6C(hi) and Ly6C(lo) monocytosis and almost abolishes atherosclerosis in hypercholesterolemic mice. *Circulation*. 117, 1649-1657

Czuprynski, C. J., J. F. Brown, N. Maroushek, R. D. Wagner and H. Steinberg. (1994) Administration of anti-granulocyte mAb RB6-8C5 impairs the resistance of mice to Listeria monocytogenes infection. *J Immunol*. 152, 1836-1846

Dahl, R., J. C. Walsh, D. Lancki, P. Laslo, S. R. Iyer, H. Singh and M. C. Simon. (2003) Regulation of macrophage and neutrophil cell fates by the PU. 1:C/EBPalpha ratio and granulocyte colony-stimulating factor. *Nat Immunol*. 4, 1029-1036

Dai, X. M., G. R. Ryan, A. J. Hapel, M. G. Dominguez, R. G. Russell, S. Kapp, V. Sylvestre and E. R. Stanley. (2002) Targeted disruption of the mouse colony-stimulating factor 1 receptor gene results in osteopetrosis, mononuclear phagocyte deficiency, increased primitive progenitor cell frequencies, and reproductive defects. *Blood*. 99, 111-120

Dunay, I. R., R. A. Damatta, B. Fux, R. Presti, S. Greco, M. Colonna and L. D. Sibley. (2008) Gr1(+) inflammatory monocytes are required for mucosal resistance to the pathogen Toxoplasma gondii. *Immunity*. 29, 306-317

Egen, J. G., A. G. Rothfuchs, C. G. Feng, N. Winter, A. Sher and R. N. Germain. (2008) Macrophage and T cell dynamics during the development and disintegration of mycobacterial granulomas. *Immunity*. 28, 271-284

Eidsmo, L., R. Allan, I. Caminschi, N. van Rooijen, W. R. Heath and F. R. Carbone. (2009) Differential migration of epidermal and dermal dendritic cells during skin infection. *J Immunol*. 182, 3165-3172

Ersland, K., M. Wuthrich and B. S. Klein. (2010) Dynamic interplay among monocyte-derived, dermal, and resident lymph node dendritic cells during the generation of vaccine immunity to fungi. *Cell Host Microbe*. 7, 474-487

Fleming, T. J., M. L. Fleming and T. R. Malek. (1993) Selective expression of Ly-6G on myeloid lineage cells in mouse bone marrow. RB6-8C5 mAb to granulocyte-differentiation antigen (Gr-1) detects members of the Ly-6 family. *J Immunol*. 151, 2399-2408

Flores-Villanueva, P. O., J. A. Ruiz-Morales, C. H. Song, L. M. Flores, E. K. Jo, M. Montano, P. F. Barnes, M. Selman and J. Granados. (2005) A functional promoter polymorphism in monocyte chemoattractant protein-1 is associated with increased susceptibility to pulmonary tuberculosis. *J Exp Med*. 202, 1649-1658

Fogg, D. K., C. Sibon, C. Miled, S. Jung, P. Aucouturier, D. R. Littman, A. Cumano and F. Geissmann. (2006) A clonogenic bone marrow progenitor specific for macrophages and dendritic cells. *Science*. 311, 83-87

Geissmann, F., S. Jung and D. R. Littman. (2003) Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity*. 19, 71-82

Ginhoux, F., F. Tacke, V. Angeli, M. Bogunovic, M. Loubeau, X. M. Dai, E. R. Stanley, G. J. Randolph and M. Merad. (2006) Langerhans cells arise from monocytes in vivo. *Nat Immunol*. 7, 265-273

Gonzalez-Mejia, M. E. and A. I. Doseff. (2009) Regulation of monocytes and macrophages cell fate. *Front Biosci*. 14, 2413-2431

Hall, J. D., S. L. Kurtz, N. W. Rigel, B. M. Gunn, S. Taft-Benz, J. P. Morrison, A. M. Fong, D. D. Patel, M. Braunstein and T. H. Kawula. (2009) The impact of chemokine receptor CX3CR1 deficiency during respiratory infections with Mycobacterium tuberculosis or Francisella tularensis. *Clin Exp Immunol*. 156, 278-284

Hohl, T. M., A. Rivera, L. Lipuma, A. Gallegos, C. Shi, M. Mack and E. G. Pamer. (2009) Inflammatory monocytes facilitate adaptive CD4 T cell responses during respiratory fungal infection. *Cell Host Microbe*. 6, 470-481

Holt, P. G., S. Haining, D. J. Nelson and J. D. Sedgwick. (1994) Origin and steady-state turnover of class II MHC-bearing dendritic cells in the epithelium of the conducting airways. *J Immunol*. 153, 256-261

Humphreys, I. R., G. R. Stewart, D. J. Turner, J. Patel, D. Karamanou, R. J. Snelgrove and D. B. Young. (2006) A role for dendritic cells in the dissemination of mycobacterial infection. *Microbes Infect*. 8, 1339-1346

Iijima, N., M. M. Linehan, S. Saeland and A. Iwasaki. (2007) Vaginal epithelial dendritic cells renew from bone marrow precursors. *Proc Natl Acad Sci U S A*. 104, 19061-19066

Inaba, K., M. Inaba, N. Romani, H. Aya, M. Deguchi, S. Ikehara, S. Muramatsu and R. M. Steinman. (1992) Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J Exp Med*. 176, 1693-1702

Jakubzick, C., F. Tacke, F. Ginhoux, A. J. Wagers, N. van Rooijen, M. Mack, M. Merad and G. J. Randolph. (2008) Blood monocyte subsets differentially give rise to CD103+ and CD103- pulmonary dendritic cell populations. *J Immunol*. 180, 3019-3027

Jiao, X., R. Lo-Man, P. Guermonprez, L. Fiette, E. Deriaud, S. Burgaud, B. Gicquel, N. Winter and C. Leclerc. (2002) Dendritic cells are host cells for mycobacteria in vivo that trigger innate and acquired immunity. *J Immunol*. 168, 1294-1301

Kahnert, A., U. E. Hopken, M. Stein, S. Bandermann, M. Lipp and S. H. Kaufmann. (2007) Mycobacterium tuberculosis triggers formation of lymphoid structure in murine lungs. *J Infect Dis*. 195, 46-54

Kamath, A. T., S. Henri, F. Battye, D. F. Tough and K. Shortman. (2002) Developmental kinetics and lifespan of dendritic cells in mouse lymphoid organs. *Blood*. 100, 1734-1741

Kamphorst, A. O., P. Guermonprez, D. Dudziak and M. C. Nussenzweig. (2010) Route of antigen uptake differentially impacts presentation by dendritic cells and activated monocytes. *J Immunol*. 185, 3426-3435

Kang, S. J., H. E. Liang, B. Reizis and R. M. Locksley. (2008) Regulation of hierarchical clustering and activation of innate immune cells by dendritic cells. *Immunity*. 29, 819-833

Khader, S. A., J. Rangel-Moreno, J. J. Fountain, C. A. Martino, W. W. Reiley, J. E. Pearl, G. M. Winslow, D. L. Woodland, T. D. Randall and A. M. Cooper. (2009) In a murine tuberculosis model, the absence of homeostatic chemokines delays granuloma formation and protective immunity. *J Immunol*. 183, 8004-8014

Kurihara, T., G. Warr, J. Loy and R. Bravo. (1997) Defects in macrophage recruitment and host defense in mice lacking the CCR2 chemokine receptor. *J Exp Med*. 186, 1757-1762

Laouar, Y., T. Welte, X. Y. Fu and R. A. Flavell. (2003) STAT3 is required for Flt3L-dependent dendritic cell differentiation. *Immunity*. 19, 903-912

Leon, B. and C. Ardavin. (2008) Monocyte migration to inflamed skin and lymph nodes is differentially controlled by L-selectin and PSGL-1. *Blood*. 111, 3126-3130

Leon, B., M. Lopez-Bravo and C. Ardavin. (2007) Monocyte-derived dendritic cells formed at the infection site control the induction of protective T helper 1 responses against Leishmania. *Immunity*. 26, 519-531

Lin, Y., J. Gong, M. Zhang, W. Xue and P. F. Barnes. (1998) Production of monocyte chemoattractant protein 1 in tuberculosis patients. *Infect Immun*. 66, 2319-2322

Lindquist, R. L., G. Shakhar, D. Dudziak, H. Wardemann, T. Eisenreich, M. L. Dustin and M. C. Nussenzweig. (2004) Visualizing dendritic cell networks in vivo. *Nat Immunol*. 5, 1243-1250

Liu, K. and M. C. Nussenzweig. (2010) Origin and development of dendritic cells. *Immunol Rev*. 234, 45-54

Liu, K., G. D. Victora, T. A. Schwickert, P. Guermonprez, M. M. Meredith, K. Yao, F. F. Chu, G. J. Randolph, A. Y. Rudensky and M. Nussenzweig. (2009) In vivo analysis of dendritic cell development and homeostasis. *Science*. 324, 392-397

Liu, K., C. Waskow, X. Liu, K. Yao, J. Hoh and M. Nussenzweig. (2007) Origin of dendritic cells in peripheral lymphoid organs of mice. *Nat Immunol*. 8, 578-583

McCurley, N. and I. Mellman. (2010) Monocyte-derived dendritic cells exhibit increased levels of lysosomal proteolysis as compared to other human dendritic cell populations. *PLoS One*. 5, e11949

McKenna, H. J., K. L. Stocking, R. E. Miller, K. Brasel, T. De Smedt, E. Maraskovsky, C. R. Maliszewski, D. H. Lynch, J. Smith, B. Pulendran, E. R. Roux, M. Teepe, S. D. Lyman and J. J. Peschon. (2000) Mice lacking flt3 ligand have deficient hematopoiesis affecting hematopoietic progenitor cells, dendritic cells, and natural killer cells. *Blood*. 95, 3489-3497

Mohrs, K., A. E. Wakil, N. Killeen, R. M. Locksley and M. Mohrs. (2005) A two-step process for cytokine production revealed by IL-4 dual-reporter mice. *Immunity*. 23, 419-429

Murray, E., R. Webb and M. Swann. (1926) A disease of rabbits characterized by a large mononuclear monocytosis, caused by a hitherto undescribed bacillus Bacterium monocytogenes. *J Pathol Bacteriol*. 29, 407-439

Nahrendorf, M., F. K. Swirski, E. Aikawa, L. Stangenberg, T. Wurdinger, J. L. Figueiredo, P. Libby, R. Weissleder and M. J. Pittet. (2007) The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. *J Exp Med*. 204, 3037-3047

Naik, S. H., D. Metcalf, A. van Nieuwenhuijze, I. Wicks, L. Wu, M. O'Keeffe and K. Shortman. (2006) Intrasplenic steady-state dendritic cell precursors that are distinct from monocytes. *Nat Immunol*. 7, 663-671

Nakano, H., K. L. Lin, M. Yanagita, C. Charbonneau, D. N. Cook, T. Kakiuchi and M. D. Gunn. (2009) Blood-derived inflammatory dendritic cells in lymph nodes stimulate acute T helper type 1 immune responses. *Nat Immunol*. 10, 394-402

Niess, J. H., S. Brand, X. Gu, L. Landsman, S. Jung, B. A. McCormick, J. M. Vyas, M. Boes, H. L. Ploegh, J. G. Fox, D. R. Littman and H. C. Reinecker. (2005) CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science*. 307, 254-258

Park, S. J., M. D. Burdick, W. K. Brix, M. H. Stoler, D. S. Askew, R. M. Strieter and B. Mehrad. (2010) Neutropenia Enhances Lung Dendritic Cell Recruitment in Response to Aspergillus via a Cytokine-to-Chemokine Amplification Loop. *J Immunol*.

Peters, W., H. M. Scott, H. F. Chambers, J. L. Flynn, I. F. Charo and J. D. Ernst. (2001) Chemokine receptor 2 serves an early and essential role in resistance to Mycobacterium tuberculosis. *Proc Natl Acad Sci U S A*. 98, 7958-7963

Randolph, G. J. (2006) Migratory dendritic cells: sometimes simply ferries? *Immunity*. 25, 15-18

Randolph, G. J., S. Beaulieu, S. Lebecque, R. M. Steinman and W. A. Muller. (1998) Differentiation of monocytes into dendritic cells in a model of transendothelial trafficking. *Science*. 282, 480-483

Randolph, G. J., K. Inaba, D. F. Robbiani, R. M. Steinman and W. A. Muller. (1999) Differentiation of phagocytic monocytes into lymph node dendritic cells in vivo. *Immunity*. 11, 753-761

Randolph, G. J., C. Jakubzick and C. Qu. (2008) Antigen presentation by monocytes and monocyte-derived cells. *Curr Opin Immunol*. 20, 52-60

Ravindran, R., L. Rusch, A. Itano, M. K. Jenkins and S. J. McSorley. (2007) CCR6-dependent recruitment of blood phagocytes is necessary for rapid CD4 T cell responses to local bacterial infection. *Proc Natl Acad Sci U S A*. 104, 12075-12080

Reljic, R., C. Di Sano, C. Crawford, F. Dieli, S. Challacombe and J. Ivanyi. (2005) Time course of mycobacterial infection of dendritic cells in the lungs of intranasally infected mice. *Tuberculosis (Edinb)*. 85, 81-88

Robben, P. M., M. LaRegina, W. A. Kuziel and L. D. Sibley. (2005) Recruitment of Gr-1+ monocytes is essential for control of acute toxoplasmosis. *J Exp Med*. 201, 1761-1769

Russell, D. G. (2007) Who puts the tubercle in tuberculosis? *Nat Rev Microbiol*. 5, 39-47

Rydstrom, A. and M. J. Wick. (2007) Monocyte recruitment, activation, and function in the gut-associated lymphoid tissue during oral Salmonella infection. *J Immunol*. 178, 5789-5801

Sallusto, F. and A. Lanzavecchia. (1994) 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. *J Exp Med*. 179, 1109-1118

Schreiber, H. A. and M. Sandor. (2010) The role of dendritic cells in mycobacterium-induced granulomas. *Immunol Lett*. 130, 26-31

Schreiber, H. A., P. D. Hulseberg, J. Lee, J. Prechl, P. Barta, N. Szlavik, J. S. Harding, Z. Fabry and M. Sandor. (2010) Dendritic cells in chronic mycobacterial granulomas restrict local antibacterial T cell response in a murine model. *PLoS One*. 5, e11453

Schreiber, O., K. Steinwede, N. Ding, M. Srivastava, R. Maus, F. Langer, J. Prokein, S. Ehlers, T. Welte, M. D. Gunn and U. A. Maus. (2008) Mice that overexpress CC chemokine ligand 2 in their lungs show increased protective immunity to infection with Mycobacterium bovis bacille Calmette-Guerin. *J Infect Dis*. 198, 1044-1054

Scott, H. M. and J. L. Flynn. (2002) Mycobacterium tuberculosis in chemokine receptor 2-deficient mice: influence of dose on disease progression. *Infect Immun*. 70, 5946-5954

Serbina, N. V. and E. G. Pamer. (2006) Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nat Immunol*. 7, 311-317

Serbina, N. V., T. Jia, T. M. Hohl and E. G. Pamer. (2008) Monocyte-mediated defense against microbial pathogens. *Annu Rev Immunol*. 26, 421-452

Serbina, N. V., W. Kuziel, R. Flavell, S. Akira, B. Rollins and E. G. Pamer. (2003a) Sequential MyD88-independent and -dependent activation of innate immune responses to intracellular bacterial infection. *Immunity*. 19, 891-901

Serbina, N. V., T. P. Salazar-Mather, C. A. Biron, W. A. Kuziel and E. G. Pamer. (2003b) TNF/iNOS-producing dendritic cells mediate innate immune defense against bacterial infection. *Immunity*. 19, 59-70

Steinman, R. M. and Z. A. Cohn. (1973) Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J Exp Med*. 137, 1142-1162

Steinman, R. M. and Z. A. Cohn. (1974a) Identification of a novel cell type in peripheral lymphoid organs of mice. II. Functional properties in vitro. *J Exp Med*. 139, 380-397

Steinman, R. M., D. S. Lustig and Z. A. Cohn. (1974b) Identification of a novel cell type in peripheral lymphoid organs of mice. 3. Functional properties in vivo. *J Exp Med*. 139, 1431-1445

Sunderkotter, C., T. Nikolic, M. J. Dillon, N. Van Rooijen, M. Stehling, D. A. Drevets and P. J. Leenen. (2004) Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response. *J Immunol*. 172, 4410-4417

Tacke, F., D. Alvarez, T. J. Kaplan, C. Jakubzick, R. Spanbroek, J. Llodra, A. Garin, J. Liu, M. Mack, N. van Rooijen, S. A. Lira, A. J. Habenicht and G. J. Randolph. (2007) Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques. *J Clin Invest*. 117, 185-194

Tacke, F. and G. J. Randolph. (2006) Migratory fate and differentiation of blood monocyte subsets. *Immunobiology*. 211, 609-618

Tian, T., J. Woodworth, M. Skold and S. M. Behar. (2005) In vivo depletion of CD11c+ cells delays the CD4 +T cell response to Mycobacterium tuberculosis and exacerbates the outcome of infection. *J Immunol*. 175, 3268-3272

Tsou, C. L., W. Peters, Y. Si, S. Slaymaker, A. M. Aslanian, S. P. Weisberg, M. Mack and I. F. Charo. (2007) Critical roles for CCR2 and MCP-3 in monocyte mobilization from bone marrow and recruitment to inflammatory sites. *J Clin Invest*. 117, 902-909

Ulrichs, T. and S. H. Kaufmann. (2006) New insights into the function of granulomas in human tuberculosis. *J Pathol*. 208, 261-269

Varol, C., L. Landsman, D. K. Fogg, L. Greenshtein, B. Gildor, R. Margalit, V. Kalchenko, F. Geissmann and S. Jung. (2007) Monocytes give rise to mucosal, but not splenic, conventional dendritic cells. *J Exp Med*. 204, 171-180

Volkman, H. E., H. Clay, D. Beery, J. C. Chang, D. R. Sherman and L. Ramakrishnan. (2004) Tuberculous granuloma formation is enhanced by a mycobacterium virulence determinant. *PLoS Biol*. 2, e367

Wakim, L. M., J. Waithman, N. van Rooijen, W. R. Heath and F. R. Carbone. (2008) Dendritic cell-induced memory T cell activation in nonlymphoid tissues. *Science*. 319, 198-202

Waskow, C., K. Liu, G. Darrasse-Jeze, P. Guermonprez, F. Ginhoux, M. Merad, T. Shengelia, K. Yao and M. Nussenzweig. (2008) The receptor tyrosine kinase Flt3 is required for dendritic cell development in peripheral lymphoid tissues. *Nat Immunol*. 9, 676-683

Whitelaw, D. M. (1966) The intravascular lifespan of monocytes. *Blood*. 28, 455-464

Wolf, A. J., L. Desvignes, B. Linas, N. Banaiee, T. Tamura, K. Takatsu and J. D. Ernst. (2007a) Initiation of the adaptive immune response to Mycobacterium tuberculosis depends on antigen production in the local lymph node, not the lungs. *J Exp Med*.

Wolf, A. J., B. Linas, G. J. Trevejo-Nunez, E. Kincaid, T. Tamura, K. Takatsu and J. D. Ernst. (2007b) Mycobacterium tuberculosis infects dendritic cells with high frequency and impairs their function in vivo. *J Immunol*. 179, 2509-2519

Zhan, Y., Y. Xu, S. Seah, J. L. Brady, E. M. Carrington, C. Cheers, B. A. Croker, L. Wu, J. A. Villadangos and A. M. Lew. (2010) Resident and monocyte-derived dendritic cells become dominant IL-12 producers under different conditions and signaling pathways. *J Immunol*. 185, 2125-2133