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Review

Role of inflammatory dendritic cells in innate and adaptive immunity

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The major role of cells of the dendritic family in immunity and tolerance has been amply documented. Since their discovery in 1973, these cells have gained increasing interest from immunologists, as they are able to detect infectious agents, migrate to secondary lymphoid tissue, and prime naive T lymphocytes, thereby driving immune responses. Surprisingly, they can also have the opposite function, that is, preventing immune responses, as they are involved in central and peripheral tolerance. Most dendritic cells (DCs) derive from a common precursor and do not arise from monocytes and are considered "conventional" DCs. However, a new population of DCs, namely "inflammatory" DCs, has recently been identified, which is not present in the steady state but differentiates from monocytes during infection/inflammation. In this review, we summarize the role of these "inflammatory" DCs in innate and adaptive immunity.

Keywords: Antigen presentation · Inflammatory dendritic cells · In vivo animal models · T helper cell development

Discovery of monocyte-derived dendritic cells (DCs)

In 1998, Randolph and colleagues reported a surprising finding: they cultured blood mononuclear cells with monolayers of human endothelial cells grown on a collagen matrix, and found that the cells that had reverse transmigrated acquired phenotypic and functional features of DCs. In particular, they appeared to be potent stimulators of allogeneic T cells [1]. Monocytes that did not transmigrate and remained in the collagen, differentiated into macrophage-like cells. The same research group [2] further examined the fate of latex microspheres injected into the skin and noticed that by 18 h after injection most microspheres were phagocytosed. Of note, a large population of cells containing FITC-latex accumulated in the draining lymph nodes (LNs), mainly in the T-cell area. These cells phenotypically resembled DCs and were absent from monocyte-deficient op/op mice. These observations suggested that a subset of monocyte-derived DCs could play a major role in presentation of particulate antigens and were reminiscent of old studies showing that DCs could be obtained in culture from human PBMCs [3, 4]. Although the precursors were not formally identified, they were plentiful in human blood and adherent, suggesting a monocytic lineage.

More recently, Geissmann and colleagues [5] examined blood monocytes in mice and humans and identified two functional subsets as defined by the level of expression of CX_3CR1. Resident CX_3CR1^{high} monocytes were found in the blood and noninflamed peripheral organs where they homed in a CX_3CR1-dependent way. CX_3CR1^{low} monocytes were short-lived, were actively recruited to inflamed tissues independently of their CX_3CR1 genotype, and differentiated into functional DCs that had the ability to stimulate naive T cells. Although the authors proposed the term "inflammatory monocytes" for the immediate precursors of antigen-presenting DCs, we will name them "inflammatory DCs," unless discussing monocyte differentiation to DCs, as subsequent reports clearly indicate that most "inflammatory monocytes" differentiate into $CD11c^+$ cells displaying functional properties of the dendritic family. Other identified inflammatory monocytes such as $Ly6C^{high}$ or $CCR2^+$ monocytes are discussed later in this review in the sections Th2-type immunity and Th1-type immunity.

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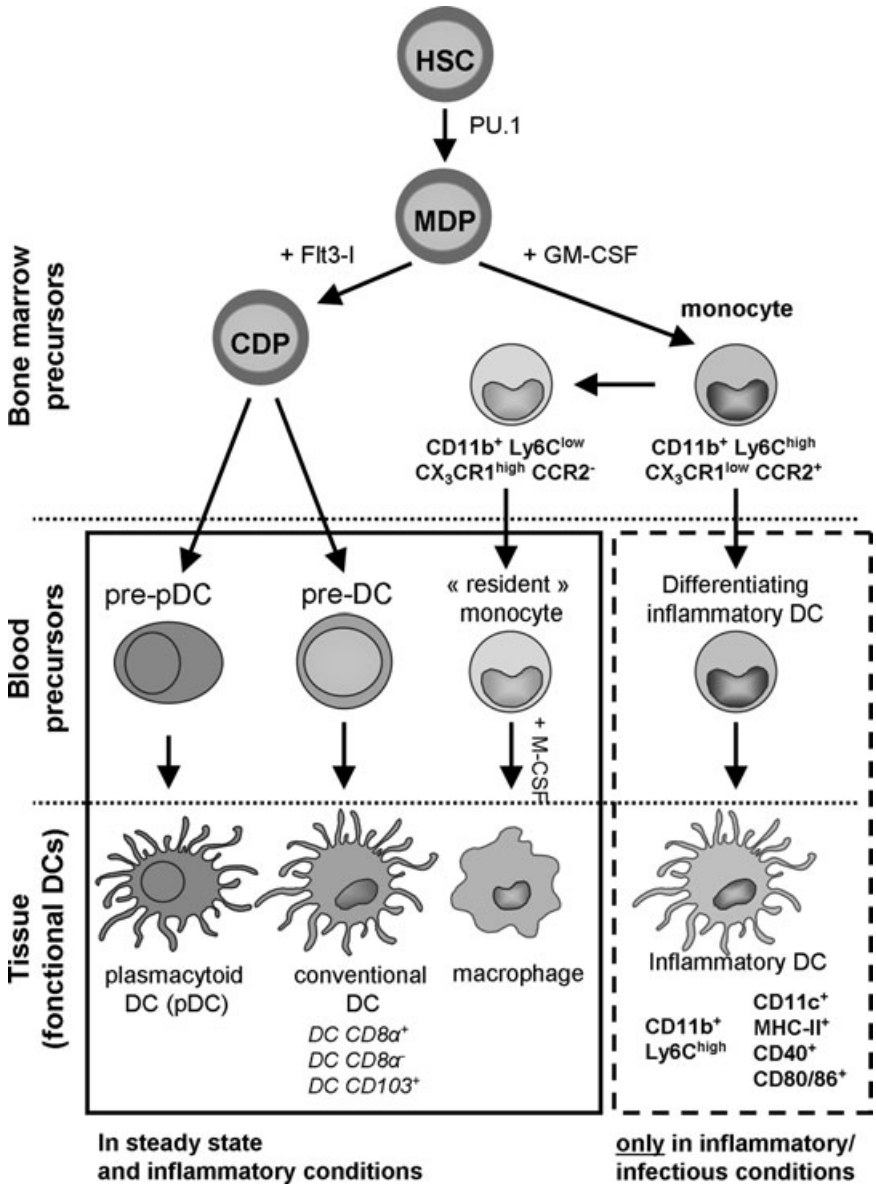

Figure 1. Origin and development of conventional and inflammatory dendritic cells (DCs). Inflammatory and conventional DCs share the common features of DCs, that is, morphology, migratory properties, DC surface markers (CD11c, MHC II, costimulatory molecules CD80/86, CD40), and ability to prime naïve T cells. However, they differ in their lineage origin at an early step of hematopoietic stem cell (HSC) differentiation in the bone marrow. PU.1-expressing HSCs differentiate into macrophage/DC progenitors (MDPs) that represent the latest common developmental stage between conventional and inflammatory DCs. In the presence of Flt-3 ligand, MDPs give rise to the common DC progenitor (CDP). CDPs further produce plasmacytoid and precursor DCs (pre-DCs) that travel through the blood to lymphoid and nonlymphoid tissues in steady state and inflammatory conditions. In the presence of GM-CSF, MDPs differentiate into monocytes expressing high levels of the Ly6C marker. Ly6C^{high} monocytes can further give rise to Ly6C^{low} monocytes that circulate through the blood and colonize peripheral tissues to become mainly macrophages in the steady state. Only in case of inflammation/infection, Ly6C^{high} monocytes emigrate from the bone marrow by a CCR2-dependent mechanism, travel through the blood and reach inflamed/infected tissues where they fully differentiate into inflammatory DCs.

These observations identified a novel population of DCs, which do not derive from a MDP (macrophage/DC precursor)/CDP (common DC progenitor) as shown for so-called classical DCs such as conventional and plasmacytoid DCs, but rather from monocytes in inflammatory conditions (Fig. 1). This raises the question of the respective role of conventional versus inflammatory DCs in innate and adaptive immune responses.

Inflammatory DCs in innate immunity

The observation that monocytes may, in some conditions, differentiate into DCs was confirmed by Serbina et al. [6], in a study published back-to-back with that of Geissmann and colleagues [5]. In the course of the study by Serbina et al. [6], which aimed to identify the source of nitric oxide (NO) and tumor necrosis factor (TNF) (essential mediators produced by monocytes and DCs for the control of *Listeria monocytogenes*), the authors showed that infection with this bacteria induced the recruitment of a novel TNF- and iNOS-producing DC subset to the spleen. In addition, the authors compared cellular responses in wild-type (WT) mice and mice lacking the CCR2 chemokine receptor, and found that the number of CD11c^{+} cells increased in WT but not CCR2$^{-/-}$ (a *Listeria* susceptible strain [7]) mice following *Listeria monocytogenes* infection; CD11b^{int} CD11c^{int} cells constituted 3.6% of the total splenocyte population 48 h after infection of WT mice, and displayed upregulated CD80, CD86, CD40, and MHC class II expression as well as a DC morphology.

Serbina et al. [6] further showed that the production of TNF-α and NO was markedly reduced in CCR2$^{-/-}$ mice, an observation in-line with the high susceptibility of these mice to *Listeria monocytogenes* infection, whereas CD8^{+} and CD4^{+} T-cell responses

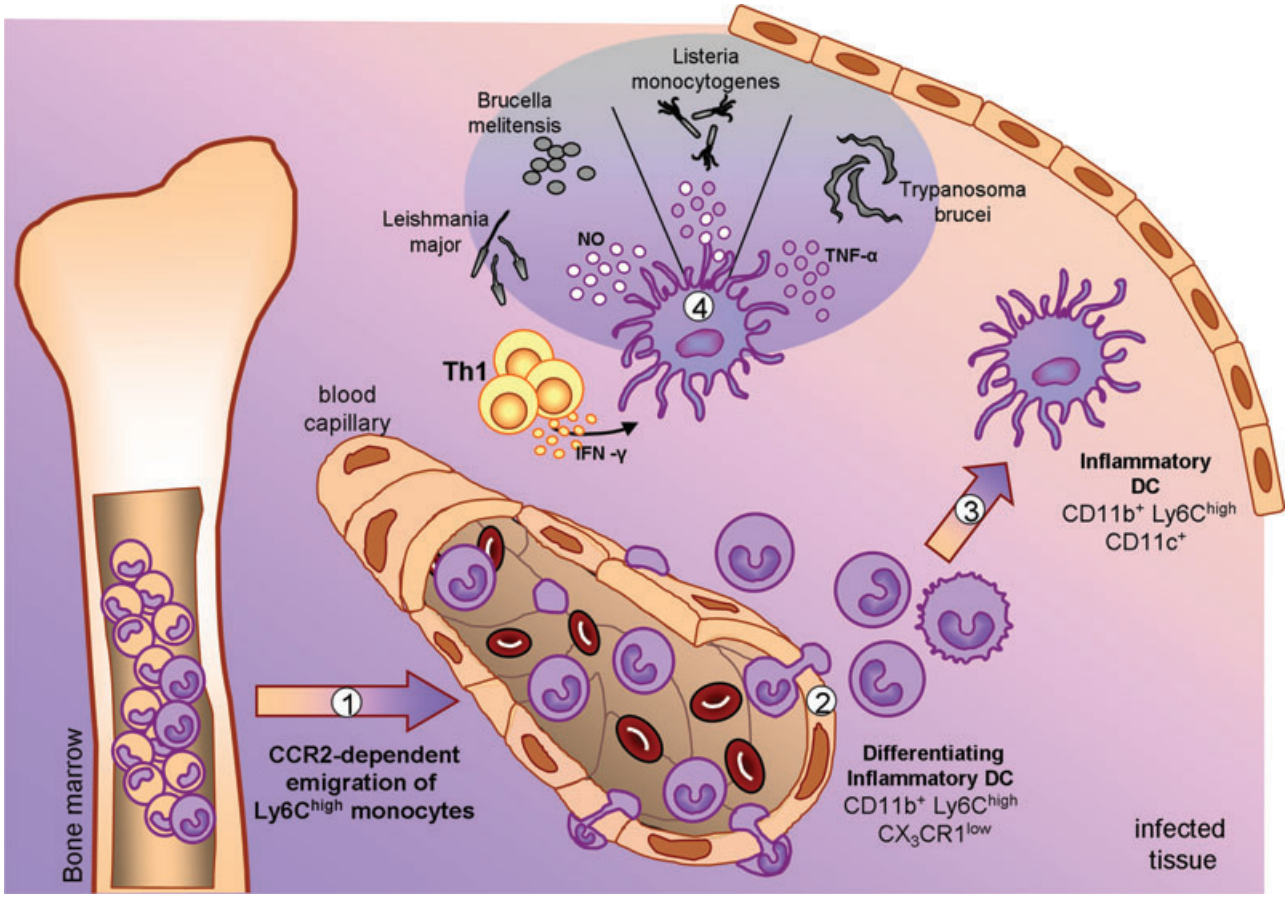

Figure 2. Inflammatory dendritic cells (DCs) in innate immunity. During infection, progenitors/monocytes emigrate from the bone marrow to the blood circulation through a mechanism depending on CCR2 signaling. CD11b$^+$ Ly6C^{high} CX$_3$CR1^{low} monocytes then migrate from the blood to the infected tissues where they differentiate into CD11b$^+$ Ly6C^{high} CD11c$^+$ inflammatory DCs characterized by their production of tumor necrosis factor-α (TNF-α) and nitric oxide (NO). Clearance of *Brucella melitensis* [9], *Leishmania major* [8], *Listeria monocytogenes* [10], and *Trypanosoma brucei* [11] infection has been shown to depend on the recruitment of inflammatory DCs and their expression of NO and/or TNF-α. IFN-γ secreted by Th1 lymphocytes may be involved in the differentiation and/or function of inflammatory DCs upon infection of *B. Melitensis* and *Leishmania major*.

were preserved. The identified monocyte-derived DCs were named TIP (TNF-iNOS producing) DCs, and were shown to play a crucial role in early antimicrobial defense, with their recruitment requiring CCR2 [6]. Of note, these TIP-DCs were not directly infected with *Listeria monocytogenes* and therefore are probably not involved in bacterial transport to the spleen [6]. Interestingly, in another study, the resistance to *Leishmania major* infection (in C57BL/6 mice) was associated with the presence of iNOS-producing inflammatory DCs that depend on a Th1 microenvironment, that is, IFN-γ-producing CD4$^+$ T cells. By contrast, STAT-6-deficient BALB/c mice, which are defective in IL-4 and IL-13 signaling, displayed higher recruitment of iNOS-DC in LN following *Leishmania major* infection [8]. Similarly, inflammatory DCs were shown to be the main iNOS-producing cells in the spleen and peritoneal cavity of mice infected with *Brucella melitensis* and their activation required TLR4- or TLR9-mediated MYD88-dependent triggering [9] (Fig. 2).

Although these inflammatory DCs have been shown to play a beneficial role in intracellular pathogen clearance, they may also mediate immune pathology during parasitic infection [11]. In *Trypanosoma brucei brucei* infected mice, bone marrow derived monocytes were found to be recruited to the spleen, LNs, and liver where they differentiated into mature inflammatory DCs and represented a major cellular source of TNF and iNOS. Infected IL-10 KO mice had a higher proportion of inflammatory DCs but this increased population was associated with enhanced liver injury and early death of the host. Collectively, these observations [8, 11] show that Th1-type cytokines favor the differentiation of inflammatory DCs at the site of infection, whereas IL-10, IL-4, and IL-13 act as negative regulators.

Monocyte emigration from the bone marrow in steady state conditions and during *Listeria monocytogenes* infection has been shown to be dependent on CCR2 signaling, but CCR2 appears not to be required for migration from the blood to the tissues [12]. Thus, in CCR2$^{-/-}$ mice, monocytes are retained in the bone marrow and resemble the inflammatory DCs that are normally recruited to the spleens of WT mice infected with *Listeria monocytogenes*. Ly6C$^+$ monocytes recruited to the site of inflammation,

however, require CCR2 for entry into HSV-2-infected tissue; indeed, a recent report [13] demonstrates that CCR2$^{-/-}$ mice, challenged intravaginally with HSV-2, sustain higher virus titers in the vaginal secretion, as compared with WT mice. The authors further showed that type I interferons, produced by nonmonocytic cells, induced CCR2 ligand expression on monocytes leading to recruitment of monocytes to the infected tissues.

Collectively, the observations described in this section indicate that monocytes are recruited from the bone marrow to the blood during infection and that they differentiate into cells displaying properties shared by cells of the dendritic family. These "inflammatory dendritic cells," through NO and TNF-α production, have a major role in the clearance of infectious agents. Notably, NO, which is generated by the actions of iNOS, has remarkable microbicidal properties, altering pathogen metabolism: NO can interact with oxygen species to form oxidant derivatives causing DNA deamination, strand breaks, and other alterations [14]; and it can inhibit the metabolic activity and function of some trypanosomal proteins by chemically modifying their cysteine residues and/or by binding to metalloproteins that mediate crucial metabolic processes [15]. TNF-α, on the other hand, presents a lectin-like domain that binds specific glycoproteins in the flagellar pocket of *T. brucei* disturbing the osmoregulatory capacity of the pathogen and leading to its lysis [16, 17]. TNF-α has also been shown to bind gram-negative bacteria through specific TNF-α receptors expressed on the bacteria that differ from TNFR1 and TNFR2 expressed by eukaryotic cells. In the case of TNF-α/*Shigella flexneri* complexes, their phagocytic uptake by human and mouse macrophage cell lines has been shown to be increased two- to five-fold as compared with untreated bacteria [18].

Inflammatory DCs in adaptive immunity

In 2007, two reports clearly suggested that these monocyte-derived DCs may also be involved in the next phase of the immune response, that is, adaptive immunity. Leon et al. [19] reported that, during *Leishmania major* infection, two de novo formed DC subsets were found in popliteal LNs. One population derived from monocytes that had been recruited to the dermis and had subsequently migrated to the LNs, whereas the other population developed from monocytes directly recruited to the LNs. Among the DC subsets present in the popliteal LNs, only these two monocyte-derived subsets were infected by *Leishmania major*, suggesting a role in T-cell immunity. Although both identified DC subsets were able to promote IFN-γ production by T cells and expressed I-A^{d}-LACK complexes, only the DC subset derived from the monocytes that were first recruited to the infection site (the skin) before migration to the LNs appeared to be essential for the induction of pathogen-specific T-cell responses.

At the same time, Tezuka et al. [20] highlighted the role of inflammatory DCs in IgA production in the mucosa-associated lymphoid tissues. The authors noticed that serum IgA levels, as well as secretory IgA production in the gut, were lower in iNOS$^{-/-}$ as compared with WT mice. The authors further showed that iNOS was elevated in DCs in mucosa-associated lymphoid tissues and that these DCs resembled inflammatory DCs, as determined by their expression of TNF-α and iNOS. Interestingly, iNOS was shown to control B-cell expression of TGF-βRII as well as DC-derived APRIL and BAFF; TGF-β and APRIL/BAFF being required for T-cell-dependent and independent IgA production, respectively. The number of iNOS^{+} DCs was strongly reduced in MyD88$^{-/-}$, germ-free and TLR2$^{-/-}$, TLR4$^{-/-}$, TLR9$^{-/-}$ mice, and was associated with impaired IgA production, suggesting that iNOS-producing DCs are activated through recognition of commensal bacteria [20]. A later report demonstrated that pulmonary CD4^{+} T cell responses to inhaled spores required CCR2^{+} Ly6C^{hi} monocytes and derivative CD11b^{+} DCs [21]. In this report, Hohl et al. [21] generated a CCR2 depleter mouse using the diphtheria toxin induced cell ablation strategy and showed a reduction in the transport of *Aspergillus fumigatus* from the lungs to the draining LNs, diminished CD4^{+} T-cell priming, and impaired fungal clearance.

These reports [18–21] implicate monocyte-derived inflammatory DCs as players in the early steps of adaptive immunity, but do not formally demonstrate that these cells prime naive T cells in vivo. Additional experiments using mice lacking conventional DCs will be required to test whether inflammatory DCs transport antigen to the LNs and/or activate specific T lymphocytes. An interesting question is whether inflammatory DCs govern the development of T helper cells, as has previously been shown for conventional DCs [22].

Th2-type immunity

The analysis of the effect of the widely used alum hydroxide (alum) was the first evidence for a role of inflammatory DCs in the induction of Th2-type immunity. In a first report, Kool et al. [23] noticed that intraperitoneal injection of OVA-alum induced rapid recruitment of CD11b^{+}F4/80^{int}Ly6C^{high} "inflammatory monocytes" to the peritoneal cavity within 6 h after injection. When fluorescent OVA was mixed with alum, it could be determined that these inflammatory monocytes took up antigen in large amounts and the fluorescently labeled monocytes could be found 24 h after immunization in the mediastinal LNs, where a high proportion of the cells converted to DCs. Indeed, virtually all cells that acquired the fluorescent antigen and migrated to the LN expressed CD11c 36 h after OVA/alum i.p. injection. The authors further showed that antigen transport to, and presentation by, both inflammatory Ly6C^{high} monocytes and converted DCs in the LNs occurred when alum was injected with antigen, whereas injection of antigen alone resulted mainly in presentation by LN-resident DCs that acquired the antigen via the afferent lymph. Addition of alum adjuvant to OVA has been shown to lead to stronger, more persistent Th2 immune responses, as compared with the effect of OVA alone. [23]. Of note, the increase in DCs carrying antigenic cargo was strongly reduced in NALP3$^{-/-}$ mice, suggesting that alum, in contrast with bacteria-derived adjuvants, activates the NALP3 inflammasome rather than TLRs [24].

Finally, inhaled house dust mite extracts have been shown to induce the recruitment to MLNs of FcγRI$^+$ inflammatory type DCs that appeared to be necessary and sufficient, as APCs, for the development of Th2-type inflammation. This observation clarifies a controversy regarding the role of DCs versus basophils in Th2 priming [25–27] and suggests that basophils may amplify, rather than induce, Th2 immunity to house dust mite allergen [28].

Th1-type immunity

The observations discussed in the previous section suggest that, in some conditions (when alum is used as an adjuvant or upon intranasal administration of house dust mite antigen), inflammatory DCs may induce Th2-type immune responses. However, inflammatory DCs also appear to be critical for host resistance in several infectious models where Th1-type responses are protective. In particular, oral infection with the enteric pathogen *Toxoplasma gondii* has been shown to provoke the recruitment of CCR2$^+$ inflammatory monocytes, a process that was associated with the control of infection. These inflammatory monocytes homed to the lamina propria where they expressed IL-12, TNF-α, and iNOS, but not CD11c. These observations indirectly suggest that inflammatory monocytes may gain the capacity to trigger Th1 immunity.

The analysis of *plt* mice clearly demonstrated that inflammatory DCs can potently stimulate Th1 responses. These mice display the "paucity of lymph node T cell" mutation, that is, deletion of the *Ccl19* and *Ccl21* genes [29]. Surprisingly, although these mice have strongly reduced migration of T cells and DCs, these mice have increased numbers of antigen-specific T cells and increased delayed-type hypersensitivity responses [30]. Nakano et al. reported that the DC-subset composition was altered in *plt* LNs: the frequency of CD11b^{hi}Gr-1$^+$ inflammatory DCs was higher in resting LNs and increased considerably after immunization or viral infection, as compared with the frequencies in WT mice [30]. These CD11b^{hi}Gr-1$^+$ inflammatory DCs produced IL-12p70 upon stimulation in vitro and stimulated T-cell production of IFN-γ; their paucity in CCR2$^{-/-}$ mice correlated with much lower IFN-γ production, suggesting that blood-derived inflammatory DCs were critical for the development of Th1 responses [30]. Using an anti-mouse DC-SIGN mAb to distinguish monocyte-derived DCs from conventional DCs in tissues, Cheong et al. [31] reported that LPS rapidly recruited, to the T-cell area of LNs, DC-SIGN$^+$ cells that were distinct from other DCs and were derived from monocytes. These cells efficiently presented proteins and bacteria captured in vivo to T cells, and had the capacity to induce strong production of IFN-γ and IL-2 by CD4$^+$ T cells in vitro.

Iijima et al. [13] confirmed the critical role of monocyte-derived DCs for the generation of Th1 immunity and provided evidence suggesting that tissue-resident DCs and inflammation-induced monocyte-derived DCs may play distinct biological roles. These authors used a murine model of genital herpes infection and showed that CCR2$^{-/-}$ mice, infected intravaginally with a sublethal dose of HSV-2, had more severe pathology than WT mice. They further showed that CCR2 was required for the entry of monocytes into the vaginal tissue, by a mechanism that depended on type I IFN production (by local nonmonocytic cells), which induced expression of chemokine ligands. Of note, IFN-γ secretion by CD4$^+$ T cells in response to HSV-2 antigens was similar in WT and CCR2$^{-/-}$ mice, and the recruitment of specific effector CD4$^+$ and CD8$^+$ T cells into the infected mucosa was normal, indicating that priming, recruitment, and the effector functions of Th1 cells were intact in the CCR2$^{-/-}$ hosts. By contrast, IFN-γ levels in the vaginal mucous secretion were strongly diminished in CCR2$^{-/-}$ hosts, as compared with WT mice, suggesting that monocyte-derived APCs may be required to reactivate Th1-type cells in the virally infected tissue. In support of this conclusion, CD11b$^+$CD11c$^+$ cells, purified from vaginal tissue of WT mice at day 5 post infection, were able to activate effector CD4$^+$ T cells in culture without added antigen.

A synergy between conventional DCs and monocyte-derived DCs was also recently reported in a murine model of Salmonella infection [32]. The authors analyzed the DC populations accumulating in the T-cell zone of responding lymphoid tissue and found a rapid accumulation of F4/80$^+$ CD11c$^+$ inflammatory DCs, a higher proportion of which were infected as compared with conventional DCs. Depletion of monocytes using clodronate liposomes prevented the accumulation of monocyte-derived DCs in the T-cell zone (while sparing conventional DC accumulation), and resulted in impaired CD4$^+$ T-cell priming. Both DC populations were individually able to present the antigen acquired in vivo to CD4$^+$ T cells ex vivo and to induce the proliferation and IFN-γ production of CD4$^+$ T cells, furthermore they synergized when they were cultured together. Collectively, these observations indicate that inflammatory DCs may be involved in Th1 priming in infectious conditions.

Th17-type immunity

It is still unclear whether inflammatory DCs may trigger the differentiation of Th17-type cells. Further studies on the role of DC subsets in the lamina propria will probably help to clarify this issue. Indeed, Varol et al. [33] have shown, using a combination of conditional cell ablation and precursor cell engraftment, that CD103$^+$ CX_3CR1^- lamina propria DCs originate through a DC-committed precursor (i.e. a conventional DC) in an Flt3L-dependent way, whereas CD11b$^+$$CX_3CR1^+$ DCs derive from Ly6C$^+$ monocytes under the control of GM-CSF. Interestingly, these subsets not only have different origins, but also distinct functions. In particular, the monocyte-derived CD11b$^+$ lamina propria DCs exacerbated colitis by secreting TNF-α, as mice engrafted with *Tnf*$^{-/-}$ monocytes developed milder colitis upon dextran sulfate sodium (DSS) treatment than recipients of WT monocytes. Although the authors have not further analyzed the T helper cell activation, DSS colitis has been shown to involve Th1/Th17-mediated acute inflammation, thereby indirectly suggesting a role for inflammatory DCs in Th17 activation. Siddiqui et al. [34] recently identified a subset of E-cadherin$^+$ DCs (E-cadherin is the receptor of CD103), which

accumulated in a T-cell transfer, but not innate, model of colitis. This E-cadherin$^+$ subset arose from monocytes and produced colitogenic cytokines upon activation in vitro. The authors transferred DCs generated in vitro from bone marrow into mice undergoing T-cell-mediated colitis, and found that recipients of E-cadherin$^+$ DCs developed a more severe pathology and higher frequencies of IL-17$^+$ CD4$^+$ T cells in the intestine and the gut-associated lymphoid tissues, in comparison with recipients of E-cadherin$^-$ DCs, suggesting indirectly that a subset of inflammatory DCs may promote Th17-type responses in vivo. Moreover, in the lung, Fei et al. [35] examined the mechanisms underlying *Aspergillus*-induced neutrophilia and airway inflammation, and reported that TNF-α from inflammatory DCs acted as a molecular switch to regulate neutrophil/eosinophil influx and regulated the level of IL-17.

Finally, in 2000, a report demonstrated that CCR2 expression on host-derived mononuclear cells but not on transferred myelin oligodendrocyte glycoprotein (MOG)-specific T lymphocytes, was required for the induction of experimental autoimmune encephalomyelitis [36], but the role of inflammatory DCs was not studied. It was subsequently shown [37] that CNS glial expression of CCL2 (ligand for CCR2) was required for maximum disease development. Using chimeric mice, the authors demonstrated that CCL2 deficiency in CNS (but not leukocytes) resulted in a reduction in the number of macrophages and "myeloid" DCs expressing iNOS and TNF (presumably inflammatory DCs) in the CNS. However, equal frequencies of both IFN-γ- and IL-17-producing T cells were measured in WT and CNS-CCL2-deficient mice, suggesting that recruited inflammatory APCs do not influence experimental autoimmune encephalomyelitis by altering Th1/Th17 differentiation [37].

An interesting observation was made in humans [38]: a subset of CD14$^+$ monocytes was shown to migrate in a Boyden chamber in which human BBB-endothelial cells separate the upper and lower chambers. A total of 15% of the CD14$^+$ monocytes seeded on BBB-endothelial cells transmigrated to the lower chamber, whereas 45% were associated with Blood-brain-barrier (BBB)-endothelial cells in the subendothelial space. These endothelial-associated cells acquired a partial DC phenotype, had the ability to secrete IL-6, IL-12p70, and TGF-β, and favored the production of IL-17 or IFN-γ by CD4$^+$ T lymphocytes in an allo-MLR assay in vitro. It is interesting that endothelial-associated cells were detected in infiltrated and demyelinated areas in multiple sclerosis lesions, but not in inactive plaques. This observation, together with the presence of numerous CD4$^+$ T lymphocytes expressing IL-17 in the active lesions, may validate the biological relevance of the in vitro data and suggest that monocyte-derived DCs may polarize cytokine secretion toward a Th1 or Th17 phenotype.

Collectively, the observations noted in the sections Th2-type immunity, Th1-type immunity, and Th17-type immunity indicate that inflammatory DCs have the capacity to trigger the development of distinct Th-cell subsets. It is likely that the inflammatory stimulus (nature of the infection, adjuvant, presence of TLR ligands, or activators of inflammasomes) and the tissue microenvironment (regulatory mechanism) may determine their function in situ (Fig. 3).

CD8$^+$ T lymphocytes

Cross-presentation is critical for the induction of immunity or tolerance to antigens not expressed by DCs, that is, for tumor antigens, some viral antigens, and some autoantigens. One report has investigated the role of the cross-presentation pathway in monocyte-derived DCs, as compared with that of the classical cross-presenters CD8α$^+$ DCs [39]. The authors used a murine model of GM-CSF-dependent inflammatory peritonitis, and the spleens of the diseased mice were found to contain a population of CD11c^{int} MHC II^{hi} Ly6C$^+$ CD11b$^+$ cells. These cells, when isolated and injected intravenously with soluble OVA into OT-I mice, were able to activate OT-I T cells. Of note, the cross-presentation of soluble OVA was impaired in MR$^{-/-}$ and IRAP$^{-/-}$ mice, indicating that the endosomal pathway was critical; interestingly, distinct pathways seem to mediate cross-presentation by CD8α$^+$ DCs and by inflammatory DCs, as MR and IRAP were dispensable for cross-presentation by splenic CD8α$^+$ DCs.

Role of inflammatory versus conventional DCs

The relative role of conventional versus inflammatory DCs is still unclear but may differ quantitatively and/or qualitatively. First, inflammatory DCs may act as safeguards in the case of uncontrolled infection and be recruited to reinforce the function of conventional DCs. This sequence of events would ensure that the intensity of the immune response would be adapted to the level of infection. In favor of this hypothesis, it was shown that, in the case of infection with the highly pathogenic influenza A, excessive recruitment of inflammatory DCs promoted immune-induced pathology [40]. However, the complete elimination of these cells was also detrimental as influenza-specific CD8$^+$ T cell numbers were significantly reduced in the lungs (but not the LNs) of CCR2$^{-/-}$ animals, an observation in-line with the capacity of these inflammatory DCs to serve as APCs for CD8$^+$ T cells in the lung of mice infected with influenza A viruses. The authors showed that reducing inflammatory DC accumulation resulted in reduced mortality. It should be noted that the inflammatory DCs may replace conventional DCs that migrate from peripheral tissues or undergo apoptosis after maturation in the LNs.

Second, besides the quantitative aspect discussed in the first point, the function of conventional and inflammatory DCs may be triggered by distinct mechanisms. Host type I signaling on CD8α$^+$ DCs has been shown to be required for cross-presentation and activation of antitumor CD8$^+$ T cells [41, 42]. It may not, however, be critical for cross-priming by inflammatory DCs.

Third, there is increasing evidence that conventional DCs are critical for tolerance to self. Indeed, targeting an antigen on DCs through the DC-restricted endocytic receptor DEC-205 at the steady state (i.e. in the absence of additional stimuli) provokes a state of tolerance [43] and constitutively DC-depleted mice or mice in which DCs are defective in the uptake of apoptotic cellular antigen develop autoimmunity [44, 45]. These opposing functions

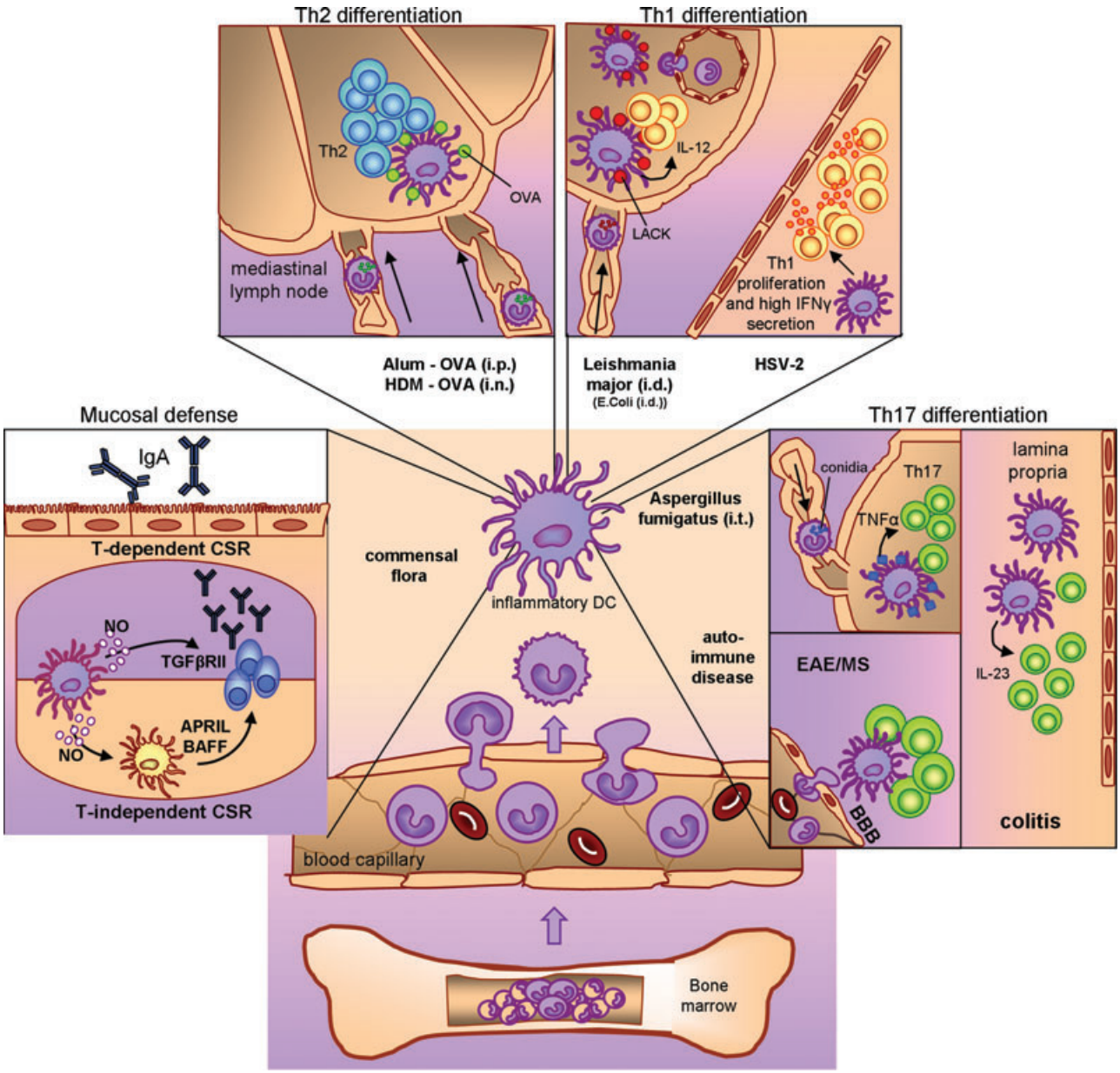

Figure 3. Examples of inflammatory dendritic cells (DCs) in adaptive immunity. A "naturally occurring" inflammatory DC population in the mucosa-associated lymphoid tissues (MALTs) produces nitric oxide (NO) upon activation by gut flora and triggers T-cell dependent and independent IgA production by B lymphocytes [20]. Monocyte-derived inflammatory DCs induce the differentiation of Th2 lymphocytes after intraperitoneal immunization with Alum-OVA [23] or intratracheal exposure to HDM-OVA [28]. Two subsets of inflammatory DCs are recruited in the popliteal LNs of *Leishmania major* infected mice, migrating directly from the blood monocytes through high endothelial venules (HEVs), or indirectly after differentiation in the infected tissues, leading to Th1 priming [19]. During Herpes simplex virus-2 (HSV-2) infection, inflammatory DCs are required to reactivate IFN-γ secretion by Th1 cells in the infected vaginal tissue [13]. Inflammatory DCs may induce Th17 responses in case of fungal infection [21] (*Aspergillus fumigatus*) promoting tumor necrosis factor-α (TNF-α) dependent pathological neutrophilia [35], autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE)/multiple sclerosis (MS) [38] and intestinal inflammation [34].

of conventional DCs, that is, their capacity to induce either immunity or tolerance, have not been described for inflammatory DCs; thus the two subsets may drive different responses.

Therefore, it seems likely that conventional and inflammatory DCs may play complementary roles in vivo and synergize in the case of infection/inflammation. Conventional DCs appear critical for tolerance to self and for triggering specific immunity, whereas inflammatory DCs are mainly involved in innate defense and in T-cell activation. Whether both cell types synergize for optimal T cell priming in vivo remains to be determined. The elucidation of the molecular mechanisms underlying the adjuvant properties of both cell types and their respective contribution in T-cell activation in vivo is an important issue for optimal vaccine design.

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Abbreviations: **CDP**: common DC progenitor · **MDP**: macrophage/DC precursor

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