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Brief Reviews

Macrophages as APC and the Dendritic Cell Myth
David A. Hume

Dendritic cells have been considered an immune cell type that is specialized for the presentation of Ag to naive T cells. Considerable effort has been applied to separate their lineage, pathways of differentiation, and effectiveness in Ag presentation from those of macrophages. This review summarizes evidence that dendritic cells are a part of the mononuclear phagocyte system and are derived from a common precursor, responsive to the same growth factors (including CSF-1), express the same surface markers (including CD11c), and have no unique adaptation for Ag presentation that is not shared by other macrophages. The Journal of Immunology, 2008, 181: 5829–5835.

The early 1970s were among the most exciting periods in the history of cellular immunology, with the recognition that T lymphocyte activation requires the processing of Ag into peptides that are presented in the context of self class I or class II major histocompatibility molecules. Several groups recognized that presentation of exogenous Ags was a specialized function of adherent cells. A novel cell population of cells in the spleen and lymph nodes appeared to be adapted to the process of Ag presentation. These cells, dubbed “dendritic cells” (DC), were nonphagocytic cells considered to be quite distinct from “macrophages.” Evolution of the DC concept required the recognition that “immature DC” internalize and store Ag and subsequently mature into effective APC. Recognition of different forms of T cell activation (Th1, Th2, Th17, Treg) necessitated the definition of DC subsets specialized to activate T cell subsets (3–6). Because Ag presentation per se could not easily be viewed in vivo, surface markers were used as surrogates to identify DC. Among these markers, in the mouse the expression of the integrin CD11c (CR4, Itgax; p150,95) on the cell surface (7) or that of a CD11c reporter transgene (8) has become a de facto definition of DC that distinguishes them from macrophages. The use of such markers, as well as the discovery of culture systems that drive the production of relatively pure populations of DC, led many to talk of DC lineage or indeed multiple DC lineages (4, 5).

The mononuclear phagocyte system (MPS) is a family of cells that comprises committed precursors in the bone marrow, circulating blood monocytes, and tissue macrophages in every organ in the body (9). Many subpopulations of macrophages have been defined based upon surface markers (10, 11). In this review, I will examine the evidence that DC are a part of the MPS and will argue that there are no pathways of development, markers, or even functions in Ag presentation that distinguish them from macrophages.

Differentiation in the mononuclear phagocyte system
The definition of the MPS was originally intended to separate cells of this family from lymphoid cells (T and B lymphocytes), granulocytes (which are polymorphonuclear rather than mononuclear), and endothelial cells (which had previously been grouped together with macrophages under the definition of the reticuloendothelial system). Each of these separations has been challenged by subsequent observations: the progenitor cells infiltrating the thymus retain myeloid potential; fetal liver can give rise to mixed colonies of macrophages and B cells during fetal development; macrophage-like cells derived from the yolk sac proliferate extensively without going through an obvious monocyte stage; and granulocytes can be transformed into macrophages and monocytes into endothelial cells (9, 12, 13). Most tissues contain substantial macrophage populations, up to 15% of total cells, that share a “dendritic” morphology, defined cellular location, and expression of surface markers and gene expression profiles (9, 12). Fig. 1A shows an example of F4/80 staining of mouse intestinal lamina propria. Note the way that F4/80-positive cells spread along the basal lamina of the epithelium. This location is typical of macrophages associated with simple epithelia throughout the body; a particularly striking example is shown in Fig. 1B in the renal medulla and many more images can be viewed at www.macrophages.com. We have recently generated transgenic animals (MacGreen mice) in which all of these cells express enhanced GFP (EGFP) by using the promoter of the csf1r gene (14). Fig. 1C shows a comparable view to Fig. 1A of the gut with this transgene, and Fig. 1D, displays a view of the lung showing the remarkable frequency of myeloid cells in this organ. Macrophages also line the entire microvasculature, in this instance spreading longitudinally along...
the vessel wall. In a movie of the capillaries of the cremaster muscle in MacGreen mice (Ref. 15 and see www.macrophages.com) we can observe these cells extending processes into the blood flow in real time. The ability of vessel-lining macrophages to sample the luminal contents was first reported in the 1950s (16, 17). A special subset of vessel-lining macrophages also extends along lymphatic vessels (not shown), and recently their importance in Ag capture in the subcapsular sinus of lymph nodes has been recognized (18).

The MPS of individual hemopoietic and lymphoid organs is diverse, with several subcompartments and functions (19). In the spleen, for example, there are interdigitating cells of the T cell areas (which some would consider the classical DC), vascular-lining macrophages of the red pulp, sinusoidal macrophages of the red pulp in direct contact with the blood (the major function of which is elimination of effect RBC), marginal zone macrophages and metallophils, subcapsular macrophages, immature monocytes derived from hemopoiesis in the red pulp, and active tingible body macrophages of germinal centers that eliminate dying T and B cells. Each of these subpopulations can be distinguished by surface marker expression. So, even in a single organ, resident tissue macrophages can be very heterogeneous. During development and in sterile wounds or tissue injury the resident macrophage is supplemented from the circulation and the activities of the inflammatory cells are biased toward phagocytosis, extracellular proteolysis, and the production of growth factors that promote growth and repair (20). Infectious agents and/or immunological stimuli attract macrophages that differ greatly depending on the precise nature of the challenge. These phenotypes have been classified into two classes of “activation”: classical activation is directed toward antimicrobial and tumoricidal effector function with the Th1 product IFN-γ being the archetypal activating factor, whereas alternative activation, in which the Th2 product IL-4 is implicated, is associated with the suppression of classical activation and responses to parasites and tumors (11). Literally hundreds of surface markers and endocytic receptors display heterogeneous expression on particular resident or activated macrophage populations or subsets. Given the diversity of tissue and inflammatory macrophages, it is misleading to identify a single typical macrophage population as a comparator to a DC in studies of Ag presentation.

CSF and the production of mononuclear phagocytes

Part of the support for DC as a separate cell type from macrophages has come from efforts to show that different growth factors promote their development. The production of mononuclear phagocytes from progenitor cells is directed by CSF, including M-CSF (CSF-1), GM-CSF, and Fms-like tyrosine kinase 3 ligand (Flt3L). Deficiency of CSF-1 in the mouse (op/op) and rat (sit/sit) highlights the importance of tissue macrophages in many aspects of normal development (21, 22). Initially, analysis of the op/op mutant suggested that CSF-1 was not required for DC development, but subsequent knockout of the CSF-1 receptor gene in the mouse produced a more penetrant phenotype (23), perhaps due in part to the existence of a second ligand for the CSF-1 receptor IL-34 (24). Both the CSF-1 and the CSF-1R mutations cause significant reductions in populations of DC and their presumptive predecessors (23, 25). CSF-1R (and the csf1r transgene) is expressed by all classical and immature DC (25, 26). Injection of CSF-1 into mice expanded the circulating CD11b/CD11c-positive mononuclear cells in blood (27) and the splenic population of CD11c-positive cells as well as plasmacytoid DC (28). As the sole stimulus in vitro, CSF-1 promotes the growth of pure cultures of proliferating macrophages from mouse bone marrow; only a subset of the resulting cells generally expresses the Ag-representing apparatus, although it can be induced to do so by IFN-γ (29). These cells resemble more closely the cells that infiltrate sterile inflammatory sites, exemplified by peritoneal exudate cells elicited by a stimulus such as thioglycollate broth (30, 31).

A key discovery in the DC field was the observation that GM-CSF (alone or in combination with IL-4) can promote the expansion/differentiation of bone marrow and blood monocytes into cells with APC potential that can be matured further with stimuli such as microbial products (32). This finding has led to numerous studies in which GM-CSF-stimulated cells are equated with DC and are contrasted to CSF-1-stimulated cells (macrophages) in terms of their ability to present Ag. Interestingly, macrophage biology groups have studied GM-CSF-stimulated cells as a model of alternative macrophage development (33). Because CSF-1 is present in the circulation and in all tissues in vivo (22) whereas GM-CSF is inducible by stimuli such as LPS or T cell mitogens, GM-CSF presumably always acts in vivo in the presence of CSF-1. One of the earliest events in the response of macrophages to any microbial agonist is the cleavage of the CSF-1 receptor mediated by a TNF-α-converting enzyme and the consequential regulation of CSF-1-dependent genes (30, 34). Cells differentiated in GM-CSF express splice variants of the CSF-1 receptor that may encode a soluble decoy receptor (35, 36). So, both GM-CSF and APC maturation signals may act in part by opposing CSF-1 action. The other key factor to consider is IFN-γ, which is a profound activator of macrophage cytoidal function and class II MHC expression and is normally produced alongside GM-CSF by activated T cells (37). IFN-γ as a sole stimulus is myelosuppressive and blocks CSF-1 action; yet, in combination with GM-CSF and CSF-1 it actually promotes mononuclear phagocyte proliferation (38).
Like GM-CSF, Flt3L can expand immature or mature mouse and human APC populations in bone marrow cultures, although the phenotypic characteristics may be somewhat distinct and, like CSF-1, it can greatly expand the population of CD11c-positive cells in spleen and lymph nodes when injected into mice (39). Knockout of Flt3L causes a significant loss of CD11c-positive cells in spleen and lymph node and lung parenchyma, although the phenotype is difficult to interpret given a more global defect in leukocyte cellularity in marrow and blood (40, 41). Interestingly, the deficiency in parenchymal DC in lung had no impact on lung-associated immune responses (42).

Macrophage deactivation is a crucial step in the resolution of all inflammatory lesions controlled by “inflammation suppressor genes” (43). As a subset of the activation response, APC activity is also likely to be switched off and be replaced by the wound repair macrophage phenotype required for resolution. Human monocyte-derived APC grown in GM-CSF and IL-4 retain the ability to reprogram to a macrophage phenotype in response to CSF-1 and, vice versa, monocyte-derived macrophages can acquire APC activity (44–47). In the mouse, all tissue DC express a CSF-1R-EGFP transgene (14, 25), and CSF-1 treatment of so-called immature DC such as Langerhans cells can maintain them in the immature state and prevent induction of CD11c, class II MHC, and costimulators (48).

In summary, MPS cells are exposed during their differentiation and migration to combinations of growth factors such as CSF-1/IL34, GM-CSF, IL-4, IFN-γ, and Flt3L, each of which contribute to their cellular phenotype. Cells grown in any one of these factors on their own probably have no counterpart in vivo, and there is no evidence to identify a growth factor that specifically supports differentiation of DC nor separates them from the MPS.

Precursors and monocyte subpopulations

Alongside efforts to identify DC-specific growth factors, there has been considerable effort to identify cells in bone marrow, blood, or tissues that are committed to becoming DC as opposed to macrophages. The lineage relationship between macrophages and DC in vivo was resolved with the identification of a common clonogenic progenitor cell in the bone marrow called the macrophage-DC progenitor (MDP). This cell is the precursor of blood monocytes and tissue macrophages and DC in many tissues (49, 50). MDP were not efficient at generating resident DC in lymphoid organs that probably proliferate locally but gave rise to the so-called inflammatory and TNF/inducible NO synthase-producing DC (26, 49, 51, 52). The conclusion that many tissue CD11c-positive cells arise from blood monocytes was supported in detailed studies of the origins of the Langerhans cells of the skin and their dependence upon CSF-1R signaling (53). In keeping with studies on MDP in the steady state, the CD11c-positive cell populations of the spleen and probably the lymph node were not replenished directly from blood monocytes (49, 50), unlike the CD11c-positive populations of intestine and lung under the same conditions. Randolph et al. (54) suggested that monocyte fate (macrophage vs DC) was determined following trans-endothelial migration, but we now recognize at least two functional monocyte subsets, distinguished by size and the level of expression of markers, such as F4/80, Ly6C (Gr-1), CD43, and the chemokine receptor CX3CR1. One of the most exciting observations in the field was the visualization of a subset of monocytes that patrol the endothelial surface (55). Some monocyte subsets may already have a distinct tendency to acquire APC activity. Jakubzick et al. (56) claimed that distinct monocyte subsets contribute to different DC populations in the lung, distinguished mainly by expression of another integrin, CD103. In the rat, the CX3CR1/CD43-high population can give rise to so-called veiled cells, the APC of afferent lymph, following transplantation (57). However, there is no strong evidence to support the existence of a committed DC-monocyte, and their derivation from monocytes places DC clearly within the definition of the MPS.

Macrophages as suppressor cells

Steinman and Witmer (58) argued that purified DC account for all of the stimulatory activity of the spleen in an allogeneic MLR. This conclusion has evolved to a point where DC has become synonymous with APC, and biochemical explanations for the superior APC activity of DC have been sought. Trombetta and Mellman (3) review the pathways for uptake, intracellular trafficking, and processing of potential Ags in APC. A central theme, based upon the work of Delamarre et al. (59), is that DC are actively phagocytic but degrade engulfed Ag more slowly and retain it for subsequent presentation to T cells. The apparent difference between DC and macrophages in Ag-processing and presentation may be mouse strain specific. The commonly used mouse strain C57BL/6 has a mutation affecting the expression of cathepsin E (60), and a knockout of cathepsin E on the 129/Sv background impairs Ag presentation by macrophages but not by DC (61). Even allowing that one can enrich for APC activity, the claim that DC are uniquely endowed with the ability to stimulate naïve T cells is fundamentally flawed. Within the much larger macrophage population there are class II-positive and class II-negative cells that are able to suppress the activation of T cells (62). Alternatively activated F4/80-positive macrophages generated in parasite infection can powerfully suppress Ag-specific T cell activation (63). Similarly, Dillon et al. (64) found that the TLR2 agonist zymosan stimulated marginal zone and red pulp macrophages and generated TGF-β-dependent suppression of T cell activation. Among the splenic myeloid population there are so-called inhibitory myeloid cells that express high levels of CSF-1R, F4/80, CD11b, and Ly6C (65, 66), and their expansion in response to CSF-1 is immunosuppressive (67, 68). Interestingly, CSF-1-dependent purified F4/80-positive myeloid suppressors can differentiate into functional CD11c-positive DC when cultivated in GM-CSF (66).

The selective removal of suppressor macrophages provides an alternative explanation for the enrichment of APC activity in DC fractions and the ability of the purified DC to act on naive cells. In fact, because APC activity in the DC fraction is actually not enriched above the total activity of spleen, the large majority of APC must reside with the “macrophages,” compromised by suppressors in the same fraction. In a typical MLR or Ag-presentation assay, populations of APC and T cell responders are all placed together in a closed system, whereas in vivo suppressors and activators may be segregated and soluble suppressors may be continuously removed. Suppressive macrophages act selectively on cells that respond to low doses of mitogen (62), and self-tolerance requires very stringent and selective
control of naive or primary T cell activation. So, to activate naive T cells efficiently, it may be necessary to remove suppressor macrophages. Aside from their ability to suppress primary T cell activation nonspecifically, class II MHC-positive macrophages contribute Ag-specific inactivation and peripheral tolerance. In mice that lacked the macrophage-specific marker protein F4/80 there was a failure of both inducible Ag-specific tolerance and oral tolerance (69). Activated CD11b on the surface of macrophages has also been found to generate an inhibitory signal to T cells (70). Recently, Denning et al. (71) purified APCs in the intestinal lamina propria. In addition to conventional DC, there was a class II MHC-positive, CD11c- and CD11b-positive cell that selectively activated CD25-negative regulatory T cells. They did not apparently isolate the F4/80-positive, CD11b-negative, class II MHC-positive, CD11c-positive population (Fig. 1A; see also Ref. 50). These cells would likely exert Ag-nonspecific suppression (72).

An additional variable to consider in studies of APC activity in vitro is the nature of the T cell response being measured. Rothoeft et al. (73) compared APC activity of human monocyte-derived DC (grown in GM-CSF) and monocyte-derived macrophages (grown in CSF-1). Both cell types clustered with naive T cells and formed an immunological synapse. The “DC” were somewhat more efficient at inducing proliferation and IL-2 and IFN-γ production, while the “macrophages” selectivity induced IL-4 production.

**Macrophages can present Ag in a primary response**

When we compare macrophages and DC in APC activity in vivo rather than in closed systems in vitro, the differences in APC activity are less compelling. Pozzi et al. (29) compared the activity of Ag-pulsed, CD11c-negative, bone marrow-derived macrophages (grown in CSF-1) and DC (grown in GM-CSF plus IL-4) by direct injection into mice. Both populations migrated to lymph nodes and activated CD8 T cell proliferation. In contrast to the lack of stimulatory activity of the bulk population of splenic macrophages, McCormack et al. (74–76) showed that cloned macrophage cells from spleen presented alloantigen to naive CD8-positive cells. Immortalized clonal macrophage cell lines (CD14, F4/80, CD11b-positive), when stimulated with IFN-γ to induce class II MHC and pulsed with Ag, could elicit Th1-polarized primary immune responses in mice (77). Moser (78) also reported that Ag-pulsed macrophages could generate primed T cells in vivo but, in comparison to DC, selectively activated Th2 responses. Finally, extending the discussion above of macrophage-mediated suppression, Guan et al. (79) reported that CSF-1-treated peritoneal macrophages exerted Ag-specific suppression of alloreactivity and autoimmune responses in mice.

**CD11c is not a DC marker in mice or humans**

The argument as to whether macrophages can present Ag is partly a semantic one, presuming that DC and macrophages are distinguishable by any marker that is not directly required for Ag-presentation. There are a number of candidate endocytic receptors, including DEC-205, DCL1, and DC-HIL/gp100, that probably function in Ag uptake (31, 80, 81). The most-studied candidate DC marker in the mouse is the integrin CD11c. There is no evidence for any direct function of CD11c in T cell activation based upon a knockout phenotype (82). CD11c in humans is a marker for the mononuclear phagocyte system (83) and an active complement receptor (CR4) that is induced during macrophage maturation (84). The original description of mouse CD11c (7) detected the Ag on interdigitating cells in T cell areas. The more recent studies of CD11c-yellow fluorescent protein transgenic mice, focused solely on the lymph node, did not support the distinction between macrophages and DC (8). In fact, CD11c in the mouse is an endocytic receptor that is widely expressed and inducible during inflammation in macrophages, granulocytes, DC, and T cells and has no direct role in T cell activation. The effect of a CD11c-null mutation on the pathology of experimental autoimmune disease is not considered to be due to any defect in Ag presentation (85).

The CD11c-positive cells of the lamina propria of the gut can extend processes between epithelial cells and sample the luminal contents (86, 87). Their location and morphology is indistinguishable from that of the F4/80-positive, CSF-1R-positive macrophages in Fig. 1. Vallon-Eberhard et al. (88) isolated intestinal lamina propria cells from rag-/- mice, and identified two major populations of myeloid cells, both expressing class II MHC and CD11c but differing in expression of CX3CR1 and CD14. Both populations expressed the macrophage marker F4/80. In the kidney, Soos et al. (89) rediscovered the interstitial network of stellate F4/80-positive cells (Fig. 1B), confirmed their expression of class II MHC, and showed that they express CX3CR1-EGFP and CD11c. In our own array studies, CD11c is highly expressed in thiglycollate-elicted peritoneal macrophages (our unpublished observations) and, as noted below, all of the lung macrophages express CD11c. Taken together, these data and many other studies indicate that CD11c is expressed by many macrophages in the mouse, as it is in human, especially on the macrophage population associated with epithelia and on all inflammatory macrophages.

***CD11c-diphtheria toxin receptor (DTR) transgenic depletion method proves only that CD11c-positive cells are involved in immunity***

A CD11c-DTR transgenic mouse was developed as a tool to especially ablate DC (90). But, diphtheria toxin administration to CD11c-DTR mice was later shown to ablate all of the marginal zone macrophage populations of spleen and the sinusoidal macrophages from the lymph nodes, as well as F4/80-positive cells from the red pulp of spleen (91). Neuenbahn et al. (92) used the model to demonstrate that CD11c-positive cells in the spleen are required for infection with the macrophage-trophic infection *Listeria monocytogenes*. Inter alia, they confirm that CD11c-EGFP from the same transgene is expressed on a network of stellate cells in the red pulp. In the lung, van Rijt et al. (93) showed that the large majority of alveolar macrophages and interstitial myeloid cells in the lung express CD11c and class II MHC and were ablated following diphtheria toxin administration in the CD11c-DTR model. They do not appear to have analyzed the substantial population of interstitial macrophages, identified by von Garnier et al. (94), that express both CD11c and F4/80 but lack class II MHC and apparently has suppressive activity.

The use of the CD11c-DTR model indicates that CD11c-positive mononuclear phagocytes are required for Ag presentation, confirms that CD11c is expressed on resident macrophages in lung and gut, and provides no evidence that this activity resides in a subset that one could classify as DC.
Markers and the infinite number of MPS subpopulations

Gene expression in mammalian cells is a stochastic process, and each individual allele at any locus has its own intrinsic probability of being induced at the level of individual cells (95, 96). As a consequence, surface markers commonly appear to divide populations of cells into two clear sets on a FACS profile. This principal is very well illustrated by a study on the APC populations of the mouse uterus, which used an extensive panel of putative macrophage and DC markers as well as class II MHC (97). Although there were three distinct populations with distinct forward and side scatter properties, namely small immature monocyte-like cells (F4/80-positive, CD11b-class II-negative), large mature macrophages (F4/80-positive, class II-positive), and DC (F4/80-negative, class II MHC positive), each population could be further subdivided. Similarly, a study of myeloid cells from the Peyer’s patch showed that the CD11c-positive cells could be subdivided based upon expression of F4/80, CD11b, FCr, CD44, and ICAM-1 (98). The only conclusion one can make from these studies is that the number of subpopulations is an exponential function of the number of markers, and no marker distinguishes DC from macrophages.

Conclusion

The literature constructs a picture of a typical DC and emphasizes its importance in every aspect of immunity (for example, see Ref. 99). Arguments are put forward to separate DC from macrophages. Even if one accepts the dogma that presentation of Ag to naive T cells is a specialized function of cells called DC, the evidence reviewed herein indicates that all macrophages are “immature DC” and all DC are “immature macrophages.” But in my view, DC as a separate cell type with unique capacity or destiny do not actually exist. They are simply a heterogeneous subset of mononuclear phagocytes. The machinery required to present Ag to T cells is not strictly coexpressed with any other marker. The plasticity of mononuclear phagocytes and their diversity may be the key to the initiation, the nature, and the outcome of cellular immunity.

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Disclosures

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