Differentiation and heterogeneity in the mononuclear phagocyte system

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Cells of the mononuclear phagocyte system (MPS) are found in large numbers in every organ of the body, where they contribute to innate and acquired immunity and homeostasis. This review considers the locations of MPS cells, surface markers that distinguish subsets of monocytes and macrophages, the pathways of MPS differentiation, and the growth factors and transcription factors that guide them. Although the number of MPS sub-populations that can be defined is infinite, the features that unite the MPS remain compelling. Those features clearly include antigen-presenting dendritic cells within the MPS and argue against any basis for separating them from macrophages.

INTRODUCTION

The concept of the mononuclear phagocyte system (MPS) is derived from research by van Furth and co-workers^{1–4} in the late 1960s and early 1970s. MPS embraces a family of cells that includes committed precursors in the bone marrow, circulating blood monocytes and tissue macrophages, and dendritic cells (DCs) in every organ in the body (reviewed in ref. ⁵ and ref. ⁶). Numerous anti-macrophage antibodies were produced, with a consequent subdivision of macrophages into many sub-populations.^{7,8} Many, but not all, tissue macrophage populations in the mouse were recognized by the anti-macrophage antibody, F4/80.^{5,9–14} The use of F4/80 led to the recognition that macrophages in tissues are numerous and they occupy a precise anatomical niche in relation to other tissue cell types.⁵

Figure 1 contains some examples of images from these studies. Macrophages have a particularly intimate relationship with epithelial and endothelial cells. In simple epithelia and throughout the capillary and lymphatic circulation, tissue macrophages spread along basement membranes; in stratified and pseudostratified epithelia such as skin, trachea, and cervix, they are integrated within the epithelium. Sinusoidal macrophages, such as those of liver, spleen, and some endocrine organs, have direct contact with the blood. But the separation by endothelium does not prevent pericapillary macrophages from extending processes into the lumen and sampling the blood contents.^{15,16} The ability of macrophages to extend processes across epithelia and into lymphatic vessels has also been recognized.^{17–19} We commonly think of macrophages as cells of the immune system and forget their central function in many other aspects of embryonic

development, homeostasis, and wound repair.^{5,6,20} As an example, the macrophages of the epidermis, known as Langerhans cells, form the center of the so-called epidermal proliferative units and control the proliferation and differentiation of keratinocytes.^{21–23} Those lining the surfaces of bone control osteoblast differentiation and calcification,²⁴ and those in the embryo can control the development and nephron endowment in the kidney.²⁵ The central importance of macrophages in development is highlighted by the many systems affected by macrophage depletion in the colony-stimulating factor-1 (CSF-1)-deficient *op/op* mouse, including somatic growth, development of the pancreas and nervous system, and male and female fertility.⁵ Resident macrophages become adapted to perform particular functions in different organs; as a result of this, brain macrophages (microglia) are very different from alveolar macrophages of the lung, Kupffer cells of the liver, or the largest tissue macrophage population of those lining the wall of the gut.

Of course, macrophages are essential for both innate and acquired immunity. A reasonable definition of a pathogen is a microorganism that evades constitutive killing by macrophages; one effector mechanism for the acquired immune system is to increase the microbicidal activity of macrophages, in the process increasing bystander damage and pathology in the host. Mononuclear phagocytes may also become adapted to present antigen to T lymphocytes and can control the precise nature of the T-cell response. A subset of MPS cells express the endocytic receptors, intracellular trafficking and processing pathways, and cell surface major histocompatibility complex (MHC) molecules required to present antigen to T lymphocytes. A subset of these

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Figure 1 Common locations and morphologies of MPS cells as defined by immunolocalization of F4/80 antigen. These sections are from tissues perfusion-fixed with glutaraldehyde and paraffin-embedded, which preserves the ramified processes of tissues of MPS cells. (a) The F4/80+ macrophages in the walls of the afferent and efferent arterioles of the juxtaglomerular complex, where blood flow to the glomerulus glom is regulated. (b) The lamina propria of the intestine, demonstrating the remarkable density of macrophages in this region. Three distinct locations can be seen; cells spread in the plane of the epithelial basement membranes indicated by the long arrow, along the walls of vessels including major vessels indicated by the short arrow and monocyte-like cells indicated by the arrowhead, which are presumably recently arrived. (c) Graphical representation of the non-random distribution of macrophages in tissues; in this case microglia spread in two dimensions in the inner plexiform layer of the retina. (d) A section of bone showing the complete absence of F4/80 from osteoclasts Ocl; the large ramified macrophages that form the center of hemopoietic islands indicated by the long arrow and macrophages within the canopy layer on the bone surface indicated by the short arrow. (e) A major role of macrophages in development; in this case a macrophage that has migrated through retina to engulf a dying neuron in the developing retina. MPS, mononuclear phagocyte system. Original studies.^{5,9-14} Other images with detailed annotation can be seen at <http://www.macrophages.com>.

cells is referred to as DCs; whether there is a genuine functional distinction between DCs and other MPS cells in terms of antigen-presenting activity has been debated elsewhere.⁵ Cells of the MPS may also act as powerful suppressors of T-cell activation and probably have a major function in the development of central and peripheral tolerance. In this review, we will consider evidence of similarities and differences between macrophage and DC differentiation; their relative importance in T-cell activation will not be considered.

HETEROGENEITY AND MARKERS IN THE MONONUCLEAR PHAGOCYTE SYSTEM

Although macrophages in tissues have many features in common, including extensive lysosomes, stellate morphology, and location relative to epithelia, they are nevertheless extremely heterogeneous in terms of function and surface marker expression. Our knowledge of this plasticity is most extensive for mouse.²⁶

Two different transgenic lines have been used to delineate the MPS: the *CX3CR1-EGFP*^{27,28} and *csf1r* transgenes; MacGreen/MacBlue.²⁹⁻³¹ Both appear to highlight the large majority of tissue cells that we would regard as belonging to the MPS, although both also express in other cells. *CX3CR1* is expressed in some endothelial and epithelial cells.²⁷ *Csf1r-EGFP* is expressed in granulocytes.³⁰ Granulocytes are excluded by definition from the MPS, being “polymorphonuclear” as opposed to “mononuclear,” although as noted below, they clearly derived from a common committed myeloid progenitor. The expression of *csf1r-EGFP* transgene in granulocytes is actually a reflection of the fact that *csf1r*, alongside many other macrophage-specific genes, is expressed at the mRNA level in these cells, but the proteins are not translated. In this and several other papers referenced therein, granulocytes could be converted to macrophages in culture.³⁰ Whether this occurs *in vivo*, in the context of inflammation, remains to be proven or disproven. The advan-

Figure 2 The uses of the MacGreen transgene. In the MacGreen transgene, the *csf1r* promoter drives the expression of EGFP. Panel a–d at left show EGFP fluorescence in spleen (a), liver (b), lung (c) and small intestine (d). Note the high frequency of positive cells that can be detected by fluorescence-activated cell sorting following enzymatic digestion of tissues. At the right, the remarkable frequency of positive cells in the embryo, especially in areas of high cell death, such as the interdigital area in the left panel and the yolk sac in the right panel. EGFP, enhanced green fluorescent protein.

tage of fluorescent transgenes is that they permit ready isolation of tissue macrophage populations (see **Figure 2**). We used this feature to isolate and characterize the large macrophage population from developing mouse embryo, which have features in common with tumor-associated macrophages.²⁵ The functions and ontogeny of macrophages in the embryo have been reviewed elsewhere.³² An interesting subset of these cells are derived from the yolk sac, do not pass through a monocyte stage, and are not affected by mutation of the macrophage-specific transcription factor, PU.1.³³ Macrophages in different adult organs differ in their gene expression profiles; for example, *csf1r-EGFP*(+) cells isolated from the lung express genes such as tartrate-resistant acid phosphatase, matrix metalloproteinase 9, and cathepsin K in common with osteoclasts (unpublished). Another widely studied MPS reporter gene is a *CD11c-GFP/YFP* transgene.³⁴ *CD11c*, along with several candidate endocytic receptors including *DEC-205*, *DC-SIGN*, *DCL1*, and *DC-HIL/gpnm* that probably function in antigen uptake, has been advocated as a marker that distinguishes DC from macrophages.^{35–37} *CD11c* in humans was originally defined as a marker for the MPS³⁸ and was subsequently shown to be an active complement receptor 4 that is induced during macrophage maturation.³⁹ The original description of mouse *CD11c*⁴⁰ showed that the antigenic epitope detected by a hamster monoclonal antibody was enriched on interdigitating cells in T-cell areas. The more recent studies of *CD11c-YFP* transgenic mice focused solely on the lymph node, where the transgene permits the study of antigen presentation and antigen-presenting cell (APC) dynamics by live imaging,

but did not provide evidence for distinction between macrophages and DCs.³⁴ Most other evidence suggests that *CD11c* is present on the large majority of macrophages associated with epithelia–mucosal surfaces. The lung contains two major macrophage populations, the alveolar and interstitial cells, that differ in many functions.⁴¹ *CD11c* appears to be expressed on both populations.⁴² The *CD11c*-positive cells of the lamina propria of the gut became especially interesting when it was shown, partly through the use of a *CD11c-EGFP* transgene, that they can extend processes between epithelial cells and sample the luminal contents^{17,18,43,44} and appear to specifically express *tlr5*.⁴⁵ Vallon-Eberhard *et al.*⁴⁶ 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 the expression of *CX3CR1* and *CD14*. Both populations expressed the macrophage marker *F4/80*. In fact, the large majority of lamina propria *F4/80*-positive/class II MHC-positive phagocytic macrophages are not active APCs, but are powerfully suppressive.⁴⁷ *CD11c* also overlaps substantially, if not completely, with *F4/80* and *CX3CR1-EGFP* in the kidney.^{9,48} Similarly, in the brain, a subset of microglia,^{49–51} and in the liver, a subset of Kupffer cells,⁵² express *CD11c* antigen or the *CD11c-EGFP/EYFP* transgene, and in both locations *CD11c* is clearly expressed/induced on inflammatory macrophages.

Molecules expressed on the cell surface are of particular functional interest because they determine the ability of MPS cells to interact with pathogens and with other cell types to generate an appropriate innate and acquired immune response.²⁶ With

the possible exception of the CSF-1 receptor,⁵³ which is cleaved selectively from the cell surface in response to Toll-like receptor signaling,^{54,55} there are no markers that are expressed specifically and ubiquitously on all MPS cells. Surface markers of mouse MPS cells can be divided into two categories; those that are heterogeneously expressed on individual cells within any one location and those that are enriched on defined functional sub-populations of cells in specific organs or locations within organs.⁵⁶ Considering the latter category, Taylor *et al.*²⁶ highlight the distinct distributions of F4/80 and various scavenger receptors and C-type lectins between white pulp, red pulp, marginal zone, marginal zone metalophil, and tingible body macrophages in the spleen. Examples of location-specific expression appear to become less well defined with more detailed observations. For example, langerin (CD207) was originally considered as a marker for Langerhans cells of the skin and appears to be necessary and sufficient for the formation of the Birbeck granules, which are characteristic of these cells.^{57,58} A *langerin-DT* transgene was created and used to demonstrate functions of Langerhans cells in contact sensitivity.⁵⁹ Subsequently, we find that CD207 is more widely expressed outside the skin and draining lymph nodes,^{60,61} and even within skin, on large populations of dermal cells that derive from blood monocytes rather than Langerhans cells.^{62,63} In addition, to make matters worse, the distribution of langerin/CD207 appears to vary between mouse strains.⁶⁴ Similarly, sialoadhesin, which was recently shown to be identical to the MOMA-1 antigen, was originally shown to be expressed most strongly on the marginal metalophilic macrophages of the spleen and the macrophages that form the centers of hematopoietic islands. However, it is also expressed on subcapsular sinus macrophages in lymph nodes, most macrophages of intestinal lamina propria, and Peyer's patch and subsets in central nervous system,⁶⁵ and is widely expressed on macrophages in the lung,⁶⁶ as well as uterus.⁶⁷

Most studies of macrophage marker expression in tissues, or on isolated MPS cell populations, have used only one or two markers and seldom examined them on the same section. One exception was a thorough study of the large dermal macrophage population,⁶⁸ which demonstrated that most coexpress *F4/80*, *CD11b*, and *mMGL* (a C-type lectin). Subsets expressed sialoadhesin, CD11c, class II MHC, and mSIGN-R1. A recent publication examined the expression of four markers, F4/80, CD68/macrosialin, CD11b, and CD11c in spleen, and normal and inflamed liver, by three-color immunofluorescence.⁵² Essentially, all combinations of single-, double-, triple-, or quadruple-positive cells were possible in different locations or circumstances. Studies on isolated myeloid cell populations or tissue sections from the skin, brain, lung, gut, ovary, testis, kidney, and lymph node/Peyer's patch^{8–10,13,41,43,47,48,51,56,60,64,67,69–88} reveal similar diversity. In any two-color immunofluorescence profile, one finds all possible null, single-, and double-positive populations. In many cases, the incidence of double-positive cells is no greater than that expected from random intersection of the two frequencies of single-positive cells. Many published studies refer to subsets of cells, e.g., CD11c (hi), as DCs and distinguish them from macrophages. But, it is not clear that there

is any one marker, or even a combination of markers, that can provide this distinction. One interesting study examined such heterogeneity in the external muscle layer of the intestine. The authors observed a regular dense planar network of cells with dendritic–stellate morphology.⁶⁰ These cells are evident in earlier studies of F4/80¹³ and class II MHC⁷⁹ immunolocalization, and are obvious in the external muscle and/or mesothelial layers of many internal organs such as bladder, gut, uterus, peritoneal wall, and diaphragm of the MacGreen transgene unpublished. Although direct comparison is not possible and there is the formal possibility that there is a parallel unrelated network of stellate cells, the large majority of these cells must express *csf1r* and *F4/80*, and by inference would be considered MPS cells. On account of the regular two-dimensional array of these cells, one can assess the relative frequency of positive cells per unit area in a flat mount. Other macrophage markers, CD14 and Fc receptors, were detected at around 60–70% of the frequency of MHC-II-positive cells, where CD11c or DEC205 was detected at 20–30% frequency, and somewhat less expressed detectable langerin CD207. Since there was no two colours analysis, this study cannot assess the extent of overlap of these markers on individual cells.

If one considers the full set of surface markers that display heterogeneous expression in macrophages, which also include the macrophage scavenger receptors CD36, CD14, SIRP α , Toll-like receptors, other integrins, EGF-TM7 proteins, other Ig superfamily receptors (Siglecs), and multiple C-type lectins,²⁶ the set of combinations and sub-populations is essentially infinite. This is especially true if one identifies subsets of cells on a fluorescence-activated cell sorting profile that express high or low levels of a marker. At least some of this heterogeneity arises because of the stochastic nature of transcriptional regulation, so that there is a genuinely random assortment of surface markers.⁸⁹ One could take the view that this presents potential pathogens with a formidable arsenal of potential host defense in which every macrophage is unique, especially as we know that some receptors that have functional polymorphisms, such as Toll-like receptor 4, are expressed monoallelically in individual cells.⁹⁰ In other cases, a particular combination of surface markers determines potential function (e.g., the antigen uptake, presenting, and costimulatory molecules such as CD80, CD86, and CD40) but even in this case, different combinations may provide different T-cell subsets with distinct signals. Surface markers such as the chemokine receptors and integrins must act in concert to determine recruitment and location in a tissue. As discussed below, monocyte subsets differ in their propensity to be recruited. There must be a degree of determination that ensures that certain sets of genes are coexpressed at the right time and place, and this in turn determines a cellular function. The appropriate timing is an important issue, because much of the heterogeneity we see in tissue reflects the fact that macrophages within the tissue are in different stages of their cycle of life and death, migration, development, and function, responding to an infinite combination of signals. Many apparent subsets, such as the CD8-positive and CD8-negative APC of spleen, are now known to be interconvertible and derived from a common progenitor.⁹¹ Similarly,

CD11b⁺ and F4/80⁺ myeloid suppressor cells in spleen can be stimulated with granulocyte macrophage (GM)-CSF to become functional CD11c-positive APCs.⁹² So, surface marker expression cannot be taken as the sole indication of lineage, function, or destiny.

DIFFERENTIATION IN THE MONONUCLEAR PHAGOCYTE SYSTEM

Committed cells within the mononuclear phagocyte lineage progress through a series of defined morphologically distinct stages; a common myeloid progenitor shared with granulocytes giving rise to monoblasts, promonocytes, and thence monocytes, which migrate into tissues.⁵ The production of mononuclear phagocytes from progenitor cells is directed by CSFs, to some extent lineage restricted and hierarchical in their actions. They include macrophage CSF (CSF-1), granulocyte-macrophage CSF (GM-CSF), and *fms*-like tyrosine kinase 3 ligand. Natural mutations in the *CSF-1* gene provided the evidence that CSF-1 is required for the production of a substantial subset of tissue macrophages in the mouse (*op/op*) and rat (*tl/tl*), at the same time highlighting the importance of tissue macrophages in many aspects of normal development.^{93,94} Initially, analysis of the *op/op* mutation suggested that CSF-1 was not required for DC development, but subsequent knockout of the *CSF-1* receptor gene in the mouse produced an even more penetrant phenotype,⁹⁵ perhaps due in part to the existence of a second ligand for the CSF-1 receptor, interleukin (IL)-34.⁹⁶ Both the CSF-1 and CSF-1R mutations do cause significant reductions in populations of CD11c-positive cells and their presumptive precursors.^{95,97} Injection of CSF-1 into mice expanded the blood monocyte and tissue macrophage populations,⁹⁸ and more recently was shown to expand circulating CD11b/CD11c-positive mononuclear cells in blood⁹⁹ and the splenic population of CD11c-positive cells as well as plasmacytoid DCs (ref. ¹⁰⁰; DA Hume, unpublished). A key discovery in the DC field was the observation that GM-CSF alone, or its combination with IL-4, can promote the expansion–differentiation of bone marrow and blood monocytes into cells with potent APC activity.^{101–108} Interestingly, combined null mutations in CSF-1, GM-CSF, and G-CSF do not completely ablate macrophage numbers or their recruitment in mice.¹⁰⁹ This may, in part, be attributable to substitution by IL-34 which acts on the CSF-1R.⁹⁶ In addition, *fms*-like tyrosine kinase 3 ligand¹¹⁰ and vascular endothelial growth factor-A,^{111,112} both of which act through tyrosine kinase receptors related to the CSF-1R, are partly responsible for age-dependent compensation of the osteopetrotic phenotype of CSF-1-deficient mice, and *fms*-like tyrosine kinase 3 ligand can expand circulating myeloid cell and CD11c-positive APC numbers.¹¹³

The cells of the MPS are produced from pluripotent progenitor cells in the bone marrow. These cells require combined stimulus from CSF-1 and factors including IL-1, IL-3, GM-CSF, and interferon- γ to produce colonies in semi-solid medium.^{114,115} Early studies of these cells¹¹⁶ demonstrated that the colony-forming cells lacked surface CSF-1 receptors and the costimulatory factors probably act to induce the receptor on the cell surface. Nevertheless, in a detailed study of the CSF-1R locus,

the promoter region was found to be in active chromatin and mRNA is expressed at low levels, in these early progenitors.¹¹⁷ Subsequent studies suggest that macrophage-specific transcription factor PU.1, which acts on elements in the promoter and a key enhancer in the first intron,^{118,119} is involved in initiating chromatin rearrangement and commitment.¹²⁰ PU.1 is not absolutely required for early macrophage differentiation,³³ and the effect of the knockout depends upon mouse genetic background,¹²¹ perhaps because its actions can be compensated by other members of the Ets family.^{119,122} The *CSF-1R* gene is the best understood MPS-specific gene, and its transcriptional regulation has been reviewed in detail elsewhere.⁹³ Like many myeloid promoters, it has a novel proximal promoter architecture, lacking a TATA box or GC-rich elements, and instead has tandemly repeated purine-rich elements. Alongside of PU.1 and other Ets factors, it is responsive to runx1 AML1 and C/EBP factors as activators, and differentiation from primitive precursors apparently involves downregulation of repressors including *c-myb*, *pax5*, *id2*, and *foxP1*. Each of the regulators that acts upon the *csf1r* has been attributed functions in myeloid lineage commitment; this area has been reviewed recently.¹²³ Alongside the genes that control what might be called the generic MPS program, there are, undoubtedly, factors that direct subsets of MPS cells to particular phenotypes. Some are surprisingly specialized. The macrophage-specific transcription factor, TFE-C, acts upon a small subset of target genes induced by IL-4, including the G-CSF receptor.^{124,125} The related transcription factor, microphthalmia-associated transcription factor, is specifically required for the maturation of osteoclasts.¹²⁶ The MafB transcription factor was attributed a role in the divergence of macrophage and DC phenotypes, but the knockout in mice had a surprisingly subtle phenotype, including selective loss of F4/80 and disordered cytoskeletal function.^{127–129} In fact, genome-wide mouse transcriptome analysis revealed that macrophages are among the most diverse single sources of mRNA¹³⁰ and express some 60–70% of all predicted nuclear proteins in different states of activation.¹³¹ So, if there is a deterministic basis for the plasticity of MPS cells, the underlying control network is exceedingly complex.

PRECURSORS OF TISSUE MONONUCLEAR PHAGOCYTES

There are a number of different issues surrounding the origins of heterogeneity in MPS, in particular the level of determination of fate during differentiation, the roles of local proliferation in tissues vs. monocyte recruitment, and the lineage relationship between macrophages and DCs. Inaba *et al.*¹⁰⁶ reported that granulocytes, macrophages, and DCs can all be found within single colonies grown in GM-CSF *in vitro*. Nikolic *et al.*¹³² showed similarly that in liquid culture, precursors of progressive commitment retained the ability to produce macrophage or DC when switched between GM-CSF- and CSF-1-containing medium. However, this does not mean that the colony-forming cells are uniform and functionally equivalent. Witsell and Schook¹³³ reported that individual colonies produced in GM-CSF or CSF-1 varied widely in their ability to respond to inflammatory stimuli and in the sets of cytokines they produced. The

common lineage relationship between macrophages and DCs *in vivo* was confirmed with the identification of a common clonogenic progenitor cell in the bone marrow called the macrophage–DC progenitor. Grafting studies indicated that this cell is an immediate precursor of blood monocytes and of tissue macrophages and DCs in many tissues.^{91,134} Macrophage–DC progenitors were not efficient at generating resident DC in lymphoid organs, but gave rise to so-called inflammatory or TipDC.^{91,135–137} 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.¹³⁸ A contrary view came from Onai *et al.*,¹³⁹ who identified a population of cells that they claimed were both clonogenic and “DC-restricted”. The flaw in this study lies in the use of an antibody against the CSF-1R (CD115) to purify the population. The antibody used, AFS98, was originally isolated on the basis of its ability to block CSF-1 signaling, and we showed recently that CSF-1 does, indeed, compete directly with the antibody for binding to cells.³⁰

In keeping with studies on macrophage–DC progenitor, in the steady state, the CD11c-positive cell populations of the spleen and probably the lymph node were not replenished directly from blood monocytes,^{91,134,140,141} supporting the view that there is a proliferating local progenitor population. By contrast, monocytes were able to replenish the CD11c-positive populations of the intestine and lung under the same conditions. The presence of locally proliferating macrophage populations has been known for many years. In fact, van Furth and Diesselhoff-den Dulk¹⁴² recognized that around half of the splenic MPS cells are derived from local proliferation in the steady state. In studies of bone marrow chimeras, using a *ROSA-26-lacZ* reporter transgene, mature monocyte–macrophages engrafted into tissues such as the spleen, liver, and lung and formed clusters suggestive of continued local proliferation. Interestingly, engraftment and replacement of tissue macrophages, especially in the brain, was relatively slow.^{143,144} This may indicate that resident macrophages occupying specific anatomical niches turn over very slowly. It could also argue in favor of local proliferation, but again earlier studies suggest that in the lung at least, there is little evidence of labeled thymidine uptake, and the half-lives of interstitial and alveolar macrophages in mice are in the order of a month.¹⁴⁵

MONOCYTE SUBSETS

The issue of whether certain monocytes have a determined fate has also been clarified recently. On the basis of a model system, Randolph *et al.*¹⁴⁶ initially suggested that monocyte fate (macrophage vs. DC) was determined following transendothelial migration. However, a major body of work now recognizes the existence within peripheral blood of at least two functional monocyte states, distinguished by size and the level of expression of markers such as F4/80, ly6C (Gr-1), CD11b, CD43, and the chemokine receptor, CX3CR1.¹⁴⁷ They are believed to be the functional equivalent of human monocyte populations distinguished based upon expression of CD14 and CD16.¹⁴⁷ The key observation in the field was based upon monocyte adop-

tive transfer experiments, in which ly6C(+) and CX3CR1(lo) monocytes were selectively recruited to the peritoneal cavity as thioglycollate-elicited exudate cells, where ly6C(–) and CX3CR1(hi) cells were more inclined to replenish resident tissue MPS populations.¹⁴⁷ Selective recruitment of the ly6C(+) inflammatory monocyte probably relates to their selective expression of the chemokine receptor, CCR2, and the adhesion molecule, P-selectin. Sunderkotter *et al.*¹⁴⁸ showed that during replenishment of blood monocyte pools following toxic liposome administration, the “inflammatory” ly6C(+) population appears first, and they argue that the two populations represent a differentiation sequence. Their studies of *in vitro* culture of bone marrow in GM-CSF or CSF-1 support this view,¹³² and in our hands, the expression of ly6C mRNA in CSF-1-stimulated bone marrow cultures is rapidly downregulated as cells become adherent (DA Hume and JA Hamilton, unpublished). As discussed in detail elsewhere¹³⁶, the ly6C(+) cells have a very short half-life and can be depleted selectively without loss of the ly6C(–) population. So, even if they are able to differentiate, the normal fate of the “immature” cells may be to leave the circulation before they have an opportunity to become “mature” or “resident” type cells.

The definition of the ly6C(–) monocytes as “resident” was confounded by subsequent findings; the visualization of a subset of monocytes that patrol the endothelial surface.¹⁴⁹ These cells were ly6C(–) and CX3CR1(hi), but in response to a microbial or other challenge they extravasated rapidly. Furthermore, these cells displayed a selective ability to produce inflammatory cytokines such as tumor necrosis factor- α . The latter finding was essentially confirmed by Burke *et al.*,¹⁵⁰ who separated blood monocytes based upon the *csf1r-EGFP* transgene and CD43 expression. This study showed that both populations are depleted from blood in response to microbial or lipopolysaccharide challenge.

The “resident” vs. “inflammatory” definition must also take account of evidence that the monocyte subsets may have different propensities to give rise to particular resident MPS populations, including DCs. Jakubzick *et al.*⁸³ claimed that the “inflammatory” monocytes repopulate a resident DC sub-population in the lung, distinguished mainly by the expression of another integrin, CD103. “Inflammatory” monocytes are certainly recognized as precursors of so-called inflammatory DCs (reviewed in ref. 136.) In rats, Yrlid *et al.*¹⁵¹ identified equivalent monocyte sub-populations, suggested that they can interconvert, and also provided evidence that the CX3CR1/CD43-high population can give rise to the so-called veiled cells, APCs of afferent lymph, following transplantation. These might be regarded as resident or inflammatory, depending upon whether the gut mucosa is considered a chronic inflammatory environment.

What is clearly the case is that blood monocytes are heterogeneous in terms of their expression of key molecules, chemokine receptors, and cell adhesion molecules that determine exactly how they will respond to stimuli that recruit them into tissues. What is not yet clear is the extent to which the monocyte subsets can be further subdivided in terms of their effector functions

and fates, and whether distinct populations are recruited to distinct stimuli and distinct locations.

CONCLUSION

MPS is a complex system that can be viewed in different ways. It can be unified based upon a common origin of the cells from pluripotent progenitors, the coexpression of certain markers, dependence upon growth factors especially CSF-1, and common location and morphology of the cells of the system. It can be divided into essentially infinite subsets because of the plasticity of gene expression profiles and the incredible diversity of different stimuli that MPS cells can encounter and respond to (reviewed in refs. ^{5,6}). As in so many intellectual pursuits, whether one sees MPS subsets as useful constructs depends in some measure upon whether you are a lumpster or a splitter,¹⁵² and arguments for splitting are generally more contentious. In facing this challenge, it is worth remembering that the MPS functions as a system within individual organs and indeed within the whole body; a diverse population of interacting cells. We may not gain insight or therapeutic benefit by focusing on arbitrarily defined sub-populations.

DISCLOSURE

The authors declare no conflict of interest.

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