Homeostasis in the mononuclear phagocyte system

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The mononuclear phagocyte system (MPS) is a family of functionally related cells including bone marrow precursors, blood monocytes, and tissue macrophages. We review the evidence that macrophages and dendritic cells (DCs) are separate lineages and functional entities, and examine whether the traditional view that monocytes are the immediate precursors of tissue macrophages needs to be refined based upon evidence that macrophages can extensively self-renew and can be seeded from yolk sac/foetal liver progenitors with little input from monocytes thereafter. We review the role of the growth factor colony-stimulating factor (CSF)1, and present a model consistent with the concept of the MPS in which local proliferation and monocyte recruitment are connected to ensure macrophages occupy their well-defined niche in most tissues.

Introduction

The MPS was originally defined as a family of cells derived from a pluripotent progenitor in the bone marrow and includes committed bone marrow progenitors, blood monocytes, and tissue macrophages; the latter comprising around 10% of total cells in every organ in the body [1,2]. MPS cells share many features, notably their phagocytic activity, but are also extremely plastic in their patterns of gene expression, defying identification based upon surface markers [3]. The proliferation and differentiation of MPS cells is controlled by macrophage CSF1 and interleukin (IL)-34, acting through a common receptor, CSF1R [4]. The traditional view of the MPS is that the major proliferative compartment is within the progenitors in the marrow, whereas blood monocytes provide the immediate precursors to replace tissue macrophages. This view has been challenged by several recent studies that, in effect, divide the MPS into separate cell lineages arising at different stages of embryonic development. The relationship of DCs with the MPS continues to be controversial; overlap of function and marker expression with monocyte-derived cells has made these cells difficult to delineate from macrophages, particularly in nonlymphoid tissues. Recent advances have provided evidence of a

distinct precursor for DCs that would allow their definition as a separate haematopoietic lineage. It is therefore timely to ask whether these findings necessitate a complete revision of the MPS concept.

The complex relation between DCs and macrophages

One challenge to the concept of the MPS was the identification of the antigen-presenting DCs. Should these cells be included in the MPS, and if not, how can they be distinguished from macrophages? Efforts to define DCs have been based on function, molecular markers, and dependence upon growth and transcription factors. Each of the criteria are discussed below.

The definition of a macrophage, or a mononuclear phagocyte, as a professional phagocyte is relatively straightforward and owes its origins to Metchnikoff. When first described, DCs were obviously distinct, nonphagocytic by definition, and corresponded to the interdigitating cells in T cell areas in lymphoid organs and migratory cells in afferent lymph. We refer to these cells defined by Steinman and Cohn as 'classical' DCs, while recognising that even this strict definition may in some circumstances combine several cell types of different developmental origin. With time, the definition of classical DCs in the mouse expanded to include phagocytes, and was subdivided into numerous functional subsets, including crosspresenting, migratory, myeloid, lymphoid, tolerogenic, and inflammatory or TipDCs, that differ in expression of surface markers including CD11c, CD8, CD103, F4/80, and CD11b, and may also differ between tissues [5]. DCs are believed to have a unique ability to present antigen to naïve T cells; this has in turn led to the implicit view that any cell with antigen-presenting cell (APC) activity is a DC by definition. The alternative view is that subsets of macrophages can also present antigen to naïve T cells [6]. This remains a divisive issue in immunology. As noted by Randolph [7], the question effectively divides the community into the macrophage and DC believers, who do not communicate because they disagree on basic principles.

The view that classical DCs are unique as APCs requires a definition of a classical DC. That in turn depends upon the identification of markers to distinguish clearly all classical DCs from all macrophages. As reviewed previously, it is not clear that any surface marker fulfills this criterion, partly because no one marker is perfectly correlated with any other [3,6,7]. Recent systematic analysis of large gene expression arrays by the ImmGen Consortium identified molecular markers that distinguish DCs from a

set of prototypical macrophages, and similarly identified macrophage-specific markers, notably the Fc receptor CD64 and the signalling molecule Mer tyrosine kinase receptor (MerTK) [8,9]. Re-analysis of the same data, and a large meta-analysis of many datasets in the public domain for both mice and humans, using a network clustering approach, did not support the identification of these definitive DC or macrophage markers [10–12]. Instead, that analysis divided the myeloid APCs into two major clusters based upon selective expression of genes associated with endocytosis and lysosomal degradation. On the basis of this analysis, rather than distinguishing DCs from macrophages, the authors suggested a dichotomy between phagocytic APCs (antigen-presenting macrophages) and nonphagocytic APCs (classical DC). The phagocytic APCs included cells derived from the growth of bone marrow in CSF2 [granulocyte–macrophage (GM)-CSF], which have been termed bone-marrow-derived DCs (BMDCs). Many of the transcription factors associated with classical DCs, such as helix–loop–helix (HLH) transcription factor inhibitor of DNA binding or differentiation 2 (Id2), zinc finger and BTB domain containing 46 (zbtb46), nuclear factor, interleukin 3 regulated (Nfil3), and basic leucine zipper transcription factor, ATF-like 3 (BATF3), may act primarily as repressors of phagocytic differentiation (reviewed in [13]), and have been found to be functionally redundant for generation of CD8+ DCs in bone marrow reconstitution experiments [14]. So, one might take the view that a classical DC is the default option in MPS differentiation when the phagocyte gene expression cluster is repressed. Zbtb46 has been suggested as a definitive classical DC marker in several studies (e.g., [15]), but it does not fall within a DC-specific gene expression cluster. The proposed functional divide between macrophages and DCs as APCs has been further compromised with the recent recognition that blood monocytes can enter tissues and capture and transport antigen to lymph nodes without acquiring characteristics of classical DCs [16].

A separate question from the functional distinction is whether classical DCs, if they can be strictly defined, constitute a distinct cell lineage. Macrophages and granulocytes are clearly both phagocytes, and share a committed GM progenitor, but are commonly regarded as separate lineages, even though they may be interconverted [17]. Indeed, a recent paper claimed that granulocytes can also differentiate into DCs (or perhaps more accurately, active APCs) [18]. Classical DCs certainly share growth factors and committed progenitors with monocytes and macrophages. The differentiation of precursors of both macrophages and classical DCs is regulated by stem cell factor (SCF/c-kit), macrophage CSF (CSF1), IL-34, GM-CSF (CSF2), granulocyte CSF (CSF3), IL-3, and fms-related tyrosine kinase ligand. Flt3 ligand administration to mice produces a selective increase in the numbers of classical DCs in lymphoid and peripheral tissues [19], whereas CSF1 can expand tissue macrophage populations [20]. As discussed below, antibodies against the receptors for these factors are commonly used to identify progenitors, but there is considerable redundancy in their actions and the factors interact in complex ways. Mutations in CSF1 or IL-34, or their shared receptor (csf1r, CD115) cause a

substantial reduction in particular tissue macrophage populations, including bone-resorbing osteoclasts, microglia, and Langerhans cells [21–23]. A CSF2, or CSF2R knockout (KO) had a selective effect on lung macrophage populations, corresponding to the human disease, alveolar proteinosis [24–26]. CSF1 also contributes to lung macrophage populations, as evident from the impact of the double KO with CSF2 [24]. Mutations of SCF/c-kit or Flt3L had severe effects on haematopoiesis, reflecting expression of their receptors on multipotent progenitors. Mutation of Flt3L produces a selective loss of both lymphoid and nonlymphoid tissue classical DCs [27,28], whereas CSF2R deletion produces a loss in many tissues of a subset of classical DCs defined by the CD103 (Itgae) surface marker. There was less effect of CSF2R deletion on DCs that express CD11b [25]. In mouse marrow, a high proliferative potential macrophage colony-forming unit requires costimulation with CSF2 or IL-3 plus CSF1 [29]. There are likely to be other factors that substitute for CSF2 or IL-3, because mice that lack the common receptors for these two factors still produce macrophage and classical DC progenitors [25]. Similarly, although CSF1R is expressed at low levels on progenitors, whereupon it can influence fate decision [30], it is not essential for monocyte generation [31]. Taken together, the data do not provide a definitive view of the separation of macrophage and DC lineages based upon growth-factor dependence, but do suggest a broad distinction between cells that depend upon Flt3L and/or GM-CSF versus those that depend upon CSF1 (or IL-34).

Accordingly, the growth factor receptors, c-kit (CD117), Flt3 (CD135) and Csf1r (CD115) have been used as markers to purify bone marrow progenitor cells [32]. The DC/macrophage lineages were unified through the identification of a shared clonogenic progenitor, the macrophage and DC precursor (MDP) [33] (Figure 1A). These cells, purified based upon coexpression of Csf1r and Flt3, gave rise to classical DCs, plasmacytoid DCs, monocytes, and macrophages following adoptive transfer [34]. The concept of ontogenically distinct precursors of DCs and macrophages arose upon identification of a committed dendritic cell progenitor, the common dendritic cell progenitor (CDP), in the bone marrow, that did not give rise to monocytes upon intravenous adoptive transfer, but was proposed to derive from the MDP [35] (Figure 1A). A circulating precursor that seeded classical DCs and not macrophages/monocytes, termed the pre-DC, was subsequently identified following a similar adoptive transfer strategy [5], and indeed, fate-mapping studies indicate that classical DCs are not derived from mature monocytes but from this phenotypically distinct pre-DC (Figure 1A). Jakubzick *et al*. [36] first used a lysM-cre dependent reporter strain to demonstrate that resident classical DCs in lymphoid tissues are unlikely to derive from monocytes. More recently, Schraml *et al*. [37] have reported that the C-type lectin domain family 9, member A (CLEC9A) is expressed in CDP and pre-DC but not MDP or several conventional DC populations. Subsequent fate-mapping of CLEC9A-expressing cells using *Clec9a-cre* mice showed progeny within classical lymphoid and nonlymphoid tissue DC subsets, but not in blood monocytes, plasmacytoid DCs, nor in