CORRECTED 3 DECEMBER 2010; SEE LAST PAGE

Development of Monocytes, Macrophages, and Dendritic Cells

Frederic Geissmann,[1]* Markus G. Manz,[2] Steffen Jung,[3] Michael H. Sieweke,[4] Miriam Merad,[5] Klaus Ley[6]*

Monocytes and macrophages are critical effectors and regulators of inflammation and the innate immune response, the immediate arm of the immune system. Dendritic cells initiate and regulate the highly pathogen-specific adaptive immune responses and are central to the development of immunologic memory and tolerance. Recent in vivo experimental approaches in the mouse have unveiled new aspects of the developmental and lineage relationships among these cell populations. Despite this, the origin and differentiation cues for many tissue macrophages, monocytes, and dendritic cell subsets in mice, and the corresponding cell populations in humans, remain to be elucidated.

White blood cells, or leukocytes, are a diverse group of cell types that mediate the body's immune response. They circulate through the blood and lymphatic system and are recruited to sites of tissue damage and infection. Leukocyte subsets are distinguished by functional and physical characteristics. They have a common origin in hematopoietic stem cells and develop along distinct differentiation pathways in response to internal and external cues. The mononuclear phagocyte system represents a subgroup of leukocytes originally described as a population of bone marrow–derived myeloid cells that circulate in the blood as monocytes and populate tissues as macrophages in the steady state and during inflammation (*1*). In different tissues, they can show substantial heterogeneity with respect to phenotype, homeostatic turnover, and function. The discovery of dendritic cells (DCs) as a distinct lineage of mononuclear phagocytes, specialized in antigen presentation to T cells and the initiation and control of immunity (*2*), revealed additional roles of these cells in shaping the immune response to pathogens, vaccines, and tumors, as well as additional heterogeneity. Whereas a detailed map of the relationship between monocytes and DCs and their progenitors begins to emerge, other areas like the origin and renewal of tissue macrophage subsets remain less defined.

Monocytes

Monocytes (Fig. 1A) circulate in the blood, bone marrow, and spleen and do not proliferate in a steady state (*3*, *4*). Monocytes represent immune effector cells, equipped with chemokine receptors and adhesion receptors that mediate migration from blood to tissues during infection. They produce inflammatory cytokines and take up cells and toxic molecules. They can also differentiate into inflammatory DCs or macrophages during inflammation and possibly, less efficiently, in the steady state. Migration to tissues and differentiation to inflammatory DCs and macrophages are likely determined by the inflammatory milieu and pathogen-associated pattern-recognition receptors (*5*).

Macrophages

Macrophages (Fig. 1A) are resident phagocytic cells in lymphoid and nonlymphoid tissues and are believed to be involved in steady-state tissue homeostasis, via the clearance of apoptotic cells, and the production of growth factors. Macrophages are equipped with a broad range of pathogen-recognition receptors that make them efficient at phagocytosis and induce production of inflammatory cytokines (*6*). The developmental origin and the function of tissue macrophage subsets, such as microglia (macrophages in the central nervous system), dermal macrophages, and splenic marginal zone and metallophilic macrophages remain insufficiently understood.

Classical DCs

Classical DCs (cDCs) are specialized antigen-processing and -presenting cells, equipped with high phagocytic activity as immature cells and high cytokine-producing capacity as mature cells (*7*, *8*). Although present in human circulation, cDCs are rare in mouse blood. cDCs are highly migratory cells that can move from tissues to the T cell and B cell zones of lymphoid organs via afferent lymphatics and high endothelial venules. cDCs regulate T cell responses

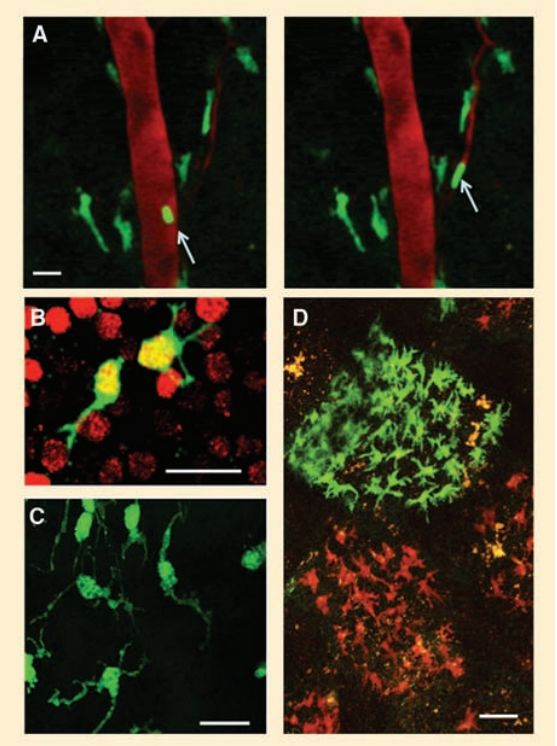


Fig. 1. (**A**) Still frames from time-lapse intravital confocal microscopy of a crawling monocyte (arrow) and perivascular macrophages in the dermis. Scale bar indicates 20 μm, initial magnification 20×. [Credit: F. Geissmann; for details, see (*52*)] (**B**) Dividing LCs in the epidermis. Scale bar, 20 μm; initial magnification 20×. [Credit: I. Chorro and F. Geissmann; for details, see (*35*)] (**C**) Confocal micrograph of aortic whole mount from a *Cx3cr1*$^{GFP/GFP}$ *Apoe*$^{-/-}$ mouse viewed from endothelial side. Scale bar, 20 μm; initial magnification 20×. [Credit: K. Ley] (**D**) Intravital two photon microscopic image of intestinal villi of CD11c^{+} cell–depleted mice reconstituted by grafts of bone marrow monocytes expressing red and green fluorescent reporters and yielding red and green fluorescent lamina propria cells, respectively. Scale bar, 20 μm; initial magnification 40×. [Credit: S. Jung and G. Shakhar; for details, see (*26*)]

[1]Centre for Molecular and Cellular Biology of Inflammation, Division of Immunology, Infection, and Inflammatory Diseases, King's College London, Great Maze Pond, London SE1 1UL, UK. [2]Division of Hematology, Department of Internal Medicine, Zürich University Hospital, CH-8091 Zürich, Switzerland. [3]Department of Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel. [4]Centre d'Immunologie de Marseille-Luminy (CIML), Université Aix Marseille, Campus de Luminy, Case 906, 13288 Marseille Cedex 09, France; Institut National de la Santé et de la Recherche Médicale (INSERM), U631 Marseille, France; Centre National de la Recherche Scientifique (CNRS), UMR6102, Marseille, France. [5]Department of Gene and Cell Medicine and Institute for Immunology, Icahn Medical Institute, Mount Sinai School of Medicine, New York, NY 10029, USA. [6]Division of Inflammation Biology, La Jolla Institute for Allergy and Immunology, La Jolla, CA 92037, USA.

*To whom correspondence should be addressed. E-mail: frederic.geissmann@kcl.ac.uk (F.G.); klaus@liai.org (K.L.)

Downloaded from www.sciencemag.org on May 7, 2012

PETITIONER EXHIBIT 1069
Page 1 of 7

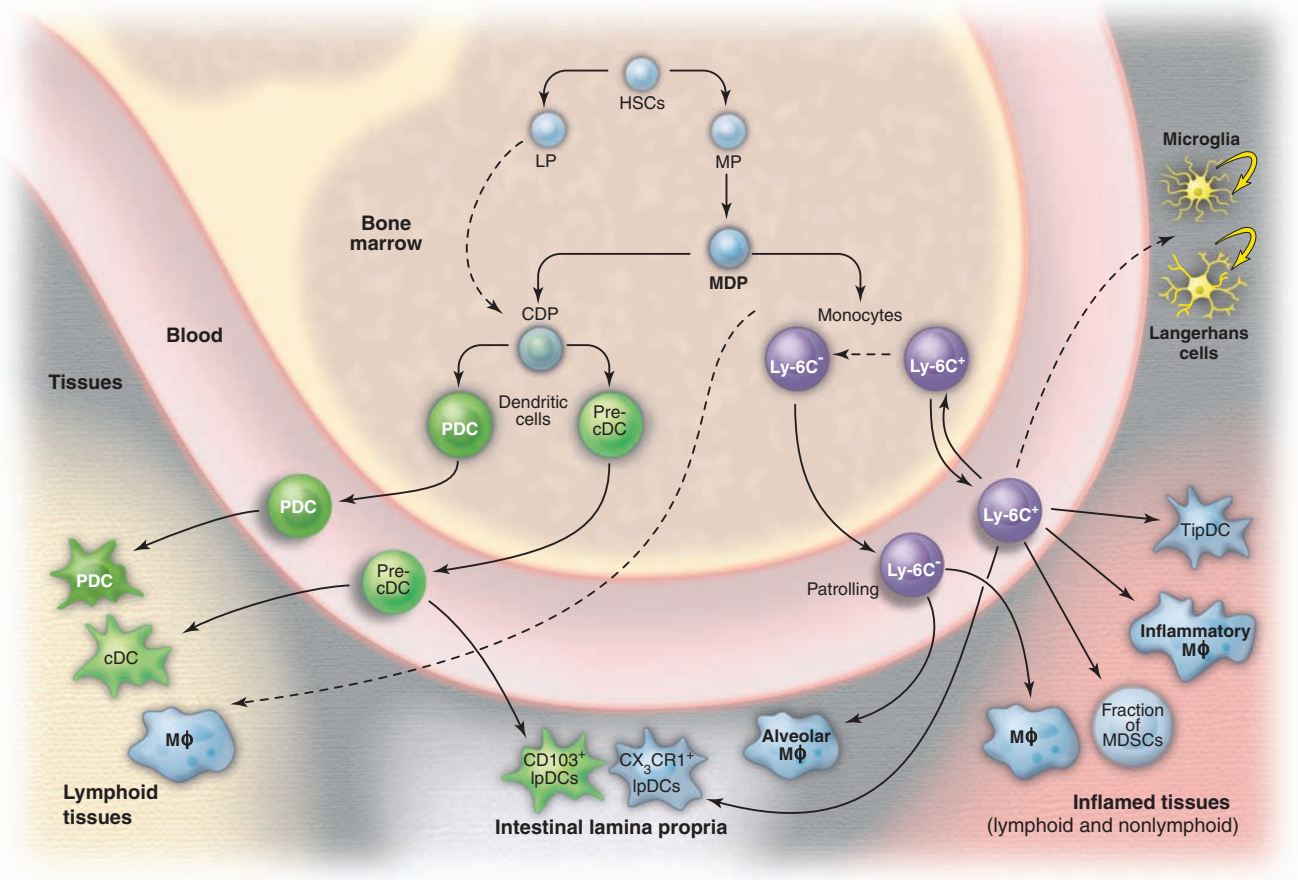


Fig. 2. Differentiation of DCs and macrophages in mice. In the bone marrow, hematopoietic stem cells (HSCs) produce myeloid (MP) and lymphoid (LP) committed precursors. MPs give rise to monocyte, macrophage, and DC precursors (MDPs). MDPs give rise to monocytes, some populations of macrophages, and common DC precursors (CDPs). Two monocyte subsets, Ly-6C$^+$ and Ly-6C$^-$, leave the bone marrow to enter the blood. CDPs give rise to preclassical dendritic cells (pre-cDCs) and plasmacytoid dendritic cells (PDCs). Pre-cDCs circulate in blood and enter lymphoid tissue, where they give rise to CD8α^+ and CD8α^- cDCs, and nonlymphoid tissues, where they may give rise to CD103$^+$ lamina propria DCs (lpDCs). Under homeostatic conditions, Ly-6C$^-$ monocytes may contribute to alveolar macrophages (MΦ), and Ly-6C$^+$ monocytes can become CX$_3$CR1$^+$ lpDCs in nonlymphoid tissues. During inflammation, Ly-6C$^+$ monocytes give rise to monocyte-derived DCs, for example, tumor necrosis factor (TNF) and inducible nitric oxide synthase (iNOS)–producing dendritic cells (TipDCs), inflammatory macrophages, and may contribute to myeloid-derived suppressor cells (MDSCs) associated with tumors. They are also suspected (dashed arrow) to contribute to microglia and Langerhans cells in selected experimental conditions. Microglia and Langerhans cells can renew independently from the bone marrow (curved arrow). HSCs can also leave their bone marrow niche (dashed arrow) and enter peripheral tissues, where they differentiate to myeloid cells during inflammation. It is unclear at this time whether LPs contribute substantially to PDCs and cDCs (dashed arrow).

both in the steady state and during infection. They are generally short-lived and replaced by blood-borne precursors (*9*, *10*). Of note, they are distinct from Langerhans cells (Fig. 1B) (DCs found in the epidermis), which are not replaced by blood-borne cells at steady state (*11*). Individual myeloid cell populations may share features of DCs and macrophages and can be difficult to ascribe to one or the other cell type (Fig. 1, C and D).

Plasmacytoid DCs

Plasmacytoid DCs (PDCs) differ from cDCs in that they are relatively long-lived and a proportion of them carry characteristic immunoglobulin rearrangements (*12*). They are present in the bone marrow and all peripheral organs. PDCs are specialized to respond to viral infection with a massive production of type I interferons (IFNs); however, they also can act as antigen-presenting cells and control T cell responses (*13*).

The development of the mononuclear phagocyte system is controlled by cytokines: small secreted proteins that promote cell-cell communication and can act as growth and differentiation factors. The generation of monocytes and macrophages, and to some extent DCs, is dependent on the cytokine and hematopoietic growth factor receptor Csf1r (also known as c-fms, M-CSFR, and CD115), expressed in monocytes, macrophages, and mononuclear phagocyte precursors (*14*–*17*). Characterization of *op/op* mice, a spontaneous mutant lacking a functional *Csf1* gene, has revealed both the role of Csf1 in the development of mononuclear phagocytes and also their broad functions (*17*). The known ligands of Csf1r, Csf1/M-CSF (*18*) and interleukin (IL)-34 (*19*), are likely both important for the development of the mononuclear phagocyte lineage, because M-CSF–deficient mice have a milder phenotype than Csf1r-deficient mice. Another cytokine receptor closely related to Csf1r, fms-related tyrosine kinase 3 (FLT3, also known as Flk2) receptor, is critical for the development of cDCs and PDCs (*10*, *20*–*22*).

Following on the early work on Csf1r, a large amount of work has been devoted in the past 20 years to investigating the origin and differentiation pathways of monocytes, macrophages, and DCs. This recently culminated in a series of studies that unveiled what is likely to be the main pathway for the develop-

Downloaded from www.sciencemag.org on May 7, 2012

PETITIONER EXHIBIT 1069
Page 2 of 7

ment of DCs, monocytes, and macrophages from bone marrow progenitors both in the steady state and during inflammation.

What Are the Precursors of Monocytes, Macrophages, and DCs?

The use of multicolor fluorescence-activated cell sorting has allowed identification of progenitors and differentiated cell populations on the basis of the expression of multiple cell surface proteins (table S1). These cells can then be isolated from mouse bone marrow, blood, or spleen and grafted into recipient mice to determine lineage relationships. This methodology was recently used to delineate the DC developmental program in vivo (*3*, *9*, *23*–*26*). Current models propose that blood monocytes, many macrophage subsets, and most DCs originate in vivo from hematopoietic stem cell–derived progenitors with myeloid-restricted differentiation potential (Fig. 2). Successive commitment steps in the bone marrow include common myeloid progenitors (CMPs), granulocyte-macrophage precursors (GMPs), and macrophage/DC progenitors (MDPs). MDPs are a subset of proliferating cells in the bone marrow that share phenotypic characteristics with myeloid precursor populations and give rise to many macrophages and DC subsets (*24*) (table S1) but largely cannot differentiate into granulocytes, another cell type of the myeloid lineage. Within the bone marrow, MDPs differentiate to monocytes and to the common DC precursors (CDPs).

The CDPs are proliferating cells that differentiate into PDCs and the precursors for cDCs (pre-cDCs) in the bone marrow but have lost the potential to give rise to monocytes (*9*, *23*, *27*). At steady state, pre-cDCs are found in the bone marrow, blood, and spleen (*9*, *28*). They enter lymph nodes from the blood through high endothelial venules and integrate into the DC network, where they acquire a mature cDC surface phenotype and morphology. cDC development also involves cell division in lymphoid organs, which is controlled in part by regulatory T cells (*9*), Flt3 (*10*), and the lymphotoxin-β receptor (*29*). Pre-cDCs can also differentiate into $CD103^+$ mucosal DCs in the lamina propria (*26*, *30*).

Monocytes exit to the blood and can enter tissues under inflammatory conditions. They give rise to subsets of macrophages and to inflammatory DCs that share many of the phenotypic features and functions of DCs, such as the ability to process and present antigen to T cells (*3*, *5*, *26*, *31*, *32*); however, it is now widely accepted that monocytes do not give rise to cDCs and PDCs (*9*, *24*).

Are There Exceptions to the Classical Developmental Pathways?

Although many macrophages and DC subsets are renewed from bone marrow progenitors, there are notable exceptions. For example, neither microglia nor Langerhans cells (LCs) are dependent on the bone marrow for their renewal in the steady state and possibly during inflammation (*33*, *34*). LCs were recently shown to develop from an embryonic precursor that colonizes the epidermis before birth, differentiates in situ, and then proliferates during the first week of life to establish the LC network (*35*). Adult LCs self-renew in situ and can massively proliferate during inflammation (*35*). Thus, epidermal LCs appear to be their own progenitors. In contrast, hematopoietic stem cells and precursors recirculate between the periphery and the bone marrow in adults (*36*) and may contribute directly to the differentiation of certain myeloid cells in inflamed tissues (*37*). Lymphoid-restricted progenitors contribute little to peripheral lymphoid organ or tissue DCs, with the possible exception of the thymus. There, developing T cells and potentially a proportion of thymic macrophages, DCs, PDCs, and B cells might be derived directly from blood-borne early thymocyte progenitors (*38*, *39*).

What Are the Origins of Nonlymphoid Tissue Resident Dendritic Cells?

Recent studies have established the existence of two distinct DC populations in the lung and dermis [reviewed in (*34*)], which are characterized as $CD103^+$ and CX_3CR1^+ DCs. Likewise, recent studies have revealed phenotypically definable $CD11c^+$ $CD103^+$ and CX_3CR1^+ subpopulations in the intestinal lamina propria (*26*, *30*, *40*) (table S1), which are derived from pre-cDCs and $Ly6C^+$ monocytes, respectively (*26*). Mice reconstituted exclusively with CX_3CR1^+ lamina propria cells developed severe intestinal inflammation when challenged in an innate colitis model (*26*). Thus, the balance of lamina propria DC subsets is likely important for gut homeostasis. Of note, only $CD103^+$ cells emigrate from the lamina propria to the mesenteric lymph nodes (*30*, *41*). CX_3CR1^+ cells are poor T cell stimulators (*41*) and thus may represent lamina propria macrophages rather than DCs.

What Is the Developmental Relationship Between Mature Monocyte Subsets?

Differentiation of MDPs into monocytes and macrophages is less well understood than their differentiation into DCs. Among differentiated "mature" monocytes in the bone marrow, blood, and spleen, at least two phenotypic and functional subsets can be identified [reviewed in (*3*)], whose developmental relationship is still unclear. Adoptive transfer experiments demonstrate that bone marrow $Gr1^+$/$Ly\text{-}6C^{high}$ monocytes can shuttle between the blood and the bone marrow and lose Ly-6C expression (*25*, *42*), suggesting that they give rise to $Gr1^-$/$Ly\text{-}6C^{low}$ monocytes. However, neither a genetic defect in nor antibody-mediated depletion of $Gr1^+$/$Ly\text{-}6C^{high}$ monocytes affected the generation of $Gr1^-$/$Ly\text{-}6C^{low}$ monocytes (*43*–*46*). When bone marrow is used as a source of monocytes, current adoptive transfer protocols may not always distinguish mature $Gr1^+$/$Ly\text{-}6C^{high}$ monocytes from their proliferating precursors because pre-monocytes are ill-defined. Therefore, a better characterization of monocyte precursors and new lineage tracking studies will be needed to establish the developmental relationship between monocyte subsets.

Are Monocytes Effector Cells or Circulating Precursors?

Monocytes have long been considered as a developmental intermediate between bone marrow precursors and tissue macrophages. It is now clear, however, that many DCs and tissue macrophages do not originate from monocytes in a steady state. Conversely, monocytes carry out specific effector functions during inflammation (*3*). "Inflammatory" $Gr1^+$/$Ly\text{-}6C^{high}$ monocytes are referred to as such because they give rise to macrophages and DCs in a variety of infectious models. During infection with *Listeria monocytogenes*, $Gr1^+$/$Ly\text{-}6C^{high}$ monocytes differentiate into DCs that produce inflammatory mediators such as tumor necrosis factor α, nitric oxide, and reactive oxygen species (known as TipDCs) (*5*, *47*). $Gr1^+$/$Ly\text{-}6C^{high}$ monocytes are required for resistance to the pathogen *Toxoplasma gondii* but in this case differentiate into mucosal macrophages, which differ in surface phenotype and inflammatory mediator production from TipDCs (*48*, *49*). In some tumors, up to half of the myeloid-derived suppressor cells [cells that can protect tumors from immune attack (*50*)] are $Gr1^+$/$Ly\text{-}6C^{high}$. Upon spinal cord injury, recruited $Gr1^+$/$Ly\text{-}6C^{high}$ monocytes differentiate into macrophages that critically contribute to the recovery (*51*). Thus, $Gr1^+$/$Ly\text{-}6C^{high}$ monocytes can differentiate into a variety of macrophages and DCs subtypes that can both activate and inhibit the immune response, depending on local or systemic cues received and the nature of encountered pathogen.

Are $Gr1^-$/$Ly\text{-}6C^{low}$ Monocytes a New Cell Subset with a Role in Tissue Repair?

$Gr1^-$/$Ly\text{-}6C^{low}$ monocytes represent a functionally distinct monocyte subset (*52*). They are characterized by a smaller size, lack of expression of CCR2 and L-selectin, but higher expression of the chemokine receptor CX_3CR1 and the integrin LFA-1 (*31*) (table S1). $Gr1^-$/$Ly\text{-}6C^{low}$ monocytes were initially termed "resident" in mice because of their longer half-life in vivo and their localization to both resting and inflamed tissues after adoptive transfer. Intravital microscopy revealed that $Gr1^-$/$Ly\text{-}6C^{low}$ monocytes exhibit long-range crawling on the luminal side of the vascular endothelium and are ideally located to survey endothelial cells and surrounding tissues for damage or infection (*52*). Moreover, $Gr1^-$/$Ly\text{-}6C^{low}$ monocytes are recruited at a late phase to the ischemic myocardium, where they express vascular-endothelial growth factor (VEGF), and are proposed to promote healing via myofibroblast accumulation, angiogenesis, and deposition of collagen (*53*). Transcriptional profiling of extravasated monocytes retrieved from the peritoneum

Downloaded from www.sciencemag.org on May 7, 2012

PETITIONER EXHIBIT 1069
Page 3 of 7

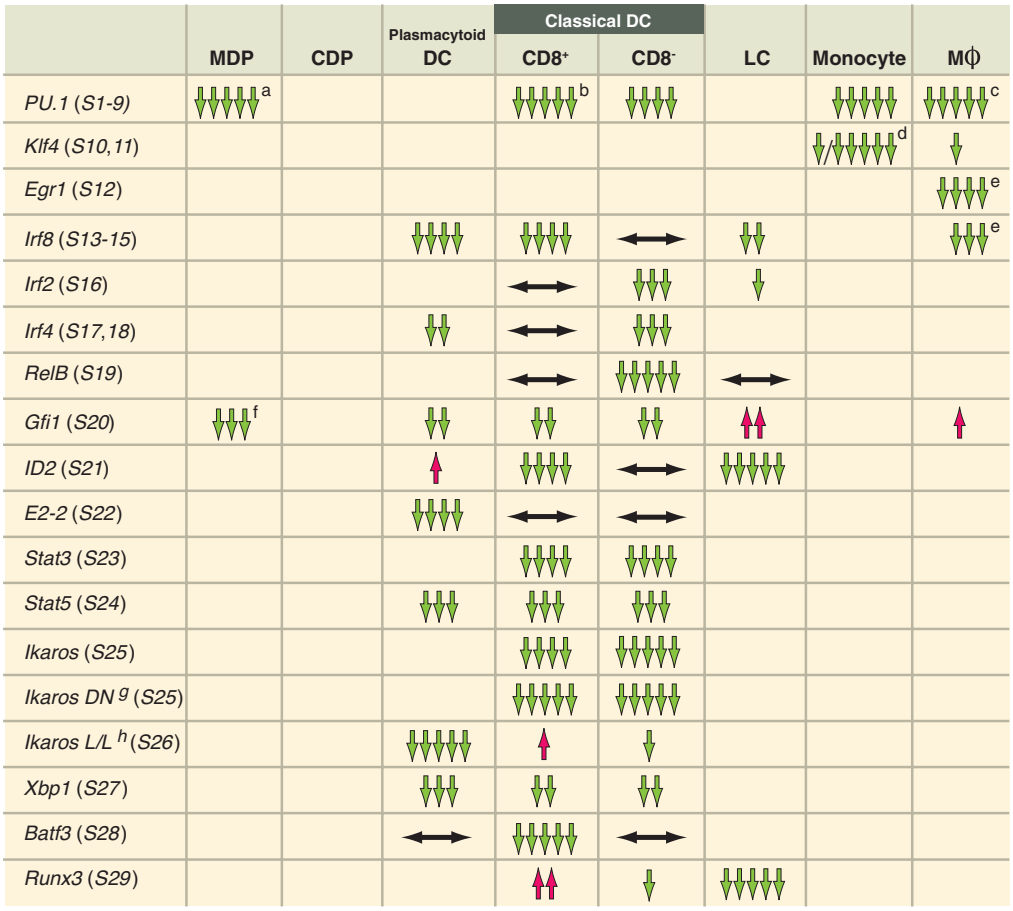


Fig. 3. Phenotype of transcription factor knockout (KO) mice in different populations of the MPs. The ↓ indicates a reduction in KO; ↑ is an increase in KO; and ↔ means unchanged numbers of the populations, suggesting a positive (green), negative (red), or no (black) effect on the development of the respective population. Number of arrows indicates the relative strength of the effect. Specific notes are as follows: (a) MDPs have not been specifically analyzed but PU.1-deficient mice lack all myeloid progenitors, of which MDPs are a subpopulation; (b) in one study (*S7*), $CD8^+$ $CD11c^+$ cells were detected in E16.5 embryos; (c) some macrophage subpopulations are present in the embryo; (d) $LyC6^-/LyC6^+$, respectively; (e) M-CSF–dependent differentiation in culture; (f) MDPs were not specifically studied, but the population of lin^- $ckit^+$ $Flt3^+$ progenitors, which includes MDPs, was reduced; (g) dominant negative allele; and (h) low-level expression. References *S1* to *S29* are found in the supporting online material (SOM).

after *L. monocytogenes* infection (*3*, *52*) indicates that $Gr1^-/Ly\text{-}6C^{low}$ monocytes initiate a macrophage differentiation program that resembles the one described for "alternatively activated macrophages," cells that are proposed to be involved in tissue repair (*54*) (also known as M2 macrophages). Conversely $Gr1^+/Ly\text{-}6C^{high}$ monocytes that entered the peritoneum at the same time initiate a more inflammatory "classical" transcriptional program that resembles TipDCs or M1 macrophage differentiation. Direct experimental evidence that monocytes are involved in tissue repair is missing, however, and the differential contributions of monocyte subsets to neoangiogenesis (*55*, *56*) need further study.

What Determines Monocyte Differentiation Potential?

The emerging evidence that monocyte subsets exert distinct functions and have distinct fates, such as the differentiation into cells with features of M1 and M2 macrophages, seemingly contradicts the notion of a general plasticity of monocytes and macrophages, which holds that monocytes respond to their environment by differentiating into a variety of macrophages and DC-like cells [reviewed in (*57*)]. This concept of plasticity is largely based on the effects of cytokines on monocytes in vitro. Exposure to granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 induces differentiation of human and mouse monocytes into DCs, irrespective of their subset (*58*, *59*). Moreover, addition of transforming growth factor-β1 confers the phenotype of LCs (*60*), whereas exposure to M-CSF induces monocytes to differentiate into macrophages. Addition of IFNγ (or lipopolysaccharide) to M-CSF induces the differentiation of M1-like macrophages, whereas addition of IL-4 induces the differentiation of M2-like macrophages (*54*, *61*, *62*). M2 macrophages have been further subsetted depending on the culture conditions (*62*). The chemokine CXCL4 induces another macrophage phenotype with possible relevance to vascular diseases (*63*).These results suggest that monocytes are phenotypically polarized by the microenvironment to mount specific functional programs. M1 macrophages, whose prototypical activating stimuli are IFNγ and LPS, exhibit potent microbicidal properties and promote strong IL-12–mediated T helper 1 responses. In contrast, M2 macrophages support T helper 2–associated effector functions and may play a role in resolution of inflammation through endocytic clearance and trophic factor synthesis (*54*). In vitro studies may not, however, fully recapitulate in vivo differentiation, and thus heterogeneity of monocytes may mediate in part their plasticity in vivo, with $Gr1^-/Ly\text{-}6C^{low}$ and $Gr1^+/Ly\text{-}6C^{high}$ monocytes being committed to differentiate more readily into M2-like cells or inflammatory DCs and M1-like macrophages, respectively.

Transcriptional Control of Lineage Commitment in the Mononuclear Phagocyte System

The successively more restricted lineage potentials of MDPs, CDPs, pre-DCs, monocytes, macrophages, and DCs involve the selection of specific gene expression programs. For some pathways, the transcription factors responsible for these cell fate choices have recently begun to be elucidated. Although several transcription factor knockout mice show deficiencies in the mononuclear phagocyte system, they often display broad effects in multiple cell types (*3*, *34*, *64*) (Fig. 3). A few exceptions have been identified, such as the loss of PDCs in mice deficient for the basic helix-loop-helix transcription factor E2-2 (*65*) or the loss of $CD8\alpha^+$ cDCs in mice deficient for the basic leucine zipper transcription factor Batf3 (*66*). Similarly selective transcription factor requirements may be responsible for monocyte (*44*) and macrophage subtypes and will require further investigation.

However, broad effects on many lineages do not necessarily exclude important functions in specific commitment events. An instructive example is the myeloid transcription factor

Downloaded from www.sciencemag.org on May 7, 2012

PETITIONER EXHIBIT 1069
Page 4 of 7

PU.1. Although PU.1 is required for the earliest steps of myeloid lineage commitment in hematopoietic stem cells (*67*) and its absence results in general myeloid lineage deficiencies (*3*, *34*), PU.1 has additional key selector gene functions at several branch points of myeloid lineage diversification, particularly during the macrophage-versus-DC choice of monocytes (*68*). The function of PU.1 at particular progenitor stages depends on its balance with antagonistic factors driving alternative fates. Intermediate expression of PU.1 in GMPs can overcome the neutrophil fate-inducing effects of the basic leucine zipper transcription factor C/EBPα and activate the macrophage-specifying zinc finger transcription factors Egr-1 and Egr-2 (*3*, *69*). In contrast, high expression of PU.1 is required to induce a DC fate in monocytes and to antagonize the macrophage-inducing transcription factors c-Maf and MafB (*3*, *68*). Of interest, the transcription factor balance specifying M2 or DC fate of monocytes in culture correlates with cell fate choice in vivo, as outlined above for the programs initiated by $Ly6C^{low}$ and $Ly6C^{high}$ monocytes upon *L. monocytogenes* infection of the peritoneum (*52*, *68*).

In general, gain-of-function experiments have been informative in defining transcription factor potential in driving particular cell fate choices. For example, ectopic expression of the transcription factors MafB, c-Maf, Egr1, ICSBP/IRF8, KLF4, and PU.1 in early progenitors can drive monocyte/macrophage fates. PU.1 and RelB induce DC differentiation via monocytic intermediates and E-box proteins (likely mimicking endogenous E2-2) or SpiB can induce PDC fates (*3*). In most of these experiments, however, undefined or early progenitor-enriched populations were used. Given that the function of any factor will depend on other cooperating and antagonistic transcription factors expressed at each specific progenitor stage (*70*), this makes it difficult to precisely identify the affected commitment event. Such differentiation stage-specific manipulation of transcription factors will require models that faithfully reproduce defined differentiation stages in culture, the development of new genetic tools of inducible gene regulation in vivo, or adoptive transfer of genetically marked, defined cell populations.

Another aspect of emerging importance is the coupling of cytokine receptor signaling with specific transcription factors in lineage commitment. For example, signal transducer and activator of transcription 3 (STAT3) is required for Flt3L- but not GM-CSF–mediated DC differentiation (*21*, *34*, *64*). Similarly, Id2 and Runx3 mediate TGFβ signals in LC differentiation (*34*, *64*). Furthermore, MafB limits M-CSF signals and inhibits PU.1 activation in HSCs (*67*), whereas MafB and c-Maf together inhibit M-CSF–dependent proliferative signals in mature monocytes and macrophages and assure their withdrawal from cell cycle (*71*).

Besides transcription factors, epigenetic modification, with large-scale histone and DNA modifications like acetylation and methylation, and micro-RNAs have recently been reported to regulate important functions of mononuclear phagocytes (*72*, *73*) and are also emerging as important determinants of lineage choice but will not be discussed here.

Perspectives and Future Challenges

Although the studies discussed above have shed light on the in vivo development and homeostasis of the mononuclear phagocyte system, mechanisms that control replacement of many macrophage subsets and resident cells remain mysterious. Most organs contain resident tissue macrophages, many of which have long half-lives. After irradiation and other forms of tissue injury, bone marrow–derived cells frequently repopulate the injured organs. Whether bone marrow–derived cells under inflammatory conditions assume the same phenotype and function as resident cells that populate tissues under steady state conditions remains largely unclear. Fate mapping experiments investigating steady state developmental potential will therefore have to avoid experimental inflammation. Toward this end, Cre recombinase expression under the control of promoters for key markers (such as lysozyme) have been used to activate conditional reporter genes so that all downstream daughter cells express a stable marker (*9*). It has to be cautioned, however, that "key" markers are seldom completely specific; progenitor cells can express low levels of the marker and marker-positive cells may not uniformly turn on the reporter, all of which may complicate the interpretation of such lineage-marking experiments (*74*, *75*). Parabiotic mice and adoptive transfer into mice in which cell populations have been depleted by genetic means may provide complementary approaches.

The transcription factors controlling monocyte and macrophage subtype-specific programs in vivo also need to be identified. Models that faithfully reproduce defined differentiation stages in culture and the development of new genetic tools of inducible gene regulation that enable precise differentiation by stage-specific manipulation of transcription factors in vivo could accelerate discovery in this area. The use of new model systems for the study of phagocytes, such as *Drosophila melanogaster*, which lack lymphocytes but possess a powerful innate immune system and are perfectly suited for genetic studies and in vivo imaging, has the potential to accelerate discovery of genetic pathways that control the development and functions of phagocytes in vivo (*76*–*78*).

Another challenge will be to translate knowledge obtained from mouse and other models into a better understanding of the mononuclear phagocyte system and its role in inflammation and associated diseases in humans. A large number of studies have attempted to recapitulate in vitro some of the heterogeneity of DCs and macrophages. On the basis of these studies, functional subsets of macrophages and DCs (e.g., tolerogenic DCs) inside tissues have been proposed to mediate important functions in vivo, such as tissue repair, angiogenesis, or tolerance to self. Laser capture dissection techniques are being developed to obtain gene expression data from macrophages isolated from tissues (*79*). Flow cytometry–based cell sorting approaches also hold promise for isolating pure macrophage and DC populations from various tissues and organs. These techniques are especially important in order to translate some of the findings made in mice into the human system. Global expression profiling of mouse DC, macrophages, and monocyte subsets might assist in the identification of corresponding human populations. Human hemato-lymphoid-system mouse models that will provide in vivo DC and macrophage readouts from human hematopoietic stem cells and progenitor cells will also be important (*80*). Lastly, studies of patients with deficiencies for genes involved in myeloid cell differentiation and functions will be critical to assess the relevance of models derived from studies in the mouse and in vitro experiments in human cells (*81*).

References and Notes

1. R. van Furth, Z. A. Cohn, *J. Exp. Med.* **128**, 415 (1968).
2. R. M. Steinman, D. S. Lustig, Z. A. Cohn, *J. Exp. Med.* **139**, 1431 (1974).
3. C. Auffray, M. H. Sieweke, F. Geissmann, *Annu. Rev. Immunol.* **27**, 669 (2009).
4. F. K. Swirski *et al.*, *Science* **325**, 612 (2009).
5. N. V. Serbina, T. Jia, T. M. Hohl, E. G. Pamer, *Annu. Rev. Immunol.* **26**, 421 (2008).
6. S. Gordon, *Cell* **111**, 927 (2002).
7. J. Banchereau, R. M. Steinman, *Nature* **392**, 245 (1998).
8. I. Mellman, R. M. Steinman, *Cell* **106**, 255 (2001).
9. K. Liu *et al.*, *Science* **324**, 392 (2009); published online 12 March 2009 (10.1126/science.1170540).
10. C. Waskow *et al.*, *Nat. Immunol.* **9**, 676 (2008).
11. M. Merad *et al.*, *Nat. Immunol.* **3**, 1135 (2002).
12. L. Corcoran *et al.*, *J. Immunol.* **170**, 4926 (2003).
13. M. Colonna, G. Trinchieri, Y. J. Liu, *Nat. Immunol.* **5**, 1219 (2004).
14. R. T. Sasmono *et al.*, *Blood* **101**, 1155 (2003).
15. X. M. Dai *et al.*, *Blood* **99**, 111 (2002).
16. M. G. Cecchini *et al.*, *Development* **120**, 1357 (1994).
17. W. Wiktor-Jedrzejczak, S. Gordon, *Physiol. Rev.* **76**, 927 (1996).
18. E. S. Kawasaki *et al.*, *Science* **230**, 291 (1985).
19. H. Lin *et al.*, *Science* **320**, 807 (2008).
20. H. J. McKenna *et al.*, *Blood* **95**, 3489 (2000).
21. N. Onai, A. Obata-Onai, R. Tussiwand, A. Lanzavecchia, M. G. Manz, *J. Exp. Med.* **203**, 227 (2006).
22. A. D'Amico, L. Wu, *J. Exp. Med.* **198**, 293 (2003).
23. N. Onai *et al.*, *Nat. Immunol.* **8**, 1207 (2007).
24. D. K. Fogg *et al.*, *Science* **311**, 83 (2006); published online 1 December 2005 (10.1126/science.1117729).
25. C. Varol *et al.*, *J. Exp. Med.* **204**, 171 (2007).
26. C. Varol *et al.*, *Immunity* **31**, 502 (2009).
27. S. H. Naik *et al.*, *Nat. Immunol.* **8**, 1217 (2007).
28. S. H. Naik *et al.*, *Nat. Immunol.* **7**, 663 (2006).
29. K. Kabashima *et al.*, *Immunity* **22**, 439 (2005).
30. M. Bogunovic *et al.*, *Immunity* **31**, 513 (2009).
31. F. Geissmann, S. Jung, D. R. Littman, *Immunity* **19**, 71 (2003).
32. C. Auffray *et al.*, *J. Exp. Med.* **206**, 595 (2009).
33. B. Ajami, J. L. Bennett, C. Krieger, W. Tetzlaff, F. M. Rossi, *Nat. Neurosci.* **10**, 1538 (2007).
34. M. Merad, M. G. Manz, *Blood* **113**, 3418 (2009).

Downloaded from www.sciencemag.org on May 7, 2012

PETITIONER EXHIBIT 1069
Page 5 of 7

35. L. Chorro *et al.*, *J. Exp. Med.* **206**, 3089 (2009).
36. D. E. Wright, A. J. Wagers, A. P. Gulati, F. L. Johnson, I. L. Weissman, *Science* **294**, 1933 (2001).
37. S. Massberg *et al.*, *Cell* **131**, 994 (2007).
38. L. Wu, K. Shortman, *Semin. Immunol.* **17**, 304 (2005).
39. J. Li, J. Park, D. Foss, I. Goldschneider, *J. Exp. Med.* **206**, 607 (2009).
40. E. Jaensson *et al.*, *J. Exp. Med.* **205**, 2139 (2008).
41. O. Schulz *et al.*, *J. Exp. Med.* **206**, 3101 (2009).
42. U. Yrlid, C. D. Jenkins, G. G. MacPherson, *J. Immunol.* **176**, 4155 (2006).
43. M. W. Feinberg *et al.*, *EMBO J.* **26**, 4138 (2007).
44. J. K. Alder *et al.*, *J. Immunol.* **180**, 5645 (2008).
45. J. C. Scatizzi *et al.*, *Am. J. Pathol.* **168**, 1531 (2006).
46. A. Mildner *et al.*, *Nat. Neurosci.* **10**, 1544 (2007).
47. E. Narni-Mancinelli *et al.*, *J. Exp. Med.* **204**, 2075 (2007).
48. I. R. Dunay *et al.*, *Immunity* **29**, 306 (2008).
49. P. M. Robben, M. LaRegina, W. A. Kuziel, L. D. Sibley, *J. Exp. Med.* **201**, 1761 (2005).
50. K. Movahedi *et al.*, *Blood* **111**, 4233 (2008).
51. R. Shechter *et al.*, *PLoS Med.* **6**, e1000113 (2009).
52. C. Auffray *et al.*, *Science* **317**, 666 (2007).
53. M. Nahrendorf *et al.*, *J. Exp. Med.* **204**, 3037 (2007).
54. F. O. Martinez, L. Helming, S. Gordon, *Annu. Rev. Immunol.* **27**, 451 (2009).
55. M. Grunewald *et al.*, *Cell* **124**, 175 (2006).
56. M. De Palma *et al.*, *Cancer Cell* **8**, 211 (2005).
57. P. R. Taylor, S. Gordon, *Immunity* **19**, 2 (2003).
58. F. Sallusto, A. Lanzavecchia, *J. Exp. Med.* **179**, 1109 (1994).
59. F. Geissmann *et al.*, *Immunol. Cell Biol.* **86**, 398 (2008).
60. F. Geissmann *et al.*, *J. Exp. Med.* **187**, 961 (1998).
61. M. Stein, S. Keshav, N. Harris, S. Gordon, *J. Exp. Med.* **176**, 287 (1992).
62. F. O. Martinez, A. Sica, A. Mantovani, M. Locati, *Front. Biosci.* **13**, 453 (2008).
63. C. A. Gleissner *et al.*, *Circ. Res.* **106**, 203 (2010).
64. M. Zenke, T. Hieronymus, *Trends Immunol.* **27**, 140 (2006).
65. B. Cisse *et al.*, *Cell* **135**, 37 (2008).
66. K. Hildner *et al.*, *Science* **322**, 1097 (2008).
67. S. Sarrazin *et al.*, *Cell* **138**, 300 (2009).
68. Y. Bakri *et al.*, *Blood* **105**, 2707 (2005).
69. P. Laslo *et al.*, *Cell* **126**, 755 (2006).
70. M. H. Sieweke, T. Graf, *Curr. Opin. Genet. Dev.* **8**, 545 (1998).
71. A. Aziz *et al.*, *Mol. Cell. Biol.* **26**, 6808 (2006).
72. R. M. O'Connell *et al.*, *J. Exp. Med.* **205**, 585 (2008).
73. R. M. O'Connell, K. D. Taganov, M. P. Boldin, G. Cheng, D. Baltimore, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 1604 (2007).
74. T. Miyamoto *et al.*, *Dev. Cell* **3**, 137 (2002).
75. H. Shigematsu *et al.*, *Immunity* **21**, 43 (2004).
76. J. A. Martinez-Agosto, H. K. Mikkola, V. Hartenstein, U. Banerjee, *Genes Dev.* **21**, 3044 (2007).
77. D. T. Babcock *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 10017 (2008).
78. L. Cuttell *et al.*, *Cell* **135**, 524 (2008).
79. E. Trogan *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 2234 (2002).
80. M. G. Manz, *Immunity* **26**, 537 (2007).
81. J. L. Casanova, L. Abel, *Annu. Rev. Immunol.* **20**, 581 (2002).

82. The diagram depicted in Fig. 2 is the result of a collective discussion at a recent workshop: "Monocytes, Macrophages and Dendritic Cell Heterogeneity," 2 to 7 March 2009, Paris, France, organized by the Fondation des Treilles (www.les-treilles.com/newsite/index.html). We thank V. Bronte, I. Charo, J. Van Ginderachter, G. MacPherson, A. Mantovani, E. Pamer, R. Steinman, and F. Swirski for their input into Fig. 1 and for their ideas that we have incorporated into this review. Any misrepresentation is the responsibility of the authors. Work by F.G. and K.L. on the development of monocytes, macrophages, and dendritic cells is funded by Medical Research Council UK grant G0900867 to F.G. and grant NIH R01 HL058108 to K.L.

Supporting Online Material

www.sciencemag.org/cgi/content/full/327/5966/656/DC1
Table S1
References

10.1126/science.1178331

Downloaded from www.sciencemag.org on May 7, 2012

PETITIONER EXHIBIT 1069
Page 6 of 7

ERRATUM

Post date 3 December 2010

Review: "Development of monocytes, macrophages, and dendritic cells" by F. Geissmann *et al*. (5 February, p. 656). Reference 71 was incorrect. It should be A. Aziz *et al., Science* **326**, 867 (2009).

Downloaded from www.sciencemag.org on May 7, 2012

PETITIONER EXHIBIT 1069
Page 7 of 7