Studying the mononuclear phagocyte system in the molecular age

Andrew Chow, Brian D. Brown‡§ and Miriam Merad*‡¶*

Abstract | The mononuclear phagocyte system (MPS) comprises monocytes, macrophages and dendritic cells. Tissue phagocytes share several cell surface markers, phagocytic capability and myeloid classification; however, the factors that regulate the differentiation, homeostasis and function of macrophages and dendritic cells remain largely unknown. The purpose of this manuscript is to review the tools that are currently available and those that are under development to study the origin and function of mononuclear phagocytes.

Monocytes
Monocytes are mononuclear phagocytes that circulate in the blood. Monocytes are thought to differentiate into macrophages and some dendritic cells in peripheral tissues. They consist of two subsets: classical monocytes and non-classical monocytes.

*Departments of Oncological Sciences and Medicine, Mount Sinai School of Medicine, New York, New York 10029, USA.
‡Immunology Institute, Mount Sinai School of Medicine, New York, New York 10029, USA.
§Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, New York 10029, USA.
¶Tisch Cancer Institute, Mount Sinai School of Medicine, New York, New York 10029, USA.
Correspondence to M.M.
e-mail: miriam.merad@mssm.edu
doi:10.1038/nri3087

The mononuclear phagocyte system (MPS) is composed of monocytes, macrophages and dendritic cells (DCs) and has crucial roles in maintaining organismal homeostasis. In addition, the diverse activities of the MPS are relevant in inflammation, autoimmunity, infection, cancer and organ transplantation. Commitment to the mononuclear phagocyte lineage is determined at the stage of the macrophage and DC progenitor (MDP), at which point, erythroid, megakaryocyte, lymphoid and granulocyte fates have been precluded (FIG. 1). MDPs give rise to monocytes and common DC progenitors (CDPs)[1,2]. Whereas monocytes can directly participate in effector immune responses or differentiate into macrophages or DCs, the differentiation potential of CDPs is restricted to the DC lineage (FIG. 1). CDPs give rise to plasmacytoid DCs and pre-DCs, which subsequently give rise to DCs[3–5]. Plasmacytoid DCs have been recently reviewed and will not be further covered in this manuscript[6]. How cell fate is decided, including the factors that drive MDP differentiation and the genes responsible, is still not well known.

What is becoming increasingly clear is that monocytes, macrophages and DCs are not homogenous populations. Just as CD4$^+$ T cells further differentiate into distinct subsets (such as T helper 1 (T_H1), T_H2, T_H17 and regulatory T (T_{Reg}) cells), monocytes, macrophages and DCs can also differentiate into discrete functional subsets. The monocyte population is composed of two main subsets in mice and humans, designated classical monocytes and non-classical monocytes, and these cells are found primarily in the circulation, bone marrow and spleen[7]. Macrophages are found in all tissues throughout the body from embryonic to adult life. Macrophages are well known for their functions in protecting the host from pathogens and their roles in clearing dead cells, but they also have unique functions that are influenced by their locations in the body. For example, bone marrow macrophages promote stromal retention of haematopoietic stem and progenitor cells[8], and lymph node subcapsular sinus macrophages prevent central nervous system (CNS) invasion after peripheral infection with a neurotropic virus[9].

DCs were originally distinguished from macrophages by their increased ability to activate T cells in an antigen-dependent manner, and these cells are also found in lymphoid and non-lymphoid tissues. Whereas macrophage heterogeneity is classified based on tissue location, the two main DC subsets are characterized by differential requirements for transcription factors and distinct origins. The transcription factors inhibitor of DNA binding 2 (ID2), interferon-regulatory factor 8 (IRF8) and basic leucine zipper transcriptional factor ATF-like 3 (BATF3) are the basis for this distinction. Although lymphoid tissue CD8$^+$ DCs and non-lymphoid tissue CD103$^+$ DCs (collectively termed BATF3-dependent DCs) require ID2, IRF8 and BATF3 for their development, lymphoid tissue CD8$^-$ DCs and non-lymphoid tissue CD11b$^+$ DCs (collectively termed BATF3-independent DCs) develop independently of these factors[10–14]. The BATF3-dependent DC subset is completely derived from a circulating pre-DC, but at least some BATF3-independent DCs arise from circulating monocytes[11,12] (FIG. 1).

There are a number of outstanding questions about the development, homeostasis and function of MPS cells. Although several subsets of monocytes, macrophages and DCs have been identified, the individual contributions of these subsets to health and disease are not well known, and it is probable that additional, functionally distinct subsets exist. Moreover, few of the relevant genes involved in the development and function of MPS cells have been characterized. Over the last few decades, a number of tools have been developed to address these

© 2011 Macmillan Publishers Limited. All rights reserved

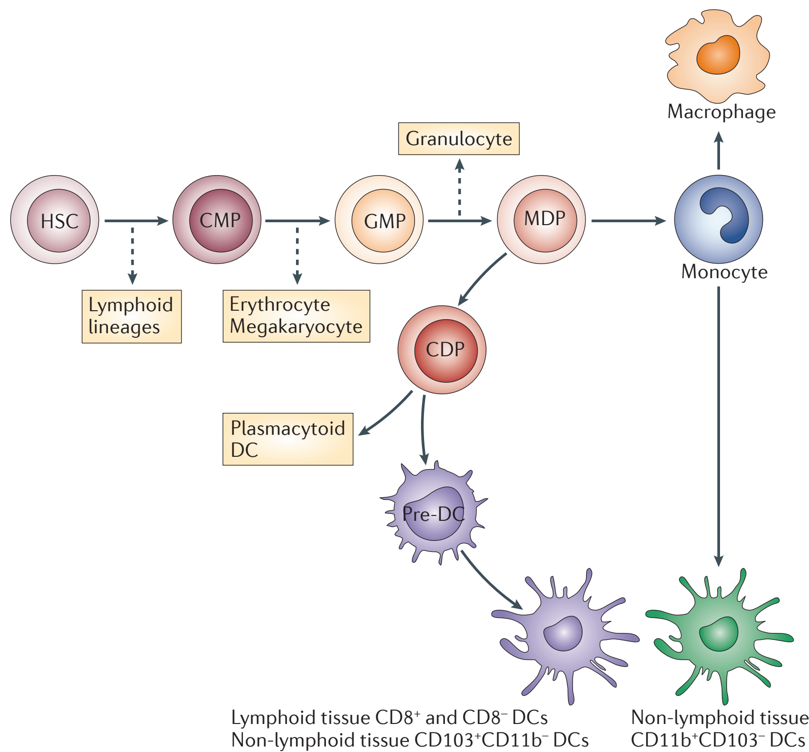

Figure 1 | **Lineage of mononuclear phagocytes.** Commitment to differentiation into a monocyte, macrophage or dendritic cell (DC) occurs at the stage of the macrophage and DC progenitor (MDP). MDPs can give rise to common DC progenitors (CDPs) or monocytes. CDPs are committed to the DC lineage and give rise to both $CD8^+$ and $CD8^-$ DCs in lymphoid tissues and $CD103^+CD11b^-$ DCs in peripheral non-lymphoid tissues. In addition, monocytes can give rise to some $CD11b^+CD103^-$ DCs or to macrophages. CMP, common myeloid progenitor; GMP, granulocyte and macrophage progenitor; HSC, haematopoietic stem cell.

Macrophages
Macrophages are tissue-resident phagocytes that specialize in the capture and clearance of damaged cells. Macrophages also capture and clear microorganisms and secrete pro-inflammatory molecules in response to microbial infection, and thus have a crucial role in host defence.

Dendritic cells
(DCs). DCs are tissue-resident phagocytes that specialize in the presentation of antigens to T cells to promote immunity to foreign antigens and tolerance to self antigens.

important questions. Methods have been developed to ablate and track specific cell types, and several strategies exist for gain- and loss-of-function genetic analyses, including targeted gene knockouts and RNA interference. However, the broad activity of some of the available approaches, which often affect multiple MPS subsets, presents limitations. This has led to efforts to develop better genetic tools and animal models that enhance our precision in manipulating particular mononuclear phagocyte populations. Here, we review the tools that are currently available and those that are under development to study the origin and function of mononuclear phagocytes, as well as the genes that make the system work (TABLE 1). These new tools will help us to answer some of the lingering questions about the ontogenic relationship (BOX 1) and functional diversity (BOX 2) of mononuclear phagocytes and, potentially, to design more targeted therapies for diseases that involve MPS cells.

Gene deficiency models

Animals deficient in various transcription factors, cytokines and cytokine receptors have been essential in building our understanding of the developmental and homeostatic requirements for mononuclear phagocytes in various tissues of the body. The discovery that severely reduced numbers of macrophages are present in the lymphoid and non-lymphoid tissues of osteopetrotic mice (*Csf1*$^{op/op}$ mice)[15] — which have a frameshift mutation in the gene encoding the cytokine colony-stimulating factor 1 (CSF1; also known as M-CSF) — established the role of CSF1 in macrophage differentiation. In addition, the findings that mice deficient in FMS-related tyrosine kinase 3 (FLT3) or its ligand, FLT3L, have marked reductions in lymphoid and non-lymphoid DC populations[16] have helped to establish the key role of FLT3L in the commitment and differentiation of the DC lineage.

The dissection of the mechanisms that control the differentiation of the DC lineage and DC subsets has also been bolstered by the study of animals deficient in particular transcription factors. The numbers of DCs are reduced in all DC subsets in *Stat3*$^{-/-}$, *Gfi*$^{-/-}$, *Stat5*$^{-/-}$, *Ikaros*$^{-/-}$ and *Xbp1*$^{-/-}$ mice[16]. Lymphoid tissue $CD8^+$ DCs and non-lymphoid tissue $CD103^+CD11b^-$ DCs are specifically reduced in number in mice lacking BATF3, IRF8 or ID2, whereas the numbers of lymphoid tissue $CD8^-$ DCs are specifically reduced in *Irf2*$^{-/-}$ and *Irf4*$^{-/-}$ mice[16].

Although the study of mice deficient in transcription factors or cytokines has provided insight into the physiological and pathological relevance of monocytes, macrophages and DCs, the broad impact of these gene deficiencies makes it difficult to pin down functions to a particular mononuclear phagocyte population. Hence, animal models to specifically deplete particular populations (TABLE 2) and cell-type specific knockdown of various proteins are essential to further our knowledge of mononuclear phagocytes.

Non-genetic depletion models

Depleting a cell lineage or specific cell type provides an excellent means for determining its relevance and function. Several methods exist to non-genetically deplete MPS cells and to study the consequences of their removal from the organism. Clodronate-encapsulated liposomes are one of the most effective agents at depleting mononuclear phagocyte populations in rodents[17]. The liposomes are taken up by MPS cells as a result of the phagocytic activities of these cells and, once inside a cell, the clodronate bisphosphonate induces apoptosis. Provided that there is no issue with access of the liposomes, it appears that all mononuclear phagocytes are sensitive to depletion by clodronate-encapsulated liposomes (TABLE 2), including monocytes, macrophages and DCs.

Neutralizing antibodies specific for the CSF1 receptor (CSF1R)[18] have also been used to deplete macrophage populations[19,20] (TABLE 2). Moreover, CSF1R signalling can be blocked downstream of the receptor using small molecules that interfere with kinase activity. Several oral kinase inhibitors, such as CYC10268 (REF. 21), Ki20227 (REF. 22) and GW2580 (REF. 23), have been developed to successfully reduce populations of mononuclear phagocytes (reviewed in REF. 24) and are being considered for translation into patients.

A downside of these agents is that they deplete a broad range of mononuclear phagocyte cell types, and this can lead to erroneous conclusions. One example of this occurred when macrophages and DCs were

© 2011 Macmillan Publishers Limited. All rights reserved

Table 1 | **Pros and cons of different models for understanding the MPS**

Model	Uses	Examples	Pros	Cons
Non-genetic depletion	Cell loss of function	Clodronate liposomes, CSF1R-specific antibody	Inducible, efficient and efficacious in all mouse strains	Currently available non-genetic depletion methods have widespread effects on MPS cells, precluding precise functional characterization of MPS subsets. Inducible cell death of mononuclear phagocytes can be inflammatory
Gene knockout mice	Gene function assessment, cell loss of function	*Csf1*$^{op/op}$, *Csf1r*$^{-/-}$, *Flt3*$^{-/-}$, *Csf2r*$^{-/-}$, *Batf3*$^{-/-}$	Efficient gene deletion and permits functional identification of essential genes for MPS subsets	Potential for compensation from absence of cellular population from birth. Deletion of gene might have broad effects on multiple MPS subsets and even on non-MPS subsets
DTR transgenic mice	Cell loss of function	*Cd11b*-DTR, *Cd11c*-DTR, *Cd169*-DTR, *Langerin*-DTR	Inducible and efficient	The promoters used in some existing models are expressed in multiple MPS subsets and/or non-MPS lineages. Mice can develop immunity to diphtheria toxin, precluding long-term depletion. Inducible cell death of mononuclear phagocytes can be inflammatory
DTA mice	Cell loss of function	*Cd11c*-Cre x *Rosa26*-stopflox-DTA, *Langerin*-DTA	Efficient and no development of immunity to diphtheria toxin	The promoters used in some existing models are expressed in multiple MPS subsets and/or non-MPS lineages. Potential for compensation from absence of cell population from birth
Reporter mice	*In vivo* cell tracking	*Cx3cr1*-GFP, *Lyz2*-GFP, *Cd115*-GFP, *Cd11c*-YFP, *Langerin*-GFP	Permits cell tracking without the need for antibodies. A side benefit has been the labelling of chemokine and cytokine receptors that are difficult to stain with currently available antibodies	Models are not available for tracking many MPS subsets. The promoters used in some existing models are expressed in multiple MPS subsets and/or non-MPS lineages
Cre-recombinase and floxed mice	Gene function assessment, cell loss of function (if crossed to inducible DTR or inducible DTA) and *in vivo* cell tracking (if crossed to *Rosa26*-reporter mice)	*Lyz2*-Cre x *Rosa26*-stopflox-EGFP, *Cd11c*-Cre x *Dicer*$^{fl/fl}$	*LoxP*-flanked genes are only excised in cells expressing the promoter driving Cre-recombinase. Flexibility in intercrossing mice lines, rather than generating new lines for cell type-specific knockdown or overexpression	Requires considerable breeding efforts. The promoters used in some existing models are expressed in multiple MPS subsets and/or non-MPS lineages
Lineage tracing	*In vivo* assessment of cell origin	*Runx1*-Cre^{ERT2} x stopflox-EGFP	Useful for determining progenitor-mature cell lineage relationships	There is a paucity of promoters that are specific for MPS progenitors
Humanized mice	*In vivo* human MPS assessment	Human haemato-lymphoid system (HHLS) mice	Permits *in vivo* study of human mononuclear phagocytes and human MPS-specific pathogens	Generating these mice is expensive and laborious. Although improving, most HHLS models have poor reconstitution of MPS cell types, particularly macrophages
RNA interference (RNAi)	Gene function assessment	Small hairpin RNA (shRNA), small interfering RNA (siRNA), and RNAi libraries	More rapid gene knockdown than knockout mice. shRNA libraries permit high-throughput unbiased screening and identification of genes essential for MPS functions	Vectors expressing shRNA have variable efficiency and multiple constructs may have to be tested to find suitable levels of gene knockdown. RNAi is more difficult to employ for *in vivo* studies, and there can be off-target effects
Molecular profiling	Cell population comparison, gene function assessment	Microarrays, deep sequencing, proteomics	Permits relatively unbiased comparison of various immune cell populations. Identifies candidate genes essential to MPS functions	Transcriptome profiling may not always be representative of protein levels; functional validation is still required
Zebrafish	*In vivo* cell tracking, gene function knockdown	mhc2dab:GFP zebrafish	Ease of gene knockdown and cell visualization	Compared with mice, fewer tools are currently available and MPS subsets are less well described

Batf3, basic leucine zipper transcriptional factor ATF-like 3; *Csf1*, colony-stimulating factor 1; *Csf1r*, colony-stimulating factor 1 receptor; *Cx3cr1*, CX_3C chemokine receptor 1; DTA, diphtheria toxin fragment A; DTR, diphtheria toxin receptor; EGFP, enhanced green fluorescent protein; *Flt3*, FMS-related tyrosine kinase 3; GFP, green fluorescent protein; *Lyz2*, lysozyme M; MPS, mononuclear phagocyte system; YFP, yellow fluorescent protein.

both implicated in aggravating graft-versus-host disease (GVHD), as both were eliminated by systemic treatment with clodronate-encapsulated liposomes[25]. However, in a recent study using a CSF1R-specific antibody and a low-dose of liposomal clodronate, it was shown that although DCs aggravate GVHD, host lymphoid tissue-resident macrophages are in fact able to limit GVHD induction and severity[20]. In addition, it is known that antibodies specific for CSF1R can persist in the circulation for weeks, and this can lead, potentially, to

© 2011 Macmillan Publishers Limited. All rights reserved

Box 1 | **Tools needed to further our understanding of MPS origin and homeostasis**

What are the homeostatic requirements for the steady-state maintenance of the various cells of the MPS?
Until recently, one of the dogmas in the field stated that tissue macrophages and dendritic cells (DCs) were terminally differentiated cells that proliferated minimally in tissues. Recent studies have revealed that macrophages and DCs can proliferate *in situ* and, in some cases, can self-renew throughout life[82,103,104]. However, the exact mechanisms that control the maintenance of tissue phagocytes in the steady state remain unclear. The use of new mouse models, generated by crossing mice that express Cre specifically in macrophages or DCs with mice that have a floxed cytokine receptor gene (such as *Csf1r*$^{flox/flox}$ mice[105]), could help to reveal key homeostatic requirements for the maintenance of tissue phagocytes.

What are the definitive ontogenic relationships among mononuclear phagocytes?
Several studies have established that macrophage and DC progenitors (MDPs) give rise to monocytes and steady-state splenic macrophages following adoptive transfer into naive animals[1,2,106]. Whether the MDP transits through a monocyte intermediate before giving rise to splenic macrophages is unclear. Furthermore, the contribution of MDPs and monocytes to other steady-state macrophage populations, including those from non-lymphoid tissues and those found in lymph nodes, has not been established. The identification of gene promoters that are active only in particular circulating populations of mononuclear phagocyte system (MPS) cells (such as MDPs, GR1^{hi} monocytes or GR1^{low} monocytes) should help to trace the lineage and determine the origin of peripheral tissue macrophages in the steady state, as these promoters can be used to drive the expression of the Cre recombinase. Also, although it is established that monocytes can give rise to macrophages during inflammation, the extent to which monocytes contribute to the post-inflammation reconstitution of the macrophage compartment is unclear.

Classical monocytes
(Also known as inflammatory monocytes). This subset of monocytes is important in innate immune protection against infectious pathogens. During infectious challenge, these cells produce pro-inflammatory cytokines and can give rise to TNF- and iNOS-producing (TIP) dendritic cells, which contribute to the development of adaptive immune responses. In mice, these monocytes are characterized by high-level expression of LY6C, CCR2 and L-selectin (CD62L). In humans, classical monocytes are CD14^{hi}CD16^{-}.

Non-classical monocytes
This subset of monocytes patrols the blood circulation and has been shown to promote tissue healing. There is evidence that these cells are derived from classical monocytes and can give rise to tissue-resident macrophages. In mice, these cells are characterized by low-level expression of LY6C and high-level expression of CX$_3$CR1, LFA1 and CD43. In humans, non-classical monocytes are CD14^{-}CD16^{+} or CD14^{low}CD16^{+}.

Graft-versus-host disease
(GVHD). Tissue damage in a recipient of allogeneic tissue (usually a bone-marrow transplant) that results from the activity of donor cytotoxic T lymphocytes that recognize the tissues of the recipient as foreign. GVHD varies markedly in extent, but it can be life threatening in severe cases. Damage to the liver, skin and gut mucosa are common clinical manifestations.

misinterpretation concerning the relevance of a particular mononuclear phagocyte population. Consequently, with broad-spectrum depleting agents, one must be careful to verify in other depletion models of more precise specificity that a biological phenomenon is indeed due to depletion of a particular cell type. In addition, one can deliver the agent in a tissue-restricted manner, as has been done with clodronate-encapsulated liposomes to selectively deplete dermal macrophages[26], subcapsular sinus macrophages[9,27] and lung[28–30] and colonic[31] mononuclear phagocytes.

Genetic depletion mouse models

Conditional ablation approaches. The human diphtheria toxin receptor (*DTR*) gene provides a genetic means to deplete specific populations of mouse cells. The human DTR is 10^3 to 10^5 times more sensitive to diphtheria toxin than the mouse DTR, and thus when the human DTR is expressed in a mouse cell, that cell will die in the presence of diphtheria toxin[32]. Transgenic mice have been generated that express the human DTR under the control of the mouse *Cd11b* promoter[33], and this model has been used to deplete myeloid populations in the peripheral blood[34–36], bone marrow[8] and spleen[37], as well as in non-lymphoid sites, such as the peritoneal cavity[33,36], liver[33,38,39], kidney[36,40–44], reproductive organs[36,45,46], pancreas[37], atherosclerotic plaques[34] and perivascular fat[47] (TABLE 2). This method of depletion results in a broad depletion of myeloid cells, as CD11b is expressed on neutrophils, monocytes, eosinophils, macrophages and some DCs. This is useful for understanding the role of myeloid cells in various disease settings, but it can confound the interpretation of results. For example, in the setting of carbon tetrachloride-induced hepatic injury, depletion of CD11b^{+} cells during injury ameliorated the extent of tissue damage, whereas their depletion after injury slowed tissue repair[33]. This was probably a result of depleting two different CD11b-expressing populations at two distinct phases of the injury.

Another genetic conditional depletion model with broad specificity is the 'MAFIA' (macrophage FAS-induced apoptosis) mouse line, which expresses a suicide construct based on FK506-binding protein (*FKBP*) and *FAS* (also known as *CD95*) downstream of the *Csf1r* promoter[48]. When mice are treated with the FKBP-dimerizing drug AP20187, cells that express CSF1R are ablated[48,49]. This technique has been used to deplete monocytes and numerous populations of macrophages (TABLE 2). Although more selective depletion of MPS cells is achieved with MAFIA mice than in *Cd11b*-DTR mice, one of the drawbacks of using this model is the substantial inflammation that arises using the 5-day drug treatment protocol, as exemplified by bone marrow neutrophilia[8].

Cd169-DTR mice[50] allow for specific depletion of CD169-expressing macrophage populations, which include marginal metallophilic and marginal zone macrophages in the spleen[50], lymph node macrophages[51] and bone marrow macrophages[8] (TABLE 2). This model was used to demonstrate that splenic marginal zone macrophages suppress immune responses elicited by apoptotic cell-associated antigens[50]; that lymph node CD11c^{+}CD169^{+} macrophages cross-present cell-associated antigens after tumour cell death[51]; and that bone marrow-resident macrophages promote the retention of haematopoietic stem and progenitor cells[8]. This mouse model is an important advance for delineating the specific roles of CD169^{+} macrophages in physiology and disease, as it does not deplete monocytes[8], DCs[50] or neutrophils (A.C. and M.M., unpublished observations). Notably, macrophages that do not express CD169 — such as microglia, dermal macrophages and peritoneal macrophages — are not depleted in this model.

Cd11c-DTR mice[52] have been used extensively to assess conditional depletion of classical DCs. Although initially thought to be specific for DCs, it is now known that alveolar macrophages and splenic marginal zone and metallophilic macrophages are depleted efficiently in this model, and that CD11c^{+} plasmablasts and a subset of activated CD8^{+} T cells are ablated as well[32,53]. Notably,

© 2011 Macmillan Publishers Limited. All rights reserved

Box 2 | **Tools needed to further our understanding of MPS function**

What are the differential roles of infiltrating macrophages and tissue-resident macrophages?
Steady-state tissue-resident macrophages have many trophic and tissue homeostatic roles in addition to their functions in providing protection against pathogens[107]. However, the vast majority of the literature on macrophages focuses on their contribution to inflammation in pathological disease models. In many of these cases, the macrophages that are described are not tissue-resident macrophages, but are derived from monocytes that recently infiltrated the tissue, as highlighted in the case of peritoneal inflammation[108]. The identification of definitive markers for infiltrating monocyte-derived macrophages would be valuable in clarifying the differential functions of infiltrating monocyte-derived macrophages and tissue-resident macrophages. Clearly, the array of phenotypical markers currently in use — such as CD11b, F4/80 (also known as EMR1), CD11c, MHC class II molecules and CX_3C-chemokine receptor 1 (CX_3CR1) — is insufficient; new genes specific to each macrophage population will allow us to generate new tracking and depletion models to distinguish these two populations. Furthermore, developing humanized mouse models with improved engraftment of tissue-resident macrophages should help to illuminate the functions of the human equivalents of these populations.

What are the differential functions of tissue DC subsets?
Our progress in understanding the specialization of the dendritic cell (DC) lineage has accelerated owing to the identification of the transcription factors basic leucine zipper transcriptional factor ATF-like 3 (BATF3), interferon-regulatory factor 8 (IRF8) and inhibitor of DNA binding 2 (ID2) as crucial for the development of $CD8^+$ and $CD103^+$ DCs, and as a result of the generation of a BATF3-deficient mouse[10]. However, the field is still in need of mice in which the *Batf3* promoter drives the expression of the Cre recombinase in order to achieve specific gene expression or knockdown in $CD8^+$ and $CD103^+$ DCs without affecting $CD8^-$ or $CD11b^+$ DCs. XC-chemokine receptor 1 (XCR1) appears to be expressed exclusively by $CD8^+$ and $CD103^+$ DCs and is crucial for the cross-presentation of antigens to $CD8^+$ T cells[109] (J. Helft and M.M., unpublished observations). Thus, the generation of an *Xcr1*-Cre^{ERT2} mouse could be another strategy to specifically target $CD8^+$ and $CD103^+$ DCs. Similarly, the discovery of novel promoters that are active specifically in $CD8^-$ and $CD11b^+$ DCs (or subpopulations within these) is also much needed to clarify the contributions of these DC subsets to tissue immunity.

DT administration to transgenic mice under the short 5.5-kb *Cd11c* promoter does not lead to depletion of monocyte-derived DCs[53], plasmacytoid DCs[54] or natural killer (NK) cells[55,56]. The insights acquired from this model and the challenges of its use have been covered in other reviews[32,53]. One of the limitations described for this mouse strain is the invariable lethality associated with multiple injections of diphtheria toxin; this precludes long-term depletion studies unless *Cd11c*-DTR–wild-type bone marrow chimaeras are generated. A second *Cd11c*-DTR model was developed, using the full-length *Cd11c* promoter, in which lethality was not observed at the normal range of diphtheria toxin dosing (8 ng per g), although mortality was seen at higher doses (64 ng per g)[57]. Both models were similarly efficient in the ablation of DCs, splenic marginal zone macrophages, $CD11c^{hi}$ T cells and $CD11c^{hi}$ B cells but, owing to the use of the full-length promoter, $CD11c^{hi}$ NK and NKT cells were also ablated in this model.

Another DTR model, the langerin-DTR mouse, which was independently generated by two groups[58,59] (reviewed in REF. 32), can be used to deplete a more restricted population of DCs. These animals have a DTR–EGFP (enhanced green fluorescent protein) gene cassette knocked into the mouse gene that encodes langerin (also known as CLEC4K and CD207), and this enables specific depletion of langerin-expressing DCs, including epidermal Langerhans cells[58–62], langerin-expressing $CD8^+$ DCs in lymphoid tissue[58,63] and $CD103^+$ DCs in peripheral tissues[60–62,64] (TABLE 2).

Constitutive ablation with DTA. One of the limitations of the DTR models is that most MPS cells are quickly replaced; thus, diphtheria toxin must be repeatedly administered. To overcome this limitation and to achieve constitutive cell depletion, diphtheria toxin fragment A (DTA) can be used instead of the DTR. In this model, a stopflox-DTA gene cassette is knocked into the constitutively expressed *Rosa26* locus. The stop cassette prevents translation of DTA. In the presence of Cre recombinase, the stop cassette, which is flanked by *loxP* sites, is excised, and DTA is expressed, which leads to cell death. A model of constitutive ablation of DCs was generated using *Cd11c*-Cre animals crossed to stopflox-DTA mice to assess the global function of DCs[53]. One report demonstrated a myeloproliferative syndrome without autoimmunity[65], whereas another group reported a break in self tolerance[66]. Notably, a recent report using this model in MRL.Fas^{lpr} mice that are susceptible to a lupus-like syndrome demonstrated that the animals were in fact protected from the progression of autoimmunity[67]. The stark contrast in outcomes could reflect the contribution of the environment in these models, but also indicates the need for more DC-specific ablation models for more definitive studies on the relevance of distinct DC populations in T cell homeostasis and autoimmune disease progression. Epidermal Langerhans cells have also been constitutively ablated using this approach[32].

Cell type-specific gene expression and knockout

Various genes that are relevant to MPS cell development and function have been identified using gene-overexpression or gene-knockout models. However, with the appreciation that the MPS comprises a number of distinct cell subsets with unique gene expression patterns, there is a need for tools to overexpress or knock out particular genes in a cell type-specific manner.

The simplest way to overexpress a gene in a specific cell type is to place the gene downstream of a reconstituted promoter that has an expression pattern that is restricted to the cell type of interest. For example, a transgene encoding the CSF3 receptor (CSF3R) was

Langerhans cells
Dendritic cells that inhabit the epidermis. They are best distinguished by their high-level expression of the C-type lectin receptor langerin and its associated Birbeck granules. In contrast to other dendritic cells, Langerhans cells self-renew locally and are not depleted by high doses of X-ray irradiation.

Cre recombinase
Cre is a site-specific recombinase that recognizes and binds specific DNA sequences known as *loxP* sites. Two *loxP* sites recombine in the presence of Cre, enabling excision of the intervening DNA sequence.

© 2011 Macmillan Publishers Limited. All rights reserved

Table 2 | **Efficacy of various models for depleting mononuclear phagocytes in mice**

Cell type	Cell subset	Depletion strategy and efficacy of each method						
		Clodronate liposomes[17]	CSF1R-specific antibodies[18]	*Cd11b*-DTR[33]	MAFIA[48]	*Cd169*-DTR[50]	*Cd11c*-DTR[52,57]	Langerin-DTR[58,59]
Monocyte	GR1^hi cells from peripheral blood	+++[110,111]	–[19]; ++[20]	+++[34–36]	++[48]; +++[49]	–*	ND	ND
	GR1^low cells from peripheral blood	+++[110,111]	++[20]; +++[19]			–*	ND	ND
	Bone marrow GR1^hi cells	+++[8]	+*	++[8]	+++[8]	–[8]	–*	ND
	Bone marrow GR1^low cells	+++[8]	+*	++[8]	+++[8]	–[8]	–*	ND
Macrophage	Bone marrow	+++[8]	+*	+[8]	+++[8]	+++[8]	–[8,59]	ND
	Lymph node	+++[9,27]	+++[20]	ND	+++[49]	+++[51]	+++[9]	ND
	Peritoneum	+++[112,113]	+++[19]	+++[33,36]	+++[48,49]	–*	ND	ND
	Splenic red pulp	+++[114,115]	++[20]	ND	+++*	++*	–[116]	ND
	Splenic marginal metallophilic	+++[114]	+++[20]	ND	ND	+++[50]	+++[57,116]	ND
	Splenic marginal zone	+++[114]	ND	ND	ND	+++[50]	+++[116]	ND
	Lung	++[117,118]; +++[29,30,119,120]	–[19]	–[36]; +++[35]	+++[48,49]	ND	+++[121]	–[64]
	Liver	++[25]; +++[122]	+++[19]	–[36]	ND	ND	ND	ND
	Gut	++[123]; +++[31]	+++[19]	ND	+++[31]	ND	ND	ND
	CNS	+++[124]	–[19]	ND	–[125]	–*	ND	ND
	Dermis	+++[26]	+++[19]	ND	ND	ND	ND	ND
Dendritic cell	Lymph node	–[25]	–[20]	ND	ND	–[51]	++[126]; +++[57]	–[127]; ++[58]
	Spleen CD8^+	+++[115]	–[20]	ND	ND	–[50]	+++[52,57,116]	+++[63]
	Spleen CD8^–	+++[115]		ND	ND			–[63]
	Lung CD103^+ CD11b^–	–[29]	ND	ND	ND	–*	++[64,128]	+++[64]
	Lung CD103^– CD11b^+	–[29]	ND	ND	ND	–*		–[64]
	Liver CD103^+ CD11b^–	ND	ND	ND	ND	–*	+++[129]	ND
	Liver CD103^– CD11b^+	ND	ND	ND	ND	–*		ND
	Gut CD103^+ CD11b^–	+++[31]	ND	ND	ND	–*	+++[130]	ND
	Gut CD103^– CD11b^+		ND	ND	ND	–*		ND
	Dermis CD103^+ CD11b^–	ND	ND	ND	ND	–*	++[126]	+++[60–62]
	Dermis CD103^– CD11b^+	ND	ND	ND	ND	–*		–*
	Langerhans cells	–[26,131]	+++*	ND	ND	–*	–/+[32]	+++[58–62]

The table only includes data from reports in which depletion takes place in mice under steady-state conditions. Data from rats and other species or depletion demonstrated in the context of other manipulations (such as infection, cancer or transplantation) were excluded. Population subset boxes are merged when data does not distinguish between subsets. –, <20% of population depleted; +, 20–50% of population depleted; ++, 50–80% of population depleted; +++, >80% of population depleted; CSF1R, colony-stimulating factor 1 receptor; DTR, diphtheria toxin receptor; MAFIA, macrophage FAS-induced apoptosis; ND, not determined. *Unpublished observations (A.C., D. Hashimoto and M.M.).

recently cloned downstream of the *Cd68* promoter and used to generate transgenic mice. This demonstrated that CSF3R expression on CD68$^+$ monocytic cells is sufficient to mobilize haematopoietic stem and progenitor cells in response to CSF3 (also known as G-CSF)[68].

It is also possible to achieve cell type-specific or conditional gene knockout in mice. This is done by knocking in two *loxP* sites at distant introns within a given gene, which is known as 'floxing' the gene. The mouse is then crossed with a mouse that expresses the Cre recombinase from a cell type-specific promoter. In the cells in which Cre is expressed, the portion of the gene encompassed by the two *loxP* sites is excised and the gene is no longer expressed. This type of model is very useful for determining the precise function of a gene in a given cell type without the confounding action of knockout in other cell types. This model is also particularly useful when global loss of the gene causes embryonic lethality. For example, to overcome the embryonic lethality that results from deletion of the gene encoding Dicer, and to restrict the deletion to CD11c$^+$ cells, mice with a floxed *Dicer* gene were crossed with *Cd11c*-Cre mice. This resulted in the depletion of Langerhans cells, revealing a role for Dicer in the maintenance of this DC population, whereas other DC populations were unaffected[69]. The specific depletion of Langerhans cells in this model may be due to the slow turnover of these cells, whereas other DC populations have a much more rapid turnover once they express CD11c.

Dicer
Dicer is an endoribonuclease that cleaves double-stranded RNA and is important for processing pre-microRNAs.

© 2011 Macmillan Publishers Limited. All rights reserved

Mice with cell type-specific Cre expression also provide a way to overexpress a gene in particular cell types. This is achieved by crossing the promoter-Cre mice with mice in which a copy of the gene to be overexpressed is preceded by a stopflox cassette and knocked into the ubiquitously expressed *Rosa26* locus. Thus, in cells that express Cre, the stopflox cassette will be excised and the transgene will be expressed. The main advantage of this approach is that it does not require a unique transgenic mouse to be generated for every promoter–transgene combination. Instead, a single Cre-transgenic animal can be crossed with any line that has a *loxP*-flanked stop codon upstream of a gene encoding DTR[70], DTA[71,72] or a tracking protein (such as green fluorescent protein (GFP), red fluorescent protein (RFP), the fluorescent protein tdTomato or LacZ) to achieve cell depletion (see above) or tracking (see below) in a promoter-specific manner.

One can also achieve temporal control over protein expression or knockdown using the Cre^{ERT2} system. In this system, the Cre recombinase enzyme is linked to the mutated ligand-binding domain of the oestrogen receptor. Only when tamoxifen, an oestrogen receptor antagonist, is administered to the animal does Cre enter the nucleus, where it can excise the *loxP*-flanked sequence. This additional layer of temporal control is crucial when gene expression or knockout in a particular cell type causes embryonic lethality.

Reporter mice and lineage-tracing mouse models

Reporter mice with transgenic or knock-in expression of fluorescent proteins have added to our understanding of the origin and function of mononuclear phagocytes. Traditionally, these models have been generated by placing a reporter, such as LacZ or EGFP, downstream of a reconstituted promoter or in an appropriate bacterial artificial chromosome, or by knocking the reporter into a specific genomic locus. Various mouse models have been created to allow antibody-free tracking of mononuclear phagocytes. These include lysozyme M (*Lyz2*)-EGFP mice[73], *Csf1r*-EGFP (MacGreen[74] and MAFIA[48]) mice, CX_3C-chemokine receptor 1 (*Cx3cr1*)-EGFP mice[75], CC-chemokine receptor 2 (*Ccr2*)-RFP mice[76], MHC class II-EGFP mice[77], *Cd11c*-DTR/GFP mice[52], *Cd11c*-EYFP (enhanced yellow fluorescent protein) mice[78], langerin-EGFP mice and langerin-DTR-GFP mice[58,59]. The uses and pitfalls of most of these models have been recently reviewed[79].

When used in tandem with antibodies in flow cytometry, these reporter models are very useful in identifying specific subpopulations of monocytes, macrophages or DCs, especially for markers to which commercial antibodies are either suboptimal or not available. However, when used singly for imaging purposes, the main limitation of these reporter mice is their inability to precisely track specific populations of cells owing to overlapping activities of the promoters among mononuclear phagocytes. For example, EGFP is expressed by neutrophils as well as mononuclear phagocytes in *Lyz2*-EGFP[73], MacGreen[74,80,81] and MAFIA[11,12,48] mice. *Cx3cr1*-EGFP animals have been extensively used to study the MPS, because CX_3CR1 is expressed throughout mononuclear phagocyte development and is absent from granulocytes[75]. Expressed on early myeloid precursors in the embryo[82] and adult myeloid progenitors (such as MDPs, CDPs and pre-DCs)[83], CX_3CR1 remains expressed on circulating monocytes, as well as on some tissue macrophages and DCs[75]. However, CX_3CR1 is not a specific marker of the MPS, as it can also be expressed by lymphoid cells (such as NK cells[75]) and by mouse epidermal γδ T cells (F. Ginhoux and M.M., unpublished observations). Similarly, *Ccr2*-RFP animals have RFP-expressing monocytes, with classical monocytes showing higher RFP expression than non-classical monocytes, but NK cells and T cells[76] are also labelled in these mice.

MHC class II-EGFP knock-in animals can be used to identify DCs, but there is also EGFP expression on B cells and macrophages[77,84]. *Cd11c*-DTR/GFP and *Cd11c*-EYFP mice have been used to track DCs[52,78]; however, although there are no reports of fluorescence in other cell types, subsets of monocytes, macrophages, NK cells, B cells and T cells also express CD11c, and it remains to be seen if these subpopulations are also labelled in CD11c reporter mice. Reporter animals based on the langerin promoter selectively label langerin-expressing DCs[58,59], allowing for direct visualization of epidermal Langerhans cells and $CD103^+$ DCs in an antibody-free system. New models with such a restricted expression of a reporter gene by only certain subpopulations of monocytes, macrophages or DCs will be invaluable for imaging mononuclear phagocytes *in vivo*.

To better understand the developmental origins of MPS cells, lineage-tracing models have been developed. These mice are generated by crossing *Rosa26*-stopflox-EGFP or *Rosa26*-stopflox-LacZ animals with mice that express Cre from a specific promoter. In cells in which the promoter is active, Cre is expressed, the floxed stop cassette is excised and the reporter is then expressed. As this reaction is irreversible, the cells continue to express the reporter even if they differentiate and the promoter that drives Cre expression is turned off. Thus, cells with a common gene expression pattern or heritage can be identified. Recently, *Runx1*-Cre^{ERT2} mice were crossed with *Rosa26*-stopflox-EGFP or *Rosa26*-stopflox-LacZ animals to trace the lineage of yolk sac-derived haematopoietic cells[85]. Using this mouse line for fate-mapping analyses, it was determined that adult microglia are derived from yolk sac macrophages[82]. Similar fate-mapping approaches to label other cell types, such as MDPs and monocytes, will be instrumental in helping us to identify the origins of tissue macrophages and DCs.

The limitation of this approach is that the tracing is defined by the expression pattern of the specific promoter driving Cre expression. If this promoter is active in the progenitors of unrelated lineages, this can confound interpretations. Thus, it is important to identify promoters with high fidelity to the cell type of interest.

© 2011 Macmillan Publishers Limited. All rights reserved

Improving targeting

For achieving cell type-specific expression of transgenes, including that encoding Cre, there are several useful promoters (such as those from the *Lyz2*, *Cd11b*, *Cd11c* and langerin genes) that have been reconstituted or can be inserted into a bacterial artificial chromosome. However, although these promoters are helpful for broad, although sometimes incomplete, targeting to macrophages or DCs, they are not useful for targeting specific subsets[79]. Therefore, we need to identify regulatory elements that enable transgene expression to be more specifically targeted to distinct mononuclear phagocyte subpopulations. In addition, as similar DC and macrophage subsets may have diverse roles depending on the tissue environment, it would be extremely useful to generate methods that enable cell type-specific ablation in particular organs and tissues. In this way, the tissue-specific role of a subset can be better determined. Transcriptional profiling provides an *in silico* means to find new regulatory elements that can be used to drive cell type-specific transgene expression. Of course, few promoters or enhancers are restricted to a single cell type, so achieving exquisite targeting can require additional actions.

An alternative and complementary strategy is to use microRNA-based targeting. To do this, synthetic target sites for a microRNA that is expressed in a specific cell type are placed downstream of the transgene[86,87]. This results in suppression of the transgene in cells in which the microRNA is expressed. This 'de-targeting' approach has been used to specifically eliminate transgene expression in a variety of cell types (including T cells, mature DCs and haematopoietic stem cells), and it can be a powerful addendum to improve transgene targeting in transgenic systems that rely on cell type-specific promoters[88,89].

Molecular profiling

Molecular profiling involves the use of technologies that globally characterize gene expression and protein levels, such as microarrays, next-generation sequencing and proteomics. It is being used to answer some of the outstanding questions about MPS biology; for example, to define the differences between DCs and macrophages, and between their subsets. Because of their low frequency and broad distribution throughout tissues, it has been difficult to isolate different *in vivo* DC and macrophage populations at sufficient purity and quantity for profiling. Quantitative proteomics has been particularly challenging; however, Mann and colleagues recently reported that a label-free approach could be used to determine the abundance of more than 5,000 proteins in four different subsets of splenic DCs isolated from mice[90]. Their results confirmed earlier transcriptional profiling studies indicating distinct gene expression signatures between DC subsets, and even showed that the differential expression of components of pattern recognition pathways leads to differences in the functions of splenic DCs.

One of the most ambitious attempts at understanding the molecular programming of MPS cells and other haematopoietic cells has been established by the Immunological Genome Project (ImmGen). ImmGen is a US National Institutes of Health (NIH)-funded consortium, which we are part of, that has isolated almost every known subset of immune cells (including more than 20 different populations of DCs and macrophages) and has performed microarray-based transcriptional profiling on each. The ImmGen data (available from the ImmGen website) enables a broad comparison of gene expression signatures across haematopoietic cells. Although gain- and loss-of-function experiments will still be necessary to determine the relevance of many of these genes to MPS cells, this compilation of data should help to identify the core gene expression signatures that define subtypes of monocytes, macrophages and DCs and the progenitors from which they arise (that is, haematopoietic stem cells, multipotent progenitors, common myeloid progenitors, granulocyte and macrophage progenitors, MDPs and CDPs).

High-throughput screening

RNA interference is the most rapid means to identify gene function in human and mouse cells, and has been effective in elucidating gene functions in macrophages and DCs. A large-scale example was recently reported by Paul *et al.*[91]. They performed a genome-wide screen to identify genes that regulate MHC class II expression by transfecting a model cell line, which was distributed in dozens of 96-well plates, with small interfering RNAs (siRNAs) targeted against 21,245 human genes. High-throughput fluorescence-activated cell sorting (FACS) and confocal microscopy were used to monitor the impact of each gene knockdown on representative markers for MHC class II expression and peptide loading, as well as for MHC class II localization. Their screen identified 250 genes with a previously unappreciated role in MHC class II regulation, including a new axis that links cell signalling with an actin-based mechanism involved in redistributing MHC class II molecules on the cell surface during DC maturation.

Hacohen and colleagues used a lentiviral vector-based small hairpin RNA (shRNA) library to investigate the regulatory role of 125 genes in the response of mouse bone marrow-derived DCs to lipopolysaccharide (LPS) or polyinosinic–polycytidylic acid (polyI:C). Instead of looking at particular phenotypic markers following gene knockdown, as Paul *et al.* did, Hacohen and colleagues used transcriptome profiling to uncover the set of genes controlled by a putative regulatory gene[92]. This type of approach allows for a relatively unbiased interrogation of gene function and enabled them to identify a core network of 24 regulatory genes (including several unexpected cell cycle genes, such as *Jun* and *Timeless*) that regulates the larger network of genes involved in the response of bone marrow-derived DCs to pathogens.

At present, high-throughput screening is only feasible in cell culture, which limits the approach to *in vitro* models of macrophage and DC function. Current high-throughput screening studies use cell lines, such as human THP-1 pro-monocytic cells, or cells derived from progenitor cell cultures, such as macrophages induced by CSF1 and DCs induced by CSF2 (also known as GM-CSF) or FLT3L. Certain subsets — such as CD103^{+} DCs, red pulp

© 2011 Macmillan Publishers Limited. All rights reserved

macrophages, lymph node subcapsular macrophages and alveolar macrophages — cannot be easily manipulated in culture. However, as we learn more about the developmental requirements of macrophages and DCs, it should be possible to generate better *in vitro* models for performing gain- and loss-of-function studies.

The high cost of genome-wide RNA interference screens will lead many investigators to choose a candidate gene approach rather than an unbiased, comprehensive screen. An alternative is to use pooled libraries of cDNAs or shRNAs, introduce these into cells *en masse* and use selection techniques (for example, FACS based on a phenotypic marker) followed by deconvolution of the library in the selected cells to identify the cDNAs or shRNAs, and by inference the genes, that drove the selected phenotype. This type of approach, which is relatively cheap, has been used in cancer biology (for example, to identify genes involved in chemoresistance[93]) and may be amenable to the study of macrophage and DC development and function.

The creation of better siRNA and shRNA designs and validated libraries is starting to increase the use of RNA interference. The Broad Institute, for one, is validating the efficiency of target knockdown for >50,000 publicly available lentiviral vector-based shRNA constructs[94], and the Hannon and Lowe laboratories recently generated a mouse model that can be used to create conditional shRNA-transgenic mice[95]. These new tools will help to facilitate genomic analyses of the MPS.

Humanized mice to study the MPS

Differences between mouse and human immunology, including in MPS cells, have been previously described[96]. Moreover, human strains of certain pathogens that infect MPS cells — such as HIV, hepatitis B virus and hepatitis C virus — do not cause disease in murine systems owing to differences in the susceptibility of murine and human MPS cells to infection. This precludes studies of these human pathogens *in vivo* in rodent models. Collectively, these factors have driven the development of human-haemato-lymphoid-system (HHLS) mice (reviewed in REF. 97). The general concept is to reconstitute a human haematopoietic system in mice by transplanting human CD34$^+$ haematopoietic stem and progenitor cells into newborn immunocompromised mice that have been sublethally irradiated. The first generation of these models suffered from impaired haematopoietic stem cell engraftment and, consequently, reduced human cell chimerism after 4–6 months, and the engraftment of human myeloid cells was also poor[97]. In order to achieve greater engraftment of human MPS cells in HHLS mice, human cytokines — such as thrombopoietin, interleukin-3, CSF1 and CSF2 — have been introduced into these mice through various modalities, including straightforward injection, transgenic expression or knock-in expression[97,98]. Modification of the mouse signal-regulatory protein-α (*Sirpa*) gene has also been used to yield higher engraftment of human cells[99]. These approaches have allowed for more durable myeloid chimerism of human MPS cells in mice and will facilitate the study of human myeloid cells in a physiological *in vivo* setting.

Use of zebrafish to study MPS cell development

New mouse models will greatly advance our understanding of macrophage and DC development. However, identifying the role of the thousands of genes expressed in these cells requires models that are more amenable to accelerated analysis. The identification of macrophages and DCs in zebrafish may make this a useful model to study the development of these cells[100]. These aquatic vertebrates make it comparatively easy to study the development, migration and localization of a cell lineage because the embryos, and now even a strain of adults[101], are translucent. A zebrafish model was recently generated with a GFP reporter under the control of the MHC class II gene promoter, and this established similarities in the tissue distribution of MPS cells in zebrafish and higher order mammals[102]. It will now be useful to generate strains of zebrafish with GFP reporters specific for macrophages and DCs, and to cross these strains with the many zebrafish gene mutants that are currently being generated.

Conclusions

The tools that are currently available have been instrumental in establishing the field of mononuclear phagocytes. In order to push the field to the next stage and to answer many of the lingering questions (see BOX 1 and BOX 2), the next generation of tools must be developed to more precisely dissect the MPS.

1. Fogg, D. K. *et al.* A clonogenic bone marrow progenitor specific for macrophages and dendritic cells. *Science* **311**, 83–87 (2006).
This study was the first to characterize a dedicated macrophage and DC progenitor.
2. Auffray, C. *et al.* CX3CR1$^+$ CD115$^+$ CD135$^+$ common macrophage/DC precursors and the role of CX3CR1 in their response to inflammation. *J. Exp. Med.* **206**, 595–606 (2009).
3. Onai, N. *et al.* Identification of clonogenic common Flt3$^+$M-CSFR$^+$ plasmacytoid and conventional dendritic cell progenitors in mouse bone marrow. *Nature Immunol.* **8**, 1207–1216 (2007).
4. Naik, S. H. *et al.* Development of plasmacytoid and conventional dendritic cell subtypes from single precursor cells derived *in vitro* and *in vivo*. *Nature Immunol.* **8**, 1217–1226 (2007).
References 3 and 4 were the first characterizations of a DC-restricted progenitor (termed the CDP) that gives rise to classical DCs and plasmacytoid DCs.
5. Liu, K. *et al. In vivo* analysis of dendritic cell development and homeostasis. *Science* **324**, 392–397 (2009).
This was the first characterization of pre-DCs and how they are related to MDPs and CDPs.
6. Reizis, B., Bunin, A., Ghosh, H. S., Lewis, K. L. & Sisirak, V. Plasmacytoid dendritic cells: recent progress and open questions. *Annu. Rev. Immunol.* **29**, 163–183 (2011).
7. Ingersoll, M. A., Platt, A. M., Potteaux, S. & Randolph, G. J. Monocyte trafficking in acute and chronic inflammation. *Trends Immunol.* 10 Jun 2011 (doi:10.1016/j.it.2011.05.001).
8. Chow, A. *et al.* Bone marrow CD169$^+$ macrophages promote the retention of hematopoietic stem and progenitor cells in the mesenchymal stem cell niche. *J. Exp. Med.* **208**, 261–271 (2011).
9. Iannacone, M. *et al.* Subcapsular sinus macrophages prevent CNS invasion on peripheral infection with a neurotropic virus. *Nature* **465**, 1079–1083 (2010).
10. Hildner, K. *et al.* Batf3 deficiency reveals a critical role for CD8α$^+$ dendritic cells in cytotoxic T cell immunity. *Science* **322**, 1097–1100 (2008).
11. Ginhoux, F. *et al.* The origin and development of nonlymphoid tissue CD103$^+$ DCs. *J. Exp. Med.* **206**, 3115–3130 (2009).
12. Bogunovic, M. *et al.* Origin of the lamina propria dendritic cell network. *Immunity* **31**, 513–525 (2009).
13. Edelson, B. T. *et al.* Peripheral CD103$^+$ dendritic cells form a unified subset developmentally related to CD8α$^+$ conventional dendritic cells. *J. Exp. Med.* **207**, 823–836 (2010).
This was the first report that BATF3 is a crucial transcription factor for both CD8α$^+$ and CD103$^+$ DCs, a basis for the future classification of DC subsets.
14. Hashimoto, D., Miller, J. & Merad, M. Characterizing DC and macrophage heterogeneity *in vivo*. *Immunity* **35**, 323–335 (2011).

© 2011 Macmillan Publishers Limited. All rights reserved

15. Chitu, V. & Stanley, E. R. Colony-stimulating factor-1 in immunity and inflammation. *Curr. Opin. Immunol.* **18**, 39–48 (2006).
16. Merad, M. & Manz, M. G. Dendritic cell homeostasis. *Blood* **113**, 3418–3427 (2009).
17. van Rooijen, N. & van Kesteren-Hendrikx, E. Clodronate liposomes: perspectives in research and therapeutics. *J. Liposome Res.* **12**, 81–94 (2002).
18. Sudo, T. *et al.* Functional hierarchy of c-kit and c-fms in intramarrow production of CFU-M. *Oncogene* **11**, 2469–2476 (1995).
19. MacDonald, K. P. *et al.* An antibody against the colony-stimulating factor 1 receptor depletes the resident subset of monocytes and tissue- and tumor-associated macrophages but does not inhibit inflammation. *Blood* **116**, 3955–3963 (2010).
20. Hashimoto, D. *et al.* Pretransplant CSF-1 therapy expands recipient macrophages and ameliorates GVHD after allogeneic hematopoietic cell transplantation. *J. Exp. Med.* **208**, 1069–1082 (2011).
21. Irvine, K. M. *et al.* A CSF-1 receptor kinase inhibitor targets effector functions and inhibits pro-inflammatory cytokine production from murine macrophage populations. *FASEB J.* **20**, 1921–1923 (2006).
22. Ohno, H. *et al.* A c-fms tyrosine kinase inhibitor, Ki20227, suppresses osteoclast differentiation and osteolytic bone destruction in a bone metastasis model. *Mol. Cancer Ther.* **5**, 2634–2643 (2006).
23. Conway, J. G. *et al.* Inhibition of colony-stimulating-factor-1 signaling *in vivo* with the orally bioavailable cFMS kinase inhibitor GW2580. *Proc. Natl Acad. Sci. USA* **102**, 16078–16083 (2005).
24. Burns, C. J. & Wilks, A. F. c-FMS inhibitors: a patent review. *Expert Opin. Ther. Pat.* **21**, 147–165 (2011).
25. Zhang, Y. *et al.* APCs in the liver and spleen recruit activated allogeneic CD8⁺ T cells to elicit hepatic graft-versus-host disease. *J. Immunol.* **169**, 7111–7118 (2002).
26. Wang, H. *et al.* Activated macrophages are essential in a murine model for T cell-mediated chronic psoriasiform skin inflammation. *J. Clin. Invest.* **116**, 2105–2114 (2006).
27. Junt, T. *et al.* Subcapsular sinus macrophages in lymph nodes clear lymph-borne viruses and present them to antiviral B cells. *Nature* **450**, 110–114 (2007).
28. McGill, J., Van Rooijen, N. & Legge, K. L. Protective influenza-specific CD8 T cell responses require interactions with dendritic cells in the lungs. *J. Exp. Med.* **205**, 1635–1646 (2008).
29. Mircescu, M. M., Lipuma, L., van Rooijen, N., Pamer, E. G. & Hohl, T. M. Essential role for neutrophils but not alveolar macrophages at early time points following *Aspergillus fumigatus* infection. *J. Infect. Dis.* **200**, 647–656 (2009).
30. Traeger, T. *et al.* Selective depletion of alveolar macrophages in polymicrobial sepsis increases lung injury, bacterial load and mortality but does not affect cytokine release. *Respiration* **77**, 203–213 (2009).
31. Qualls, J. E., Kaplan, A. M., van Rooijen, N. & Cohen, D. A. Suppression of experimental colitis by intestinal mononuclear phagocytes. *J. Leukoc. Biol.* **80**, 802–815 (2006).
32. Bennett, C. L. & Clausen, B. E. DC ablation in mice: promises, pitfalls, and challenges. *Trends Immunol.* **28**, 525–531 (2007).
33. Duffield, J. S. *et al.* Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. *J. Clin. Invest.* **115**, 56–65 (2005).
34. Stoneman, V. *et al.* Monocyte/macrophage suppression in CD11b diphtheria toxin receptor transgenic mice differentially affects atherogenesis and established plaques. *Circ. Res.* **100**, 884–893 (2007).
35. Cailhier, J. F. *et al.* Resident pleural macrophages are key orchestrators of neutrophil recruitment in pleural inflammation. *Am. J. Respir. Crit. Care Med.* **173**, 540–547 (2006).
36. Cailhier, J. F. *et al.* Conditional macrophage ablation demonstrates that resident macrophages initiate acute peritoneal inflammation. *J. Immunol.* **174**, 2336–2342 (2005).
37. Saxena, V., Ondr, J. K., Magnusen, A. F., Munn, D. H. & Katz, J. D. The countervailing actions of myeloid and plasmacytoid dendritic cells control autoimmune diabetes in the nonobese diabetic mouse. *J. Immunol.* **179**, 5041–5053 (2007).
38. Devey, L. *et al.* Tissue-resident macrophages protect the liver from ischemia reperfusion injury via a heme oxygenase-1-dependent mechanism. *Mol. Ther.* **17**, 65–72 (2009).
39. Fallowfield, J. A. *et al.* Scar-associated macrophages are a major source of hepatic matrix metalloproteinase-13 and facilitate the resolution of murine hepatic fibrosis. *J. Immunol.* **178**, 5288–5295 (2007).
40. Duffield, J. S. *et al.* Conditional ablation of macrophages halts progression of crescentic glomerulonephritis. *Am. J. Pathol.* **167**, 1207–1219 (2005).
41. Henderson, N. C. *et al.* Galectin-3 expression and secretion links macrophages to the promotion of renal fibrosis. *Am. J. Pathol.* **172**, 288–298 (2008).
42. Lin, S. L., Castano, A. P., Nowlin, B. T., Lupher, M. L. & Duffield, J. S. Bone marrow Ly6C^{high} monocytes are selectively recruited to injured kidney and differentiate into functionally distinct populations. *J. Immunol.* **183**, 6733–6743 (2009).
43. Machida, Y. *et al.* Renal fibrosis in murine obstructive nephropathy is attenuated by depletion of monocyte lineage, not dendritic cells. *J. Pharmacol. Sci.* **114**, 464–473 (2010).
44. Qi, F. *et al.* Depletion of cells of monocyte lineage prevents loss of renal microvasculature in murine kidney transplantation. *Transplantation* **86**, 1267–1274 (2008).
45. Chua, A. C. L., Hodson, L. J., Moldenhauer, L. M., Robertson, S. A. & Ingman, W. V. Dual roles for macrophages in ovarian cycle-associated development and remodelling of the mammary gland epithelium. *Development* **137**, 4229–4238 (2010).
46. Jasper, M. J. *et al.* Macrophage-derived LIF and IL1B regulate α(1,2)fucosyltransferase 2 (Fut2) expression in mouse uterine epithelial cells during early pregnancy. *Biol. Reprod.* **84**, 179–188 (2011).
47. Withers, S. B. *et al.* Macrophage activation is responsible for loss of anticontractile function in inflamed perivascular fat. *Arterioscler. Thromb. Vasc. Biol.* **31**, 908–913 (2011).
48. Burnett, S. H. *et al.* Conditional macrophage ablation in transgenic mice expressing a Fas-based suicide gene. *J. Leukoc. Biol.* **75**, 612–623 (2004).
49. Burnett, S. H. *et al.* Development of peritoneal adhesions in macrophage depleted mice. *J. Surg. Res.* **131**, 296–301 (2006).
50. Miyake, Y. *et al.* Critical role of macrophages in the marginal zone in the suppression of immune responses to apoptotic cell-associated antigens. *J. Clin. Invest.* **117**, 2268–2278 (2007).
51. Asano, K. *et al.* CD169-positive macrophages dominate antitumor immunity by crosspresenting dead cell-associated antigens. *Immunity* **34**, 85–95 (2011).
52. Jung, S. *et al. In vivo* depletion of CD11c⁺ dendritic cells abrogates priming of CD8⁺ T cells by exogenous cell-associated antigens. *Immunity* **17**, 211–220 (2002).
53. Bar-On, L. & Jung, S. Defining dendritic cells by conditional and constitutive cell ablation. *Immunol. Rev.* **234**, 76–89 (2010).
54. Sapoznikov, A. *et al.* Organ-dependent *in vivo* priming of naive CD4⁺, but not CD8⁺, T cells by plasmacytoid dendritic cells. *J. Exp. Med.* **204**, 1923–1933 (2007).
55. Plaks, V. *et al.* Uterine DCs are crucial for decidua formation during embryo implantation in mice. *J. Clin. Invest.* **118**, 3954–3965 (2008).
56. Lucas, M., Schachterle, W., Oberle, K., Aichele, P. & Diefenbach, A. Dendritic cells prime natural killer cells by trans-presenting interleukin 15. *Immunity* **26**, 503–517 (2007).
57. Hochweller, K., Striegler, J., Hammerling, G. J. & Garbi, N. A novel CD11c.DTR transgenic mouse for depletion of dendritic cells reveals their requirement for homeostatic proliferation of natural killer cells. *Eur. J. Immunol.* **38**, 2776–2783 (2008).
58. Kissenpfennig, A. *et al.* Dynamics and function of Langerhans cells *in vivo*: dermal dendritic cells colonize lymph node areas distinct from slower migrating Langerhans cells. *Immunity* **22**, 643–654 (2005).
59. Bennett, C. L. *et al.* Inducible ablation of mouse Langerhans cells diminishes but fails to abrogate contact hypersensitivity. *J. Cell Biol.* **169**, 569–576 (2005).
60. Nagao, K. *et al.* Murine epidermal Langerhans cells and langerin-expressing dermal dendritic cells are unrelated and exhibit distinct functions. *Proc. Natl Acad. Sci. USA* **106**, 3312–3317 (2009).
61. Ginhoux, F. *et al.* Blood-derived dermal langerin⁺ dendritic cells survey the skin in the steady state. *J. Exp. Med.* **204**, 3133–3146 (2007).
62. Bursch, L. S. *et al.* Identification of a novel population of Langerin⁺ dendritic cells. *J. Exp. Med.* **204**, 3147–3156 (2007).
63. Farrand, K. J. *et al.* Langerin⁺ CD8α⁺ dendritic cells are critical for cross-priming and IL-12 production in response to systemic antigens. *J. Immunol.* **183**, 7732–7742 (2009).
64. GeurtsvanKessel, C. H. *et al.* Clearance of influenza virus from the lung depends on migratory langerin⁺CD11b⁻ but not plasmacytoid dendritic cells. *J. Exp. Med.* **205**, 1621–1634 (2008).
65. Birnberg, T. *et al.* Lack of conventional dendritic cells is compatible with normal development and T cell homeostasis, but causes myeloid proliferative syndrome. *Immunity* **29**, 986–997 (2008).
66. Ohnmacht, C. *et al.* Constitutive ablation of dendritic cells breaks self-tolerance of CD4 T cells and results in spontaneous fatal autoimmunity. *J. Exp. Med.* **206**, 549–559 (2009).
67. Teichmann, L. L. *et al.* Dendritic cells in lupus are not required for activation of T and B cells but promote their expansion, resulting in tissue damage. *Immunity* **33**, 967–978 (2010).
68. Christopher, M. J., Rao, M., Liu, F., Woloszynek, J. R. & Link, D. C. Expression of the G-CSF receptor in monocytic cells is sufficient to mediate hematopoietic progenitor mobilization by G-CSF in mice. *J. Exp. Med.* **208**, 251–260 (2011).
69. Kuipers, H., Schnorfeil, F. M., Fehling, H. J., Bartels, H. & Brocker, T. Dicer-dependent microRNAs control maturation, function, and maintenance of Langerhans cells *in vivo*. *J. Immunol.* **185**, 400–409 (2010).
70. Buch, T. *et al.* A Cre-inducible diphtheria toxin receptor mediates cell lineage ablation after toxin administration. *Nature Methods* **2**, 419–426 (2005).
71. Brockschnieder, D., Pechmann, Y., Sonnenberg-Riethmacher, E. & Riethmacher, D. An improved mouse line for Cre-induced cell ablation due to diphtheria toxin A, expressed from the Rosa26 locus. *Genesis* **44**, 322–327 (2006).
72. Voehringer, D., Liang, H. E. & Locksley, R. M. Homeostasis and effector function of lymphopenia-induced "memory-like" T cells in constitutively T cell-depleted mice. *J. Immunol.* **180**, 4742–4753 (2008).
73. Faust, N., Varas, F., Kelly, L. M., Heck, S. & Graf, T. Insertion of enhanced green fluorescent protein into the lysozyme gene creates mice with green fluorescent granulocytes and macrophages. *Blood* **96**, 719–726 (2000).
74. Sasmono, R. T. *et al.* A macrophage colony-stimulating factor receptor–green fluorescent protein transgene is expressed throughout the mononuclear phagocyte system of the mouse. *Blood* **101**, 1155–1163 (2003).
75. Jung, S. *et al.* Analysis of fractalkine receptor CX_3CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. *Mol. Cell. Biol.* **20**, 4106–4114 (2000).
76. Saederup, N. *et al.* Selective chemokine receptor usage by central nervous system myeloid cells in CCR2–red fluorescent protein knock-in mice. *PLoS ONE* **5**, e13693 (2010).
77. Boes, M. *et al.* T-cell engagement of dendritic cells rapidly rearranges MHC class II transport. *Nature* **418**, 983–988 (2002).
78. Lindquist, R. L. *et al.* Visualizing dendritic cell networks *in vivo*. *Nature Immunol.* **5**, 1243–1250 (2004).
79. Hume, D. A. Applications of myeloid-specific promoters in transgenic mice support *in vivo* imaging and functional genomics but do not support the concept of distinct macrophage and dendritic cell lineages or roles in immunity. *J. Leukoc. Biol.* **89**, 525–538 (2011).
80. MacDonald, K. P. *et al.* The colony-stimulating factor 1 receptor is expressed on dendritic cells during differentiation and regulates their expansion. *J. Immunol.* **175**, 1399–1405 (2005).
81. Sasmono, R. T. *et al.* Mouse neutrophilic granulocytes express mRNA encoding the macrophage colony-stimulating factor receptor (CSF-1R) as well as many other macrophage-specific transcripts and can transdifferentiate into macrophages *in vitro* in response to CSF-1. *J. Leukoc. Biol.* **82**, 111–123 (2007).

© 2011 Macmillan Publishers Limited. All rights reserved

82. Ginhoux, F. *et al.* Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* **330**, 841–845 (2010).
This report showed that adult microglia are derived from primitive macrophages that arise prior to embryonic day 8.
83. Helft, J., Ginhoux, F., Bogunovic, M. & Merad, M. Origin and functional heterogeneity of non-lymphoid tissue dendritic cells in mice. *Immunol. Rev.* **234**, 55–75 (2010).
84. Mempel, T. R., Scimone, M. L., Mora, J. R. & von Andrian, U. H. *In vivo* imaging of leukocyte trafficking in blood vessels and tissues. *Curr. Opin. Immunol.* **16**, 406–417 (2004).
85. Samokhvalov, I. M., Samokhvalova, N. I. & Nishikawa, S. Cell tracing shows the contribution of the yolk sac to adult haematopoiesis. *Nature* **446**, 1056–1061 (2007).
This was the first published technique for the fate mapping of yolk sac-derived haematopoietic cells in adult mice.
86. Brown, B. D., Venneri, M. A., Zingale, A., Sergi Sergi, L. & Naldini, L. Endogenous microRNA regulation suppresses transgene expression in hematopoietic lineages and enables stable gene transfer. *Nature Med.* **12**, 585–591 (2006).
This was the first demonstration that microRNAs can be used for de-targeting transgenes and vectors.
87. Brown, B. D. & Naldini, L. Exploiting and antagonizing microRNA regulation for therapeutic and experimental applications. *Nature Rev. Genet.* **10**, 578–585 (2009).
88. Brown, B. D. *et al.* Endogenous microRNA can be broadly exploited to regulate transgene expression according to tissue, lineage and differentiation state. *Nature Biotech.* **25**, 1457–1467 (2007).
89. Gentner, B. *et al.* Identification of hematopoietic stem cell-specific miRNAs enables gene therapy of globoid cell leukodystrophy. *Sci. Transl. Med.* **2**, 58ra84 (2010).
90. Luber, C. A. *et al.* Quantitative proteomics reveals subset-specific viral recognition in dendritic cells. *Immunity* **32**, 279–289 (2010).
91. Paul, P. *et al.* A genome-wide multidimensional RNAi screen reveals pathways controlling MHC class II antigen presentation. *Cell* **145**, 268–283 (2011).
92. Amit, I. *et al.* Unbiased reconstruction of a mammalian transcriptional network mediating pathogen responses. *Science* **326**, 257–263 (2009).
93. Bivona, T. G. *et al.* FAS and NF-κB signalling modulate dependence of lung cancers on mutant EGFR. *Nature* **471**, 523–526 (2011).
94. Root, D. E., Hacohen, N., Hahn, W. C., Lander, E. S. & Sabatini, D. M. Genome-scale loss-of-function screening with a lentiviral RNAi library. *Nature Methods* **3**, 715–719 (2006).
95. Premsrirut, P. K. *et al.* A rapid and scalable system for studying gene function in mice using conditional RNA interference. *Cell* **145**, 145–158 (2011).
96. Mestas, J. & Hughes, C. C. Of mice and not men: differences between mouse and human immunology. *J. Immunol.* **172**, 2731–2738 (2004).
97. Willinger, T., Rongvaux, A., Strowig, T., Manz, M. G. & Flavell, R. A. Improving human hemato-lymphoid-system mice by cytokine knock-in gene replacement. *Trends Immunol.* **32**, 321–327 (2011).
98. Rathinam, C. *et al.* Efficient differentiation and function of human macrophages in humanized CSF-1 mice. *Blood* **118**, 3119–3128 (2011).
This paper demonstrated remarkable improvement in engraftment of human myeloid cells when human *CSF1* was knocked into *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ recipient mice.
99. Strowig, T. *et al.* Transgenic expression of human signal regulatory protein α in Rag2$^{-/-}$γc$^{-/-}$ mice improves engraftment of human hematopoietic cells in humanized mice. *Proc. Natl Acad. Sci. USA* **108**, 13218–13223 (2011).
This paper demonstrated remarkable improvement in engraftment of human haematopoietic cells when human *SIRPA* was genetically engineered into *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ recipient mice.
100. Lugo-Villarino, G. *et al.* Identification of dendritic antigen-presenting cells in the zebrafish. *Proc. Natl Acad. Sci. USA* **107**, 15850–15855 (2010).
This was the first report to characterize a DC population in zebrafish.
101. White, R. M. *et al.* Transparent adult zebrafish as a tool for *in vivo* transplantation analysis. *Cell Stem Cell* **2**, 183–189 (2008).
102. Wittamer, V., Bertrand, J. Y., Gutschow, P. W. & Traver, D. Characterization of the mononuclear phagocyte system in zebrafish. *Blood* **117**, 7126–7135 (2011).
103. Jenkins, S. J. *et al.* Local macrophage proliferation, rather than recruitment from the blood, is a signature of T_H2 inflammation. *Science* **332**, 1284–1288 (2011).
This was the first publication to show that, in the context of nematode challenge, macrophages at the site of infection are derived from local populations, rather than from recruited blood precursors.
104. Merad, M. *et al.* Langerhans cells renew in the skin throughout life under steady-state conditions. *Nature Immunol.* **3**, 1135–1141 (2002).
105. Li, J., Chen, K., Zhu, L. & Pollard, J. W. Conditional deletion of the colony stimulating factor-1 receptor (c-fms proto-oncogene) in mice. *Genesis* **44**, 328–335 (2006).
106. Varol, C. *et al.* Monocytes give rise to mucosal, but not splenic, conventional dendritic cells. *J. Exp. Med.* **204**, 171–180 (2007).
107. Pollard, J. W. Trophic macrophages in development and disease. *Nature Rev. Immunol.* **9**, 259–270 (2009).
108. Ghosn, E. E. *et al.* Two physically, functionally, and developmentally distinct peritoneal macrophage subsets. *Proc. Natl Acad. Sci. USA* **107**, 2568–2573 (2010).
109. Dorner, B. G. *et al.* Selective expression of the chemokine receptor XCR1 on cross-presenting dendritic cells determines cooperation with CD8$^+$ T cells. *Immunity* **31**, 823–833 (2009).
110. Sunderkotter, C. *et al.* Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response. *J. Immunol.* **172**, 4410–4417 (2004).
111. Engel, D. R. *et al.* CCR2 mediates homeostatic and inflammatory release of Gr1^{high} monocytes from the bone marrow, but is dispensable for bladder infiltration in bacterial urinary tract infection. *J. Immunol.* **181**, 5579–5586 (2008).
112. Kolaczkowska, E. *et al.* Resident peritoneal leukocytes are important sources of MMP-9 during zymosan peritonitis: superior contribution of macrophages over mast cells. *Immunol. Lett.* **113**, 99–106 (2007).
113. Leendertse, M. *et al.* Peritoneal macrophages are important for the early containment of *Enterococcus faecium* peritonitis in mice. *Innate Immun.* **15**, 3–12 (2009).
114. van Rooijen, N., Kors, N. & Kraal, G. Macrophage subset repopulation in the spleen: differential kinetics after liposome-mediated elimination. *J. Leukoc. Biol.* **45**, 97–104 (1989).
115. Nikolic, T., Geutskens, S. B., van Rooijen, N., Drexhage, H. A. & Leenen, P. J. Dendritic cells and macrophages are essential for the retention of lymphocytes in (peri)-insulitis of the nonobese diabetic mouse: a phagocyte depletion study. *Lab. Invest.* **85**, 487–501 (2005).
116. Probst, H. C. *et al.* Histological analysis of CD11c-DTR/GFP mice after *in vivo* depletion of dendritic cells. *Clin. Exp. Immunol.* **141**, 398–404 (2005).
117. Hickman-Davis, J. M., Michalek, S. M., Gibbs-Erwin, J. & Lindsey, J. R. Depletion of alveolar macrophages exacerbates respiratory mycoplasmosis in mycoplasma-resistant C57BL mice but not mycoplasma-susceptible C3H mice. *Infect. Immun.* **65**, 2278–2282 (1997).
118. Leemans, J. C. *et al.* Depletion of alveolar macrophages exerts protective effects in pulmonary tuberculosis in mice. *J. Immunol.* **166**, 4604–4611 (2001).
119. Zhang-Hoover, J., Sutton, A., van Rooijen, N. & Stein-Streilein, J. A critical role for alveolar macrophages in elicitation of pulmonary immune fibrosis. *Immunology* **101**, 501–511 (2000).
120. Bem, R. A. *et al.* Depletion of resident alveolar macrophages does not prevent Fas-mediated lung injury in mice. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **295**, L314–L325 (2008).
121. Landsman, L., Varol, C. & Jung, S. Distinct differentiation potential of blood monocyte subsets in the lung. *J. Immunol.* **178**, 2000–2007 (2007).
122. Sitia, G. *et al.* Kupffer cells hasten resolution of liver immunopathology in mouse models of viral hepatitis. *PLoS Pathog.* **7**, e1002061 (2011).
123. Zhao, A. *et al.* Th2 cytokine-induced alterations in intestinal smooth muscle function depend on alternatively activated macrophages. *Gastroenterology* **135**, 217–225 (2008).
124. Carmichael, M. D. *et al.* Role of brain macrophages on IL-1β and fatigue following eccentric exercise-induced muscle damage. *Brain Behav. Immun.* **24**, 564–568 (2010).
125. Steel, C. D. *et al.* Distinct macrophage subpopulations regulate viral encephalitis but not viral clearance in the CNS. *J. Neuroimmunol.* **226**, 81–92 (2010).
126. Tang, H. *et al.* The T helper type 2 response to cysteine proteases requires dendritic cell–basophil cooperation via ROS-mediated signaling. *Nature Immunol.* **11**, 608–617 (2010).
127. King, I. L., Kroenke, M. A. & Segal, B. M. GM-CSF-dependent, CD103$^+$ dermal dendritic cells play a critical role in Th effector cell differentiation after subcutaneous immunization. *J. Exp. Med.* **207**, 953–961 (2010).
128. van Rijt, L. S. *et al.* *In vivo* depletion of lung CD11c$^+$ dendritic cells during allergen challenge abrogates the characteristic features of asthma. *J. Exp. Med.* **201**, 981–991 (2005).
129. Plitas, G. *et al.* Dendritic cells are required for effective cross-presentation in the murine liver. *Hepatology* **47**, 1343–1351 (2008).
130. Vallon-Eberhard, A., Landsman, L., Yogev, N., Verrier, B. & Jung, S. Transepithelial pathogen uptake into the small intestinal lamina propria. *J. Immunol.* **176**, 2465–2469 (2006).
131. Kurimoto, I., van Rooijen, N., Dijkstra, C. D. & Streilein, J. W. Role of phagocytic macrophages in induction of contact hypersensitivity and tolerance by hapten applied to normal and ultraviolet B-irradiated skin. *Immunology* **83**, 281–287 (1994).

Acknowledgements

A.C. is funded by a fellowship from the US National Institutes of Health (NIH) National Heart, Lung, and Blood Institute (5F30HL099028-02). B.D.B. is supported by an NIH Pathfinder Award (DP2DK083052-01 and 1R56AI081741-01A1) and funding from the Juvenile Diabetes Research Foundation (17-2010-770). M.M. is supported by NIH grants CA112100, HL086899 and AI080884.

Competing interests statement

The authors declare no competing financial interests.

FURTHER INFORMATION

Brian D. Brown's homepage: http://research.mssm.edu/brownlab/Brown_Lab/Home.html
Miriam Merad's homepage: http://www.mssm.edu/profiles/miriam-merad
ImmGen website: http://www.immgen.org

ALL LINKS ARE ACTIVE IN THE ONLINE PDF

© 2011 Macmillan Publishers Limited. All rights reserved