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Citation for published version:
Hume, D, Summers, K & Rehli, M 2016, 'Transcriptional regulation and macrophage differentiation' Microbiology Spectrum, vol. 4, no. 3, MCHD-0024-2015. DOI: 10.1128/microbiolspec.MCHD-0024-2015

Digital Object Identifier (DOI):
10.1128/microbiolspec.MCHD-0024-2015

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Microbiology Spectrum

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Microbiology Spectrum
American Society for Microbiology Press

Transcriptional Regulation and Macrophage Differentiation

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ABSTRACT Monocytes and macrophages are professional phagocytes that occupy specific niches in every tissue of the body. Their survival, proliferation, and differentiation are controlled by signals from the macrophage colony-stimulating factor receptor (CSF-1R) and its two ligands, CSF-1 and interleukin-34. In this review, we address the developmental and transcriptional relationships between hematopoietic progenitor cells, blood monocytes, and tissue macrophages as well as the distinctions from dendritic cells. A huge repertoire of receptors allows monocytes, tissue-resident macrophages, or pathology-associated macrophages to adapt to specific microenvironments. These processes create a broad spectrum of macrophages with different functions and individual effector capacities. The production of large transcriptomic data sets in mouse, human, and other species provides new insights into the mechanisms that underlie macrophage functional plasticity.

THE CELLS OF THE MONONUCLEAR PHAGOCYTE SYSTEM

The mononuclear phagocyte system (MPS) was originally defined by van Furth and Cohn (1) as a family of cells of the innate immune system derived from hematopoietic progenitor cells under the influence of specific growth factors (2, 3). Differentiated cells of the MPS, monocytes and macrophages, are effectors of innate immunity, engulfing and killing pathogens. They are also needed for tissue repair and resolution of inflammation and for the generation of an appropriate acquired immune response. Their biology and differentiation have been reviewed by a number of authors (2, 4–8). The original definition of the MPS considered an essentially linear sequence from pluripotent progenitors, through committed myeloid progenitors shared with granulocytes, to promonocytes and blood monocytes, and thence to tissue macrophages (2, 4–8). Resident macrophages differ in function between tissues, and within tissues they occupy a specific niche (9). In some locations, for example, associated with epithelia, they clearly have individual identifiable territories that form a regular pattern (2, 3).

THE BIOLOGY OF BLOOD MONOCYTE SUBSETS

Monocytes in peripheral blood have been subdivided into subsets based on certain surface markers (4, 9–13). The seminal study in the area of monocyte subset function (14) segregated mouse peripheral blood monocytes based on their expression of chemokine receptors and behavior on adoptive transfer; those expressing CCR2 (and the marker Ly6C) were recruited to inflammatory sites, whereas those expressing CX3CR1 were selectively recruited to noninflammatory sites. Subsequent mouse studies have indicated that Ly6C^{hi} monocytes replenish the large resident macrophage population of the gastrointestinal tract (15–18) and patrol the extravascular space in many other organs (19). The more mature Ly6C^{lo} populations, which have a much longer half-life in the circulation, may perform a patrolling

Received: 14 October 2015, **Accepted:** 23 October 2015, **Published:** 3 June 2016
Editor: Siamon Gordon, Oxford University, Oxford, United Kingdom
Citation: Hume DA, Summers KM, Rehli M. 2016. Transcriptional regulation and macrophage differentiation. *Microbiol Spectrum* 4(3): MCHD-0024-2015. doi:10.1128/microbiolspec.MCHD-0024-2015.
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function in the circulation and sense nucleic acids and viruses (16, 20, 21). The subsets have also been referred to as "classical" (Ly6C^{hi} in mice or CD14^{hi} in humans) and "nonclassical" (Ly6C^{lo} in mice or CD16^{hi} in humans) under a proposed unifying nomenclature (11, 13). Given the lack of correlation between markers, and species differences, it is likely that these populations could be further subdivided into different and/or smaller subsets using additional markers. Rather than give the subsets names, it may be better to refer specifically to the markers used. Expression array profiling of human and mouse monocyte subsets defined by the markers described above (Ly6C and CD14/16) supported the idea that these subsets were functionally equivalent across species, although there were many species-specific expression differences (22). One major difference between mice and humans is the relative abundance of the subsets. In mice, the major populations are equally abundant, whereas in humans, the CD16^{hi} subset is a minor subpopulation that also varies widely with disease states. CD16 as a marker is also difficult to interpret since there is extensive copy number variation in the gene encoding this marker, *FCG3RB*, underlying inflammatory disease susceptibility in humans (23). In humans, an "intermediate" population (CD14^{int}, CD16^{int}) has been identified and attributed specialized functions (10–13). As discussed further below, the definition of the intermediate population depends somewhat on the position of gates on the fluorescence-activated cell sorter, and the use of other markers such as SLAN and TIE2 may produce distinct subsets (10, 12). Bovine monocytes have also been subdivided based on CD14 and CD16 as markers, with CD172a (SIRPA) and CD163 providing additional markers that varied between subpopulations (24). Pig peripheral blood monocytes, which were more human-like than were mouse macrophages in their overall gene expression profiles, could also be subdivided into roughly equal subsets, depending on reciprocal expression of CD14 and CD163, but appeared to lack a genuine "nonclassical" population (25). In rats, a separate marker, CD43, separates monocytes into two populations (26). As in the mouse, these populations differ in their expression of chemokine receptors CCR2 and CX3CR1 (26). CD43 expression also differs between the mouse monocyte subpopulations (27). Adoptive transfer and lineage trace studies in the mouse and rat confirmed that the mature (Ly6^{lo}CD43^{hi}) cells derive from the Ly6C^{hi}CD43^{lo} cells (26, 28, 29). The monocyte subpopulations are therefore likely to be the extremes of a differentiation series, in which the time spent in transition is relatively short.

THE DEVELOPMENT OF THE MPS

During embryonic development in the mouse, macrophages are first identifiable as a distinct cell type in the yolk sac, and from that location they migrate and infiltrate all of the tissues of the body. In mice, the first appearance occurs in Reichert's membrane (30). The yolk sac-derived macrophages are apparently generated as mature macrophages, without an obvious monocyte-like intermediate, and are highly motile, actively phagocytic, and proliferative (31–33). In outbred mice, these yolk sac-derived macrophages lacked detectable expression of the macrophage-specific transcription factor PU.1, and the null mutation of the *Spi1* gene encoding PU.1 did not compromise their development (32, 33). Subsequent studies demonstrated that the impact of the *Spi1* knockout mutation depends strongly on genetic background; in some strains the knockout is midgestation lethal, whereas in others it is myelodeficient at birth (34). The initial view, based on studies in the chick, was that yolk sac-derived macrophages are replaced later in development by the products of definitive hematopoiesis (35). This view was strongly supported by recent cellular transplantation studies in the chicken, where yolk sac cells injected into early embryos produced macrophages that were lost by the time of hatch, but bone marrow-derived cells produced long-term chimerism (36). Unexpectedly, this chimerism was restricted to the macrophage lineage, suggesting the existence of a macrophage-restricted progenitor cell in the bone marrow that has self-renewal capacity. There is also some evidence for such a progenitor in the mouse (37–39).

In the mouse, an emerging consensus is that populations of tissue macrophages, notably the microglia of the brain (40), the epidermal macrophages (Langerhans cells) of the skin (41, 42), and alveolar macrophages of the lung (43), are seeded from the yolk sac or fetal liver during development, and thereafter are maintained by self-renewal (reviewed in reference 38). However, this consensus depends on assumptions about the validity of the models used. Almost all of these studies use a single inbred mouse strain, C57BL/6, because most knockouts and conditional reporters have been made on this background. By contrast to the outbred mice, in the C57BL/6 line yolk sac macrophages were apparently dependent on PU.1, which was expressed in the yolk sac, and were independent of the expression of c-MYB, which is expressed in definitive hematopoietic stem cells (HSCs) (44). The view that the yolk sac is a major source of tissue macrophages in the adult has been extended to the point where blood monocytes are no longer believed

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to make a significant contribution to any tissue macrophage population (29, 44, 45) other than that of the gastrointestinal tract (46). The vast majority of adult tissue-resident macrophages in adult mouse liver, brain, skin, and lung were proposed to originate from yolk sac erythromyeloid progenitors, distinct from HSCs that colonize the fetal liver, with minimal replacement from HSC progeny (via blood monocytes) even by 1 year of age (47, 48).

Other authors, using different experimental models, have reached a different conclusion about the role of the yolk sac and embryonic macrophages in adult macrophage populations. Epelman et al. (49) reported that ~50% of macrophages in most organs, other than brain, were labeled with a conditional reporter driven by the *Flt3* promoter, which is expressed in HSCs. Neither *Flt3* mRNA nor the conditional reporter was detected in the yolk sac, and they were relatively low even in fetal liver. Sheng et al. (50), using a conditional reporter gene based on the *Kit* locus, which is expressed only in HSCs, also concluded that the large majority of tissue macrophages derive from definitive progenitors. On the basis of these data, one would conclude that definitive marrow-derived macrophages gradually replace those of embryonic origin. The most recent contribution to this emerging field made the interesting observation that the yolk sac-derived macrophages could be ablated with injection of antibody to macrophage colony-stimulating factor receptor (CSF-1R) into the embryo, and they were apparently replaced by distinct c-MYB-dependent fetal liver-derived monocytes by the end of gestation (51). These authors used inducible lineage trace markers to support the view that the large majority of tissue macrophages are derived from fetal liver-derived monocytes that seed tissue in the embryo and then self-renew. They reiterate the view that microglia are derived exclusively from the yolk sac macrophage population. However, in mice treated with anti-CSF-1R the microglia were repopulated, presumably from the monocytic source, by the time of birth. This finding may relate to much earlier observations, using outbred mice, that monocytes entered the brain, and especially the retina, and clearly transdifferentiated into microglia, around the time of birth (52, 53).

HOMEOSTASIS AND THE MACROPHAGE NICHE

As noted above, the distribution of macrophages in tissues is very regular; individual cells occupy a specific niche and seldom overlap processes with each other. Each of the models used to infer the origins and turnover of tissue macrophages in mice makes the assumption that the methods used do not disturb the steady state, and this assumption may also not be valid. The proliferation and differentiation of macrophages is controlled by CSF-1R, which is activated by two ligands, macrophage colony-stimulating factor (CSF-1) and interleukin-34 (IL-34) (7, 54). Administration of CSF-1 to mice promoted expansion of the monocyte pool and increased monocyte infiltration into tissues and tissue-resident macrophage proliferation (55–57). CSF-1 is cleared from the circulation through receptor-mediated endocytosis by the CSF-1 receptor, involving both the macrophages of the liver and spleen (58) and blood monocytes (29). Accordingly, blockade of the CSF-1R with a monoclonal antibody causes a massive increase in circulating CSF-1 (59). The central role of CSF-1 in macrophage homeostasis is evident from the CSF-1-deficient osteopetrotic (*Csf1*$^{op/op}$) mouse and toothless rat (*Csf1*$^{tl/tl}$), which have gross deficiencies of tissue macrophages and many pleiotropic consequences of that deficiency (60). Treatment with anti-CSF-1R antibody also depletes most tissue macrophage populations in adult mice, indicating that CSF-1 dependence is maintained throughout life. Anti-CSF-1R does not deplete monocytes (59), nor does it impact on monocytopoiesis (61), but it does prevent differentiation to form the Ly6C^{lo} population (29, 59).

Based on the available data, there is an intrinsic CSF-1/CSF-1R feedback loop that controls monocyte numbers and differentiation, monocyte recruitment, and tissue macrophage numbers through the local and systemic availability of CSF-1. When an individual macrophage niche becomes vacant, CSF-1 is no longer consumed and the local CSF-1 concentration rises to promote either local proliferation or recruitment of blood monocytes. Studies of development and turnover of mononuclear phagocytes need to take account of this homeostatic loop. Inducible Cre reporters, bone marrow transplantation, parabiosis, and monocyte depletion/mutations are all very likely to alter the homeostatic balance by altering the availability of CSF-1 (17). For example, the estrogen analog tamoxifen is commonly used in lineage trace experiments. There is evidence that CSF-1 expression is regulated by estrogen and is elevated in ovariectomized mice and in pregnancy (62, 63). Furthermore, hematopoietic progenitor cells respond directly to estrogen, which modulates their differentiation in the presence of myeloid growth factors (64). When embryos are pulsed with tamoxifen to induce recombinase activity, the same treatment may induce or repress CSF-1 in the embryo or

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the mother and alter the relative contribution of yolk sac phagocytes. Once a macrophage niche has been occupied by a yolk sac-derived progenitor, it may be less available to cells of definitive origin. Similarly, the lack of any impact on tissue macrophage numbers when monocytes are depleted (for example, in MYB or CCR2 knockout mice) could be explained by the increased availability of circulating CSF-1 (29), leading to the compensatory proliferation of the tissue-resident macrophages.

TRANSCRIPTOMIC ANALYSIS OF THE RELATIONSHIP BETWEEN MACROPHAGES AND DENDRITIC CELLS

The functional definition of a macrophage is in the name, "big eater," coined by Elie Metchnikoff (65). One might expect that the set of genes required to make up a professional phagocyte would be coexpressed in macrophages, and this might also provide clues to the function of genes for which there is poor annotation. The principle of guilt by association has been confirmed many times in analysis of large data sets. Genes that are coexpressed at the mRNA level commonly encode protein products that participate in the same pathway or process (66–68). Since the pioneering efforts of Su et al. (69) to generate the SymAtlas (now BioGPS; http://biogps.org) from sets of microarray data from mouse and human tissues, there have been numerous gene expression "atlases" across multiple tissues and within tissues across cell types and developmental time. The network tool BioLayout *Express*^{3D} was developed to allow the visualization of coexpression relationships in large data sets (70, 71) and was used to dissect the mouse BioGPS data set (67) and subsequently in a meta-analysis of publicly available mouse (72) and human data (73) relating to hematopoietic differentiation and macrophage biology and in a preliminary pig expression atlas (66). BioLayout analysis was used to identify a large set of coexpressed genes that were most highly expressed in phagocytes and that encoded proteins associated with lysosomes, including all of the components of the vacuolar ATPase proton pump and lysosomal hydrolases. This lysosomal/endocytosis cluster of coexpressed genes included those encoding transcription factors such as PU.1 and C/EBP (CCAAT/enhancer-binding protein) that likely contribute to transcriptional control in macrophage differentiation, and indeed can drive differentiation to a phagocyte phenotype when expressed in fibroblasts (74). The promoters of these phagocyte-enriched genes, in common with those of known human lysosomal proteins (75), contained purine-rich motifs, binding sites for the macrophage transcription factor PU.1, and the recognition motifs for basic helix-loop-helix transcription factors of the microphthalmia transcription factor family: MITF, TFEB, TFEC, and TFE3. All four MITF family members are expressed in macrophages. TFEC is a macrophage-specific transcription factor and itself a PU.1 target gene (76). MITF interacts both physically and genetically with PU.1 (34) and can transactivate the promoter of the *ACP5* lysosomal enzyme gene (34).

By contrast to the clear coexpression of phagocyte-specific genes, the genes encoding surface antigen markers, recognized by monoclonal antibodies, that are commonly used to divide monocytes and macrophages into subpopulations, were not stringently coexpressed with either phagocytic function or with each other. The gene encoding the well-studied macrophage marker EMR1 (F4/80), for example, was in a very small coexpression cluster with the gene for a transcription factor, MAFB, that was subsequently shown to regulate its expression (77, 78). High expression of F4/80 was proposed as a marker for tissue macrophages that derive from yolk sac progenitors (44). The clear lack of correlation between surface markers is important because it means that we cannot predict the gene expression or surface antigen profile of individual cells based on the presence of any marker. Each of the proteins we regard as surface markers has a function and has its own intrinsic regulation.

One of the most prevalent uses of surface markers is the separation of macrophages from dendritic cells (DCs) and the segregation of both populations into subpopulations. The activation of T lymphocytes requires the presentation of the antigen on major histocompatibility complex (MHC) molecules on the surface of an antigen-presenting cell (APC). For some groups, the term "dendritic cell" has been merged with "antigen-presenting cell." The problem with this view is that antigen presentation is a regulated pathway, and the genes involved are not correlated with any other cellular function. For some significant time, the integrin CD11c (ITGAX) was considered a DC marker in mice, even though it was clearly expressed by many tissue macrophage populations (6, 79). In the cluster analysis of mouse BioGPS data, CD11c was not coexpressed with any other marker (67).

The original description of the classical DC by Steinman and Cohn made the clear distinction that "unlike macrophages, they do not appear to engage in active endocytosis" (80). Analysis of large transcriptomic data sets reveals that the *a priori* definition of cells as DCs currently groups together cells with very distinct

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transcriptional profiles (6, 17, 79, 81, 82). The Immunological Genome Consortium (ImmGen) produced transcriptomic profiles from a large number of different myeloid cell populations isolated from multiple mouse tissues based on cell surface marker expression, and proposed distinct expression signatures unique to DCs and macrophages (83, 84). Their analysis concluded that MHC class II (MHC-II) and the transcriptional regulator *CIITA* were part of a DC signature, which was predictable given the selection of MHC-II-negative macrophages for comparison. A separate analysis of the entire data set failed to identify coexpressed genes that correlated with the *a priori* definition of cells as macrophages or DCs (85). Even within this large myeloid-specific data set, there was no correlation between any of the cell surface receptors, such as CD11b, CD11c, F4/80, MHC-II, CD64, CSF-1R, and FLT3, that are commonly used to classify subpopulations or cell types of macrophages and DCs. As discussed in detail previously (6), this lack of correlation between markers is supported by both fluorescence-activated cell sorter profiling and immunohistochemistry, where the number of subpopulations that can be defined is a function of the number of markers examined.

With respect to the macrophage-DC distinction, meta-analysis of large microarray data sets from mouse and human (72, 73) produced the same conclusion as analysis of the ImmGen data; cells annotated as DCs clearly segregated into two groups. As one might have predicted based on the original definition of the DC, the expression cluster that divided all macrophage/DC-related networks into two clear classes was the cluster containing lysosomal/endocytosis genes described above. The classical DCs isolated as defined by Steinman had very low expression of all of the genes in this cluster, and lower expression of the putative regulators (PU.1, C/EBP, and MITF family), whereas there was a separate class of cells that included monocyte- and bone marrow-derived cells described as “DCs” and many MHC-II-positive myeloid cells from tissues, which were clearly capable of being active phagocytes based on their expression of the large endocytic cluster (67, 72, 81).

The only transcriptional signature associated with APC activity in both macrophages and “DCs” is a very small one, including MHC-II, CD74, and the regulator CIITA. Several recent reviews (82, 86, 87) have considered the transcriptional control in the development of the classical DC, and the BioGPS and ImmGen data confirm the separation of these cells from macrophages. The data also confirm some of the lineage-restricted transcription factors in mice that distinguish the classical, FLT3-dependent DC from the macrophage (86). The transcription factors that most clearly define classical DCs, such as BATF3 and ZBTB46, are transcriptional repressors. One of the functions of these transcription factors could be to block macrophage differentiation and expression of endocytic function (88).

The ImmGen data were also used to define a number of surface markers that positively identify “macrophages” as distinct from “DCs,” including the Fc receptor CD64 and the signaling molecule MERTK (83). These two markers did not correlate with each other in our reanalysis of the ImmGen data. CD64 is a direct target of CSF-1 signaling in mice and has been considered a marker, alongside CD163, of M2 polarization (89). CD64 is absent from elicited peritoneal macrophages, whereas MERTK is very highly expressed and further induced by lipopolysaccharide (LPS) (http://biogps.org). Therefore, like CD11c discussed above, their expression is state specific, and they cannot be considered as markers for a definitive “macrophage” or “DC.” The shortcomings of CD64 as a marker are illustrated by a study of dermal myeloid cells. CD64 was used to distinguish $CD11b^+$ macrophages from $CD11b^+$ DCs in the dermis, leading to the conclusion that only ~10% of the $CD11b^+$ myeloid cells in the dermis are macrophages (90, 91). The $CD11b^+$ “DCs” were monocyte derived and expressed most other macrophage “markers” (e.g., F4/80, CD68, and lysozyme) (90, 91). Alongside Langerhans cells of the epidermis, the vast majority of dermal myeloid cells expressed a *Csf1r*-EGFP (enhanced green fluorescent protein) transgene and were completely ablated by treatment of mice with anti-CSF-1R antibody (59). A recent proposal for a unified nomenclature that separates macrophages and DCs based on ontogeny and growth factor dependence would clearly separate the $CD11b^+$ DCs in skin, which are CSF-1 dependent, from classical DCs, which depend on FLT3 ligand (92). This distinction would also separate the putative classical DC subclasses in mouse, cDC1 and cDC2, which differ in expression of the chemokine receptor XCR1 (GPR5) and CD8 (86). Within the BioGPS data, the $CD8^+$ DCs express *Gpr5* at high levels and lack *Csf1r*. Plasmacytoid DCs also lack *Csf1r* mRNA. Conversely, the $CD8^-$ DCs lack GPR5 and expressed both *Csf1r* and *Flt3*, and detectable EMR1 (F4/80). It is not clear that the markers are expressed on the same cells within this population. What is clear is that the definition of cDC2s as distinct from macrophages is rather more contentious (86).

The separation of cell populations based on cell surface markers is clearly a mainstream technology in

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immunology. Objections to the use of markers are not solely semantic. The core problem lies with the concept of a marker. A marker is only useful if it actually predicts function. Markers expressed by T lymphocytes such as CD3, CD4, and CD8 are informative, and predictive, because they are required for the function of the cells in which they are expressed, and they are, in fact, very well correlated with expression of genes required for lineage-specific function. The endosome/lysosome gene cluster discussed above is also clearly linked to function. Any one of the genes present within that cluster has a predictive value as a marker; a cell that expresses it is likely to be phagocytic. On the other hand, CD11b and CD11c encode complement receptors that have no function in antigen presentation. They are clearly likely to be required for complement receptor function, but there is no reason *a priori* to suspect that they will be highly expressed in APCs. The obvious counterargument is that markers are useful; they can be used to purify populations of cells that are enriched for a particular function of interest. However, the expression data tell us that such populations are intrinsically heterogeneous.

GENETIC VARIATION AND MACROPHAGE BIOLOGY

Like the studies of macrophage ontogeny above, the ImmGen and BioGPS data sets and much of the literature on antigen presentation and DC/macrophage divergence derive from the C57BL/6 mouse. In mice, many tissue macrophages, and cells grown in CSF-1, lack MHC-II, whereas in most other species, including humans and pigs, monocytes and monocyte-derived macrophages (MDMs) are strongly MHC-II positive. Furthermore, the C57BL/6 mouse has only one MHC-II locus since H2-Ea is absent. This locus is inducible by LPS in macrophages from BALB/c mice (93). C57BL/6 macrophages do not express cathepsin E, which is needed for antigen processing (93). A gene promoter polymorphism in the arginine transporter, *Slc7a2*, means that C57BL/6 mice have very different rates of arginine metabolism than other strains (94). At least 65 genes distinguish the transcriptome of C57BL/6 macrophages absolutely from BALB/c macrophages (93), including the C1q components proposed as unique markers for microglia (95), which were much more highly expressed and inducible in C57BL/6 than in BALB/c mouse macrophages (93). Strain-specific variation in gene expression is also reflected in allele-specific methylation patterns in macrophages from an F1 cross between these strains (96), and there is similar diversity between other mouse strains (97, 98). This means that a view of the MPS or the macrophage transcriptome based on the biology of the C57BL/6 mouse may not be generalizable to all mice, let alone to other species.

TRANSCRIPTIONAL REGULATION AND MACROPHAGE DIFFERENTIATION

Whether or not tissue macrophages derive from definitive progenitors in the marrow, and must transit through a blood monocyte precursor, it is possible to isolate committed progenitor cells from bone marrow that express receptors for hematopoietic growth factors, and to use those growth factors to generate relatively pure populations of macrophages (in CSF-1), classical DCs (in FLT3L), or macrophages with APC activity (in granulocyte-macrophage CSF [GM-CSF]; otherwise called bone marrow-derived DCs). Some of the underlying transcriptional regulation of lineage commitment in mice has been inferred from large-scale transcriptomic profiling of purified progenitor cells (99–102). Similarly, there have been detailed transcriptomic studies of the differentiation of human blood monocytes to mature macrophages, or DCs, in the presence of CSF-1 or GM-CSF (103, 104). The process of macrophage differentiation can also be modeled to some extent using myeloid leukemia cell lines. The FANTOM4 (Functional Annotation of the Mammalian Genome) consortium used the THP-1 human monocytic leukemia line to study the cascade of transcriptional events associated with macrophage differentiation (105). Genome-scale 5′ rapid amplification of cDNA ends (5′ RACE; also known as cap analysis of gene expression [CAGE]) was used in parallel with microarrays to identify changes in promoter activity with time and to infer the transcription factors involved. One of the most interesting findings was the rapid downregulation of a large cohort of transcriptional regulators, exemplified by c-MYB, and the finding that small interfering RNA-mediated downmodulation of those factors was sufficient to drive the differentiation process (105).

The largest data sets available for human macrophage differentiation have come from the FANTOM5 consortium, which also utilized CAGE to identify the sets of promoters and enhancers utilized by hundreds of different cells and tissues (106, 107). These data sets include multiple samples of different human myeloid lineages, including multipotent progenitors (CD34$^+$ stem cells), common myeloid progenitors (CMPs), and granulocyte-macrophage progenitors (GMPs), three monocyte subsets defined by CD14 and CD16 expression, MDMs (grown in CSF-1), monocyte-derived "DCs" (MDCs;

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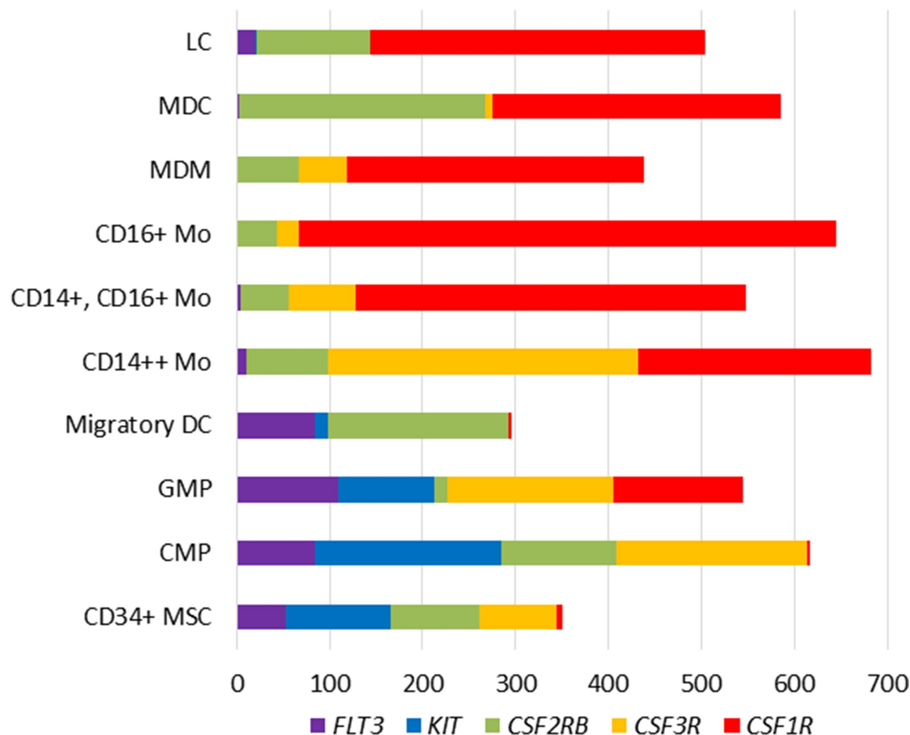
grown in GM-CSF plus IL-4), Langerhans cells (LC) and migratory DCs from skin lymphatics. Although there are subtle differences in experimental systems and purification protocols, the data complement and parallel two large data sets generated by the Blueprint consortium, one of which profiled transcriptional regulation in isolated human progenitor cells (99), and another which detailed the human monocyte response to CSF-1 and tolerization with LPS (108).

The expression profiles of myeloid lineage cells have been analyzed separately from the complete FANTOM5 data set (109) and data access provided on a separate portal at http://www.myeloidome.roslin.ed.ac.uk. This portal enables visualization of the transcription start sites and expression profiles in this set of myeloid cell types of any gene of interest, using the genome browser. It also summarizes expression profiles of long noncoding RNAs and microRNAs and cell type enhancers that correlate in their expression with promoters in the same genomic region.

Figure 1 shows the expression levels of key growth factor receptors in a macrophage-DC differentiation series extracted from the human FANTOM5 gene expression data. The combined total expression of these five growth factors was remarkably similar across all cell types, but the proportion of total expression contributed by each gene varied considerably. Consistent with the identity of the samples, *KIT* (receptor for stem cell factor), *FLT3*, *CSF2RB* (GM-CSFR), and *CSF3R* (G-CSFR) are expressed in progenitors, the CD34$^+$ multipotent stem cells (MSCs), the CMPs, and the GMPs. *Csf1r* is first expressed in the GMPs. Outside of the progenitor population, *Csf1r* and *FLT3* show opposite expression patterns; the classical DCs express *FLT3* and all other populations, including monocyte-derived "DCs" and Langerhans cells of the skin (sometimes regarded as DCs), express *Csf1r*. As expected, given the clinical use of G-CSF for stem cell mobilization, *CSF3R* is expressed in the CD34$^+$ progenitors, but interestingly, its expression is retained in CD14$^+$ mono-

FIGURE 1 Expression of selected genes encoding myeloid-restricted growth factor receptors. Stacked bars show expression of each gene in the cell type (normalized tags per million) derived from FANTOM5 CAGE data for human cells (107). Cell types are presented in the order of maturation: CD34$^+$ MSCs; CMPs; GMPs; migratory DCs; CD14^{++} monocytes (CD14^{++} Mo); CD14$^+$CD16$^+$ monocytes (CD14$^+$, CD16$^+$ Mo); CD16$^+$ monocytes (CD16$^+$ Mo); MDMs (cultured in CSF-1); monocytes cultured in GM-CSF (MDCs); and migratory DCs from skin lymphatics (LC).



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cytes and downregulated with maturation in response to CSF-1, varying inversely with *Csf1r* mRNA. The expression of *CSF3R* in monocytes and their progenitors may be related to the finding that G-CSF treatment of humans mobilizes a population of monocytes that retain expression of CD34 (110).

As we concluded from a meta-analysis of published human microarray data (73), the transcriptional profiles of monocyte-derived populations grown in different growth factors clustered together, first and foremost because of their shared expression of phagocytic genes and some endocytic receptors (109). Many transcripts were more highly expressed in monocyte-derived macrophages grown in CSF-1 (*ACP5*, *CD163*, *C1Q*, tetraspanins, *IGF1*, *VEGFB*, scavenger receptors, and *CHI3L1*) or the monocytes grown in GM-CSF (*CD1A*, *-B*, *-C*, and *-E*; *DCSTAMP*; *CLEC4A*; *CLEC10A*; *CLEC4G*; and *TNFRSF11A*, also known as *RANK*). As in other data sets discussed above, the MHC-II genes were separately clustered because they are also expressed by monocytes and their committed progenitors. As expected, the monocyte-derived "DC" cells, grown in GM-CSF, maintained higher levels of the MHC-II cluster, whereas these genes were somewhat repressed in the monocytes grown in CSF-1.

The three human monocyte populations in the FANTOM5 data set were also subjected to detailed chromatin analysis, providing global maps of H3K4me1 (promoter) and H3K27ac (enhancer) locations that largely confirmed the CAGE data (111). The CAGE-based methodology also permitted identification of regulated enhancer activity, enabling, for example, the identification of active enhancers responsible for the differential expression of *CD14* in the monocyte subpopulations. Over all, the FANTOM5 data supported the view that the monocytes are a CSF-1-dependent differentiation series, with the intermediate monocytes showing intermediate expression of the vast majority of the hundreds of genes that showed differential expression between the two extremes of monocyte phenotype. This pattern includes *Csf1r* itself, which is 3-fold elevated in $CD16^{++}$ monocytes. The one exception, consistent with findings of others (reviewed in references 10–12), is ~2-fold elevation of a subset of MHC-II (HLA-DP, HLA-DQ, and HLA-DR) transcripts in the intermediate cells relative to $CD14^{++}$ and $CD16^{++}$ extremes, despite a progressive decline in expression of the regulator CIITA. If monocytes are a differentiation series, the selective increase in the intermediate population reported in many clinical settings probably represents a change in transit time between the states.

Aside from CD14 and CD16, two other markers have been proposed to provide functional delineation of subsets of human monocytes: TIE2 and SLAN (10, 12). The former encodes a tyrosine kinase receptor for angiopoietin-2, but it was not detected in any of the monocyte preparations in FANTOM5, where it was highly expressed as expected in endothelial cells. SLAN is an antigen formed by a novel O-linked 6-sulfo-LacNac modification of P-selectin glycoprotein ligand (PSGL1). PSGL1 is itself around 2 times more highly expressed in $CD16^{++}$ monocytes than $CD14^{++}$ monocytes. However, the enzyme most likely to be required for the modification, carbohydrate sulfotransferase 2, was undetectable in $CD14^{++}$ monocytes, induced in intermediate cells, and highly expressed in $CD16^{++}$ cells. One other marker, CD43, has been used in other species as a subset marker (10, 12), and the FANTOM5 data demonstrate that it is likely to provide a similar discrimination of the $CD16^{++}$ subset in humans.

The analysis of monocyte subsets by FANTOM5 identified the coregulation of genes required for glycolytic metabolism and the metabolic burst in the classical monocytes (111). Conversely, the $CD16^{++}$ monocytes were apparently more committed to oxidative metabolism and mitochondrial energy generation. It may be that the more mature, long-lived monocytes adapt with time to the aerobic environment of the bloodstream, where the "inflammatory" monocytes are adapted to enter the relatively low-oxygen environments of tissues and inflammatory sites. Among the genes strongly downregulated in the $CD16^{++}$ monocytes is the hypoxia-inducible transcription factor *HIF1A* and the glucose transporter *GLUT3* (*SLC2A3*).

About two-thirds of the annotated transcription factors in the genome can be detected in some state of differentiation or activation of the macrophage lineage. Most do not vary greatly in their expression across myeloid lineages. About 100 transcriptional regulators showed significant variation with differentiation in the populations shown in Fig. 1. An analysis of the monocyte cell types based on expression of 108 transcription factor and growth factor receptor genes was performed using BioLayout *Express*^{3D} (70). Eleven coexpression clusters containing three or more genes were identified. The patterns of these 11 clusters are shown in Fig. 2, and their profiles are summarized in Table 1. The largest cluster consisted of the set of transcription factor genes that was expressed in progenitors and downregulated with differentiation. All of these genes were highly expressed in C57BL/6 mouse progenitor cells and downregulated with differentiation (http://biogps.org). Many

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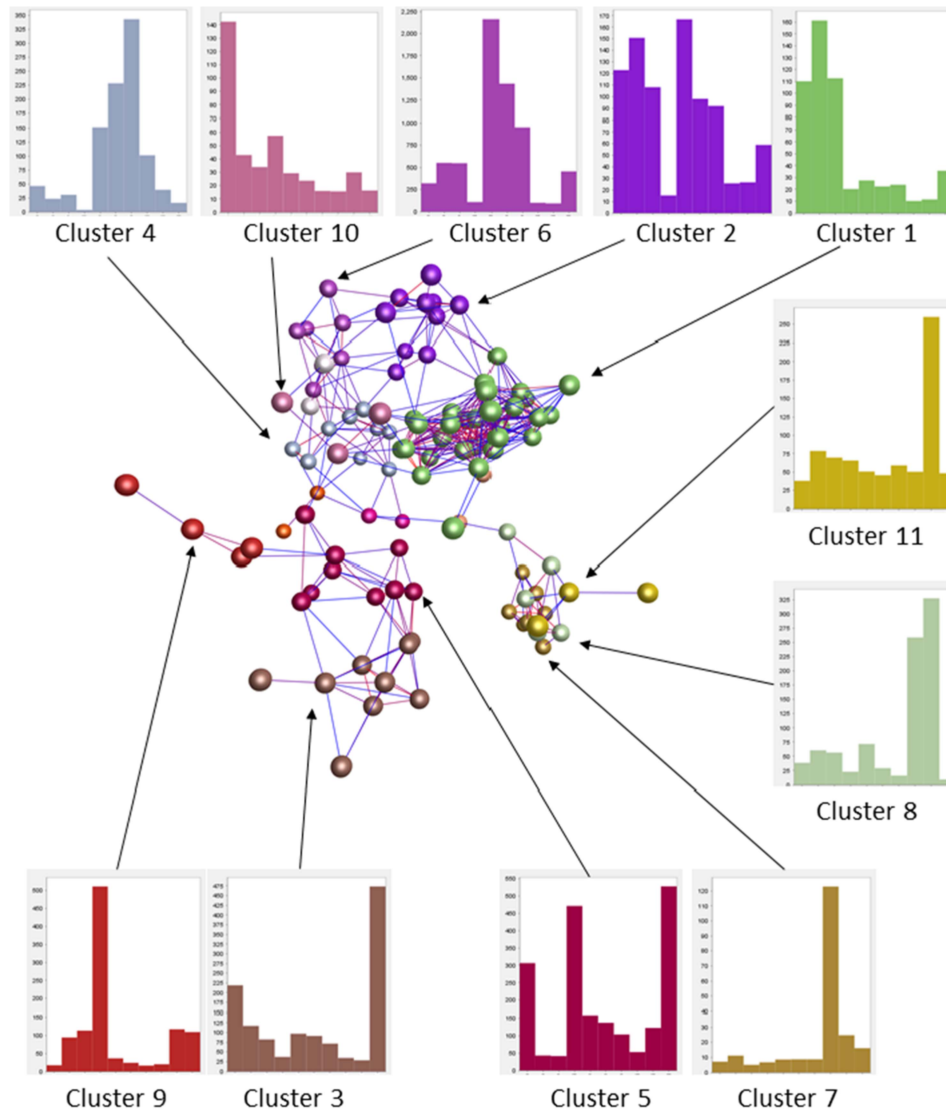

FIGURE 2 Network layout of 108 growth factor receptor and transcription factor genes in myeloid lineages. Nodes represent genes and edges correlation between expression patterns of genes at a Pearson correlation coefficient of 0.74 or greater. Nodes of the same color form a cluster. Histograms show the average expression pattern of genes within the cluster. *x* axis, cell type. Each column represents one cell type, presented in the order of maturation: $CD34^+$ mesenchymal stem cells; CMPs; GMPs; migratory DCs; $CD14^{++}$ monocytes; $CD14^+CD16^+$ monocytes; $CD16^+$ monocytes; MDMs (cultured in CSF-1); monocytes cultured in GM-CSF; and migratory DCs from skin lymphatics. Column colors are the same as the nodes in the cluster. *y* axis, average expression of genes in the cluster (normalized tags per million) derived from FANTOM5 CAGE data for human cells (107).

of them were also expressed constitutively by THP1 cells and downregulated in response to phorbol myristate acetate-induced differentiation (105).

Among these coregulated transcription factors, clusters 3 and 6 were expressed most highly in the classical monocytes and declined with maturation to the $CD16^+$ subset, and still further in response to differentiation *in vitro*. Conversely, cluster 4 included transcription factors such as *CEBPB*, *MAFB*, and *TFEB* that increased with monocyte differentiation and still further in response to CSF-1 *in vitro*. The results are broadly consistent with a recent review of monocyte differentiation

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TABLE 1 Coexpression of transcription factor genes in cells of myeloid lineages[a]

Cluster	Description	Transcription factor genes
1	High in precursors (FLT3+, KIT+)	*BACH2, DACH1, E2F1, E2F3, EGR1, ERG, ELF2, FOXP1, GFI1, GFIB, HOXA9, JUND, LMO2, MYB, MYC, MYCN, NFE2, PRDM8, RFX8, RUNX1, SOX12, TAL, TCF3, TCF4*
2	High in precursors and monocytes; down in MDMs, MDCs, and G-CSFR+	*ELF1(A), ETS2, FLI1, FRA2, KLF7, PER1, PER2, SMAD3*
3	Declines with maturation from MSCs through monocyte subsets to MDMs/MDCs; retained in LCs	*ATF3, FOSB, FRA1, IRF6, JUN, MAFF, NFATC1, NR4A1, NR4A2*
4	Highest in monocytes and MDMs, increased in CD16+ monocytes	*CEBPB, IRF7, KLF2, LMO2 (C), MAFB, NR1D1, POU2F2, TCF7L2, TFEB*
5	Highest in SCs, migratory DCs, and LCs; declines with monocyte maturation	*ARNT, BHLHE40, ETV3, ID2, NR4A3, PRDM1, REL, RELB, ZBTB46*
6	Highest in CD14+ monocytes, declines in CD16+, and suppressed in MDMs/MDCs	*CEBPD, FOS, HIF1A, JDP2, KLF4, MEF2C*
7	Induced in MDMs, low in all others	*BHLHE41, EGR2, ETV5, MAF, MITF, NR1H3* (LXRA), *PPARD*
8	Induced in both MDMs and MDCs	*CREG1, PPARG, SNAI3, TFEC*
9	High in MDCs, also increased in MDCs and LCs	*ARNTL2, CIITA, IRF4, SPIB*
10	High in SCs, down in CMPs	*ETS1, SMAD7, SOX4*
11	High in MDCs (GM-CSFR++)	*CEBPA, FOXQ1*

[a]Cluster numbers are derived from the BioLayout *Express*3D analysis shown in Fig. 2. Transcription factors are those with high expression in the cluster. MDM, monocyte-derived macrophage (cultured in CSF-1); MDC, monocyte cultured in GM-CSF plus IL-4; LC, Langerhans cell. Migratory DCs are cells isolated from skin lymphatics.

by Huber et al. (112) but greatly extend the transcriptional network. In every case, the CD14+CD16+ cells have intermediate expression, again reinforcing the view that they are a differentiation intermediate between the CD14++CD16− and CD14−CD16++ subsets. A number of genes of interest have idiosyncratic expression patterns and are not included in clusters (not shown). For example, *BATF3*, the mouse DC regulator mentioned earlier, was expressed in human monocytes and increased markedly with maturation to the CD16++ subset. It was maintained in monocytes grown in GM-CSF but ablated in those grown in CSF-1. In mice, the generation of the Ly6C−, or nonclassical, monocyte subset depends on the CSF-1-responsive early response gene *Nr4a1* (*Nur77*) (113). The *Nr4a1* gene was a member of cluster 3, and it is likely that NR4A1 protein also performs some function in maturation in human monocytes.

CSF1R, THE ARCHETYPAL MACROPHAGE-SPECIFIC GENE

The CSF-1 receptor (*Csf1r*) locus has been studied in great detail to provide an understanding of the processes of macrophage differentiation. Expression of *Csf1r* mRNA is one of the earliest markers of macrophage lineage commitment from multipotent progenitors in mouse bone marrow, as well as the appearance of macrophages in the yolk sac (32, 114). In the mouse, CSF-1 was shown to direct myeloid lineage fate in single multipotent HSCs by activating the expression of transcription factor PU.1 (115). This finding appears incompatible with human and mouse transcriptomic data (both in FANTOM5 and in BioGPS), which indicate that *Spi1* (encoding PU.1) is already highly expressed in CD34+ MSCs, whereas *Csf1r* is first detectable in GMPs (see Fig. 1). Further, the TATA-less, macrophage-specific promoter of the *Csf1r* locus in both species contains multiple PU.1/ETS binding sites that are bound by PU.1 and required for maximal activity (116, 117), and PU.1 is required for *Csf1r* expression in progenitor cells (116). Hence, a model that places PU.1 downstream of CSF-1R signaling does not fit the available expression data. Tagoh et al. (114) purified mouse c-KIT-positive progenitors and confirmed that (i) they express c-MYB and PU.1 and (ii) they lack surface CSF-1R as well as *Csf1r* mRNA. These cells require a cocktail of IL-1, IL-3, and CSF-1 to drive macrophage differentiation. The critical event that permits the expression of *Csf1r* and lineage commitment is more likely loss of c-MYB rather than induction of PU.1 (118). Detailed studies of the chromatin structure of the mouse *Csf1r* locus during differentiation of progenitors (114) revealed the occupation of both purine-rich motifs and C/EBP binding sites in the *Csf1r* promoter in the immature precursors, consistent with expression of both PU.1 and CEBP family members in these cells. These findings indicated that regulatory elements elsewhere in the locus drive transcriptional activation of *Csf1r* transcription during differentiation. Multiple analyses of transcription factor binding site occupancy and epigenetic profiles in mouse and human

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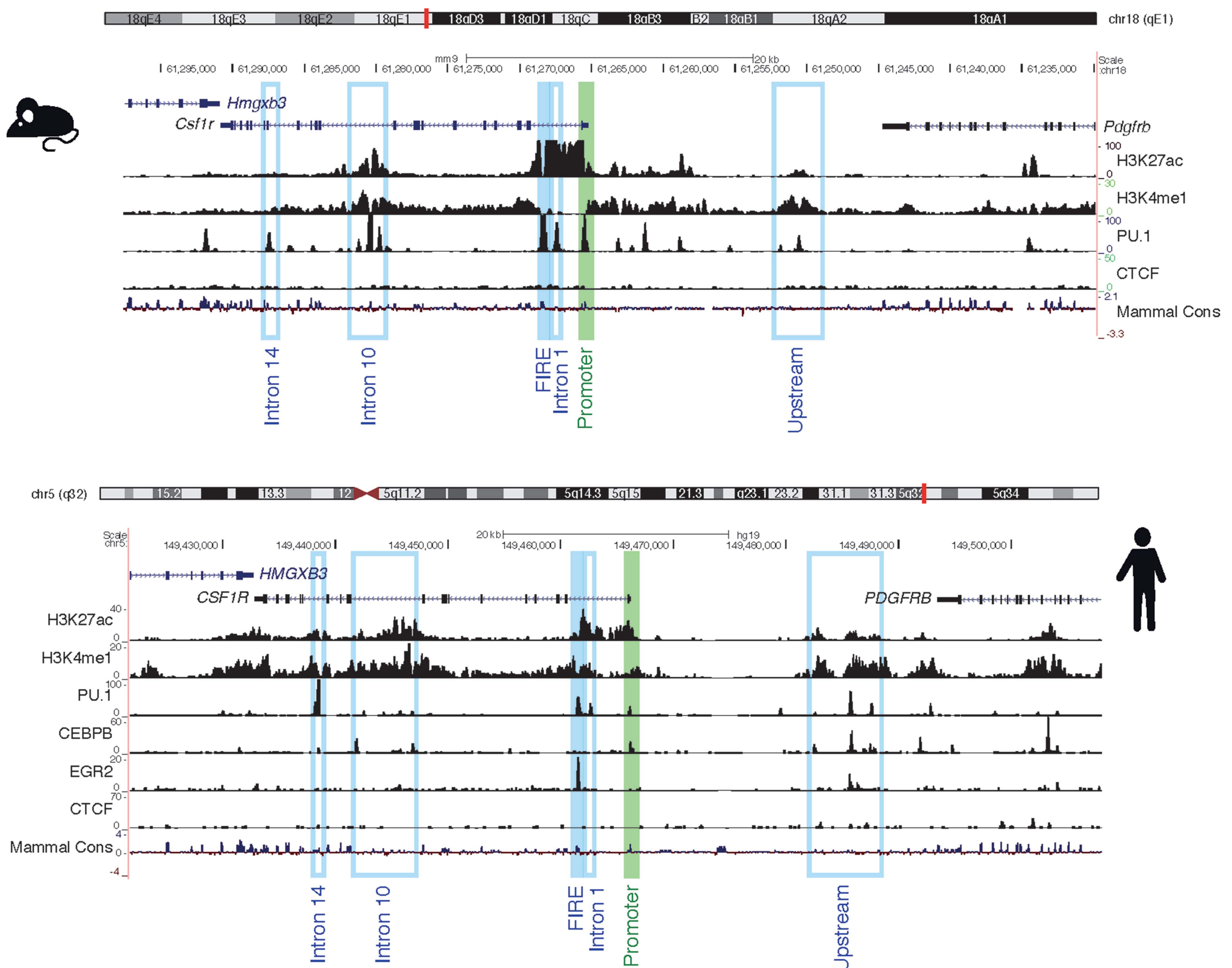
provide a consistent view of potential regulatory elements in the *Csf1r* locus (104, 111, 119, 120), summarized in Fig. 3.

The purine-rich promoter architecture of *Csf1r* is shared by other myeloid-specific promoters, and basal activity requires cooperation between PU.1 and other ETS family transcription factors (121) as well as binding of Ewing sarcoma protein or the related FUS/TLS to the transcription start site (TSS) (122). Multiple ETS family members expressed in precursors and monocytes (ERG, ELF2, ELF1, ETS2, FLI1, ETV3, and ETV5; Table 1) are likely to fulfil the role of cooperating with PU.1 to activate purine-rich promoters. Conserved candidate enhancer sites upstream of the major macrophage *Csf1r* TSS, and within several introns, are also evident in chromatin analysis (104, 111, 119, 120), and the enhancer activity was confirmed based on bidirectional promoter activity in data from both human and mouse macrophages from the FANTOM5 consortium (106, 107). All mammalian *Csf1r* genes contain a con-

FIGURE 3 Chromatin architecture of the mouse and human *Csf1r* loci. Genome browser tracks of indicated histone modifications and transcription factors associated with enhancer elements are shown. The filled green box indicates the macrophage promoter. Boxes in blue identify intergenic and intragenic enhancer candidates. FIRE is represented by a filled blue box. Chip-Seq data sets that formed the basis of this figure for human macrophages are from derived from references 104 and 159. The mouse PU.1 track is derived from reference 136, and other mouse tracks from ENCODE.



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served intronic enhancer referred as the Fms intronic regulatory element (FIRE) (123, 124). Tagoh et al. (114) demonstrated by *in vivo* footprinting that activation of mouse *Csf1r* transcription in c-KIT-positive precursors involves sequential occupation of multiple sites in the FIRE region. FIRE provides a remarkable cluster of highly conserved binding sites for many of the regulated transcription factors shown in Table 1, notably GC-rich elements likely bound by EGR1/EGR2 and KLF family members (104, 116, 125).

Csf1r reporter mice have been especially useful for studies of both CSF-1 and anti-CSF-1R treatments (55, 59, 126). A *Csf1r*-EGFP reporter containing the *Csf1r* promoter plus FIRE was detectable in all myeloid cells throughout the body, generating the MacGreen mouse (123) as well as constitutive and tamoxifen-inducible *Csf1r*-Cre transgenes used in lineage trace studies (44, 127). A 150-bp distal promoter element, containing a conserved AP1 site, is required for expression in mature macrophages and osteoclasts (128). The MacBlue transgenic line (*Csf1r*-ECFP [enhanced cyan fluorescent protein]) was generated using a promoter with this element deleted (129, 130). The reporter protein was expressed on macrophages as they appeared in the yolk sac and provided a striking picture of their abundance during embryonic development (30). Unexpectedly, the expression disappeared from the majority of tissue macrophages postnatally, whereas expression was retained in blood monocytes and their progenitors, and in the IL-34-dependent populations, the microglia and Langerhans cells in the skin (30). Even in the gut, where the macrophage population clearly derives from continuous renewal from monocytes (46), the *Csf1r*-ECFP transgene was extinguished in the majority of resident macrophages. The transgene therefore permits live imaging of monocyte trafficking (131).

Expression of the reporter genes based on the *Csf1r* locus requires the activity of FIRE (123), and the activity of FIRE is functionally conserved across mammalian species (132). FIRE is conserved also in the *Csf1r* locus of birds, and *Csf1r* reporter transgenic chickens have been produced in which all of the macrophages are labeled from the earliest yolk sac-derived cells (133). The chick provides an alternative model to study the origin of macrophages, and is the model in which the yolk sac-derived macrophages were first described (35, 134). Using transgenic reporters, we found that macrophages derived from the yolk sac are capable of extensive proliferation in response to CSF-1 *in vitro*, and in response to CSF-1 administration into the embryo. When transplanted to a nontransgenic recipient, they give rise to macrophages all over the body. However, these cells are not retained anywhere after hatching. By contrast, transplantation of cells from hatchling bone marrow into embryos prior to the onset of definitive hematopoiesis produced extensive macrophage chimerism in the embryo that was retained in mature adult birds, and which included regeneration of macrophage progenitors of donor origin in the marrow (36). We suggest that the trophic environment of the early embryo can only support the committed macrophage progenitors, perhaps similar to those of mouse (37). In keeping with the earliest findings using chick-quail yolk sac chimeras (134), these findings suggest that the contribution of the yolk sac to tissue macrophage populations is transient, and yolk sac-derived cells are replaced entirely by the progeny of definitive hematopoiesis.

TISSUE-SPECIFIC ADAPTATION AND ACTIVATION OF MACROPHAGES

Regardless of the embryonic origin, each population of tissue-resident macrophages adapts specifically to the environment in which it finds itself. Sample-to-sample comparison of the extensive ImmGen data set for the mouse demonstrated that each tissue macrophage population formed a distinct cluster (81). One recent study focused on the differentiation of microglia, the macrophages of the brain, and identified a marker set that distinguished these from blood monocytes and from tissue macrophages isolated from other locations (95). The BioGPS data, the FANTOM5 data, and the ImmGen data all include strongly validated microglial data sets. The former two confirm known microglial-enriched genes including *Aif1* (*Iba1*) and the scavenger receptors *Fcrls* and *Marco* but do not support others such as *C1q* and *Csf1r*, which are also highly expressed in other macrophage populations. A more extensive comparison combined expression profiling of isolated mouse tissue macrophage populations with extensive analysis of chromatin architecture (135, 136). Several of the genes that marked particular macrophage populations were transcription factors, notably *Sall1* and *Mef2c* in microglia, *Gata6* in peritoneal macrophages, *Lxra* in Kupffer cells, *Spic* in spleen, and *Runx3* in the gut (135). The expression of *Gata6* in mouse peritoneal macrophages appears to be required for their self-renewal and homeostasis; two groups separately reported on the select impact of myeloid-specific deletions of the gene in the resident peritoneal population (137, 138). The tissue-specific macrophage phenotypes correlated with the presence of H3K4Me1/2 enhancer profiles in their genomic

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vicinity. The role of environment in the acquisition of tissue-specific phenotypes was examined in bone marrow chimeras. The outcomes indicated not only that the tissue determines the phenotype but, *inter alia*, that monocytes derived from bone marrow progenitors can, and do, give rise to tissue-resident macrophages if the niche is available.

There is considerably less data available on tissue-specific macrophage development from other species. However, the number of macrophages in most organs is sufficiently high that we can extract a macrophage signature without isolating the cells. Within the pig gene expression atlas, we profiled lung alveolar macrophages, blood monocytes, and bone marrow-derived macrophages alongside many different tissues (66). Comparative analysis revealed that alveolar macrophages express exceptionally high levels of a wide diversity of C-type lectin receptors (SIGLEC and CLEC family members) and Toll-like receptors (TLRs), presumably to deal with inhaled particles. This pattern is also evident in the many profiles of human alveolar macrophages that can be accessed through NCBI GEO. By contrast, in the wall of the gut we could detect a clear signature of the abundant lamina propria macrophage population, but the C-type lectins and TLRs were absent.

The apparent plasticity of tissue macrophages reflects, in part, their responsiveness to numerous different stimuli, some of which are tissue specific and others shared by locations or induced in response to environmental challenges. The generic term "activation" was originally applied to the ability of recruited macrophages to acquire microbicidal and tumoricidal activity in response to products of activated T cells. A number of groups have advocated subclassification of the activation states seen in recruited macrophages, broadly into M1 and M2 or classically activated and alternatively activated (5, 139–141). The M1 and M2 nomenclature links the state of activation of the macrophages to the activation of Th1 and Th2 lymphocytes, which in turn links them to the actions of gamma interferon (IFN-γ; originally known as macrophage-activating factor) (142) and IL-4. Interestingly, although it is not commonly regarded as a macrophage growth factor, IL-4 can promote macrophage proliferation in mice *in vivo* (57, 143).

The proposed M1/M2 dichotomy is not supported by genome-scale data (73). If there were coordinated M1 or M2 "regulons," we would expect to see correlated expression of the classical marker genes for the putative M1 and M2 phenotypes across different cellular states, and this was not observed. The utility of the M1/M2 concept across species is also not clear. The gene expression profiles of "classically activated" mouse, pig, and human macrophages are very different (144), with the pig being rather more human-like (145). Similarly, the IL-4-inducible alternatively activated profiles, and putative M2 markers, are poorly conserved between mouse and human. Transglutaminase 2 was proposed as the only well-conserved marker (146). Mosser and Edwards (147) suggested that macrophage activation may better be described as a spectrum, analogous to a color wheel. Xue et al. (148) confirmed the spectrum model with an extensive comparison of human monocyte-derived macrophages grown in CSF-1 or GM-CSF and exposed to numerous distinct stimuli including IFN-γ, IFN-β, IL-4/IL-13, IL-10, glucocorticoids, TLR agonists, tumor necrosis factor, and prostaglandin. While there was a broad dichotomy between IFN-γ (M1) and IL-4 (M2) directed states, addition of further stimuli segregated the transcriptional response into many separate modules, with 49 distinct coexpression clusters containing 27 to 884 genes per module (148). The simplistic view of macrophage activation that pervades the literature is based in part on simplified models in which individual agonists are studied individually, often at a single dose, a single time point, and in a single mouse strain and/or small subset of individuals. The problem with this approach is illustrated by several findings. IFN-γ is normally made by activated T cells alongside GM-CSF. As the sole stimulus, IFN-γ causes growth arrest, whereas when GM-CSF is present, it is a mitogen (149). On the matter of dose and mouse strain, the dose response to LPS differs at the single-gene level (some genes are induced only by higher doses), and profoundly between mouse strains (93, 98). And finally, in humans it has been shown that some 80% of genes in monocytes exhibit heritable differences in gene expression and there is a major divergence in the secondary induction of IFN-β target genes in response to LPS (150).

A secondary issue is the massive heterogeneity of gene expression in macrophages at the single-cell level. Single-cell analysis of LPS-inducible genes in macrophages demonstrated essentially bimodal variation between individual cells; genes are either induced or they are not (151). The authors in this case sought causal explanations for this variation based on covariance of transcription factor expression at the single-cell level. However, an alternative view is that transcriptional activation at the individual gene level is intrinsically probabilistic (152). Stochastic variation in gene expression may occur even at the single-allele level. Indeed, the LPS receptor, TLR4, is expressed from only one allele in

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individual cells, with an allele-counting mechanism similar to that of the X chromosome (153). This finding explains the semidominance of the *Tlr4* mutation in C3H/HeJ mice, since in heterozygotes 50% of cells express the nonfunctional protein. Conversely, when one allele is deleted, all cells express *Tlr4* (from the other allele). Furthermore, because of the complex feedback loops in stimulated cells, in which LPS rapidly induces inhibitors that block signaling and degrade induced mRNAs and proteins (154, 155), individual cells show an oscillating response over relatively short time frames, eventually reaching a new steady state (156).

Against this background of complexity and heterogeneity, Murray et al. (157) proposed a set of guidelines to be used when describing macrophage activation states. They propose that descriptions of macrophage activation in disease states accurately describe the system, the way populations were isolated, and then utilize markers to enhance the description. However, even this rigorous approach is confounded if each macrophage is unique, deploying the potential arsenal of host defense weaponry in distinct combinations that change with time. The plasticity and diversity of individual macrophages may be a necessary part of their innate immune function, enabling the generation of a combinatorial diversity that cannot readily be overcome by a single pathogenicity determinant. An alternative to cell-based models is to describe diseases in terms of interacting genes. For example, if one examines very large data sets of cancer expression arrays, it is possible to extract a set of coexpressed genes that includes many known phagocyte markers, and *inter alia* indicates that there is a common tumor-associated macrophage signature, regardless of tumor type, that fits neither M1 nor M2 profiles (85).

DATABASES, WEBSITES, AND THE FUTURE

The escalating amount of data on mononuclear phagocyte biology coming from genome-scale technologies now taxes the capacity of any individual to access all of the useful information about the regulation of their favorite gene. BioGPS is an example of a new era of more user-friendly portals, as is the Immunological Genome portal (http://www.immgen.org). We established the website http://www.macrophages.com as a community website for sharing access to macrophage-related genomic and other information (158), including the massive promoter-related data sets arising from the FANTOM projects. Macrophages.com also provides macrophage-related pathway annotation data and links to the growing InnateDB (http://www.innateDB.org), which curates molecular interactions among macrophage-expressed proteins. The website also contains a curated compendium of major reviews on macrophage biology and transcriptional regulation, many of which provide much more comprehensive coverage of subtopics than this brief overview. With completed genomes, we are seeing comparable data sets available for other species, including domestic pigs, chickens, sheep, and cattle, that will underpin more-rigorous studies of the evolution of innate immunity, and also the recognition that there is very substantial genetic variation within species that underlies disease susceptibility loci.

In summary, macrophages are a very diverse cell type, expressing a subset of surface markers and derived from both yolk sac and circulating monocytes. While useful information has been gained by studies of mice, primarily the C57BL/6 strain, these are not always generalizable to humans and other animals, and more-extensive studies of larger mammals such as pigs and sheep, as well as studies utilizing the flexibility and unprecedented developmental analysis of the chicken, will be needed to clarify the role, ontogeny, and differential function of the range of myeloid cells.

ACKNOWLEDGMENTS

The Roslin Institute is supported by Institute Strategic Programme Grants from the Biotechnology and Biological Sciences Research Council.

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