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Macrophages and CSF-1
Implications for development and beyond

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Abbreviations: CSF-1, colony-stimulating factor-1; E, embryonic day; HSCs, multipotent haemopoietic stem cells; AGM, aorto-gonado-mesonephron; PDGFs, platelet-derived growth factors; TGFs, transforming growth factors; IGF-1, insulin-like growth factor-1; CCL, chemokine (C-C motif) ligand; IL, interleukin; IFN-γ, interferon gamma; TNF, tumour necrosis factor; ECM, extracellular matrix; Arg1, arginase 1; Retnla; Fizz1, resistin-like molecule alpha; Chi3l3; Ym1, chitinase 3-like 3; Mrc1, mannose receptor 1; Msr2, macrophage scavenger receptor 2; C1q, complement component 1q; Trem 2, triggering receptor expressed on myeloid cells 2; TAMS, tumour-associated macrophages; CSF-1R, CSF-1 receptor; mutation osteopetrotic; *Csf1*$^{op/op}$, CSF-1-deficient mice; GM-CSF, granulocyte-macrophage colony-stimulating factor; NGF, nerve growth factor; MMPs, matrix metalloproteinases; GH, growth hormone; BMP, bone morphogenic protein; FGF, fibroblast growth factor; LPS, lipopolysaccharide; BPD, bronchopulmonary dysplasia; YFP, yellow fluorescent protein; EAE, experimental autoimmune encephalomyelitis

Recent focus on the diversity of macrophage phenotype and function signifies that these trophic cells are no longer of exclusive interest to the field of immunology. As key orchestrators of organogenesis, the contribution of macrophages to fetal development is worthy of greater attention. This review summarizes the key functions of macrophages and their primary regulator, colony-stimulating factor (CSF)-1, during development; highlighting trophic mechanisms beyond phagocytosis and outlining their roles in a range of developing organ systems. Advances in the understanding of macrophage polarization and functional heterogeneity are discussed from a developmental perspective. In addition, this review highlights the relevance of CSF-1 as a pleiotropic developmental growth factor and summarizes recent experimental evidence and clinical advancements in the area of CSF-1 and macrophage manipulation in reproduction and organogenic settings. Interrogation of embryonic macrophages also has implications beyond development, with recent attention focused on yolk sac macrophage ontogeny and their role in homeostasis and mediating tissue regeneration.

The regulatory networks that govern development involve a complex range of growth factors, signaling pathways and transcriptional regulators arising from epithelial, mesenchymal and stromal origins. A component of the organogenic milieu common to the majority of developing organs is the tissue macrophage. These hemopoietic cells are part of the mononuclear phagocyte system regulated primarily by colony-stimulating factor (CSF)-1.[1,2]

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There is a resurgence in the field of CSF-1 and macrophage biology; where greater understanding of the heterogeneity of these cells is revealing contributions to tissue repair and regeneration beyond the phagocytic and inflammatory functions for which they were traditionally ascribed.[3–6] The accumulation of macrophages during tissue injury is no longer viewed as simply a surrogate for disease severity, with macrophages now known to be vital in governing tissue regeneration in many settings.[7–11] In particular it is the influence of CSF-1 in regulating an alternative macrophage activation state that is increasingly linked to organ repair in a range of disease models.[12–17] With many similarities drawn between organogenesis and regeneration, it is pertinent to re-examine the role of CSF-1 and macrophages in organ development.

Ontogeny of Embryonic Macrophages

Classical hemopoiesis specifies that macrophages arise from lineage-committed myeloid and subsequent monocyte precursors in the bone marrow. They are released as circulatory blood monocytes before migrating into tissues and differentiating into macrophages to replenish local populations or in response to inflammation.[1] However, this traditional dogma of macrophage ontogeny is challenged during embryonic development. First observed in the mouse embryo at embryonic day (E)7.5, the presence of primordial macrophage populations before the onset of definitive hematopoiesis indicates that alternate mechanisms of macrophage derivation in embryogenesis exist.[18,19] This first population represents a transient wave of maternally-derived macrophages, that provide scavenger functions in the embryo prior to the development of its own phagocytes, and rapidly declines by E8.5/9.[18] The first true embryonic macrophages are evident from E8 and are derived from the primitive endoderm of the yolk sac.[18,20] From E8–9, macrophages are

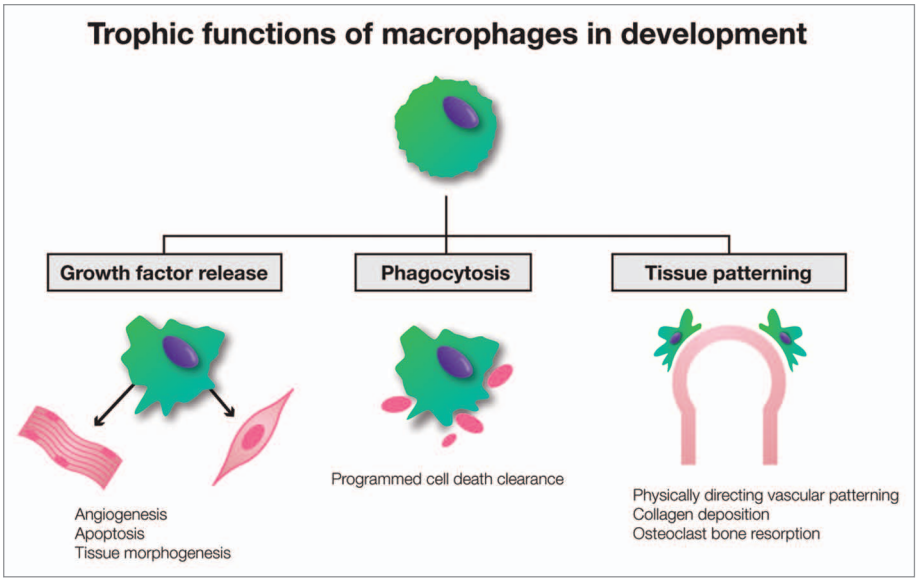

Figure 1. Key functions of macrophages in organ development. Diverse functions of macrophages contribute to the regulation of organogenesis. As potent effector cells, macrophages produce a range of growth factors that stimulate angiogenesis, induce apoptosis and regulate tissue morphogenesis. Phagocytosis and clearance of cellular debris important in programmed cell death is an essential part of the significant tissue remodelling that occurs during development. Macrophages also act to directly mediate tissue patterning through direct interactions, including physically directing vascular growth, collagen deposition in mammary bud outgrowth and the bone resorbing activity of bone-resident osteoclasts.

evident throughout the yolk sac and begin to invade the embryo proper migrating into the developing head.[18,20] Definitive hematopoiesis, defined as the derivation of hemopoietic cells from multipotent hemopoietic stem cells (HSCs), begins at approximately E10.5 from progenitors originating in the aorto-gonado-mesonephron (AGM) region.[21,22] From E12 onwards, the liver is primary site of myeloid production. Distinct from HSC-derived myeloid cells, yolk sac-derived macrophages are Myb-independent.[23]

Trophic Functions of Macrophages during Development

Large numbers of macrophages are present in virtually all developing organs, with maximum numbers correlating with key periods of organogenesis.[24] Moreover, mouse models deficient in tissue macrophages display a range of developmental abnormalities including skeletal and neurological deficiencies and impaired growth and fertility.[25-28] Furthermore, there is no viable transgenic mutant model that is totally devoid of macrophages during organogenesis,[29] highlighting the relevance of macrophages to development.

Macrophages contribute significantly to the organogenic milieu and support the morphogenic processes via a range of mechanisms (**Fig. 1**).[3,4,19,30] Most widely recognized for their phagocytic roles, macrophage-mediated clearance of cellular debris due to programmed cell death is an essential part of the significant tissue remodelling that occurs during development.[19,31] Macrophages densely populate regions of increased cell death such as the interdigital webbing during embryogenesis,[20] and inactivation of the apoptotic gene *psr* results in perturbed development of the lungs, brain and eye due to excessive tissue and impaired cellular clearance.[32] Macrophages also contribute to the induction of programmed cell death with macrophage-derived signaling directing apoptosis as part of the regulation of tissue morphogenesis.[33,34] In the developing eye, interaction between angiopoietin-2 and macrophage-derived Wnt7b is essential in driving endothelial cell apoptosis associated with vascular regression and retinal remodelling.[35,36] Another developmental function of macrophages is the provision of trophic support. As potent effector cells, macrophages produce a range of mediators including platelet-derived growth factors (PDGFs),[37] transforming growth factors (TGFs),[38] insulin-like growth factor (IGF)-1[39] and Wnts.[40-42] Macrophage-derived factors mediate cell fate decisions, as observed in β cell differentiation in the developing pancreas[43] and hepatic progenitor differentiation during liver regeneration.[40] Macrophages also contribute to development through angiogenic regulation.[30] In addition to the production of a range of pro- and anti-angiogenic factors,[36,37,42] macrophages have been shown to assist in vascular patterning by physically directing angiogenic positioning.[44]

Classification of Macrophage Phenotype

Macrophages are remarkably plastic in their ability to adapt to microenvironmental cues, evidenced by their wide-ranging locations and distinct functions.[7,45,46] Such plasticity allows them to effectively respond to environmental changes or immunological challenges in order to elicit the appropriate functional response. Classification systems have been devised to categorise macrophage phenotype, physiological activation and functional activity. Emulating the T cell field, macrophages have broadly been classified into two groups; "classically activated" M1 macrophages that are associated with host defense and pro-inflammatory outcomes, and "alternatively activated" or M2 macrophages that represent a more regulatory or reparative macrophage activation state (**Fig. 2**).[47-49]

M1 Macrophages. In response to inflammatory stimuli such as pathogenic invasion and necrosis, pro-inflammatory mediators interferon gamma (IFN-γ) and tumor necrosis factor (TNF) stimulate macrophages to take on a classical activation state.[48,50,51] These primed macrophages secrete pro-inflammatory cytokines and chemokines such as interleukin (IL)-1, TNF-α, IL-12, IL-23 and chemokine (C-C motif) ligand (CCL)8.[49,52] They also produce cytotoxic mediators including oxidative and nitrogen

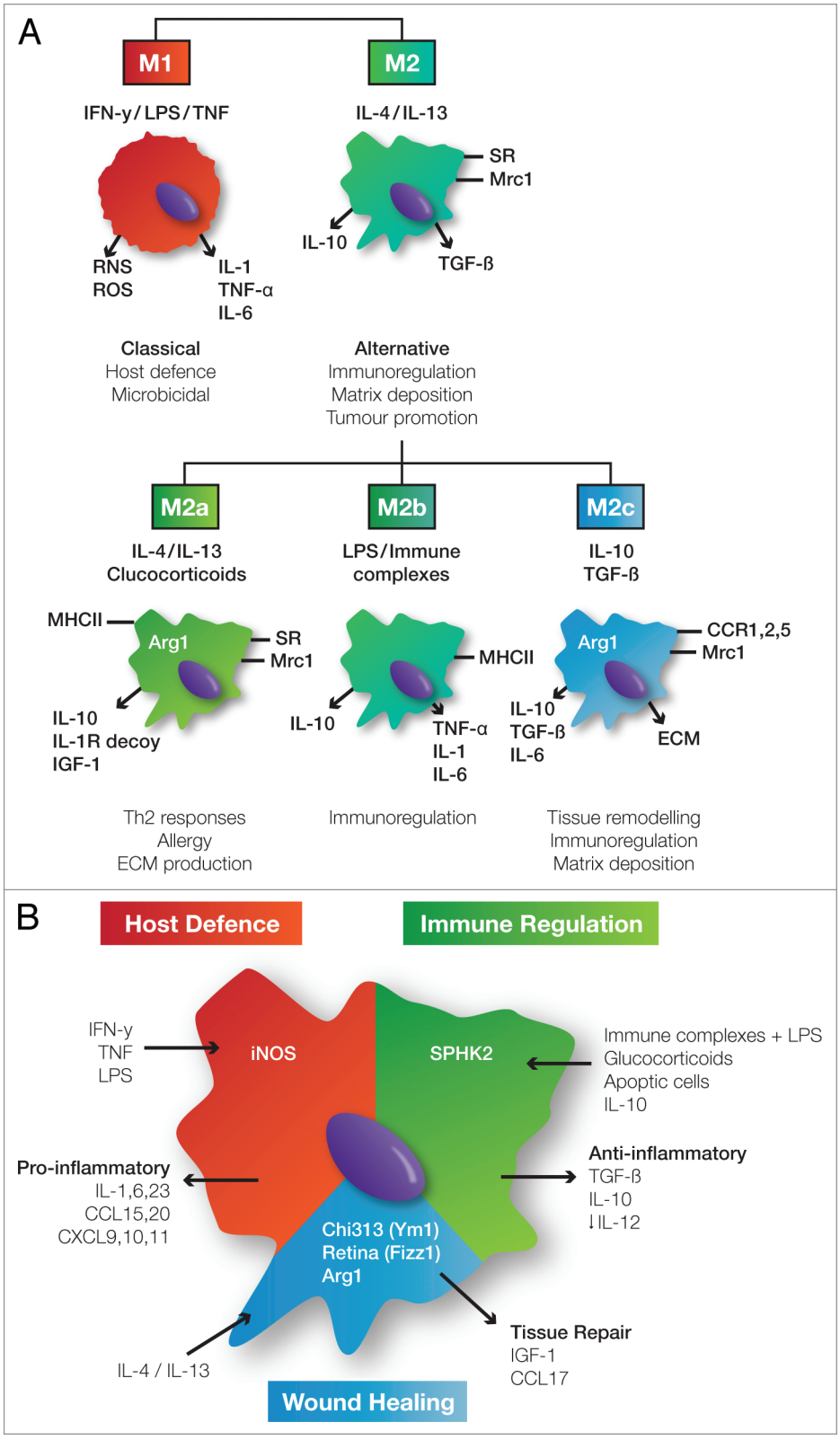

Figure 2. Macrophage heterogeneity and proposed macrophage classification systems. (**A**) Macrophage classification based on M1 "classical" activation and M2 "alternative" activation. M1 macrophages are activated by pro-inflammatory stimuli such as IFN-γ to induce production of pro-inflammatory factors and microbicidal reactive nitrogen (RNS) and oxygen species (ROS) to mediate host defense functions. Macrophages take on an alternative activation state in response to IL-4/IL-13 exposure and are characterized by mannose receptor (Mrc1) expression and release of anti-inflammatory factors such as IL-10 to mediate Th2-associated responses. Given the diversity within the alternative category, Martinez et al. proposed subclassification to include M2a, M2b and M2c which each have characteristic inducing factors, receptor expression and cytokine production to mediate Th2 responses, immunoregulation and tissue remodelling functions respectively.[48] (**B**) As opposed to discrete categories, Mosser et al. proposed a more fluid macrophage classification system based on a color wheel analogy.[45] They identify the three broad functional categories as host defense, wound healing and immunoregulation, induced in response to varied stimuli to provide characteristic receptor and cytokine production which mediate effector functions. However these are not discrete categories and macrophages have the ability to blend within broad subtypes to respond to varying microenvironmental cues, provide specific functional needs and generate extensive heterogeneity. IFN-γ, interferon gamma; LPS, lipopolysaccharide; TNF, tumor necrosis factor; iNOS, inducible nitric oxide synthase; MHCII, major histocompatibility complex class II; IL, interleukin; Th, T helper cell; SR, scavenger receptors; TGF-β, transforming growth factor β; Arg1, arginase 1; IGF-1, insulin-like growth factor 1; ECM, extracellular matrix; CCR, CC chemokine receptor; CXCL, CXC motif chemokine; Chi3l3, chitinase 3-like protein; Retnla, resistin-like molecule α; SPHK1, sphingosine kinase 1; Mrc1, mannose receptor 1.

radicals to facilitate microbicidal and tumoricidal activities.[53] This effective killing repertoire is important in host defense, however extended or inappropriate activation can lead to tissue damage as observed in chronic diseases such as rheumatoid arthritis.[50,54,55]

M2 Macrophages. The description of alternative activation has arisen from the improved understanding of macrophage diversity beyond mere pro-inflammatory phagocytes. Production of IL-4 and IL-13 as part of a Th2 response stimulates macrophages to acquire an M2 phenotype.[47,56,57] These macrophages have decreased production of pro-inflammatory cytokines and decreased microbial killing and phagocytosis activity. Instead they produce anti-inflammatory mediators such as IL-10 and TGF-β to promote dampening of immune responses.[58,59]

Alternative Macrophage Activation and Development

While the M1/M2 dichotomy has arisen from studies in adult settings of disease and repair, there are many common regulatory and molecular programs during organogenesis. In this regard, regeneration and repair of organs in the adult may represent a recapitulation of developmental programs, and congruently developmental macrophages that respond to their microenvironment and instructive cues provide important pleiotropic functions. This is evidenced by the convergent phenotype of macrophages involved in development and the M2 classification of macrophages associated with tissue repair. Importantly, Rae et al. conducted comprehensive gene expression profiling of tissue macrophages during embryonic development comparing cells derived from E12.5 kidneys, brains and lungs.[60] Interestingly, fetal macrophage abundance and gene expression was shown to be comparable regardless of the tissue of origin. Furthermore, these fetal macrophages expressed markers consistent with an M2 phenotype, including upregulation of mannose receptor 1 (Mrc1), macrophage scavenger receptor 2 (Msr2), complement component 1q (C1q), CD163, selenoprotein P, CCL24 and triggering receptor expressed on myeloid cells 2 (Trem 2).[60] In the lung, the number of macrophages is increased and they display an M2-associated activation state, indicated by upregulation of Mrc1, Arg1 and CCL17, during tissue remodelling associated with alveolar development.[61] Interestingly, but perhaps unsurprisingly, these alternatively-activated developmental macrophages are phenotypically similar to tumor-associated macrophages (TAMS).[62-64] TAMS induce angiogenesis, tissue remodelling and scavenger functions which support tumor growth and metastasis; reminiscent of M2 macrophages and their key roles essential in normal organ development.[62] Ojalvo et al. demonstrated significant overlap in the gene expression signatures of fetal macrophages and TAMS within breast cancer tissue.[64] This included genes associated with angiogenesis and other trophic functions, supporting the recapitulation of developmental events in neoplastic growth.[64]

The phenotype and function of tissue macrophages that govern extracellular matrix (ECM) production and remodelling is fundamental to organogenesis. Alternative activation of macrophages is associated with ECM production and the release of trophic factors that contribute to tissue remodelling, angiogenesis and wound healing.[47,65,66] Common alternative activation markers include expression of enzymes arginase 1 (*Arg1*), resistin-like molecule α (*Retnla; Fizz1*) and chitinase 3-like 3 (*Chi3l3; Ym1*).[56,67] Other stimuli shown to promote an alternative phenotype include glucocorticoids, IL-10 and immune complexes.[48,49,68,69] During embryonic development, ECM remodelling is a central part of the structural changes involved in branching morphogenesis and architectural establishment that is dependent on tissue macrophages. Matrix metalloproteinases (MMPs) contribute to matrix proteolysis and have positive roles in branch formation not only in the kidney[70] but also in other branching organs including the lung[71] and mammary gland.[72] MMP9 is produced by the mesenchyme, and when blocked using an anti-MMP9 Ab a decrease in ureteric bud branching in metanephric organ cultures was observed.[73] Furthermore in vivo, genetic deletion in *Mmp9*$^{-/-}$ mice resulted in impaired branching, increased mesenchymal apoptosis and decreased nephron formation.[74]

During postnatal lung development in the mouse, we have recently reported that macrophages show an upregulated expression of M2 markers (*Arg1, Ccl17,* and *Mrc1*) that correlates with the alveolarisation stage; characterized by secondary septation, alveolar wall remodelling and microvascular maturation.[61] *Mrc1* provides an important mechanism for cellular clearance associated with homeostasis and tissue reorganization,[76] and *Arg1* is associated with collagen formation and ECM production.[77] This highlights the unrecognized importance of macrophages in the alveolarisation stage of lung development, and in particular the association with an M2 activation state and tissue remodelling.[61]

CSF-1 as a Developmental Mediator

CSF-1, also known as macrophage (M)-CSF, is a hemopoietic growth factor for the mononuclear phagocyte lineage and the primary regulator of macrophage differentiation, proliferation and survival.[78-80] It elicits its effect through binding with the CSF-1 receptor (CSF-1R); a high-affinity receptor tyrosine kinase encoded by the *c-fms* proto-oncogene.[81] CSF-1 is produced by a range of cell types and acts both locally and humorally in an autocrine and paracrine manner.[78] Alternate mRNA splicing and differential proteolytic processing gives rise to three biologically-active isoforms of CSF-1: a secreted glycoprotein; a secreted proteoglycan; and a membrane spanning cell-surface glycoprotein.[82] The two major cell populations regulated by CSF-1 via expression of the CSF-1R are cells of the trophoblast lineage and hemopoietic cells of the mononuclear phagocyte system,[83] with distinct promoters specific to each lineage in humans.[84] Expression beyond these lineages is also reported in settings including Paneth cells of the small intestine,[85,86] neural stem cells[87] and kidney tubular epithelium in a setting of acute injury.[88]

CSF-1 is essential in regulating trophic macrophages during development.[4,19,28,60] CSF-1R is one of the earliest markers of these cells indicating the importance of CSF-1 in the differentiation and propagation of macrophages from the earliest stages of development.[20,83] Indeed, it is the early and consistent expression of the CSF-1R that has provided an important tool for tracking developmental macrophages.[20,83] The absence of CSF-1 results in a range of developmental abnormalities, including skeletal, neurological, growth and fertility defects,[25-28] primarily stemming from the severe deficiency in tissue macrophages. Interestingly while these developmental defects are striking, CSF-1-deficient mice (*Csf1*$^{op/op}$) exhibit few immunological defects. This is in contrast to granulocyte-macrophage colony-stimulating factor (GM-CSF), where *Gmcsf*$^{-/-}$ mutant mice are superficially healthy and fertile, with the exception of lung abnormalities associated with impaired surfactant clearance.[89] CSF-1 has non-redundant functions in development, as administration of GM-CSF was not able to correct the growth and skeletal deficiencies in CSF-1-deficient mouse.[90] Furthermore, unlike other macrophage mediators, CSF-1 levels are developmentally regulated and correlate with key periods of organogenesis.[91] Interesting, CSF-1

is associated with M2 macrophage polarization.[12,92] This is in contrast to macrophages responding to GM-CSF which have M1-associated phenotypic characteristics.[12,52,93] During tissue injury and repair, Hamilton proposes a balanced relationship between CSF-1 and GM-CSF-mediated anti- and pro-inflammatory activity.[12,15] When the ratio is tipped toward GM-CSF, a pro-inflammatory response prevails until the inflammatory stimulus diminishes and the balance shifts to a CSF-1-mediated reparative/homeostatic state.[12,15]

Macrophages in Organogenesis

CSF-1-responsive macrophages act as important regulators of organogenesis, and several examples of the important organ-specific roles of macrophages during development are evident. Microglia, the resident macrophages of the CNS, are a key cell type in brain development. From E11–12 in the mouse, they are found closely associated with ventricular surfaces, developing neuronal cells and the neural tube.[94] Macrophages contribute to neurogenesis, synaptogenesis and apoptosis involved in brain development.[34,95,96] They are also involved in remodelling arborisations of neurosecretory neurons[97] and contribute to synaptic pruning.[98] Another developmental function of macrophages has been observed during brain angiogenesis. Fantin et al. demonstrated that macrophages provide essential guidance of tip cell fusion in vessel anastomosis.[44] Mice severely devoid of macrophages through ablation of the differentiation transcription factor PU.1 displayed fewer, less complex connections.[44] Furthermore, the recruitment of angiogenic macrophages into the hindbrain was dependent on CSF-1.[44] Interestingly, it is yolk sac-derived macrophages rather than monocyte-derived macrophages that contribute to the angiogenic functions. CSF-1 has a neurotrophic effect when added to embryonic neurons in culture, promoting increased neuron survival and outgrowth, and neural function is impaired in mice deficient in CSF-1.[99] Furthermore, a more severe phenotype is observed in the brains of *Csf1r*$^{-/-}$ mutant mice, where a total absence of macrophages is associated with gross disruption of brain architecture as well as olfactory defects stemming from a highly perturbed olfactory bulb.[100] More recently, CSF-1R expression has been reported on neural progenitor cells, and it's activation linked to normal corticogenesis.[87]

Mammary gland development is one of the most elegant examples of the importance of CSF-1-responsive macrophages in branching morphogenesis. During normal development macrophages directly associate with terminal end buds, lining and directing the developing duct[101] in response to local production of CSF-1.[102,103] Macrophages promote collagen fibrillogenesis and terminal bud structural organization.[101] A lactational disturbance in *Csf1*$^{op/op}$ mice, whereby ~90% of mothers failed to feed pups, indicates alterations in mammary gland development and function in the absence of CSF-1.[104] Macrophages normally recruited to the developing ducts are absent and outgrowth and branching is reduced, resulting in atrophic, poorly branched terminal end buds,[102] a phenotype corrected by transgenic restoration of local mammary epithelium-produced CSF-1.[103]

Macrophages in the developing murine pancreas are located principally at the duct-islet interface[105] in close apposition to insulin-producing islet cells.[43] These F4/80^{+} cells are reported to display a stellate morphology.[106] Similarly in the human, macrophages cluster around developing ducts, recruited via local CSF-1 expression, and provide a supportive developmental microenvironment.[107] In the *Csf1*$^{op/op}$ mouse, macrophages are scarce and insulin mass is reduced due to reduced β cell proliferation and abnormal islet morphology.[106] In the eye, macrophages contribute to remodelling with macrophage-derived nerve growth factor (NGF) contributing to normal neuronal apoptosis.[108] Macrophages also contribute to angiogenesis of the eye. Normally located in close contact with the retinal vasculature and bridging neighboring sprouts during anastomosis, deficiency in retinal macrophages in *Csf1*$^{op/op}$ mice is associated a less complex retinal plexus.[44] The importance of CSF-1-responsive macrophages in retinal development has been elegantly demonstrated in the zebrafish, where morpholino-oligonucleotide-mediated knockdown of the CSF-1R inhibited macrophage migration and retinal colonisation, resulting in a range of developmental defects.[109] Interestingly, when depleted macrophages were allowed to return, a partial rescue of the neurogenesis and retinal growth was observed.[109]

Macrophages are also abundant in the developing heart and embryonic macrophages are essential in cardiac development. In a Xenopus study,[110] blockade of the primitive macrophage differentiation transcription factor *spib* resulted in a severe loss of macrophages and serious heart malformations. The efficiency of macrophage depletion correlated with the degree of cardiac malformation due to a lack of endocardial maturation, that could be rescued though the introduction of macrophage-containing tissue grafts.[110] In the lung, macrophages are evident as early as E10 in the mouse located within the mesenchyme and surrounding the forming lung buds and elongating bronchi.[111,112] We have previously observed that during branching morphogenesis, macrophages are located abundantly within developing branch points (**Fig. 3**).[61] Macrophage number increases during postnatal life and peaks during the alveolarisation stage.[61] In the absence of CSF-1, alveolar macrophage populations are severely depleted during postnatal development,[113,114] and in adulthood mice develop spontaneous emphysema associated with deregulated matrix MMPs and abnormal elastin deposition.[114] Furthermore, macrophages in the embryonic lung,[60] and in the postnatal lung undergoing alveolarisation[61] both demonstrate a gene expression profile indicative of a trophic M2 macrophage activation state.

IL-34—a CSF-R Ligand

The recent discovery of IL-34 as the alternate ligand for the CSF-1R raises a wealth of questions regarding the differential roles of IL-34 and CSF-1 in mediating specific effects attributed to CSF-1R signaling.[115] IL-34 mRNA has been located in a range of tissues, but is most abundant in the spleen.[115] Although the ligands share no sequence homology and have differential receptor binding kinetics,[116,117] IL-34 and CSF-1 share functional activation of the CSF-1R, with transgenic introduction of IL-34 gene

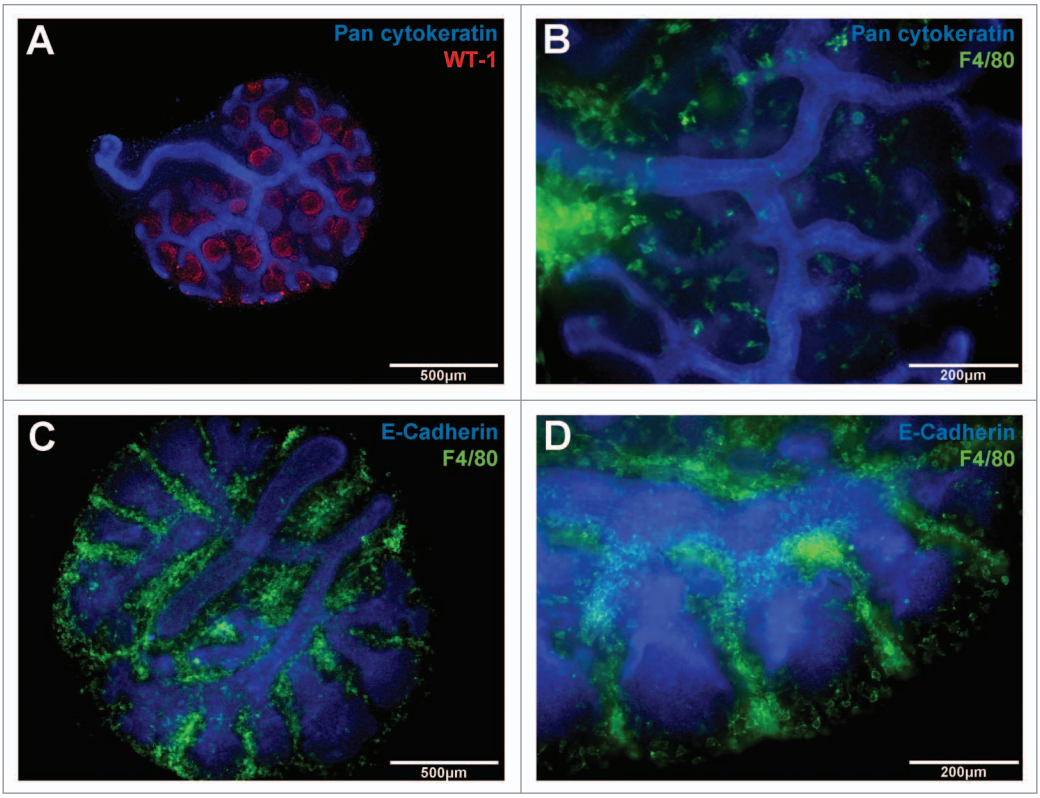

Figure 3. Macrophages are abundant in embryonic organs during development. Embryonic organs (E12.5) cultured as explants on floating membranes provide useful assays for investigating macrophages and branching morphogenesis in kidney (**A and B**) and lung (**C and D**) development. During nephrogenesis in the kidney, indicated by pan cytokeratin-labeling of branches (**A,** blue) and WT-1-labeling of developing nephrons (**A**, red), large numbers of F4/80-labeled macrophages (**B,** green) are present throughout the kidney, but with particular concentration around the central branches (**B**). Similarly in the lung, F4/80-labeled macrophages (green) are present in large numbers during branching morphogenesis (**C**). In particular, macrophages are densely located within branch points of the branching epithelium (**D,** blue).

expression under control of the CSF-1 promoter able to correct the phenotypic defects of CSF-1 deficient mice.[117] It is the differential spatiotemporal expression patterns of the two ligands that determines their complementary and non-redundant functions.[117] Embryonic mRNA expression indicates that CSF-1 may be more important in the pregnant uterus and in osteoclast regulation.[117]

IL-34 has particular importance in brain development, where IL-34 mRNA is expressed before CSF-1 mRNA and at higher levels in most regions of the developing brain.[117] Although both ligands have complementary expression in distinct regional patterns, the constitutively high expression of IL-34 in the brain supports its key role in microglial development and homeostasis,[87] and has been comprehensively examined in IL-34-deficient reporter mice.[118,119] The essential role of IL-34 has been confirmed specifically in microglial and Langerhans cell development,[119] and in microglial homeostasis in the adult.[118] Interestingly, no overt growth or osteopetrotic phenotype, and little effect on other myeloid populations including the liver and lung, were observed in IL-34 deficient animals.[118,119] Phenotypically, IL-34-mediated differentiation in vitro gives rise to macrophages with a characteristic M2 phenotype, comparable to CSF-1-responsive macrophages.[120] Furthermore, in response to GM-CSF they can be polarized to take on a characteristic M1-associated phenotype.[120]

CSF-1 and Macrophage Manipulation in Development

The diverse functions of macrophages makes them useful candidates for a range of applications including tumor killing, tissue regeneration and promoting organogenesis. The key to their utility will be a targeted, measured activation so that specific effects can be harnessed and exploited. Manipulation of CSF-1 signaling has been used clinically as an immunological mediator in settings such as cancer and infection.[121] With a growing appreciation for the importance of this pleiotropic growth factor in developmental regulation it highlights this as another area with potential clinical implications.

Reproduction. CSF-1 and macrophages have important functions in fertility and reproduction. CSF-1 is produced by the uterine epithelium during pregnancy in response to hormones such as progesterone.[122] During pregnancy, uterine production of CSF-1 increases 1000-fold compared with a 2-fold increase in serum levels.[123] The coincident expression of uterine CSF-1 and trophoblast CSF-1R supports the role of CSF-1 in placentation and placental growth.[122,124] Furthermore, local production of CSF-1 at the maternal-fetal interface is important in immunosuppression associated with pregnancy maintenance and preventing fetal rejection.[125] Defective CSF-1 production by Th2 cells

is associated with pregnancy loss[126] and women with reduced circulating CSF-1 have a higher rate of recurrent spontaneous abortion.[127] CSF-1 also mediates reproductive function through macrophage regulation. *Csf1*$^{op/op}$ mice are severely depleted in ovarian, uterine and placental macrophages.[128] Macrophages are important accessory cells in reproduction and it is the absence of CSF-1-mediated tissue macrophage regulation that is responsible for the primary fertility defects observed in *Csf1*$^{op/op}$ mice.[128]

Circulating CSF-1 protein levels may serve as a predictor for in vitro fertilization (IVF) outcomes.[129,130] Moreover, CSF-1 has been therapeutically administered as an adjunct therapy during hormonal ovarian hyperstimulation therapy.[131] Patients with documented unsuccessful responsiveness in previous hyperstimulation cycles were recruited, and CSF-1 treatment was shown to improve follicular development leading to increased numbers of mature oocytes retrieved for subsequent IVF. Concomitant CSF-1 treatment was particularly impressive in patients with low serum CSF-1 levels, and a 40% successful pregnancy rate among these 27 patients was reported.[131]

Growth and maturation. Trophic macrophages as essential regulatory mediators of development also provide a potential avenue for modulating growth and organogenesis. Geutskens et al. reported that the addition of CSF-1 to embryonic pancreas explants was associated with an increase in β cell differentiation and insulin production.[43] Furthermore, Rae et al. demonstrated that the addition of CSF-1 to embryonic kidney explants increased branching and nephrogenesis while maintaining normal morphology.[60] In these reports, enhancement of organogenesis correlated with an increase in macrophages.

The postnatal period is an important phase for CSF-1-mediated growth. In both mouse and humans, systemic CSF-1 levels normally increase in the first few days after birth.[91,132] We have previously reported that the systemic supplementation of postnatal CSF-1 in mice promoted an increase in overall organ growth and body weight, that may be due in part to increased expression of IGF-1.[13] This raises new information regarding the potential mechanism of trophic macrophage function in organogenesis and also supports an emerging link between CSF-1, macrophages and the IGF-1 growth axis.[133] Growth hormone (GH) and IGF-1 interact to provide the principle regulatory mechanism of somatic growth in mammals. The findings that hepatic-derived circulating IGF-1 (the principle source of IGF-1) contributes only minimally to body size has highlighted the significance of locally produced IGF-1 acting in an autocrine/paracrine manner during normal growth and organ development.[134-136] Macrophages can produce significant amounts of IGF-1, particularly in response to CSF-1, in vitro.[137] Interestingly, many of the growth and developmental deficiencies of *Csf1*$^{op/op}$ mice are common to IGF-1-deficient animals,[138] and in the *tl/tl* rat, dysfunctional CSF-1 production is coupled with decreased macrophages and a failure of the postnatal IGF-1 spike.[133]

Macrophage polarization, development and perturbation. Improved understanding of the key role of macrophages in organogenesis supports a more thorough examination of macrophage phenotype in governing proper development, and whether developmental perturbation may impact or be impacted upon by phenotypic and functional skewing. For example, in mouse lung, alveolar development is associated with the upregulation of M2 macrophages.[61] Examination of macrophage-mediated mechanisms of alveolar development may have particular clinical relevance for addressing lung immaturity. In particular, the alteration of trophic macrophages in bronchopulmonary dysplasia (BPD)-associated arrest of alveolarisation is underexamined, as is the macrophage implications of the ex-utero environmental changes, inflammation and therapeutic interventions in prematurity-associated lung developmental perturbation. Indeed, the inflammatory activation of macrophages during development not only contributes to tissue damage and disruption of organ development through pro-inflammatory injury, but may also skew macrophages away from their trophic functions.[111,139,140] Inflammatory activation of fetal lung macrophages through NF-κB signaling was found to upregulate pro-inflammatory mediators such as IL-1β and alter expression of Wnt7b, bone morphogenic protein (BMP)4[111] and fibroblast growth factor (FGF)-10.[139] And while inflammatory challenges such as lipopolysaccharide (LPS) or IL-1β administration in models of chorioamnionitis promote accelerated maturation, the mechanism is distinct from alveolarisation, characterized by a lung pathology associated with BPD.[141] Today, corticosteroids remain one of the most important advancements in reducing the mortality associated with preterm birth, however they are associated with precocious maturation and significant side effects. Glucocorticoids are known M2 polarizing stimuli and in a microarray study of glucocorticoid-responsive genes, the gene most significantly reduced in embryonic glucocorticoid receptor deficient mice is the M2 polarization-associated gene Chi3l3.[142] They are particularly associated with the M2a subset, which aside from Th2 responses is also linked to negative aspects including ECM production and allergy.[48,51] The effect of such medications on developmental macrophages requires examination to ascertain if inappropriate skewing of macrophage phenotype may contribute to some of the negative side effects of glucocorticoid treatment. Indeed, immunological research shows that the M1/M2 balance is a two-edged sword and that both provide important functions in their appropriate context.[7] Dysregulation of M2 macrophages is also associated with enhanced matrix production and pathological implications observed in disorders such as in asthma[143] and the immunosuppressive functions of M2s and CSF-1 is linked to tumor growth and metastasis.[144,145] CSF-1 has also been linked to the formation of atherosclerotic plaques.[146,147] Furthermore, CSF-1 is implicated in Paneth cell development and CSF-1 hyperstimulation may be associated with hyperproliferative disorders of the small intestine.[86]

Resident Macrophages and Regeneration

Post development, the preservation of a tissue resident population of macrophages is important in homeostatic maintenance and responding to pathogenic challenge. Virtually all organs contain a resident population, comprising up to 15%, which reside independent of inflammatory or immune stimulus.[20,83] The

heterogeneity of macrophages is demonstrated by the diversity of these resident populations.[3,46] In response to microenvironmental cues, macrophages display specific phenotypes and tissue-specific functions that allow them to respond to the distinct requirements relevant to the organ. While recruitment and infiltration of circulating precursors is an important strategy, particularly in host defense, whether the tissue resident population is maintained by infiltration or local proliferation remains debated.[7,148] Also of interest is the ontogeny of these resident macrophage populations. It is suggested that fetal macrophages which colonise organs during embryonic development may constitute the resident population of macrophages maintained by local proliferation in adulthood.[149,150]

One area where this hypothesis has been strengthened is microglia of the brain.[151] Elegant lineage-tracing studies, using both Runx and Myb-mediated fate mapping, have demonstrated that microglia are derived from early yolk sac macrophages.[23,150,151] Ginhoux et al. demonstrated through neonatal bone marrow transplantation and parabiosis that resident populations are maintained independently of circulating monocytes. Furthermore, activation of yellow fluorescent protein (YFP) in leukocytes at E7.5 contributed significantly to adult microglia, whereas activation later in development, when definitive hemopoiesis was predominant, resulted in little YFP observed in the brain resident macrophage population.[150] An essential role for CSF-1 in the development of yolk sac macrophages and subsequent resident macrophage populations, but not monocytes was also reported.[150] Highlighting that Runx labeling also labels a small population of HSC, Schulz et al. demonstrated the appropriateness of Myb-dependency as defining yolk sac and HSC-derived populations.[23] *Myb*$^{-/-}$ mice displayed normal levels of tissue macrophages in the brain, as well as the skin, spleen, pancreas, kidney and lung at E16.5.[23] Indeed, these F4/80^{bright} yolk sac-derived macrophages displayed the characteristic spindle-shape morphology in close association with the developing kidney and lung epithelium as described previously.[13,60] Furthermore, through pulse labeling of CSF-1R^{+} yolk sac-derived macrophages at E8, they demonstrated that at 4 weeks of age the resident populations within these organs contains macrophages derived from this early yolk sac lineage.[23]

While there is a capacity for bone marrow-derived replacement of cells in settings of depletion and injury, it remains of interest whether resident populations are maintained through in situ proliferation. Again, microglia are one resident population where such local proliferation rather than recruitment from the circulation is key.[148,152] To assess the contribution of local proliferation vs. infiltration of circulatory cells in maintaining resident populations, chimerism was induced through conditional Myb deletion and subsequent bone marrow transplantation. Although circulatory-derived donor cells contributed to replenishment in several tissues, resident macrophages in the brain remained of host origin.[23] Indeed, resident in situ proliferation has also been demonstrated in Langerhans cells of this skin.[153-155] Hoeffel et al. also indicated that Langerhans cells are of embryonic origin; initially derived from early colonisation of yolk sac-derived macrophage with precursors subsequently largely replaced by fetal liver-derived precursors.[149]

Differential ontogeny is also suggested to contribute to the alternate functions of macrophages in response to injury. Fetal macrophages possess intriguing wound healing abilities. In embryonic and PU.1-deficient neonatal mice wound healing is enhanced, with a lack of fibrotic scarring that is not associated with an inflammatory response.[156] Evidence is also increasing for a differential immune response by resident macrophage populations. Jenkins et al.[157] demonstrated that resident plural macrophages proliferate in situ in an IL-4 dependent manner and take on a more regulatory or reparative phenotype, in comparison to infiltrating cells which contribute to a more pro-inflammatory response. Similarly in the brain, proliferating resident microglia and infiltrating circulatory monocytes were shown to represent distinct populations with differential function in an experimental autoimmune encephalomyelitis (EAE) model of neuroinflammatory injury.[148] While circulatory monocyte infiltration correlated with EAE disease progression, blockade of these recruited cells reduced disease severity. Furthermore, circulatory invasion was shown to be a transitory event and these cells did not remain and contribute to the resident microglial pool.[148] The unique nature of embryonic macrophages and the suggestion that they may retain their beneficial functions as the adult resident population highlights them as potentially useful target not only in organogenesis but also in tissue repair and regeneration.

In summary, macrophages represent an important developmental cell-type essential for the regulation of normal fetal and organ development. It is clear that trophic macrophages are a versatile and potentially useful target for utilization in settings of developmental impairment, and that it will be through careful understanding of macrophage biology that safe and efficacious therapies may be devised. Furthermore, improved understanding of embryonic macrophages has implications beyond organogenesis, with these trophic macrophages in development informing ontogeny and activation important in tissue repair and regeneration.

Disclosure of Potential Conflicts of Interest

The authors have a published patent related to CSF-1 and development

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