Published in final edited form as:

Curr Top Dev Biol. 2017 ; 123: 229–275. doi:10.1016/bs.ctdb.2016.10.004.

Regulation of Embryonic and Postnatal Development by the CSF-1 Receptor

Violeta Chitu and E. Richard Stanley¹

Albert Einstein College of Medicine, Bronx, NY, United States

Abstract

Macrophages are found in all tissues and regulate tissue morphogenesis during development through trophic and scavenger functions. The colony stimulating factor-1 (CSF-1) receptor (CSF-1R) is the major regulator of tissue macrophage development and maintenance. In combination with receptor activator of nuclear factor κ B (RANK), the CSF-1R also regulates the differentiation of the bone-resorbing osteoclast and controls bone remodeling during embryonic and early postnatal development. CSF-1R-regulated macrophages play trophic and remodeling roles in development. Outside the mononuclear phagocytic system, the CSF-1R directly regulates neuronal survival and differentiation, the development of intestinal Paneth cells and of preimplantation embryos, as well as trophoblast innate immune function. Consistent with the pleiotropic roles of the receptor during development, CSF-1R deficiency in most mouse strains causes embryonic or perinatal death and the surviving mice exhibit multiple developmental and functional deficits. The CSF-1R is activated by two dimeric glycoprotein ligands, CSF-1, and interleukin-34 (IL-34). Homozygous *Csf1*-null mutations phenocopy most of the deficits of *Csf1r*-null mice. In contrast, *Il34*-null mice have no gross phenotype, except for decreased numbers of Langerhans cells and microglia, indicating that CSF-1 plays the major developmental role. Homozygous inactivating mutations of the *Csf1r* or its ligands have not been reported in man. However, heterozygous inactivating mutations in the *Csf1r* lead to a dominantly inherited adult-onset progressive dementia, highlighting the importance of CSF-1R signaling in the brain.

1. INTRODUCTION

Colony stimulating factors (CSFs) promote the proliferation and differentiation of immature bone marrow progenitor cells to form colonies of mature granulocytes and macrophages (Bradley & Metcalf, 1966; Pluznik & Sachs, 1965). The first of these to be purified was the homodimeric glycoprotein colony stimulating factor-1 (CSF-1) (also known as M-CSF), which stimulates the formation of pure macrophage colonies (Stanley, Chen, & Lin, 1978; Stanley & Heard, 1977). Using radio-labeled CSF-1, a cell surface CSF-1 receptor (CSF-1R, also known as Fms, c-Fms, CD115, FIM2, or M-CSF receptor) was identified and shown to be selectively expressed on macrophages and their progenitors (Byrne, Guilbert, & Stanley, 1981; Guilbert & Stanley, 1980, 1986). Sequencing of purified human CSF-1 led to the cDNA cloning of both human and mouse CSF-1 genes (Kawasaki et al., 1985; Ladner et al., 1987, 1988). The receptor was then purified, shown to possess tyrosine kinase activity

¹Corresponding author: richard.stanley@einstein.yu.edu.

(Yeung, Jubinsky, Sengupta, Yeung, & Stanley, 1987) and to be encoded by the *c-fms* protooncogene (Sherr et al., 1985). Subsequent sequencing of the *c-fms* cDNA revealed that it encoded a class III receptor tyrosine kinase (Coussens et al., 1986). Radioimmuno- and radioreceptor assays revealed that CSF-1 was present at biologically active concentrations in the circulation and in most tissues (Das, Stanley, Guilbert, & Forman, 1980; Stanley, 1979). When injected into the circulation, it increased blood monocyte and tissue macrophage levels (Hume, Pavli, Donahue, & Fidler, 1988), and local and circulating concentrations of CSF-1 were shown to increase dramatically in response to challenges increasing the demand for macrophages (e.g., bacterial infection) (Roth, Bartocci, & Stanley, 1997). The identification of an inactivating mutation in the *Csf1* gene (Wiktor-Jedrzejczak et al., 1990; Yoshida et al., 1990) in the *osteopetrotic* (*op*) mouse (Marks & Lane, 1976) demonstrated that CSF-1 also regulated the development of osteoclasts. Subsequently, *Csf1* deficiency was shown to cause a similar osteopetrotic phenotype in the *toothless* (*tl*) rat (Van Wesenbeeck et al., 2002). Osteoclast development was shown to depend on synergism between CSF-1 and the chemokine RANK ligand (Nakagawa et al., 1998), deficiency of which also resulted in osteopetrosis (Kong et al., 1999). In addition, the *Csf1^{op/op}* mouse was shown to exhibit developmental defects resulting in growth (Marks & Lane, 1976; Ryan et al., 2001; Wiktor-Jedrzejczak, Ahmed, Szczyluk, & Skelly, 1982), neurologic (Michaelson et al., 1996), and reproductive (Cohen, Chisholm, Arceci, Stanley, & Pollard, 1996; Cohen, Nishimura, Zhu, & Pollard, 1999; Cohen, Zhu, Nishimura, & Pollard, 2002) phenotypes. Expression of full-length CSF-1 under the control of the *Csf1* promoter rescued the phenotype of *Csf1^{op/op}* mice, confirming that CSF-1 deficiency was the sole cause of these developmental deficits in the *Csf1^{op/op}* mouse (Ryan et al., 2001).

Consistent with the pleiotropic effects of CSF-1 deficiency (Pollard & Stanley, 1996), a *Csf1* promoter lacZ transgene revealed its widespread occurrence in tissues. Several different cell types, including epithelial cells, endothelial cells, osteoblasts, and neurons, were shown to synthesize CSF-1 (Huynh et al., 2009; Nandi et al., 2012; Ryan et al., 2001). Biochemical studies revealed the existence of three distinct biologically active CSF-1 isoforms, a secreted glycoprotein (sgCSF-1) (Price, Choi, Rosenberg, & Stanley, 1992; Stanley & Heard, 1977), a secreted proteoglycan (spCSF-1) (Price et al., 1992; Suzu et al., 1992), and a membrane-spanning cell surface glycoprotein (csCSF-1) (Rettenmier et al., 1987; reviewed in Pixley & Stanley, 2004), each of which have overlapping, yet distinct effects when exclusively expressed in *Csf1^{op/op}* mice (Akcora et al., 2013; Dai, Zong, Sylvestre, & Stanley, 2004; Huynh et al., 2009; Nandi, Akhter, Seifert, Dai, & Stanley, 2006).

Apart from its expression on cells of hematopoietic origin (macrophages, osteoclasts, dendritic cells, and their precursors), the CSF-1R is also expressed on nonhematopoietic cells (reviewed in Chitu, Caescu, et al., 2015; Chitu, Gokhan, Nandi, Mehler, & Stanley, 2016; Chitu & Stanley, 2006), including Paneth cells (PC) (Huynh et al., 2009), epithelial intestinal cells of the colon (Huynh et al., 2013), renal proximal tubule epithelial cells (Menke et al., 2009), neural progenitor cells (NPCs; Nandi et al., 2012), and several subpopulations of neurons (Luo et al., 2013; Nandi et al., 2012; Wang, Berezovska, & Fedoroff, 1999), oocytes, preimplantation embryos, decidua, and trophoblastic cells (Arceci, Pampfer, & Pollard, 1992; Arceci, Shanahan, Stanley, & Pollard, 1989; Pampfer, Arceci, & Pollard, 1991; Regenstreif & Rossant, 1989; Sasmono et al., 2003). In man, two

separate *CSF1R* promoters drive expression in myeloid cells and cells of the trophoblast (Visvader & Verma, 1989).

The more severe phenotype of the *Csf1r*^{-/-} mouse compared with the *Csf1*^{op/op} mouse predicted the existence of a second ligand (Dai et al., 2002). The new ligand, interleukin-34 (IL-34), was discovered by functional screening of the secreted proteins and extracellular domains of the human proteome (Lin et al., 2008). IL-34 binds the CSF-1R with high affinity and competes with CSF-1 for binding (Lin et al., 2008; Wei et al., 2010) to the D2-D3 extracellular domains (Chen, Liu, Focia, Shim, & He, 2008; Elegheert et al., 2011; Felix et al., 2013; Ma et al., 2012). IL-34, but not CSF-1, acts via a second receptor, the chondroitin sulfate-containing proteoglycan, protein tyrosine phosphatase-zeta (Nandi et al., 2013), which interestingly, is not expressed on microglia (Lorenzetto et al., 2014) or macrophages (Nandi, S. & Stanley, E.R., personal observation) but is coexpressed with the CSF-1R on neural (Nandi et al., 2012; von Holst, Sirko, & Faissner, 2006) and hematopoietic (Himburg et al., 2012; Sarrazin et al., 2009) stem cells. IL-34, but not CSF-1, also binds another cell surface chondroitin sulfate-containing proteoglycan, syndecan-1, and this interaction enhances its ability to stimulate CSF-1R-directed macrophage migration (Segaliny, Brion, Mortier, et al., 2015). In addition, it has been suggested that IL-34 and CSF-1 homodimers interact with low affinity to form an active heterotetramer with altered receptor stimulatory activity (Segaliny, Brion, Brulin, et al., 2015). However, the significance of this heterotetramer in vivo is unclear, as the expression patterns of IL-34 and CSF-1 are mostly nonoverlapping. For example, IL-34 is highly expressed in epidermis and forebrain and low in the female reproductive tract, dermis, heart, and hindbrain, where CSF-1 is highly expressed (Chitu et al., 2016; Nandi et al., 2012; Wang et al., 2016; Wei et al., 2010).

Csf1r^{-/-} mice on most strain backgrounds die perinatally (Dai et al., 2002; Dai, Zong, Akhter, & Stanley, 2004) and the surviving mice exhibit multiple developmental deficits (Dai et al., 2002; Dai, Zong, Akhter, et al., 2004; Nandi et al., 2012). Although slightly attenuated, these phenotypes are reproduced in *Csf1*^{op/op} mice (Chitu, Caescu, et al., 2015). In contrast, *Il34*^{-/-} mice are grossly normal (Greter et al., 2012; Wang et al., 2012). Homozygous inactivating mutations of the human *Csf1r*, *Csf1*, or *Il34* genes have not been reported. However, *Csf1r* haploinsufficiency in man leads to an adult-onset progressive dementia, predominantly affecting the cerebral white matter (Konno et al., 2014; Nicholson et al., 2013; Rademakers et al., 2011) highlighting the importance of CSF-1R signaling in the nervous system (Chitu et al., 2016).

This review focuses on the function of the CSF-1R in development. We discuss its major role in the regulation of mononuclear phagocyte development, osteoclastogenesis, and bone formation and its direct regulation of other cell types. The functions of the CSF-1R in the adult have recently been discussed in a comprehensive review (Chitu, Caescu, et al., 2015). The present review complements other focused reviews from this laboratory of CSF-1R signaling in myeloid cells (Pixley & Stanley, 2004; Stanley & Chitu, 2014), CSF-1 in immunity and inflammation (Chitu & Stanley, 2006), and of the CSF-1R and its ligands in brain (Chitu et al., 2016).

2. THE CSF-1R IN MONONUCLEAR PHAGOCYTE DEVELOPMENT

2.1 Role of the CSF-1R in Monocyte and Tissue Macrophage Development

In metazoans, all tissues contain resident mononuclear phagocytes which, in the adult, represent as much as 15% of the total cells (Hume, 2008). During mouse embryonic development, macrophages (MΦ) are generated in three distinct waves (Fig. 1A). A first transient wave occurs between embryonic day (E) 7.5–8.0 and gives rise to primordial, maternally derived, yolk sac (YS) CD45^{high} c-Kit[−] MΦ (Bertrand et al., 2005; Kierdorf et al., 2013). Consistent with their maturity, these cells express the F4/80 marker and are nonproliferative (Bertrand et al., 2005). Between E7.5 and 8.0 (Fig. 1A first, left panel), the YS contains up to 100 maternally derived MΦ, located outside the blood islands, in the vicinity of the neural folds and the caudal part of the embryo (Bertrand et al., 2005) that may provide scavenger or trophic functions. Colonization of the YS by maternal MΦ is a transient process, their numbers declining to negligible levels (2–5 MΦ/YS) between E8.5 and E9 (Bertrand et al., 2005) (Fig. 1A second panel). From E7.0 to E8.0, precursors with erythromyeloid potential (EMP) emerge in the CD45[−] c-Kit⁺ YS cell fraction (Bertrand et al., 2005; Kierdorf et al., 2013; Palis, Robertson, Kennedy, Wall, & Keller, 1999) (Fig. 1A first panel). E8 EMPs represent the earliest MΦ precursor from which two subsets of YS MΦ progenitors, A1 (CD45⁺ c-Kit^{−/lo} F4/80^{lo} CX₃CR1[−]) and A2 (CD45⁺ c-Kit^{hi} F4/80^{hi} CX₃CR1⁺), arise by E9 (Hagemeyer et al., 2016; Kierdorf et al., 2013) (Fig. 1A, second panel; Fig. 1B). Depending on the detection system used, CSF-1R expression was reported to first occur at E8 in EMP (Gomez Perdiguero et al., 2015), or at E9 in A2 progenitors (Kierdorf et al., 2013). After the circulation is established at E8.5, these progenitors colonize the developing tissues giving rise to the second wave of MΦ (Hagemeyer et al., 2016; Kierdorf et al., 2013). EMPs colonizing the fetal liver give rise to fetal erythrocytes, granulocytes, and monocytes (Gomez Perdiguero et al., 2015). A2 progenitors colonize the brain giving rise to microglia (Hagemeyer et al., 2016; Kierdorf et al., 2013), meningeal, and perivascular MΦ (Goldmann et al., 2016), as well as other regions of the embryo proper, where they give rise to liver Kupffer cells and to the vast majority of F4/80^{high} CD11b^{low} tissue MΦ (Ginhoux & Prinz, 2015; Goldmann et al., 2016; Gomez Perdiguero et al., 2015; Hagemeyer et al., 2016; Schulz et al., 2012) (Fig. 1A, second and third panels; Fig. 1B). Fetal liver EMPs and all tissue MΦ are of YS origin up to E14.5. The first hematopoietic stem cells emerge in the aorta-gonad-mesonephros region after E9.0 (Golub & Cumano, 2013) (Fig. 1A, third panel). From E14.5 to E18.5 (Fig. 1A, fourth panel; Fig. 1B), HSCs replace the YS-derived myeloid progenitors in the liver and subsequently the EMP-derived fetal monocytes, granulocytes, and erythrocytes (Gomez Perdiguero et al., 2015). The contribution of the third wave of HSC-derived monocytes to tissue-resident MΦ varies with tissue and age. Monocytes give rise to F4/80^{low} CD11b^{high} tissue macrophages (Gomez Perdiguero et al., 2015; Schulz et al., 2012) and, except for the kidney, contribute only to a minor fraction of F4/80^{high} tissue MΦ, microglia, Kupffer, or Langerhans cells (LC), in the young adult (Gomez Perdiguero et al., 2015; Hagemeyer et al., 2016). In some tissues, the composition of MΦ populations changes over time from predominantly EMP– to predominantly monocyte derived. Intestinal EMP-derived MΦ are replaced by Ly6C⁺⁺ monocyte-derived MΦ within the first 2 weeks of postnatal life (Bain et al., 2014), while choroid plexus and lung alveolar MΦ are progressively replaced with age (Goldmann et al.,

2016; Gomez Perdiguero et al., 2015; Hagemeyer et al., 2016; Schulz et al., 2012). YS-derived M Φ express high levels of CSF-1R mRNA, while HSC-derived M Φ express low levels (Hagemeyer et al., 2016; Schulz et al., 2012). Germline ablation and inhibitor studies show that, consistent with its high expression in YS-derived M Φ , the CSF-1R is critical for the development of LC and microglia, partially required for the development of other tissue M Φ and not required for monocytopoiesis (Cecchini et al., 1994; Chitu et al., 2012; Dai et al., 2002; Ginhoux et al., 2010; Hoeffel et al., 2012; Lenzo et al., 2012; MacDonald et al., 2010; Merad et al., 2002; Schulz et al., 2012).

CSF-1R signaling also regulates the development and migration of fish (Hanington, Hitchen, Beamish, & Belosevic, 2009; Herbomel, Thisse, & Thisse, 2001; Wang et al., 2013), amphibian (Grayfer & Robert, 2013), and avian (Garceau et al., 2010) M Φ and CSF-1R-related receptors regulate plasmacytes, the functional equivalent of M Φ in insects (reviewed in Ratheesh, Belyaeva, & Siekhaus, 2015). However, the finding that CSF-1R-expressing YS-derived-tissue M Φ were not retained in the hatched birds, while a CSF-1R⁺ M Φ -restricted bone marrow progenitor could efficiently generate adult tissue M Φ (Garceau et al., 2015), suggests that the ontogeny of tissue M Φ may differ among vertebrates.

2.2 Regulation of Monocyte Subsets by the CSF-1R

Three subsets of monocytes—classical, intermediate, and nonclassical—with distinct functional features, have been described in mice and humans, using a recent classification approved by the Nomenclature Committee of the International Union of Immunological Societies, in which ⁺ denotes an expression level of ~10-fold, and ⁺⁺ ~100-fold, above the level of the isotype control (Ziegler-Heitbrock et al., 2010). Classical monocytes (CD14⁺⁺CD16⁻ in humans and Ly6C⁺⁺CD43⁺ in mouse) and intermediate monocytes (CD14⁺⁺CD16⁺ in humans and Ly6C⁺⁺CD43⁺⁺ in mouse) express high levels of the chemokine receptor CCR2 and are efficiently recruited to sites of injury, where they differentiate into inflammatory M Φ . Nonclassical monocytes (CD14⁺CD16⁺⁺ in humans and Ly6C⁺CD43⁺⁺ in mouse) express low levels of CCR2 but high levels of CX3CR1. Also known as patrolling monocytes, they scan the resting vasculature to promote the safe disposal of damaged endothelial cells, acting as housekeepers of the vasculature (Auffray et al., 2007; Carlin et al., 2013). Lineage tracing studies in mice have shown that circulating Ly6C⁺⁺ classical monocytes undergo stepwise differentiation into Ly6C⁺ nonclassical monocytes (Yona et al., 2013). Data from transcriptional profiling studies (Ancuta et al., 2009) suggest that this developmental relationship is conserved in humans. Conversely, blockade of CSF-1R signaling does not impair the generation of Ly6C⁺⁺ immature monocytes but leads to the selective depletion of the mature Ly6C⁺ blood monocytes (Lenzo et al., 2012; MacDonald et al., 2010; Yona et al., 2013), suggesting that the CSF-1R promotes the maturation of Ly6C⁺⁺ blood monocytes into Ly6C⁺ monocytes, or the survival of the latter. Clinical trials involving the administration of recombinant CSF-1 (Weiner et al., 1994) suggest a similar role of CSF-1R signaling in the development of the nonclassical monocyte subset in man. Furthermore, patients with adult-onset leukoencephalopathy with spheroids and pigmented glia (ALSP), a neurologic disease associated with mutations in *CSF1R* that reduce cell surface expression and perturb kinase activity (reviewed in Chitu et al., 2016), exhibit a selective reduction of the CD14⁺ CD16⁺⁺ monocytes (Hofer et al.,

2015). In mouse, the equivalent monocyte subset, Ly6C⁺, can infiltrate the brain, differentiate into perivascular MΦ, which maintain the blood–brain barrier (Audoy-Remus et al., 2008) and have neuroprotective functions following brain or spinal cord injury (Bellavance, Gosselin, Yong, Stys, & Rivest, 2015; Shechter et al., 2013). Thus, it would be of interest to establish whether the loss of the Ly6C⁺ subset contributes to the pathogenesis of ALSP in a mouse model (Chitu, Gokhan, et al., 2015).

2.3 Unique Roles of the CSF-1R Ligands, CSF-1 and IL-34, in Mononuclear Phagocyte Development

CSF-1 and IL-34 are differentially expressed both in development and in adults. In the developing mouse embryo, CSF-1, but not IL-34, is expressed in the YS (Johnson & Burgess, 1978; Wang et al., 2016). While CSF-1R signaling is crucial for the development of E12.5 YS primitive MΦ, absence of IL-34 does not affect their production or liver monocytopoiesis at E18.5 (Wang et al., 2016). These data suggest that CSF-1 is the primary regulator of embryonic MΦ development. Consistent with this, studies in *Csf1^{op/op}* mice showed that CSF-1 is a major regulator of the development and maintenance of most tissue MΦ (Cecchini et al., 1994). In contrast, *Il34^{-/-}* mice have no apparent deficits in most tissue MΦ or blood monocytes (Wang et al., 2012). IL-34 primarily regulates the development and maintenance of Langerhans cells, whereas regulation of microglia is shared with CSF-1, in a brain region-specific manner (Chitu et al., 2016; Ginhoux et al., 2010; Greter et al., 2012; Wang et al., 2012). An interesting example of nonredundant sequential regulation by CSF-1 and IL-34 has been documented in LC development (Dai et al., 2002; Wang et al., 2016). CSF-1R is essential for the development and maintenance of LC (Ginhoux et al., 2006). LC are reduced in newborn, but recover in adult *Csf1^{op/op}* mice (Cecchini et al., 1994; Dai et al., 2002), while *Il34^{-/-}* mice have a severe reduction in LC throughout life (Greter et al., 2012; Wang et al., 2012, 2016), suggesting that both CSF-1 and IL-34 contribute to LC development but that IL-34 alone is required for LC maintenance. Wang et al. (2016) showed that in the developing skin IL-34 expression commences at E17.5, is high in the epidermis and low in the dermis, while CSF-1 exhibits the opposite pattern. IL-34 is not necessary for the infiltration and survival of LC precursors in the immature dermis (possibly because of high local levels of CSF-1) but is necessary to promote the proliferation and survival of LC precursors in the mature epidermis. This CSF-1/IL-34 complementarity is conserved in the adult skin where regeneration of LC after UV irradiation is dependent on CSF-1, while their maintenance depends on IL-34 (Wang et al., 2016).

CSF-1 and IL-34 may also have unique roles in innate immunity. Adult *Csf1^{op/op}* mice exhibit only a mild reduction in monocytes and mild immunological deficits (reviewed in Chitu & Stanley, 2006). A recent study (Yamane et al., 2014) reported that follicular dendritic cell-derived IL-34, but not CSF-1, triggered the CSF-1R-dependent differentiation of splenic myeloid progenitors to follicular dendritic cell-induced monocytes, a new type of monocytic cell with B cell-stimulating activity. Other studies show that following immune challenges, IL-34 and CSF-1 are differentially expressed. In *Xenopus laevis* tadpoles infected with Ranavirus Frog virus 3 (RV FV3), IL-34 and CSF-1 have divergent patterns of expression and opposite roles in antiviral immunity, with IL-34 promoting immunity and CSF-1 increasing the susceptibility to RV FV3 infection (Grayfer & Robert, 2014, 2015). In

rainbow trout (*Oncorhynchus mykiss*), IL-34, but not CSF-1, is induced in primary head kidney MΦ by pathogen-associated molecular patterns, inflammatory cytokines, and parasitic kidney infection (Wang et al., 2013). Furthermore, TNF-α induces IL-34, rather than CSF-1 expression in fibroblast-like synovial cells isolated from patients with rheumatoid arthritis (Hwang et al., 2012). Together, these data indicate that IL-34 plays a nonredundant role in the control of MΦ activation during inflammation. Unlike CSF-1, IL-34 can also signal via a second receptor, protein tyrosine phosphatase-zeta (Nandi et al., 2013), which is coexpressed with the CSF-1R in some cell types (Chitu et al., 2016). Furthermore, IL-34 interaction with Syndecan modulates IL-34 activation of the CSF-1R (Segaliny, Brion, Mortier, et al., 2015). These distinguishing properties of IL-34 may explain some of its nonredundant biological activities.

2.4 Functional Significance of CSF-1R-Dependent Mononuclear Phagocytes

MΦ exhibit a remarkable functional plasticity reflected in their ability to adapt to developmental demands and inflammatory challenges (reviewed in Murray & Wynn, 2011; Pollard, 2009). As discussed earlier, the CSF-1R is dispensable for monocytopoiesis from HSC, suggesting that it primarily regulates the generation of YS-derived MΦ. Several studies suggest that MΦ ontogeny may also influence their responses to microenvironmental cues. EMP- and HSC-derived MΦ exhibit unique gene expression signatures. YS-derived MΦ express high levels of F4/80, CSF-1R and CX₃CR1 (Hagemeyer et al., 2016; Schulz et al., 2012), which are associated with monocyte/MΦ maturation, survival, and trophic functions (Caescu et al., 2015; Chitu, Caescu, et al., 2015; Hamilton, Zhao, Pavicic, & Datta, 2014; Nikolic, de Bruijn, Lutz, & Leenen, 2003; White, McNeill, Channon, & Greaves, 2014). In contrast, HSC-derived MΦ express low levels of F4/80, CSF-1R and CX₃CR1 mRNA, and high levels of genes responsible for antigen presentation and CCR2, a chemokine receptor that mediates recruitment to sites of inflammation (Hagemeyer et al., 2016; Schulz et al., 2012). These differences may reflect a higher propensity of YS-derived MΦ for trophic functions and of HSC-derived MΦ for inflammatory functions. Indeed, in models of tissue injury, CSF-1R-dependent resident MΦ were shown to adopt reparative functions (Leblond et al., 2015), while infiltration of CSF-1R-independent Ly6C⁺⁺ monocytes was a transient event with the potential to cause tissue damage (Ajami, Bennett, Krieger, McNagny, & Rossi, 2011; Croxford et al., 2015). Furthermore, CSF-1R signaling studies indicate that CSF-1 signaling promotes a trophic state in macrophages (Caescu et al., 2015; Fleetwood, Lawrence, Hamilton, & Cook, 2007). Induction of miR-21 downstream of CSF-1R phosphotyrosine 721-mediated signaling directly suppresses the expression of over 30 proinflammatory mRNAs, while increasing the expression of several trophic mRNAs (Caescu et al., 2015). The control of this proinflammatory to trophic switch is independent of signaling via phosphotyrosines 544, 559, and 807 that regulate the macrophage proliferative response (Yu et al., 2012), suggesting that distinct pathways regulate tissue macrophage development/maintenance and remodeling/trophic functions.

3. CSF-1-REGULATED MACROPHAGES IN TISSUE MORPHOGENESIS AND ORGANISMAL GROWTH

The dynamic changes in tissue CSF-1 levels and synthesis during development suggest that CSF-1 plays an important role in the late gestational and early postnatal periods (Roth & Stanley, 1995, 1996). In *Csf1^{op/op}* and *Csf1r^{-/-}* mice, organogenesis is relatively normal, except for disturbed skeletal development, due to the absence of osteoclasts (Dai et al., 2002; Dai, Zong, Akhter, et al., 2004; Marks & Lane, 1976) and abnormal brain development (Erblich, Zhu, Etgen, Dobrenis, & Pollard, 2011; Nandi et al., 2012). Despite this, *Csf1r^{-/-}* mutants die perinatally (Chitu et al., 2016; Dai et al., 2002; Dai, Zong, Akhter, et al., 2004). It is not clear which organ failures are responsible for lethality, although loss of direct regulation of NPCs may contribute (see later). *Csf1^{op/op}* mice survive for longer periods than *Csf1r^{-/-}* mice, and the functional consequences of this mutation have been studied in more detail. *Csf1^{op/op}* mice have reduced weight, low growth rate, extensive skeletal abnormalities, hearing, vision and olfactory deficits, abnormal intestinal organization, Paneth cell deficiency, infertility, reduced mammary gland development, altered angiogenesis and lymphangiogenesis and altered neurogenesis (Kondo et al., 2013; Ochsenbein et al., 2016; reviewed in Arnold & Betsholtz, 2013; Chitu, Caescu, et al., 2015; Chitu et al., 2016; Harvey & Gordon, 2012; Pollard, 2009; Pollard & Stanley, 1996). CSF-1 regulates tissue development through its action on both hematopoietic (macrophages and osteoclasts) and nonhematopoietic cells. Macrophages contribute to tissue and organ morphogenesis by releasing growth factors, remodeling the extracellular matrix, eliciting developmental apoptosis, and clearing apoptotic cells (reviewed in Jones & Ricardo, 2013; Pollard, 2009; Stefater, Ren, Lang, & Duffield, 2011). Mice lacking CSF-1 or the CSF-1R exhibit significant depletion of macrophages in some tissues. In other tissues, their numbers are close to normal, but their functions are impaired (Cecchini et al., 1994; Dai et al., 2002; Ginhoux et al., 2010; Roth & Stanley, 1996). Those deficits that are caused by loss of CSF-1R regulation of tissue macrophages are discussed later and summarized in Fig. 2.

3.1 Regulation of Angiogenesis and Lymphangiogenesis

3.1.1 Angiogenesis—The role of CSF-1R in developmental angiogenesis is inferred from the vascular deficits in the retina and the hindbrain of *Csf1^{op/op}* mice (reviewed in Arnold & Betsholtz, 2013). *Csf1^{op/op}* hindbrains have reduced complexity of the subventricular vascular plexus (Fantin et al., 2010), and *Csf1^{op/op}* retina exhibits significantly reduced vessel branching and insufficient arterial–venous patterning (Kubota et al., 2009). The retinal vascular phenotype was also observed after postnatal treatment with anti-CSF-1R antibody or a small molecule inhibitor (Kubota et al., 2009). Depletion and restoration studies have shown that microglia play an important role in developmental angiogenesis (Cecchin, Sennlaub, Levavasseur, Leduc, & Chemtob, 2006). Microglia are dramatically reduced in the hindbrain and retina of *Csf1^{op/op}* mice (Cecchini et al., 1994; Ginhoux et al., 2010) and the reduced retinal vascular branching recovered as development progressed (Kubota et al., 2009), concomitantly with the recovery of microglia (Cecchini et al., 1994). Although other studies suggested that macrophages promote angiogenesis by producing vascular endothelial growth factor (VEGF), neither VEGF levels nor the numbers of endothelial tip cells and filopodia were altered in *Csf1^{op/op}* retina (Kubota et al., 2009).

indicating that CSF-1 contributes to developmental vascular remodeling independently of VEGF (Kubota et al., 2009). Other macrophage-derived candidate mediators are the Wnts, which both positively and negatively regulate angiogenesis (reviewed in Newman & Hughes, 2012). However, direct cell-to-cell contacts may be necessary for directional, vascular morphogenesis in vivo (Fantin et al., 2010; Rymo et al., 2011). Fantin et al. demonstrated a close spatiotemporal relationship between macrophages and sprouting vessels, with macrophages associating at all times with endothelial tip cells and with vessel junctions and suggested that microglia promote vascular anastomosis in the developing hindbrain by physically assisting endothelial tip cell fusion through direct cell-to-cell contacts (Fantin et al., 2010) (Fig. 2). Indeed, other studies show that during retinal angiogenesis in mice, activation of Notch1 signaling in microglia by Delta-like 4 positive tip cells is important for endothelial cell anastomosis (Outtz, Tattersall, Kofler, Steinbach, & Kitajewski, 2011).

3.1.2 Lymphangiogenesis—Defects in lymphangiogenesis have been demonstrated in both *Csf1^{op/op}* and *Csf1r^{-/-}* mice. Gordon et al. found that the lymphatic vasculature was hyperplastic in *Csf1r^{-/-}* embryos (Gordon et al., 2010). In contrast, Kubota et al. showed reduced lymphatic vessel branching in the postnatal trachea and ears of *Csf1^{op/op}* mice and a slower drainage of tissue fluids in their limbs and ears (Kubota et al., 2009). Early postnatal inhibition of CSF-1 signaling in wild-type mice (P8–P15) also decreased macrophage numbers and vessel branching in the ears and trachea (Kubota et al., 2009). Focusing on postnatal lymphatic vessel development on the pleural side of the diaphragmatic muscle in *Csf1r^{-/-}* mice, Ochsenbein et al. found that macrophage ablation in *Csf1r^{-/-}* mice did not inhibit lymphatic vessel development in the diaphragm, but increased branch formation of lymphatic sprouts. Consistent with a role of macrophages in attenuating lymphatic vessel sprouting, reduced sprouting was observed in lymphatic endothelial cells cultured with conditioned medium from P7 diaphragmatic macrophages (Ochsenbein et al., 2016). Thus, while it is clear that CSF-1R-dependent macrophages regulate developmental lymphangiogenesis, they can play opposing roles, depending on the developmental stage and tissue involved.

3.2 Regulation of Neurogenesis, Neuronal Wiring, and Synaptic Pruning by Microglia

During brain development, microglia support neurogenesis, brain wiring and regulate synaptic refinement (recently reviewed in Casano & Peri, 2015; Frost & Schafer, 2016; Schafer & Stevens, 2015). Microglia are maintained through the combined action of the CSF-1R ligands (Ginhoux et al., 2010; Greter et al., 2012; Wang et al., 2012). Characterization of brain development in *Csf1r^{-/-}* mice revealed multiple structural and functional abnormalities (reviewed in Chitu et al., 2016). Studies in *Nestin-cre/+;Csf1r^{fl/fl}* mice with neural lineage-specific deletion of the *Csf1r* showed that some of these abnormalities were caused by loss of CSF-1R regulation of NPCs. Others, including increased brain mass and lateral ventricle size, atrophy of the olfactory bulb, failure of callosal axonal crossing, and decreased subcortical oligodendrocyte differentiation, were not present in the *Nestin-cre/+;Csf1r^{fl/fl}* mice and may be due to the dramatic reduction of microglia in the *Csf1r^{-/-}* mice (Nandi et al., 2012). Indeed, the finding that CSF-1 enhances oligodendrocyte differentiation in cultures of NPCs containing microglia but not in

microglia-free cultures, identifies microglia as mediators of CSF-1R effects on oligodendrogenesis (Fig. 2).

3.3 Mammary Gland Development

CSF-1 mRNA is expressed in the mammary ducts and intensely at the terminal end buds (TEBs) (Ryan et al., 2001) which grow out into the postnatal fat pad as the ducts themselves become branched during puberty. Macrophages, the only CSF-1R-expressing cells in mammary gland, are localized to the invading TEBs, are associated with collagen fibers particularly localized along the necks of the TEBs, and are motile within the collagen matrix (Gouon-Evans, Rothenberg, & Pollard, 2000; Ingman, Wyckoff, Gouon-Evans, Condeelis, & Pollard, 2006) (Fig. 2). In *Csf1^{op/op}* mammary glands, macrophages are severely depleted, gland development is retarded, and the mice have reduced TEB numbers, ductal branching, and elongation, with a rounder, less elongated, TEB shape. *Csf1^{op/op}* mice have normal estrogen levels throughout puberty, but a compromised estrogen ductal outgrowth response (Gouon-Evans et al., 2000). Abnormal mammary gland ductal development also occurs in *Csf1^{op/op}* mice during pregnancy (Pollard & Hennighausen, 1994). Local mammary ductal expression of CSF-1 specifically restores mammary gland macrophages and corrects the branching morphogenesis defect (Van Nguyen & Pollard, 2002). How macrophages mediate their effects on mammary gland development remains to be established (Brady, Chuntova, & Schwertfeger, 2016). However, as macrophages promote collagen fibrillogenesis, rather than collagen I synthesis, it appears that the collagen laid down by fibroblasts is remodeled by the macrophages to maintain TEB structure during ductal outgrowth (Ingman et al., 2006).

3.4 Pancreatic Development

Macrophages of the developing mouse pancreas are dispersed in the mesenchyme and connective tissue around vessels, ducts, islets, and interlobular septa, particularly at sites of islet neogenesis at the duct–islet interface (Charre et al., 2002). Coincident with a wave of β -cell proliferation and islet formation, the number of macrophages and CSF-1R⁺ monocytes near insulin⁺ β -cells increases from E14.5 to E17.5 (Geutskens, Otonkoski, Pulkkinen, Drexhage, & Leenen, 2005). Fetal *Csf1^{op/op}* mice are severely depleted of macrophages and have a major insulin⁺ β -cell mass deficit, due to a combination of reduced β -cell proliferation and β -cell hypotrophy (Banaei-Bouchareb et al., 2004). By P21, insulin⁺ cell mass and proliferation recover, but the hypotrophy persists. Conversely, CSF-1 added to fetal pancreas organ cultures increased the number of macrophages, which was paralleled by an increase in the number of insulin-producing cells (Geutskens et al., 2005). Consistent with a role of the CSF-1R during pancreatic development across species, CSF-1 mRNA expression is detected in ducts, aggregated epithelial cells, and vessel endothelial cells in the human embryonic pancreas (Banaei-Bouchareb, Peuchmaur, Czernichow, & Polak, 2006).

3.5 Colon Development

Basolateral immunohistochemical expression of CSF-1R has been reported by immunohistochemical methods on epithelial cells of both mouse and human colonic crypts (Huynh et al., 2013; Ramsay et al., 2004). Both *Csf1^{op/op}* and *Csf1r^{-/-}* mice were found to have abnormal colon organization, with altered enterocyte and enteroendocrine cell fates, excessive goblet cell staining, reduced cell proliferation and stem cell marker (Lgr5)

expression (Huynh et al., 2013). However, neither CSF-1R ligand elicited significant in vitro proliferative responses from immortalized mouse colonic epithelial cells, indicating that the CSF-1R does not directly regulate colonocyte proliferation in vivo. As studies of *Csf1^{op/op}* mice have revealed that macrophages are required for the amplification of mouse colonic epithelial progenitors in response to dextran sodium sulfate wounding of the epithelium (Pull, Doherty, Mills, Gordon, & Stappenbeck, 2005), the most likely mediators of this colonocyte proliferation in development are macrophages, the other major CSF-1R-expressing cell in colon.

3.6 Potential Contribution of CSF-1R to Heart and Kidney Development

CSF-1 concentrations increase in the kidney perinatally (Roth & Stanley, 1996), associated with the postnatal increase in macrophage numbers in the interstitial regions between developing tubules (Cecchini et al., 1994; Hume & Gordon, 1983; Morris, Graham, & Gordon, 1991; Rae et al., 2007). Renal proximal tubules express CSF-1 mRNA (Jang et al., 2006; Wang et al., 2015), and macrophages are severely depleted in the embryonic and postnatal kidneys of *Csf1^{op/op}* mice (Cecchini et al., 1994; Roth, Dominguez, & Stanley, 1998). CSF-1 addition to embryonic kidney explants increases renal growth and ureteric bud branching (Rae et al., 2007). However, kidney anatomy and function in *Csf1^{op/op}* mice has not been examined in detail to determine potential renal deficits.

Direct evidence for an important role of macrophages in heart development comes from studies in *X. laevis*, where loss of macrophage production leads to a failure of the myocardium to form the myocardial trough that gives rise to the heart tube. Interestingly, the knockdown of *lurp1*, a secreted macrophage Ly6 protein of unknown function, results in a similar phenotype (Smith & Mohun, 2011). CSF-1 protein and mRNA are present in both embryonic and adult mouse heart (Roth & Stanley, 1996; Ryan et al., 2001). YS-derived cardiac tissue macrophages appear in the mouse myocardium at E9.5 (Epelman et al., 2014) and CSF-1R inhibitor studies indicate that the vast majority of heart macrophages are dependent on CSF-1R signaling (Leblond et al., 2015). It remains to be established how the CSF-1R contributes to heart development.

3.7 Contribution of CSF-1 to Maintenance of Lung Tissue Integrity

CSF-1 and CSF-1 mRNA are both present in embryonic and postnatal lung, and the development of lung macrophages is highly dependent on CSF-1 (Roth et al., 1998; Roth & Stanley, 1996). The concentration of macrophages within branch points during embryonic branching morphogenesis and, postnatally, during alveolarization (P14–P21) (Jones et al., 2013) suggests that they could regulate lung development. However, despite the fact that P20 *Csf1^{op/op}* mice have reduced numbers of alveolar macrophages (Shibata, Zsengeller, Otake, Palaniyar, & Trapnell, 2001), no effect on lung morphogenesis has been reported. Rather CSF-1R signaling appears to be necessary for maintenance of tissue integrity. The alveolar macrophage deficiency in *Csf1^{op/op}* mice spontaneously corrects with age but is associated with increased lung IL-3 that promotes matrix metalloproteinase expression by alveolar macrophages and destruction of the lung tissue leading to pulmonary emphysema (Shibata et al., 2001).

3.8 Role of CSF-1-Regulated Macrophages in Growth

The reduced postnatal growth rate of *Csf1^{op/op}* and *Csf1^{Ir/-}* mice (Dai et al., 2002; Marks & Lane, 1976; Ryan et al., 2001), together with in vitro studies of the effects of CSF-1 on embryonic tissue explants (Geutskens et al., 2005; Michaelson et al., 1996; Rae et al., 2007) and in vivo (Alikhan et al., 2011), clearly indicate that CSF-1 regulates tissue growth and development. Furthermore, the correlation between CSF-1 levels and tissue macrophage densities in vivo and in explant cultures (Alikhan et al., 2011; Cecchini et al., 1994; Roth et al., 1998) suggests that the trophic effects of CSF-1 are mediated, at least in part, by macrophages. The reduced growth rate of *Csf1^{op/op}* mice is rescued by the *Csf1* promoter-first intron-driven expression of full-length CSF-1 that gives rise to both secreted isoforms and also, through incomplete proteolytic cleavage, to cell surface CSF-1 (Ryan et al., 2001). Subsequent studies showed that the secreted glycoprotein or proteoglycan isoforms of CSF-1 failed (Nandi et al., 2006), while the cell surface isoform alone was sufficient (Dai, Zong, Sylvestre, et al., 2004) to rescue growth, highlighting the importance of local regulation by CSF-1 in vivo. Several studies suggest that CSF-1 stimulates tissue growth by promoting the production of insulin-like growth factor-1 (IGF-1) by macrophages (Arkins, Rebeiz, Biragyn, Reese, & Kelley, 1993; Arkins, Rebeiz, Brunke-Reese, Minshall, & Kelley, 1995; Gow, Sester, & Hume, 2010). *Csf1^{op/op}* and *Igf1^{-/-}* mice share growth and developmental phenotypes (Liu, Yakar, & LeRoith, 2000), and circulating IGF-1 and CSF-1 levels are positively correlated (Gow et al., 2010). However, macrophage-specific *Igf1* inactivation in lean mice does not affect growth (Chang, Kim, Xu, & Ferrante, 2016), suggesting that regulation of IGF-1 production by macrophages is not a major contributor to organismal growth. Considering the limitation on size placed by the reduced skeleton (Thompson, 1942) and the complete correction of the *Csf1^{op/op}* growth deficit by osteoblast-specific promoter-driven expression of CSF-1 (Abboud, Woodruff, Liu, Shen, & Ghosh-Choudhury, 2002) or by *Csf1^{Ir}* promoter-driven expression of cell surface CSF-1, it is possible that the low growth rate is secondary to defects in bone formation.

4. REGULATION OF OSTEOCLASTS AND BONE DEVELOPMENT BY CSF-1

CSF-1R signaling plays a central role in osteoclast and bone development (Dai et al., 2002; Norgard, Marks, Reinholt, & Andersson, 2003; Pollard & Stanley, 1996; Wiktor-Jedrzejczak et al., 1990; Yoshida et al., 1990). CSF-1 plays a nonredundant function in osteoclastogenesis that cannot be substituted by GM-CSF, FLT3 ligand, or VEGF (Hodge, Kirkland, & Nicholson, 2004). Furthermore, despite the functional redundancy of IL-34 and CSF-1 in osteoclastogenesis in vitro (Wei et al., 2010), *Il-34^{-/-}* mice have no osteopetrotic phenotype (Greter et al., 2012), reflecting the differential expression of CSF-1 and IL-34 in vivo (Wei et al., 2010).

One of the most striking developmental defects in rodent *Csf1^{Ir}* or *Csf1* deficiencies is the presence of bone abnormalities, including delayed tooth eruption, auditory ossicle abnormalities, narrowing of the bone marrow cavity, increased metaphyseal bone density, reduced cortical thickness, and increased porosity (Fig. 3). These defects are associated with impaired bone remodeling due to a severe osteoclast deficiency that secondarily impairs osteoblast function as well as osteocyte survival and function (Abboud et al., 2002;

Aharinejad et al., 1999; Dai et al., 2002; Dai, Zong, Akhter, et al., 2004; Harris et al., 2012; Ryan et al., 2001; Wiktor-Jedrzejczak et al., 1990; Yoshida et al., 1990) (Fig. 3). In postnatal *Csf1^{op/op}* and *Csf1r^{-/-}* mice, osteoclast numbers progressively increase, leading to the resorption of excess trabecular bone and partial restoration of the bone marrow cavity (Begg & Bertoncello, 1993; Dai et al., 2002). The partial restoration in *Csf1^{op/op}* mice can be blocked by suppressing VEGF (Niida et al., 1999, 2005), or FLT3 (Lean, Fuller, & Chambers, 2001), signaling. However, since in contrast to FLT3, VEGF does not induce osteoclastic differentiation, nor RANK mRNA expression in vitro (Lean et al., 2001), it is likely that the actions of VEGF are indirect. Long-term CSF-1R inhibitor treatment in adult wild-type mice increases bone density (Chitu et al., 2012), suggesting that these compensatory mechanisms are limited to a narrow developmental time window.

Administration of recombinant CSF-1 partially rescued the bone phenotype of *Csf1^{op/op}* mice (Cecchini et al., 1994; Pollard & Stanley, 1996; Sundquist, Cecchini, & Marks, 1995), whereas *Csf1* promoter and first intron-driven transgenic expression of full-length CSF-1 (Ryan et al., 2001), or osteoblast-specific promoter-driven expression of CSF-1 (Abboud et al., 2002) completely rescued, emphasizing the importance of local regulation by the CSF-1R. In addition, an important role of the chondroitin sulfate chain of proteoglycan CSF-1 is indicated by the complete rescue of osteoclast development and skeletal abnormalities of *Csf1^{op/op}* mice by *Csf1* promoter and first intron-driven transgenic expression of the secreted proteoglycan isoform (Nandi et al., 2006), but not by the secreted glycoprotein or cell surface CSF-1 isoforms (Dai, Zong, Sylvestre, et al., 2004; Nandi et al., 2006).

4.1 CSF-1R Signaling in Osteoclastogenesis

In combination with RANKL, CSF-1 drives OC differentiation (Arai et al., 1999; Nakagawa et al., 1998), sustains the survival of mature OC (Bouyer et al., 2007; Fuller et al., 1993), and stimulates their spreading (Palacio & Felix, 2001; Sakai et al., 2006) and migration (Fuller et al., 1993). In vitro, the effects of CSF-1 during OC differentiation are biphasic, with low concentrations (in the normal physiological range) enhancing OC formation and high concentrations promoting macrophage differentiation (Hodge, Kirkland, & Nicholson, 2007; Perkins & Kling, 1995). Blockade of CSF-1 during the proliferative phase of OC formation dramatically inhibits osteoclastogenesis but has very little effect after the onset of OC fusion (Biskobing, Fan, & Rubin, 1995; Hodge et al., 2007), suggesting that the major role of CSF-1 is to drive the expansion of OC precursors and their maturation to a fusion-competent state but that CSF-1R signaling is not required for the process of fusion (Fig. 4). The cross talk between CSF-1R and RANK signaling pathways during OC commitment, differentiation, survival and function is illustrated in Fig. 5 and discussed below.

4.1.1 OC Precursor Proliferation—Although the precise identity of OC precursors has not been established, it is known that following exposure to CSF-1 and RANKL, both pluripotent and committed myeloid progenitors as well as circulating monocytes can activate the expression of OC-specific genes and fuse into multinucleated OC-like cells. The efficiency of OC formation is proportional to the proliferative potential and the degree of pluripotency of the progenitors (Arai et al., 1999; Hodge, Kirkland, Aitken, et al., 2004).

Studies in bone marrow cell cultures suggest that CSF-1 regulates OC precursor proliferation through several mechanisms including activation of Cdc42/PI3K/Akt (Ito et al., 2010), Erk1 (He et al., 2011; Kim, Zou, et al., 2010), and DNAX-activating protein of molecular mass 12 kDa (DAP12) pathways (Despars et al., 2013; Humphrey et al., 2004) (Fig. 5A).

Cdc42 is required for CSF-1 activation of the Akt/GSK3 β pathway, but not of ERKs. Cdc42 promotes CSF-1-driven proliferation in OC precursors (Ito et al., 2010). In vivo, OC-specific ablation of Cdc42 using cathepsin K-Cre leads to increased bone density and protection from ovariectomy-induced bone loss, while a Cdc42 gain-of-function mouse model exhibits a reciprocal phenotype (Ito et al., 2010). *Ship*^{-/-} mice have severe osteoporosis (Takeshita et al., 2002), and their bone marrow-derived macrophages exhibit increased osteoclastogenic responses due to increased CSF-1-driven proliferation of OC precursors that is associated with increased PI3K/Akt activation, but unaltered MAPK pathway activation (Zhou et al., 2006). These data suggest that the PI3K/Akt pathway plays a central role in OC precursor proliferation.

Genetic ablation of Erk1, but not Erk2, reduced bone marrow bipotent OC/macrophage progenitor cell numbers, tartrate-resistant acid phosphatase (TRAP) expression, and multinucleation and diminished bone resorption leading to an increase in bone density (He et al., 2011). SLAP is an adaptor protein that associates with the CSF-1R in lipid rafts and inhibits CSF-1-induced Erk1/2 activation and proliferation without affecting RANKL signaling. In vitro, *Slap*^{-/-} mouse bone marrow cultures exhibit increased osteoclastogenic responses but also reduced survival of mature OC following the withdrawal of CSF-1 and RANKL, features that may explain why *Slap*^{-/-} mice maintain normal OC numbers (Kim, Zou, et al., 2010).

DAP12 is a transmembrane adaptor that mediates the proliferative signals of the CSF-1R (Otero et al., 2009). DAP12-deficient mice exhibit mild osteopetrosis (Humphrey et al., 2004), while mice overexpressing DAP12 have osteopenia and increased numbers of OC (Despars et al., 2013). This is associated with greater abundance of OC precursors in the spleen. DAP12-overexpressing splenocytes hyperproliferate in the presence of CSF-1 and RANKL but do not exhibit hyperresponsiveness to increasing concentrations of RANKL (Despars et al., 2013), indicating that RANK signaling in osteoclast precursors is not regulated by DAP12.

Fyn^{-/-} mice exhibit normal basal osteoclastogenesis and bone density. However, their responses to RANKL are attenuated, indicating that although not essential in vivo, Fyn plays a regulatory role. In vitro, *Fyn* deficiency reduced CSF-1-stimulated proliferation of OC precursors, delayed their differentiation, diminished osteoclastogenesis, and increased the susceptibility of mature OC to apoptosis, without affecting the activation of Erk1/2 or Akt (Kim, Warren, et al., 2010).

4.1.2 OC Precursor Commitment—Signals emerging from the RANK–RANKL complex are crucial for the differentiation of bipotent macrophage/OC precursors to OC (Arai et al., 1999). Examination of early stages of osteoclastogenesis revealed that the

CSF-1R induces RANK expression in multipotent myeloid precursors (Arai et al., 1999) in a c-Fos-dependent manner (Arai et al., 2012) (Fig. 5B). This process is inhibited by activated Toll-like receptors, which promote matrix metalloproteinase-mediated CSF-1R shedding, thus diverting OC precursors to a monocytic fate (Ji et al., 2009). The combined actions of CSF-1 and RANKL induce high C/EBP α expression in murine bone marrow cells (Chen et al., 2013). Ectopic expression of C/EBP α induced the expression of other transcription factors essential for OC development such as c-Fos and NFATc1, and reprogrammed monocytic cells to OC-like cells in the absence of RANKL, suggesting that C/EBP α is the key transcriptional regulator of OC lineage commitment (Chen et al., 2013).

Although the precise point of osteoclastic commitment has not been defined, the upregulation of bone-resorbing enzymes, including TRAP and Cathepsin K, is likely to occur early after commitment. Inhibition of JNK at the TRAP⁺ mononuclear cell (preosteoclast, pOC) stage leads to the downregulation of CaMK and NFATc1 levels resulting in the conversion of TRAP⁺ cells to TRAP⁻ cells and concomitant upregulation of macrophage markers, phagocytic activity, and dendritic cell potential (Chang et al., 2008). Thus, JNK signaling plays a critical role in maintaining OC commitment (Fig. 5C).

4.1.3 Upregulation of OC-Specific Genes—CSF-1R-activated, Erk1/2-mediated phosphorylation of microphthalmia-associated transcription factor (Mitf) at Ser73 and RANKL-activated, p38-dependent phosphorylation of Mitf at Ser307 stimulate the upregulation of TRAP activity (Ito et al., 2010; Mansky, Sankar, Han, & Ostrowski, 2002; Weilbaecher et al., 2001) (Fig. 5C). Two adaptor proteins, PSTPIP2 and SH3BP1, have been reported to suppress the activation of Erk1/2 downstream of CSF-1R (Chitu et al., 2009; Ueki et al., 2007) and the expression of TRAP (Chitu et al., 2012; Kawaida et al., 2003; Ueki et al., 2007). While the mechanism through which SH3BP1 acts is unclear, PSTPIP2-mediated suppression of TRAP expression requires both its association with PEST-family phosphatases and its CSF-1R-induced tyrosine phosphorylation (Chitu et al., 2012). Another event critical for induction of TRAP expression is the Tbx3-dependent induction of Jun dimerization protein 2 (JDP2) expression by the CSF-1/Erk1/2 pathway (Yao et al., 2014). JDP2 mediates RANKL-induced upregulation of TRAP and Cathepsin K (Kawaida et al., 2003). It has been reported that the Src family kinase, Lyn, forms a complex with RANK, the tyrosine phosphatase, SHP-1, and the adaptor protein, Grb2-associated binder 2 (Gab2) that impedes Gab2 phosphorylation and downstream activation of JNK and NF-kappaB and subsequent OC development (Kim et al., 2009). While *Lyn*^{-/-} mice have no overt bone phenotype, they exhibit increased osteoclastogenesis in response to RANKL administration (Kim et al., 2009), indicating that Lyn could mediate bone loss in conditions associated with increased RANKL levels such as postmenopausal bone loss (Mundy, 2007).

4.1.4 Pre-OC Aggregation and Fusion—Dendritic cell-specific transmembrane protein (DC-STAMP)-deficient mice cannot develop multinucleated OCs (Yagi et al., 2005), suggesting that DC-STAMP is a crucial factor for OC fusion. Signals emerging from both CSF-1R and RANK converge to upregulate the expression of DC-STAMP (Fig. 5D). CSF-1R-induced and Erk1/2-mediated phosphorylation of Mitf at serine residue 73 stimulates Mitf transcriptional activity and OC fusion (Weilbaecher et al., 2001). RANKL

provides a permissive signal by upregulating the expression of the DEXD/H helicase family corepressor strawberry notch homologue 2 (Sbno2) (Maruyama et al., 2013). Sbno2 binds to T cell acute lymphocytic leukemia 1 (Tal1), attenuating Tal1 inhibition of DC-STAMP expression and permitting the activation of the DC-STAMP promoter by Mitf (Maruyama et al., 2013). Independently of the Mitf pathway, RANK promotes the transcription of the fusogenic receptors DC-STAMP and OC-STAMP by upregulating the expression of c-Fos and NFATc1 (Dou et al., 2014; Miyamoto et al., 2012). In addition, regulation of the actin cytoskeleton by CSF-1R and RANK also plays an important role in promoting pOC fusion. Downstream of CSF-1R, c-Src activates Rac and inhibits Rho, two small GTPases that regulate the cytoskeleton. While Rac activity is dispensable for osteoclast development and multinucleation (Croke et al., 2011), Src-mediated inhibition of Rho activity is necessary for efficient fusion (Takito et al., 2015). Activated RANK upregulates the expression of the Rho family GTP-ase Wrch (a.k.a. RhoU) (Brazier et al., 2006) which inhibits CSF-1-induced pOC migration and integrin-mediated adhesion while simultaneously promoting their aggregation and fusion (Brazier, Pawlak, Vives, & Blangy, 2009).

4.2 CSF-1R Regulation of Mature OC Migration and Bone Resorption

During bone resorption, the OCs alternate between phases of matrix degradation and nonresorptive stages of migration. Nonresorbing OCs move on the bone surface while bone-resorbing OCs are nonmotile and form an adhesion structure known as the sealing zone that separates the ruffled border from the rest of the cell membrane (Lakkakorpi & Vaananen, 1996). The sealing zone contains F-actin-rich podosomes and the $\alpha_v\beta_3$ integrin, a receptor for vitronectin and osteopontin (OPN) (Faccio et al., 2002; Insogna, Sahni, et al., 1997; Nakamura et al., 1999). Inhibition of integrin-mediated adhesion leads to the disruption of both the sealing zone and bone resorption (Nakamura et al., 1999). Paradoxically, stimulation of either human or rodent mature OCs by CSF-1 induces a rapid spreading and chemotactic response (Faccio et al., 2002; Faccio, Novack, Zallone, Ross, & Teitelbaum, 2003; Pilkington, Sims, & Dixon, 1998), while also increasing bone resorption (Faccio et al., 2002; Hodge, Collier, Pavlos, Kirkland, & Nicholson, 2011). A possible explanation resides in fact that CSF-1R collaborates with $\alpha_v\beta_3$ integrin to promote the reorganization of the OC cytoskeleton (Faccio et al., 2003). CSF-1 treatment increases OC chemotaxis to OPN coated-surfaces (Faccio et al., 2002). Like CSF-1, OPN increases both OC migration and bone resorption. It has been suggested that, via interaction with the $\alpha_v\beta_3$ integrin on the OC membrane, the OPN bound to the bone matrix mediates the anchorage of OCs to the bone, thus favoring resorption, while subsequent dephosphorylation of OPN by OC-secreted TRAP inhibits adhesion and initiates migration (Ek-Rylander & Andersson, 2010; Reinholt, Hultenby, Oldberg, & Heinegard, 1990).

In vitro, OCs plated on glass form a peripheral podosome belt which is analogous to the sealing zone. Acute stimulation with CSF-1 induces a spreading response accompanied by the dissolution of the podosome belt and the reorganization of F-actin and $\alpha_v\beta_3$ integrin in peripheral ruffles and perinuclear punctae (Faccio et al., 2007; Insogna, Tanaka, et al., 1997; Shinohara et al., 2012). While not sufficient for the transduction of CSF-1R-mediated cytoskeletal changes (Faccio et al., 2007), activation of Src by its interaction with the phosphorylated tyrosine residue 559 (Y559) of CSF-1R plays a central role in this process

(Fig. 5E). The Y559F CSF1R mutation blocks CSF-1-induced cytoskeletal reorganization (Faccio et al., 2007), and several lines of evidence suggest that c-Src is the Src family kinase (SFK) regulating the pathway mediating CSF-1R-induced actin remodelling in OC: (1) *Src*^{-/-} OC fail to form a sealing zone and their F-actin reorganization in response to CSF-1 is abolished (Insogna, Tanaka, et al., 1997); (2) Fyn or Lyn deficiencies do not affect actin organization in OC (Kim, Warren, et al., 2010; Kim et al., 2009), and (3) Hck controls podosome formation in pOC before Src expression is upregulated, but is not essential for podosome formation or bone resorption by mature OC in which Src is highly expressed (Vérollet et al., 2013). A proposed pathway through which CSF-1R Y559-dependent activation of Src regulates OC cytoskeletal organization involves Src phosphorylation of the immunoreceptor tyrosine-based activation motifs in DAP12, which triggers the recruitment and activation the nonreceptor tyrosine kinase, Syk, via Src homology region 2 (SH2) domain-mediated interactions (Zou, Reeve, Liu, Teitelbaum, & Ross, 2008) (Fig. 5E). Activated Syk phosphorylates the SH2 domain-containing leukocyte protein-76 (SLP-76) adaptor which in turn recruits and mediates the tyrosine phosphorylation of Vav3 (Faccio et al., 2005; Vérollet et al., 2013). Vav3 activates Rac in OCs downstream of both the CSF-1R and $\alpha_v\beta_3$ integrin (Faccio et al., 2005; Reeve et al., 2009). Rac1 and 2 mediate CSF-1-induced chemotactic migration in pre-OCs and mature OCs (Faccio et al., 2007; Itokawa et al., 2011; Wang et al., 2008). Deletion of DAP12, Syk, SLP-76, Vav3, or Rac1 and 2 yields OCs that fail to organize their cytoskeleton and exhibit defects in bone resorption (Croke et al., 2011; Faccio et al., 2005; Mócsai et al., 2004; Reeve et al., 2009; Zou et al., 2008; Zou, Reeve, Zhao, Ross, & Teitelbaum, 2009). In addition, the CSF-1R also initiates a negative feedback pathway leading to phosphorylation of Syk at Tyr-317, which promotes Syk association with Cbl, the ubiquitination and degradation of Syk, and attenuation of OC function (Zou et al., 2009). The Y317F mutation abolishes the association of Syk with Cbl, resulting in CSF-1-induced hyperphosphorylation of the cytoskeleton-organizing molecules, SLP-76, Vav3, and PLC γ 2, and increased resorptive capacity of the OCs (Zou et al., 2009).

Recently, the PI3K/Akt pathway has been described to regulate bone resorption downstream of CSF-1R, but not the cytoskeletal remodeling. Disruption of the CSF-1R-p85 binding site (CSF-1R Y721F) or combined targeting of the p85 α and β genes in OCs did not suppress cytoskeletal remodeling or actin ring formation (Faccio et al., 2007). However, p85 deficiency leads to an osteopetrotic phenotype caused by the absence of a ruffled border and defective transport of Cathepsin K-containing vesicles at the plasma membrane (Shinohara et al., 2012).

4.3 CSF-1R Signaling for OC Survival

CSF-1R signaling is necessary for the survival of OCs and their precursors. In OC precursors, CSF-1 exerts antiapoptotic effects through the upregulation of the antiapoptotic protein Bcl-X(L) which in turn inhibits caspase-9-mediated apoptosis (Woo, Kim, & Ko, 2002). In mature OC, CSF-1R-activated pathways that promote survival include activation of mTOR/S6 kinase, the Cbl ubiquitin ligase and the electroneutral Na/HCO₃ cotransporter, NBCn1 (Fig. 5F). Activation of mTOR/S6 kinase is regulated by multiple CSF-1R effectors, including PI3K/AKT, Erk, and geranylgeranylated proteins, such as Cdc42 (Glantschnig, Fisher, Wesolowski, Rodan, & Reszka, 2003; Kim, Zou, et al., 2010; Miyazaki et al., 2000).

Activated Cbl ubiquitinates the proapoptotic Bcl-2 family member, Bim, leading to its proteasomal degradation (Akiyama et al., 2003). CSF-1 stimulation activates NBCn1, leading to alkalinization of intracellular pH and inhibition of caspase-8-mediated apoptosis (Bouyer et al., 2007). In addition to its role in OC differentiation, Mitf is required for the expression of the antiapoptotic protein Bcl-2 in the OC lineage. Since both Mitf- and Bcl2-deficient mice are osteopetrotic (McGill et al., 2002), it is possible that Mitf activation by CSF-1 and RANKL also contributes to OC survival.

5. DIRECT REGULATION OF NONHEMATOPOIETIC CELLS BY THE CSF-1R

The CSF-1R is expressed on several nonmononuclear phagocytic cell types, including neuronal stem cells, PC, oocytes, early embryonic cells, and trophoblastic cells that are directly regulated by its ligands (Fig. 6).

5.1 Neuronal Stem Cells

Apart from microglia, the CSF-1R is expressed on NPC and several subpopulations of neurons in the brain (reviewed in Chitu et al., 2016). CSF-1, and CSF-1 and IL-34 were shown to directly regulate NPC self-renewal, differentiation, and survival in vitro (Nandi et al., 2012). Targeted deletion of the *Csf1r* in neural progenitors of *Nestin-Cre/+;Csf1r^{fl/fl}* mice did not affect the development of tissue macrophages, osteoclasts, or microglia, yet recapitulated several neurological defects of the *Csf1r^{-/-}* mouse including a smaller brain size, expansion of forebrain neural progenitors and elevated forebrain apoptosis as well as the perinatal lethality. These findings raise the possibility that neurological deficits significantly contribute to the early lethality of *Csf1r^{-/-}* mice.

5.2 Paneth Cells

PC are granulated cells located at the base of small intestinal crypts that play a critical role in the innate immune responses by releasing antimicrobial substances in response to microbial challenge. They have additional roles as regulators of detoxification, digestion, and in the regulation of stem cells as components of the stem cell niche (Clevers & Bevins, 2013; Porter, Bevins, Ghosh, & Ganz, 2002; Wehkamp & Stange, 2006). In both *Csf1^{op/op}* and *Csf1r^{-/-}* mice, there is abnormal organization of the small intestine, with marked reductions of epithelial cells, including PC and intestinal stem cells, and of interstitial macrophages of the villi (Akcora et al., 2013; Cecchini et al., 1994; Huynh et al., 2009). PCs express the CSF-1R and reside in close proximity to CSF-1-expressing epithelial cells within the crypt (Huynh et al., 2009). *Csf1* promoter-driven transgenic expression of the cell surface isoform of CSF-1 in *Csf1^{op/op}* mice rescues the macrophage and PC deficiencies, as well as stem cell activity suggesting that CSF-1 regulates intestinal development, in a juxtacrine or paracrine manner (Huynh et al., 2009). In addition, intestine-specific deletion of *Csf1r* reproduced the *Csf1r^{-/-}* phenotype, formally proving that CSF-1R cell autonomously supports PC development (Akcora et al., 2013). These studies also showed that the CSF-1R is essential for de novo generation of PC, but not for their survival and demonstrated the critical role of CSF-1R regulation of PC in the maintenance of intestinal stem cells.

5.3 Direct Regulation of Oocytes, Preimplantation Embryos, and Trophoblastic Cells

Defects in the hypothalamic–pituitary feedback system of *Csf1^{op/op}* mice lead to abnormal pubertal development and impaired fertility (reviewed in Chitu, Caescu, et al., 2015). Apart from its effects on the hypothalamic–pituitary feedback system, direct regulation by the CSF-1R in nonmononuclear phagocytic cells plays important roles in embryonic development and in the innate immune protection of the fetus at the maternal/fetal interface. CSF-1R is expressed on oocytes, early mouse embryos, and cells of the trophoblast, and CSF-1 is expressed by granulosa cells, oviduct, and highly in the uterine epithelium of pregnant mice. *Csf1^{op/op}* females have extended estrous cycles and poor ovulation rates, but once oocytes are fertilized, their implantation rates approach those of wild-type mice (Araki et al., 1996; Cohen, Zhu, & Pollard, 1997). *Csf1^{op/op}* females have fewer ovarian antral follicles and markedly reduced numbers of ovarian macrophages (Araki et al., 1996). Administration of CSF-1 to rats increased both antral follicle and ovarian macrophage numbers (Nishimura et al., 1995) suggesting that the reduced ovulation could result from loss of trophic effects of the macrophages, or loss of direct regulation of oocytes. Studies with cultured preimplantation mouse embryos showed that CSF-1 accelerates the formation of the blastocyst cavity and increases the number of trophoblast cells (Bhatnagar, Papaioannou, & Biggers, 1995; Pampfer et al., 1991). Direct CSF-1R regulation in trophoblastic cells is essential for the placental immune response to *Listeria monocytogenes*, by signaling the synthesis of chemoattractants that recruit neutrophils to the site of infection (Guleria & Pollard, 2000).

6. CONCLUSIONS

This review has focused on the developmental roles played by the CSF-1R. The CSF-1R is the central regulator of osteoclasts and microglia as reflected in the bone and neurological phenotypes of the CSF-1R-deficient mouse. Tissue-resident macrophages play an important role in organismal development. All tissue macrophages express the CSF-1R but CSF-1R-signaling is not essential for the development and survival of all macrophages. Recent studies indicate that the CSF-1R may be most critical for the development of EMP-derived tissue macrophages. However, additional studies are required to formally prove this. Of the two known CSF-1R ligands, CSF-1 is most critical for organismal development. Apart from effects mediated by its action on myeloid cells, CSF-1 also directly regulates certain nonmononuclear phagocytic cells, including neural stem cells, PC and cells of the female reproductive tract. A snapshot encapsulating both the developmental and homeostatic roles of the CSF-1R across species is summarized in Fig. 7. Initial observations showing that CSF-1 mRNA is highly expressed in cells proximal to tissue macrophages, or other CSF-1R expressing cells (Bartocci, Pollard, & Stanley, 1986; Gouon-Evans et al., 2000; Huynh et al., 2009; Nandi et al., 2012; Pollard et al., 1987; Ryan et al., 2001) suggested the importance of local regulation, which was subsequently confirmed by reconstituting CSF-1 expression either locally or systemically. Although activation of the CSF-1R on myeloid cells by either CSF-1, or IL-34, results in similar biochemical and biological outcomes, their in vivo patterns of expression are mostly distinct, both during development and inflammation, explaining the striking differences in their null phenotypes. The coexpression of the CSF-1R

with the additional IL-34 receptor, PTP ζ , on stem cells raises the possibility that the two CSF-1R ligands may elicit different biological outcomes on these cells.

What are the major unanswered questions? It would be of interest to determine whether the small differences in CSF-1R signaling between myeloid cells responding to CSF-1, IL-34, and IL-34/syndecan contribute to the specification of different tissue macrophage populations, e.g., Langerhans cells and dermal macrophages. Investigation of how the CSF-1R regulates macrophage function has been hampered by the fact that *Csf1r* deficiency affects their viability and proliferation. The recent finding that the pathways regulating macrophage survival/proliferation are distinct from those regulating their trophic/inflammatory phenotypes provides the basis for a new strategy to study the role of the CSF-1R in macrophage function. Further understanding of the signaling pathways critical for CSF-1R-regulated functions in nonmyeloid cells is also needed, especially in view of the development of CSF-1R inhibitors for long-term clinical treatments.

Acknowledgments

We thank Dr. ölen Gökhan for critically reviewing the manuscript and the editor for his helpful comments. This work was supported by NIH Grants R01 NS091519 and P01 CA100324 (to E.R.S.).

References

- Abboud SL, Woodruff K, Liu C, Shen V, Ghosh-Choudhury N. Rescue of the osteopetrotic defect in op/op mice by osteoblast-specific targeting of soluble colony-stimulating factor-1. *Endocrinology*. 2002; 143:1942–1949. [PubMed: 11956177]
- Aharinejad S, Grossschmidt K, Franz P, Streicher J, Nourani F, MacKay CA, et al. Auditory ossicle abnormalities and hearing loss in the toothless (osteopetrotic) mutation in the rat and their improvement after treatment with colony-stimulating factor-1. *Journal of Bone and Mineral Research*. 1999; 14:415–423. [PubMed: 10027906]
- Ajami B, Bennett JL, Krieger C, McNagny KM, Rossi FM. Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. *Nature Neuroscience*. 2011; 14:1142–1149. [PubMed: 21804537]
- Akcora D, Huynh D, Lightowler S, Germann M, Robine S, de May JR, et al. The CSF-1 receptor fashions the intestinal stem cell niche. *Stem Cell Research*. 2013; 10:203–212. [PubMed: 23314290]
- Akiyama T, Bouillet P, Miyazaki T, Kadono Y, Chikuda H, Chung UI, et al. Regulation of osteoclast apoptosis by ubiquitylation of proapoptotic BH3-only Bcl-2 family member Bim. *EMBO Journal*. 2003; 22:6653–6664. [PubMed: 14657036]
- Alikhan MA, Jones CV, Williams TM, Beckhouse AG, Fletcher AL, Kett MM, et al. Colony-stimulating factor-1 promotes kidney growth and repair via alteration of macrophage responses. *The American Journal of Pathology*. 2011; 179:1243–1256. [PubMed: 21762674]
- Ancuta P, Liu KY, Misra V, Wacleche VS, Gosselin A, Zhou X, et al. Transcriptional profiling reveals developmental relationship and distinct biological functions of CD16+ and CD16– monocyte subsets. *BMC Genomics*. 2009; 10:403. [PubMed: 19712453]
- Arai F, Miyamoto T, Ohneda O, Inada T, Sudo T, Brasel K, et al. Commitment and differentiation of osteoclast precursor cells by the sequential expression of c-Fms and receptor activator of nuclear factor kappaB (RANK) receptors. *The Journal of Experimental Medicine*. 1999; 190:1741–1754. [PubMed: 10601350]
- Arai A, Mizoguchi T, Harada S, Kobayashi Y, Nakamichi Y, Yasuda H, et al. Fos plays an essential role in the upregulation of RANK expression in osteoclast precursors within the bone microenvironment. *Journal of Cell Science*. 2012; 125:2910–2917. [PubMed: 22454522]

- Araki M, Fukumatsu Y, Katabuchi H, Shultz LD, Takahashi K, Okamura H. Follicular development and ovulation in macrophage colony-stimulating factor-deficient mice homozygous for the osteopetrosis (*op*) mutation. *Biology of Reproduction*. 1996; 54:478–484. [PubMed: 8788202]
- Arceci RJ, Pampfer S, Pollard JW. Expression of CSF-1/c-fms and SF/c-kit mRNA during preimplantation mouse development. *Developmental Biology*. 1992; 151:1–8. [PubMed: 1374350]
- Arceci RJ, Shanahan F, Stanley ER, Pollard JW. Temporal expression and location of colony-stimulating factor 1 (CSF-1) and its receptor in the female reproductive tract are consistent with CSF-1-regulated placental development. *Proceedings of the National Academy of Sciences of the United States of America*. 1989; 86:8818–8822. [PubMed: 2554332]
- Arkins S, Rebeiz N, Biragyn A, Reese DL, Kelley KW. Murine macrophages express abundant insulin-like growth factor-I class I *Ea* and *Eb* transcripts. *Endocrinology*. 1993; 133:2334–2343. [PubMed: 8404686]
- Arkins S, Rebeiz N, Brunke-Reese DL, Minshall C, Kelley KW. The colony-stimulating factors induce expression of insulin-like growth factor-I messenger ribonucleic acid during hematopoiesis. *Endocrinology*. 1995; 136:1153–1160. [PubMed: 7532579]
- Arnold T, Betsholtz C. Correction: The importance of microglia in the development of the vasculature in the central nervous system. *Vascular Cell*. 2013; 5:12. [PubMed: 23809768]
- Audoy-Remus J, Richard JF, Soulet D, Zhou H, Kubes P, Vallieres L. Rod-Shaped monocytes patrol the brain vasculature and give rise to perivascular macrophages under the influence of proinflammatory cytokines and angiopoietin-2. *The Journal of Neuroscience*. 2008; 28:10187–10199. [PubMed: 18842879]
- Auffray C, Fogg D, Garfa M, Elain G, Join-Lambert O, Kayal S, et al. Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. *Science*. 2007; 317:666–670. [PubMed: 17673663]
- Bain CC, Bravo-Blas A, Scott CL, Gomez Perdiguero E, Geissmann F, Henri S, et al. Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. *Nature Immunology*. 2014; 15:929–937. [PubMed: 25151491]
- Banaei-Bouchareb L, Gouon-Evans V, Samara-Boustani D, Castellotti MC, Czernichow P, Pollard JW, et al. Insulin cell mass is altered in *Csf1op/Csf1op* macrophage-deficient mice. *Journal of Leukocyte Biology*. 2004; 76:359–367. [PubMed: 15178709]
- Banaei-Bouchareb L, Peuchmaur M, Czernichow P, Polak M. A transient microenvironment loaded mainly with macrophages in the early developing human pancreas. *The Journal of Endocrinology*. 2006; 188:467–480. [PubMed: 16522727]
- Bartocci A, Pollard JW, Stanley ER. Regulation of colony-stimulating factor 1 during pregnancy. *Journal of Experimental Medicine*. 1986; 164:956–961. [PubMed: 3489064]
- Begg SK, Bertoncello I. The hematopoietic deficiencies in osteopetrotic (*op/op*) mice are not permanent, but progressively correct with age. *Experimental Hematology*. 1993; 21:493–495. [PubMed: 8462657]
- Bellavance MA, Gosselin D, Yong VW, Stys PK, Rivest S. Patrolling monocytes play a critical role in CX3CR1-mediated neuroprotection during excitotoxicity. *Brain Structure & Function*. 2015; 220:1759–1776. [PubMed: 24706067]
- Bertrand JY, Jalil A, Klaine M, Jung S, Cumano A, Godin I. Three pathways to mature macrophages in the early mouse yolk sac. *Blood*. 2005; 106:3004–3011. [PubMed: 16020514]
- Bhatnagar P, Papaioannou VE, Biggers JD. CSF-1 and mouse preimplantation development in vitro. *Development*. 1995; 121:1333–1339. [PubMed: 7789264]
- Biskobing DM, Fan X, Rubin J. Characterization of MCSF-induced proliferation and subsequent osteoclast formation in murine marrow culture. *Journal of Bone and Mineral Research*. 1995; 10:1025–1032. [PubMed: 7484277]
- Bouyer P, Sakai H, Itokawa T, Kawano T, Fulton CM, Boron WF, et al. Colony-stimulating factor-1 increases osteoclast intracellular pH and promotes survival via the electroneutral Na/HCO₃ cotransporter NBCn1. *Endocrinology*. 2007; 148:831–840. [PubMed: 17068143]
- Bradley TR, Metcalf D. The growth of mouse bone marrow cells in vitro. *Australian Journal of Experimental Biology and Medical Science*. 1966; 44:287–299. [PubMed: 4164182]

- Brady NJ, Chuntova P, Schwertfeger KL. Macrophages: Regulators of the inflammatory microenvironment during mammary gland development and breast cancer. *Mediators of Inflammation*. 2016; 2016:4549676. [PubMed: 26884646]
- Brazier H, Pawlak G, Vives V, Blangy A. The Rho GTPase *Wrch1* regulates osteoclast precursor adhesion and migration. *The International Journal of Biochemistry & Cell Biology*. 2009; 41:1391–1401. [PubMed: 19135548]
- Brazier H, Stephens S, Ory S, Fort P, Morrison N, Blangy A. Expression profile of RhoGTPases and RhoGEFs during RANKL-stimulated osteoclastogenesis: Identification of essential genes in osteoclasts. *Journal of Bone and Mineral Research*. 2006; 21:1387–1398. [PubMed: 16939397]
- Byrne PV, Guilbert LJ, Stanley ER. Distribution of cells bearing receptors for a colony-stimulating factor (CSF) in murine tissues. *The Journal of Cell Biology*. 1981; 91:848–853. [PubMed: 6276411]
- Caescu CI, Guo X, Tesfa L, Bhagat TD, Verma A, Zheng D, et al. Colony stimulating factor-1 receptor signaling networks inhibit mouse macrophage inflammatory responses by induction of microRNA-21. *Blood*. 2015; 125:e1–e13. [PubMed: 25573988]
- Carlin LM, Stamatiades EG, Auffray C, Hanna RN, Glover L, Vizcay-Barrena G, et al. Nr4a1-dependent Ly6C(low) monocytes monitor endothelial cells and orchestrate their disposal. *Cell*. 2013; 153:362–375. [PubMed: 23582326]
- Casano AM, Peri F. Microglia: Multitasking specialists of the brain. *Developmental Cell*. 2015; 32:469–477. [PubMed: 25710533]
- Cecchini MG, Dominguez MG, Mocci S, Wetterwald A, Felix R, Fleisch H, et al. Role of colony stimulating factor-1 in the establishment and regulation of tissue macrophages during postnatal development of the mouse. *Development*. 1994; 120:1357–1372. [PubMed: 8050349]
- Chang EJ, Ha J, Huang H, Kim HJ, Woo JH, Lee Y, et al. The JNK-dependent CaMK pathway restrains the reversion of committed cells during osteoclast differentiation. *Journal of Cell Science*. 2008; 121:2555–2564. [PubMed: 18650497]
- Chang HR, Kim HJ, Xu X, Ferrante AW Jr. Macrophage and adipocyte IGF1 maintain adipose tissue homeostasis during metabolic stresses. *Obesity (Silver Spring)*. 2016; 24:172–183. [PubMed: 26663512]
- Charre S, Rosmalen JG, Pelegri C, Alves V, Leenen PJ, Drexhage HA, et al. Abnormalities in dendritic cell and macrophage accumulation in the pancreas of nonobese diabetic (NOD) mice during the early neonatal period. *Histology and Histopathology*. 2002; 17:393–401. [PubMed: 11962743]
- Checchin D, Sennlaub F, Levavasseur E, Leduc M, Chemtob S. Potential role of microglia in retinal blood vessel formation. *Investigative Ophthalmology & Visual Science*. 2006; 47:3595–3602. [PubMed: 16877434]
- Chen X, Liu H, Focia PJ, Shim AH, He X. Structure of macrophage colony stimulating factor bound to FMS: Diverse signaling assemblies of class III receptor tyrosine kinases. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105:18267–18272. [PubMed: 19017797]
- Chen W, Zhu G, Hao L, Wu M, Ci H, Li YP. C/EBP α regulates osteoclast lineage commitment. *Proceedings of the National Academy of Sciences of the United States of America*. 2013; 110:7294–7299. [PubMed: 23580622]
- Chitu V, Caescu CI, Stanley ER, Lennartsson J, Ronnstrand L, Heldin CH. PDGF receptor family. In: Wheeler, DL., Yarden, Y., editors. *The receptor tyrosine kinases: Family and subfamilies*. New York: Springer Science; 2015. p. 373–538.
- Chitu V, Ferguson PJ, de Bruijn R, Schlueter AJ, Ochoa LA, Waldschmidt TJ, et al. Primed innate immunity leads to autoinflammatory disease in PSTPIP2-deficient cmo mice. *Blood*. 2009; 114:2497–2505. [PubMed: 19608749]
- Chitu V, Gokhan S, Gulinello M, Branch CA, Patil M, Basu R, et al. Phenotypic characterization of a *Csf1r* haploinsufficient mouse model of adult-onset leuko-dystrophy with axonal spheroids and pigmented glia (ALSP). *Neurobiology of Disease*. 2015; 74:219–228. [PubMed: 25497733]
- Chitu V, Gokhan S, Nandi S, Mehler MF, Stanley ER. Emerging roles for CSF-1 receptor and its ligands in the nervous system. *Trends in Neurosciences*. 2016; 39:378–393. [PubMed: 27083478]

- Chitu V, Nacu V, Charles JF, Henne WM, McMahon HT, Nandi S, et al. PSTPIP2 deficiency in mice causes osteopenia and increased differentiation of multipotent myeloid precursors into osteoclasts. *Blood*. 2012; 120:3126–3135. [PubMed: 22923495]
- Chitu V, Stanley ER. Colony-stimulating factor-1 in immunity and inflammation. *Current Opinion in Immunology*. 2006; 18:39–48. [PubMed: 16337366]
- Clevers HC, Bevins CL. Paneth cells: Maestros of the small intestinal crypts. *Annual Review of Physiology*. 2013; 75:289–311.
- Cohen PE, Chisholm O, Arceci RJ, Stanley ER, Pollard JW. Absence of colony-stimulating factor-1 in osteopetrotic (*csf1m^{OP}/csf1m^{OP}*) mice results in male fertility defects. *Biology of Reproduction*. 1996; 55:310–317. [PubMed: 8828834]
- Cohen PE, Nishimura K, Zhu L, Pollard JW. Macrophages: Important accessory cells for reproductive function. *Journal of Leukocyte Biology*. 1999; 66:765–772. [PubMed: 10577508]
- Cohen PE, Zhu L, Nishimura K, Pollard JW. Colony-stimulating factor 1 regulation of neuroendocrine pathways that control gonadal function in mice. *Endocrinology*. 2002; 143:1413–1422. [PubMed: 11897698]
- Cohen PE, Zhu L, Pollard JW. Absence of colony stimulating factor-1 in osteopetrotic (*csf1mop/csf1mop*) mice disrupts estrous cycles and ovulation. *Biology of Reproduction*. 1997; 56:110–118. [PubMed: 9002639]
- Coussens L, Van Beveren C, Smith D, Chen E, Mitchell RL, Isacke CM, et al. Structural alteration of viral homologue of receptor proto-oncogene *fms* at carboxyl terminus. *Nature*. 1986; 320:277–280. [PubMed: 2421165]
- Croke M, Ross FP, Korhonen M, Williams DA, Zou W, Teitelbaum SL. *Rac* deletion in osteoclasts causes severe osteopetrosis. *Journal of Cell Science*. 2011; 124:3811–3821. [PubMed: 22114304]
- Croxford AL, Lanzinger M, Hartmann FJ, Schreiner B, Mair F, Pelczar P, et al. The cytokine GM-CSF drives the inflammatory signature of CCR2 monocytes and licenses autoimmunity. *Immunity*. 2015; 43:502–514. [PubMed: 26341401]
- Dai XM, Ryan GR, Hapel AJ, Dominguez MG, Russell RG, Kapp S, et al. Targeted disruption of the mouse colony-stimulating factor 1 receptor gene results in osteopetrosis, mononuclear phagocyte deficiency, increased primitive progenitor cell frequencies, and reproductive defects. *Blood*. 2002; 99:111–120. [PubMed: 11756160]
- Dai XM, Zong XH, Akhter MP, Stanley ER. Osteoclast deficiency results in disorganized matrix, reduced mineralization, and abnormal osteoblast behavior in developing bone. *Journal of Bone and Mineral Research*. 2004a; 19:1441–1451. [PubMed: 15312244]
- Dai XM, Zong XH, Sylvestre V, Stanley ER. Incomplete restoration of colony-stimulating factor 1 (CSF-1) function in CSF-1-deficient *Csf1^{lop}/Csf1^{lop}* mice by transgenic expression of cell surface CSF-1. *Blood*. 2004b; 103:1114–1123. [PubMed: 14525772]
- Das SK, Stanley ER, Guilbert LJ, Forman LW. Discrimination of a colony stimulating factor subclass by a specific receptor on a macrophage cell line. *Journal of Cellular Physiology*. 1980; 104:359–366. [PubMed: 6968317]
- Despars G, Pandrurada SN, Anginot A, Domenget C, Jurdic P, Mazzorana M. DAP12 overexpression induces osteopenia and impaired early hematopoiesis. *PloS One*. 2013; 8:e65297. [PubMed: 23776468]
- Dou C, Zhang C, Kang F, Yang X, Jiang H, Bai Y, et al. MiR-7b directly targets DC-STAMP causing suppression of NFATc1 and c-Fos signaling during osteoclast fusion and differentiation. *Biochimica et Biophysica Acta*. 2014; 1839:1084–1096. [PubMed: 25123438]
- Ek-Rylander B, Andersson G. Osteoclast migration on phosphorylated osteopontin is regulated by endogenous tartrate-resistant acid phosphatase. *Experimental Cell Research*. 2010; 316:443–451. [PubMed: 19854170]
- Elegheert J, Desfosses A, Shkumatov AV, Wu X, Bracke N, Verstraete K, et al. Extracellular complexes of the hematopoietic human and mouse CSF-1 receptor are driven by common assembly principles. *Structure*. 2011; 19:1762–1772. [PubMed: 22153499]
- Epelman S, Lavine KJ, Beaudin AE, Sojka DK, Carrero JA, Calderon B, et al. Embryonic and adult-derived resident cardiac macrophages are maintained through distinct mechanisms at steady state and during inflammation. *Immunity*. 2014; 40:91–104. [PubMed: 24439267]

- Erblich B, Zhu L, Etgen AM, Dobrenis K, Pollard JW. Absence of colony stimulation factor-1 receptor results in loss of microglia, disrupted brain development and olfactory deficits. *PLoS One*. 2011;6:e26317.
- Faccio R, Grano M, Colucci S, Villa A, Giannelli G, Quaranta V, et al. Localization and possible role of two different alpha v beta 3 integrin conformations in resting and resorbing osteoclasts. *Journal of Cell Science*. 2002; 115:2919–2929. [PubMed: 12082152]
- Faccio R, Novack DV, Zallone A, Ross FP, Teitelbaum SL. Dynamic changes in the osteoclast cytoskeleton in response to growth factors and cell attachment are controlled by beta3 integrin. *The Journal of Cell Biology*. 2003; 162:499–509. [PubMed: 12900398]
- Faccio R, Takeshita S, Colaianni G, Chappel J, Zallone A, Teitelbaum SL, et al. M-CSF regulates the cytoskeleton via recruitment of a multimeric signaling complex to c-Fms Tyr-559/697/721. *The Journal of Biological Chemistry*. 2007; 282:18991–18999. [PubMed: 17420256]
- Faccio R, Teitelbaum SL, Fujikawa K, Chappel J, Zallone A, Tybulewicz VL, et al. Vav3 regulates osteoclast function and bone mass. *Nature Medicine*. 2005; 11:284–290.
- Fantin A, Vieira JM, Gestri G, Denti L, Schwarz Q, Prykhodzhiy S, et al. Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF-mediated endothelial tip cell induction. *Blood*. 2010; 116:829–840. [PubMed: 20404134]
- Felix J, Elegheert J, Gutsche I, Shkumatov AV, Wen YR, Bracke N, et al. Human IL-34 and CSF-1 establish structurally similar extracellular assemblies with their common hematopoietic receptor. *Structure*. 2013; 21:528–539. [PubMed: 23478061]
- Fleetwood AJ, Lawrence T, Hamilton JA, Cook AD. Granulocyte-macrophage colony-stimulating factor (CSF) and macrophage CSF-dependent macrophage phenotypes display differences in cytokine profiles and transcription factor activities: Implications for CSF blockade in inflammation. *Journal of Immunology*. 2007; 178:5245–5252.
- Frost JL, Schafer DP. Microglia: Architects of the developing nervous system. *Trends in Cell Biology*. 2016; 26:587–597. [PubMed: 27004698]
- Fuller K, Owens JM, Jagger CJ, Wilson A, Moss R, Chambers TJ. Macrophage colony-stimulating factor stimulates survival and chemotactic behavior in isolated osteoclasts. *Journal of Experimental Medicine*. 1993; 178:1733–1744. [PubMed: 8228819]
- Garceau V, Balic A, Garcia-Morales C, Sauter KA, McGrew MJ, Smith J, et al. The development and maintenance of the mononuclear phagocyte system of the chick is controlled by signals from the macrophage colony-stimulating factor receptor. *BMC Biology*. 2015; 13:12. [PubMed: 25857347]
- Garceau V, Smith J, Paton IR, Davey M, Fares MA, Sester DP, et al. Pivotal advance: Avian colony-stimulating factor 1 (CSF-1), interleukin-34 (IL-34), and CSF-1 receptor genes and gene products. *Journal of Leukocyte Biology*. 2010; 87:753–764. [PubMed: 20051473]
- Geutskens SB, Otonkoski T, Pulkkinen MA, Drexhage HA, Leenen PJ. Macrophages in the murine pancreas and their involvement in fetal endocrine development in vitro. *Journal of Leukocyte Biology*. 2005; 78:845–852. [PubMed: 16037409]
- Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science*. 2010; 330:841–845. [PubMed: 20966214]
- Ginhoux F, Prinz M. Origin of microglia: Current concepts and past controversies. *Cold Spring Harbor Perspectives in Biology*. 2015; 7:a020537. [PubMed: 26134003]
- Ginhoux F, Tacke F, Angeli V, Bogunovic M, Loubreau M, Dai XM, et al. Langerhans cells arise from monocytes in vivo. *Nature Immunology*. 2006; 7:265–273. [PubMed: 16444257]
- Glantschnig H, Fisher JE, Wesolowski G, Rodan GA, Reszka AA. M-CSF, TNFalpha and RANK ligand promote osteoclast survival by signaling through mTOR/S6 kinase. *Cell Death and Differentiation*. 2003; 10:1165–1177. [PubMed: 14502240]
- Goldmann T, Wieghofer P, Jordao MJ, Prutek F, Hagemeyer N, Frenzel K, et al. Origin, fate and dynamics of macrophages at central nervous system interfaces. *Nature Immunology*. 2016; 17:797–805. [PubMed: 27135602]
- Golub R, Cumano A. Embryonic hematopoiesis. *Blood Cells, Molecules & Diseases*. 2013; 51:226–231.

- Gomez Perdiguero E, Klapproth K, Schulz C, Busch K, Azzoni E, Crozet L, et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature*. 2015; 518:547–551. [PubMed: 25470051]
- Gordon EJ, Rao S, Pollard JW, Nutt SL, Lang RA, Harvey NL. Macrophages define dermal lymphatic vessel calibre during development by regulating lymphatic endothelial cell proliferation. *Development*. 2010; 137:3899–3910. [PubMed: 20978081]
- Gouon-Evans V, Rothenberg ME, Pollard JW. Postnatal mammary gland development requires macrophages and eosinophils. *Development*. 2000; 127:2269–2282. [PubMed: 10804170]
- Gow DJ, Sester DP, Hume DA. CSF-1, IGF-1, and the control of postnatal growth and development. *Journal of Leukocyte Biology*. 2010; 88:475–481. [PubMed: 20519640]
- Grayfer L, Robert J. Colony-stimulating factor-1-responsive macrophage precursors reside in the Amphibian (*Xenopus laevis*) bone marrow rather than the hematopoietic subcapsular liver. *Journal of Innate Immunity*. 2013; 5:531–542. [PubMed: 23485675]
- Grayfer L, Robert J. Divergent antiviral roles of amphibian (*Xenopus laevis*) macrophages elicited by colony-stimulating factor-1 and interleukin-34. *Journal of Leukocyte Biology*. 2014; 96:1143–1153. [PubMed: 25190077]
- Grayfer L, Robert J. Distinct functional roles of amphibian (*Xenopus laevis*) colony-stimulating factor-1- and interleukin-34-derived macrophages. *Journal of Leukocyte Biology*. 2015; 98:641–649. [PubMed: 26136505]
- Greter M, Lelios I, Pelczar P, Hoeffel G, Price J, Leboeuf M, et al. Stroma-derived interleukin-34 controls the development and maintenance of langerhans cells and the maintenance of microglia. *Immunity*. 2012; 37:1050–1060. [PubMed: 23177320]
- Guilbert LJ, Stanley ER. Specific interaction of murine colony-stimulating factor with mononuclear phagocytic cells. *The Journal of Cell Biology*. 1980; 85:153–159. [PubMed: 6965942]
- Guilbert LJ, Stanley ER. The interaction of ¹²⁵I-colony stimulating factor-1 with bone marrow-derived macrophages. *The Journal of Biological Chemistry*. 1986; 261:4024–4032. [PubMed: 3485098]
- Guleria I, Pollard JW. The trophoblast is a component of the innate immune system during pregnancy. *Nature Medicine*. 2000; 6:589–593.
- Hagemeyer N, Kierdorf K, Frenzel K, Xue J, Ringelhan M, Abdullah Z, et al. Transcriptome-based profiling of yolk sac-derived macrophages reveals a role for Irf8 in macrophage maturation. *The EMBO Journal*. 2016; 35:1730–1744. [PubMed: 27412700]
- Hamilton TA, Zhao C, Pavicic PG Jr, Datta S. Myeloid colony-stimulating factors as regulators of macrophage polarization. *Frontiers in Immunology*. 2014; 5:554. [PubMed: 25484881]
- Hanington PC, Hitchen SJ, Beamish LA, Belosevic M. Macrophage colony stimulating factor (CSF-1) is a central growth factor of goldfish macrophages. *Fish & Shellfish Immunology*. 2009; 26:1–9. [PubMed: 18984058]
- Harris SE, MacDougall M, Horn D, Woodruff K, Zimmer SN, Rebel VI, et al. Meox2Cre-mediated disruption of CSF-1 leads to osteopetrosis and osteocyte defects. *Bone*. 2012; 50:42–53. [PubMed: 21958845]
- Harvey NL, Gordon EJ. Deciphering the roles of macrophages in developmental and inflammation stimulated lymphangiogenesis. *Vascular Cell*. 2012; 4:15. [PubMed: 22943568]
- He Y, Staser K, Rhodes SD, Liu Y, Wu X, Park SJ, et al. Erk1 positively regulates osteoclast differentiation and bone resorptive activity. *PLoS One*. 2011; 6:e24780.
- Herbomel P, Thisse B, Thisse C. Zebrafish early macrophages colonize cephalic mesenchyme and developing brain, retina, and epidermis through a M-CSF receptor-dependent invasive process. *Developmental Biology*. 2001; 238:274–288. [PubMed: 11784010]
- Himburg HA, Harris JR, Ito T, Daher P, Russell JL, Quarmyne M, et al. Pleiotrophin regulates the retention and self-renewal of hematopoietic stem cells in the bone marrow vascular niche. *Cell Reports*. 2012; 2:964–975. [PubMed: 23084748]
- Hodge JM, Collier FM, Pavlos NJ, Kirkland MA, Nicholson GC. M-CSF potently augments RANKL-induced resorption activation in mature human osteoclasts. *PLoS One*. 2011; 6:e21462.
- Hodge JM, Kirkland MA, Aitken CJ, Waugh CM, Myers DE, Lopez CM, et al. Osteoclastic potential of human CFU-GM: Biphasic effect of GM-CSF. *Journal of Bone and Mineral Research*. 2004a; 19:190–199. [PubMed: 14969388]

- Hodge JM, Kirkland MA, Nicholson GC. GM-CSF cannot substitute for M-CSF in human osteoclastogenesis. *Biochemical and Biophysical Research Communications*. 2004b; 321:7–12. [PubMed: 15358207]
- Hodge JM, Kirkland MA, Nicholson GC. Multiple roles of M-CSF in human osteoclastogenesis. *Journal of Cellular Biochemistry*. 2007; 102:759–768. [PubMed: 17516513]
- Hoeffel G, Wang Y, Greter M, See P, Teo P, Malleret B, et al. Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages. *The Journal of Experimental Medicine*. 2012; 209:1167–1181. [PubMed: 22565823]
- Hofer TP, Zawada AM, Frankenberger M, Skokann K, Satz AA, Gesierich W, et al. slan-defined subsets of CD16-positive monocytes: Impact of granulomatous inflammation and M-CSF receptor mutation. *Blood*. 2015; 126:2601–2610. [PubMed: 26443621]
- Hume DA. Macrophages as APC and the dendritic cell myth. *Journal of Immunology*. 2008; 181:5829–5835.
- Hume DA, Gordon S. Mononuclear phagocyte system of the mouse defined by immunohistochemical localization of antigen F4/80. Identification of resident macrophages in renal medullary and cortical interstitium and the juxtaglomerular complex. *The Journal of Experimental Medicine*. 1983; 157:1704–1709. [PubMed: 6854206]
- Hume DA, Pavli P, Donahue RE, Fidler IJ. The effect of human recombinant macrophage colony-stimulating factor (CSF-1) on the murine mononuclear phagocyte system in vivo. *Journal of Immunology*. 1988; 141:3405–3409.
- Humphrey MB, Ogasawara K, Yao W, Spusta SC, Daws MR, Lane NE, et al. The signaling adapter protein DAP12 regulates multinucleation during osteoclast development. *Journal of Bone and Mineral Research*. 2004; 19:224–234. [PubMed: 14969392]
- Huynh D, Akcora D, Malaterre J, Chan CK, Dai XM, Bertoncello I, et al. CSF-1 receptor-dependent colon development, homeostasis and inflammatory stress response. *PloS One*. 2013;8e56951.
- Huynh D, Dai XM, Nandi S, Lightowler S, Trivett M, Chan CK, et al. Colony stimulating factor-1 dependence of paneth cell development in the mouse small intestine. *Gastroenterology*. 2009; 137:136–144. 144.e1–3. [PubMed: 19303020]
- Hwang SJ, Choi B, Kang SS, Chang JH, Kim YG, Chung YH, et al. Interleukin-34 produced by human fibroblast-like synovial cells in rheumatoid arthritis supports osteoclastogenesis. *Arthritis Research & Therapy*. 2012; 14:R14. [PubMed: 22264405]
- Ingman WV, Wyckoff J, Gouon-Evans V, Condeelis J, Pollard JW. Macrophages promote collagen fibrillogenesis around terminal end buds of the developing mammary gland. *Developmental Dynamics*. 2006; 235:3222–3229. [PubMed: 17029292]
- Insogna KL, Sahni M, Grey AB, Tanaka S, Horne WC, Neff L, et al. Colony-stimulating factor-1 induces cytoskeletal reorganization and c-src-dependent tyrosine phosphorylation of selected cellular proteins in rodent osteoclasts. *The Journal of Clinical Investigation*. 1997; 100:2476–2485. [PubMed: 9366562]
- Insogna K, Tanaka S, Neff L, Horne W, Levy J, Baron R. Role of c-Src in cellular events associated with colony-stimulating factor-1-induced spreading in osteoclasts. *Molecular Reproduction and Development*. 1997; 46:104–108. [PubMed: 8981371]
- Ito Y, Teitelbaum SL, Zou W, Zheng Y, Johnson JF, Chappel J, et al. Cdc42 regulates bone modeling and remodeling in mice by modulating RANKL/M-CSF signaling and osteoclast polarization. *The Journal of Clinical Investigation*. 2010; 120:1981–1993. [PubMed: 20501942]
- Itokawa T, Zhu ML, Troiano N, Bian J, Kawano T, Insogna K. Osteoclasts lacking Rac2 have defective chemotaxis and resorptive activity. *Calcified Tissue International*. 2011; 88:75–86. [PubMed: 21110188]
- Jang MH, Herber DM, Jiang X, Nandi S, Dai XM, Zeller G, et al. Distinct in vivo roles of colony-stimulating factor-1 isoforms in renal inflammation. *Journal of Immunology*. 2006; 177:4055–4063.
- Ji JD, Park-Min KH, Shen Z, Fajardo RJ, Goldring SR, McHugh KP, et al. Inhibition of RANK expression and osteoclastogenesis by TLRs and IFN-gamma in human osteoclast precursors. *Journal of Immunology*. 2009; 183:7223–7233.

- Johnson GR, Burgess AW. Molecular and biological properties of a macrophage colony-stimulating factor from mouse yolk sacs. *The Journal of Cell Biology*. 1978; 77:35–47. [PubMed: 307001]
- Jones CV, Ricardo SD. Macrophages and CSF-1: Implications for development and beyond. *Organogenesis*. 2013; 9:249–260. [PubMed: 23974218]
- Jones CV, Williams TM, Walker KA, Dickinson H, Sakkal S, Rumballe BA, et al. M2 macrophage polarisation is associated with alveolar formation during postnatal lung development. *Respiratory Research*. 2013; 14:41. [PubMed: 23560845]
- Kawaida R, Ohtsuka T, Okutsu J, Takahashi T, Kadono Y, Oda H, et al. Jun dimerization protein 2 (JDP2), a member of the AP-1 family of transcription factor, mediates osteoclast differentiation induced by RANKL. *The Journal of Experimental Medicine*. 2003; 197:1029–1035. [PubMed: 12707301]
- Kawasaki ES, Ladner MB, Wang AM, Van Arsdel J, Warren MK, Coyne MY, et al. Molecular cloning of a complementary DNA encoding human macrophage-specific colony-stimulating factor (CSF-1). *Science*. 1985; 230:291–296. [PubMed: 2996129]
- Kierdorf K, Erny D, Goldmann T, Sander V, Schulz C, Perdiguero EG, et al. Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. *Nature Neuroscience*. 2013; 16:273–280. [PubMed: 23334579]
- Kim HJ, Warren JT, Kim SY, Chappel JC, DeSelm CJ, Ross FP, et al. Fyn promotes proliferation, differentiation, survival and function of osteoclast lineage cells. *Journal of Cellular Biochemistry*. 2010; 111:1107–1113. [PubMed: 20717919]
- Kim HJ, Zhang K, Zhang L, Ross FP, Teitelbaum SL, Faccio R. The Src family kinase, Lyn, suppresses osteoclastogenesis in vitro and in vivo. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:2325–2330. [PubMed: 19171907]
- Kim HJ, Zou W, Ito Y, Kim SY, Chappel J, Ross FP, et al. Src-like adaptor protein regulates osteoclast generation and survival. *Journal of Cellular Biochemistry*. 2010; 110:201–209. [PubMed: 20225239]
- Kondo Y, Ramaker JM, Radcliff AB, Baldassari S, Mayer JA, Ver Hoeve JN, et al. Spontaneous optic nerve compression in the osteopetrotic (op/op) mouse: A novel model of myelination failure. *The Journal of Neuroscience*. 2013; 33:3514–3525. [PubMed: 23426679]
- Kong YY, Yoshida H, Sarosi I, Tan HL, Timms E, Capparelli C, et al. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature*. 1999; 397:315–323. [PubMed: 9950424]
- Konno T, Tada M, Tada M, Koyama A, Nozaki H, Harigaya Y, et al. Haploinsufficiency of CSF-1R and clinicopathologic characterization in patients with HDLS. *Neurology*. 2014; 82:139–148. [PubMed: 24336230]
- Kubota Y, Takubo K, Shimizu T, Ohno H, Kishi K, Shibuya M, et al. M-CSF inhibition selectively targets pathological angiogenesis and lymphangiogenesis. *The Journal of Experimental Medicine*. 2009; 206:1089–1102. [PubMed: 19398755]
- Ladner MB, Martin GA, Noble JA, Nikoloff DM, Tal R, Kawasaki ES, et al. Human CSF-1: Gene structure and alternative splicing of mRNA precursors. *The EMBO Journal*. 1987; 6:2693–2698. [PubMed: 3500041]
- Ladner MB, Martin GA, Noble JA, Wittman VP, Warren MK, McGrogan M, et al. cDNA cloning and expression of murine macrophage colony-stimulating factor from L929 cells. *Proceedings of the National Academy of Sciences of the United States of America*. 1988; 85:6706–6710. [PubMed: 2457916]
- Lakkakorpi PT, Vaananen HK. Cytoskeletal changes in osteoclasts during the resorption cycle. *Microscopy Research and Technique*. 1996; 33:171–181. [PubMed: 8845516]
- Lean JM, Fuller K, Chambers TJ. FLT3 ligand can substitute for macrophage colony-stimulating factor in support of osteoclast differentiation and function. *Blood*. 2001; 98:2707–2713. [PubMed: 11675341]
- Leblond AL, Klinkert K, Martin K, Turner EC, Kumar AH, Browne T, et al. Systemic and cardiac depletion of M2 macrophage through CSF-1R signaling inhibition alters cardiac function post myocardial infarction. *PloS One*. 2015; 10:e0137515.

- Lenzo JC, Turner AL, Cook AD, Vlahos R, Anderson GP, Reynolds EC, et al. Control of macrophage lineage populations by CSF-1 receptor and GM-CSF in homeostasis and inflammation. *Immunology and Cell Biology*. 2012; 90:429–440. [PubMed: 21727904]
- Lin H, Lee E, Hestir K, Leo C, Huang M, Bosch E, et al. Discovery of a cytokine and its receptor by functional screening of the extracellular proteome. *Science*. 2008; 320:807–811. [PubMed: 18467591]
- Liu JL, Yakar S, LeRoith D. Conditional knockout of mouse insulin-like growth factor-1 gene using the Cre/loxP system. *Proceedings of the Society for Experimental Biology and Medicine*. 2000; 223:344–351. [PubMed: 10721003]
- Lorenzetto E, Moratti E, Vezzalini M, Harroch S, Sorio C, Buffelli M. Distribution of different isoforms of receptor protein tyrosine phosphatase gamma (Ptpg-RPTP gamma) in adult mouse brain: Upregulation during neuroinflammation. *Brain Structure & Function*. 2014; 219:875–890. [PubMed: 23536318]
- Luo J, Elwood F, Britschgi M, Villeda S, Zhang H, Ding Z, et al. Colony-stimulating factor 1 receptor (CSF1R) signaling in injured neurons facilitates protection and survival. *The Journal of Experimental Medicine*. 2013; 210:157–172. [PubMed: 23296467]
- Ma X, Lin WY, Chen Y, Stawicki S, Mukhyala K, Wu Y, et al. Structural basis for the dual recognition of helical cytokines IL-34 and CSF-1 by CSF-1R. *Structure*. 2012; 20:676–687. [PubMed: 22483114]
- MacDonald KP, Palmer JS, Cronau S, Seppanen E, Olver S, Raffelt NC, et al. An antibody against the colony-stimulating factor 1 receptor depletes the resident subset of monocytes and tumor-associated macrophages but does not inhibit inflammation. *Blood*. 2010; 116:3955–3963. [PubMed: 20682855]
- Mansky KC, Sankar U, Han J, Ostrowski MC. Microphthalmia transcription factor is a target of the p38 MAPK pathway in response to receptor activator of NF-kappa B ligand signaling. *The Journal of Biological Chemistry*. 2002; 277:11077–11083. [PubMed: 11792706]
- Marks SC Jr, Lane PW. Osteopetrosis, a new recessive skeletal mutation on chromosome 12 of the mouse. *The Journal of Heredity*. 1976; 67:11–18. [PubMed: 1262696]
- Maruyama K, Uematsu S, Kondo T, Takeuchi O, Martino MM, Kawasaki T, et al. Strawberry notch homologue 2 regulates osteoclast fusion by enhancing the expression of DC-STAMP. *The Journal of Experimental Medicine*. 2013; 210:1947–1960. [PubMed: 23980096]
- McGill GG, Horstmann M, Widlund HR, Du J, Motyckova G, Nishimura EK, et al. Bcl2 regulation by the melanocyte master regulator Mitf modulates lineage survival and melanoma cell viability. *Cell*. 2002; 109:707–718. [PubMed: 12086670]
- Menke J, Iwata Y, Rabacal WA, Basu R, Yeung YG, Humphreys BD, et al. CSF-1 signals directly to renal tubular epithelial cells to mediate repair in mice. *The Journal of Clinical Investigation*. 2009; 119:2330–2342. [PubMed: 19587445]
- Merad M, Manz MG, Karsunky H, Wagers A, Peters W, Charo I, et al. Langerhans cells renew in the skin throughout life under steady-state conditions. *Nature Immunology*. 2002; 3:1135–1141. [PubMed: 12415265]
- Michaelson MD, Bieri PL, Mehler MF, Xu H, Arezzo JC, Pollard JW, et al. CSF-1 deficiency in mice results in abnormal brain development. *Development*. 1996; 122:2661–2672. [PubMed: 8787741]
- Miyamoto H, Suzuki T, Miyauchi Y, Iwasaki R, Kobayashi T, Sato Y, et al. Osteoclast stimulatory transmembrane protein and dendritic cell-specific transmembrane protein cooperatively modulate cell–cell fusion to form osteoclasts and foreign body giant cells. *Journal of Bone and Mineral Research*. 2012; 27:1289–1297. [PubMed: 22337159]
- Miyazaki T, Katagiri H, Kanegae Y, Takayanagi H, Sawada Y, Yamamoto A, et al. Reciprocal role of ERK and NF-kappaB pathways in survival and activation of osteoclasts. *The Journal of Cell Biology*. 2000; 148:333–342. [PubMed: 10648566]
- Mócsai A, Humphrey MB, Van Ziffle JA, Hu Y, Burghardt A, Spusta SC, et al. The immunomodulatory adapter proteins DAP12 and Fc receptor gamma-chain (FcRgamma) regulate development of functional osteoclasts through the Syk tyrosine kinase. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101:6158–6163. [PubMed: 15073337]

- Morris L, Graham CF, Gordon S. Macrophages in haemopoietic and other tissues of the developing mouse detected by the monoclonal antibody F4/80. *Development*. 1991; 112:517–526. [PubMed: 1794320]
- Mundy GR. Osteoporosis and inflammation. *Nutrition Reviews*. 2007; 65:S147–S151. [PubMed: 18240539]
- Murray PJ, Wynn TA. Protective and pathogenic functions of macrophage subsets. *Nature Reviews Immunology*. 2011; 11:723–737.
- Nakagawa N, Kinosaki M, Yamaguchi K, Shima N, Yasuda H, Yano K, et al. RANK is the essential signaling receptor for osteoclast differentiation factor in osteoclastogenesis. *Biochemical and Biophysical Research Communications*. 1998; 253:395–400. [PubMed: 9878548]
- Nakamura I, Pilkington MF, Lakkakorpi PT, Lipfert L, Sims SM, Dixon SJ, et al. Role of alpha(v)beta(3) integrin in osteoclast migration and formation of the sealing zone. *Journal of Cell Science*. 1999; 112:3985–3993. [PubMed: 10547359]
- Nandi S, Akhter MP, Seifert MF, Dai XM, Stanley ER. Developmental and functional significance of the CSF-1 proteoglycan chondroitin sulfate chain. *Blood*. 2006; 107:786–795. [PubMed: 16210339]
- Nandi S, Cioce M, Yeung YG, Nieves E, Tesfa L, Lin H, et al. Receptor-type protein-tyrosine phosphatase zeta is a functional receptor for interleukin-34. *The Journal of Biological Chemistry*. 2013; 288:21972–21986. [PubMed: 23744080]
- Nandi S, Gokhan S, Dai XM, Wei S, Enikolopov G, Lin H, et al. The CSF-1 receptor ligands IL-34 and CSF-1 exhibit distinct developmental brain expression patterns and regulate neural progenitor cell maintenance and maturation. *Developmental Biology*. 2012; 367:100–113. [PubMed: 22542597]
- Newman AC, Hughes CC. Macrophages and angiogenesis: A role for Wnt signaling. *Vascular Cell*. 2012; 4:13. [PubMed: 22938389]
- Nicholson AM, Baker MC, Finch NA, Rutherford NJ, Wider C, Graff-Radford NR, et al. CSF1R mutations link POLD and HDLS as a single disease entity. *Neurology*. 2013; 80:1033–1040. [PubMed: 23408870]
- Niida S, Kaku M, Amano H, Yoshida H, Kataoka H, Nishikawa S, et al. Vascular endothelial growth factor can substitute for macrophage colony-stimulating factor in the support of osteoclastic bone resorption. *The Journal of Experimental Medicine*. 1999; 190:293–298. [PubMed: 10432291]
- Niida S, Kondo T, Hiratsuka S, Hayashi S, Amizuka N, Noda T, et al. VEGF receptor 1 signaling is essential for osteoclast development and bone marrow formation in colony-stimulating factor 1-deficient mice. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102:14016–14021. [PubMed: 16172397]
- Nikolic T, de Bruijn MF, Lutz MB, Leenen PJ. Developmental stages of myeloid dendritic cells in mouse bone marrow. *International Immunology*. 2003; 15:515–524. [PubMed: 12663681]
- Nishimura K, Tanaka N, Ohshige A, Fukumatsu Y, Matsuura K, Okamura H. Effects of macrophage colony-stimulating factor on folliculogenesis in gonadotrophin-primed immature rats. *Journal of Reproduction and Fertility*. 1995; 104:325–330. [PubMed: 7473425]
- Norgard M, Marks SC Jr, Reinholt FP, Andersson G. The effects of colony-stimulating factor-1 (CSF-1) on the development of osteoclasts and their expression of tartrate-resistant acid phosphatase (TRAP) in toothless (tl-osteopetrotic) rats. *Critical Reviews in Eukaryotic Gene Expression*. 2003; 13:117–132. [PubMed: 14696961]
- Ochsenbein AM, Karaman S, Proulx ST, Goldmann R, Chittazhathu J, Dasargyri A, et al. Regulation of lymphangiogenesis in the diaphragm by macrophages and VEGFR-3 signaling. *Angiogenesis*. 2016; 19:513–524. [PubMed: 27464987]
- Otero K, Turnbull IR, Poliani PL, Vermi W, Cerutti E, Aoshi T, et al. Macrophage colony-stimulating factor induces the proliferation and survival of macrophages via a pathway involving DAPI2 and beta-catenin. *Nature Immunology*. 2009; 10:734–743. [PubMed: 19503107]
- Outtz HH, Tattersall IW, Kofler NM, Steinbach N, Kitajewski J. Notch1 controls macrophage recruitment and Notch signaling is activated at sites of endothelial cell anastomosis during retinal angiogenesis in mice. *Blood*. 2011; 118:3436–3439. [PubMed: 21795743]

- Palacio S, Felix R. The role of phosphoinositide 3-kinase in spreading osteoclasts induced by colony-stimulating factor-1. *European Journal of Endocrinology*. 2001; 144:431–440. [PubMed: 11275955]
- Palis J, Robertson S, Kennedy M, Wall C, Keller G. Development of erythroid and myeloid progenitors in the yolk sac and embryo proper of the mouse. *Development*. 1999; 126:5073–5084. [PubMed: 10529424]
- Pampfer S, Arceci RJ, Pollard JW. Role of colony stimulating factor-1 (CSF-1) and other lymphohematopoietic growth factors in mouse pre-implantation development. *Bioessays*. 1991; 13:535–540. [PubMed: 1836724]
- Perkins SL, Kling SJ. Local concentrations of macrophage colony-stimulating factor mediate osteoclastic differentiation. *The American Journal of Physiology*. 1995; 269:E1024–E1030. [PubMed: 8572192]
- Pilkington MF, Sims SM, Dixon SJ. Wortmannin inhibits spreading and chemotaxis of rat osteoclasts in vitro. *Journal of Bone and Mineral Research*. 1998; 13:688–694. [PubMed: 9556069]
- Pixley FJ, Stanley ER. CSF-1 regulation of the wandering macrophage: Complexity in action. *Trends in Cell Biology*. 2004; 14:628–638. [PubMed: 15519852]
- Pluznik DH, Sachs L. The cloning of normal “mast” cells in tissue culture. *Journal of Cellular and Comparative Physiology*. 1965; 66:319–324.
- Pollard JW. Trophic macrophages in development and disease. *Nature Reviews Immunology*. 2009; 9:259–270.
- Pollard JW, Bartocci A, Arceci R, Orlofsky A, Ladner MB, Stanley ER. Apparent role of the macrophage growth factor, CSF-1, in placental development. *Nature*. 1987; 330:484–486. [PubMed: 2446141]
- Pollard JW, Hennighausen L. Colony-stimulating factor-1 is required for mammary-gland development during pregnancy. *Proceedings of the National Academy of Sciences of the United States of America*. 1994; 91:9312–9316. [PubMed: 7937762]
- Pollard JW, Stanley ER. Pleiotropic roles for CSF-1 in development defined by the mouse mutation osteopetrotic. *Advances in Developmental Biochemistry*. 1996; 4:153–193.
- Porter EM, Bevins CL, Ghosh D, Ganz T. The multifaceted Paneth cell. *Cellular and Molecular Life Sciences*. 2002; 59:156–170. [PubMed: 11846026]
- Price LKH, Choi HU, Rosenberg L, Stanley ER. The predominant form of secreted colony stimulating factor-1 is a proteoglycan. *The Journal of Biological Chemistry*. 1992; 267:2190–2199. [PubMed: 1733926]
- Pull SL, Doherty JM, Mills JC, Gordon JI, Stappenbeck TS. Activated macrophages are an adaptive element of the colonic epithelial progenitor niche necessary for regenerative responses to injury. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102:99–104. [PubMed: 15615857]
- Rademakers R, Baker M, Nicholson AM, Rutherford NJ, Finch N, Soto-Ortolaza A, et al. Mutations in the colony stimulating factor 1 receptor (CSF1R) gene cause hereditary diffuse leukoencephalopathy with spheroids. *Nature Genetics*. 2011; 44:200–205. [PubMed: 22197934]
- Rae F, Woods K, Sasmono T, Campanale N, Taylor D, Ovchinnikov DA, et al. Characterisation and trophic functions of murine embryonic macrophages based upon the use of a Csf1r-EGFP transgene reporter. *Developmental Biology*. 2007; 308:232–246. [PubMed: 17597598]
- Ramsay RG, Micallef SJ, Williams B, Lightowler S, Vincan E, Heath JK, et al. Colony-stimulating factor-1 promotes clonogenic growth of normal murine colonic crypt epithelial cells in vitro. *Journal of Interferon & Cytokine Research*. 2004; 24:416–427. [PubMed: 15296653]
- Ratheesh A, Belyaeva V, Siekhaus DE. Drosophila immune cell migration and adhesion during embryonic development and larval immune responses. *Current Opinion in Cell Biology*. 2015; 36:71–79. [PubMed: 26210104]
- Reeve JL, Zou W, Liu Y, Maltzman JS, Ross FP, Teitelbaum SL. SLP-76 couples Syk to the osteoclast cytoskeleton. *Journal of Immunology*. 2009; 183:1804–1812.
- Regenstreif LJ, Rossant J. Expression of the c-fms proto-oncogene and of the cytokine, CSF-1, during mouse embryogenesis. *Developmental Biology*. 1989; 133:284–294. [PubMed: 2523324]

- Reinholt FP, Hultenby K, Oldberg A, Heinegard D. Osteopontin—A possible anchor of osteoclasts to bone. *Proceedings of the National Academy of Sciences of the United States of America*. 1990; 87:4473–4475. [PubMed: 1693772]
- Rettenmier CW, Roussel MF, Ashmun RA, Ralph P, Price K, Sherr CJ. Synthesis of membrane-bound colony-stimulating factor 1 (CSF-1) and down-modulation of CSF-1 receptors in NIH 3T3 cells transformed by cotransfection of the human CSF-1 and c-fms (CSF-1 receptor) genes. *Molecular and Cellular Biology*. 1987; 7:2378–2387. [PubMed: 3039346]
- Roth P, Bartocci A, Stanley ER. Lipopolysaccharide induces synthesis of mouse colony-stimulating factor-1 in vivo. *Journal of Immunology*. 1997; 158:3874–3880.
- Roth P, Dominguez MG, Stanley ER. The effects of colony-stimulating factor-1 on the distribution of mononuclear phagocytes in the developing osteopetrotic mouse. *Blood*. 1998; 91:3773–3783. [PubMed: 9573014]
- Roth P, Stanley ER. Colony-stimulating factor-1 expression in the human fetus and newborn. *Journal of Leukocyte Biology*. 1995; 58:432–437. [PubMed: 7561519]
- Roth P, Stanley ER. Colony stimulating factor-1 expression is developmentally regulated in the mouse. *Journal of Leukocyte Biology*. 1996; 59:817–823. [PubMed: 8691066]
- Ryan GR, Dai XM, Dominguez MG, Tong W, Chuan F, Chisholm O, et al. Rescue of the colony-stimulating factor 1 (CSF-1)-nullizygous mouse (Csf1 (op)/Csf1 (op)) phenotype with a CSF-1 transgene and identification of sites of local CSF-1 synthesis. *Blood*. 2001; 98:74–84. [PubMed: 11418465]
- Rymo SF, Gerhardt H, Wolfhagen Sand F, Lang R, Uv A, Betsholtz C. A two-way communication between microglial cells and angiogenic sprouts regulates angiogenesis in aortic ring cultures. *PloS One*. 2011; 6:e15846. [PubMed: 21264342]
- Sakai H, Chen Y, Itokawa T, Yu KP, Zhu ML, Insogna K. Activated c-Fms recruits Vav and Rac during CSF-1-induced cytoskeletal remodeling and spreading in osteoclasts. *Bone*. 2006; 39:1290–1301. [PubMed: 16950670]
- Sarrazin S, Mossadegh-Keller N, Fukao T, Aziz A, Mourcin F, Vanhille L, et al. MafB restricts M-CSF-dependent myeloid commitment divisions of hematopoietic stem cells. *Cell*. 2009; 138:300–313. [PubMed: 19632180]
- Sasmono RT, Oceandy D, Pollard JW, Tong W, Pavli P, Wainwright BJ, et al. A macrophage colony-stimulating factor receptor-green fluorescent protein transgene is expressed throughout the mononuclear phagocyte system of the mouse. *Blood*. 2003; 101:1155–1163. [PubMed: 12393599]
- Schafer DP, Stevens B. Microglia function in central nervous system development and plasticity. *Cold Spring Harbor Perspectives in Biology*. 2015; 7:a020545. [PubMed: 26187728]
- Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, et al. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science*. 2012; 336:86–90. [PubMed: 22442384]
- Segaliny AI, Brion R, Brulin B, Maillason M, Charrier C, Teletchea S, et al. IL-34 and M-CSF form a novel heteromeric cytokine and regulate the M-CSF receptor activation and localization. *Cytokine*. 2015a; 76:170–181. [PubMed: 26095744]
- Segaliny A, Brion R, Mortier E, Maillason M, Cherel M, Jacques Y, et al. Syndecan-1 regulates the biological activities of interleukin-34. *Biochimica et Biophysica Acta*. 2015b; 1853:1010–1021. [PubMed: 25662098]
- Shechter R, Miller O, Yovel G, Rosenzweig N, London A, Ruckh J, et al. Recruitment of beneficial M2 macrophages to injured spinal cord is orchestrated by remote brain choroid plexus. *Immunity*. 2013; 38:555–569. [PubMed: 23477737]
- Sherr CJ, Rettenmier CW, Sacca R, Roussel MF, Look AT, Stanley ER. The c-fms proto-oncogene product is related to the receptor for the mononuclear phagocyte growth factor, CSF-1. *Cell*. 1985; 41:665–676. [PubMed: 2408759]
- Shibata Y, Zsengeller Z, Otake K, Palaniyar N, Trapnell BC. Alveolar macrophage deficiency in osteopetrotic mice deficient in macrophage colony-stimulating factor is spontaneously corrected with age and associated with matrix metalloproteinase expression and emphysema. *Blood*. 2001; 98:2845–2852. [PubMed: 11675359]

- Shinohara M, Nakamura M, Masuda H, Hirose J, Kadono Y, Iwasawa M, et al. Class IA phosphatidylinositol 3-kinase regulates osteoclastic bone resorption through Akt-mediated vesicle transport. *Journal of Bone and Mineral Research*. 2012; 27:2464–2475. [PubMed: 22806988]
- Smith SJ, Mohun TJ. Early cardiac morphogenesis defects caused by loss of embryonic macrophage function in *Xenopus*. *Mechanisms of Development*. 2011; 128:303–315. [PubMed: 21515365]
- Stanley ER. Colony-stimulating factor (CSF) radioimmunoassay: Detection of a CSF subclass stimulating macrophage production. *Proceedings of the National Academy of Sciences of the United States of America*. 1979; 76:2969–2973. [PubMed: 313570]
- Stanley ER, Chen DM, Lin HS. Induction of macrophage production and proliferation by a purified colony stimulating factor. *Nature*. 1978; 274:168–170. [PubMed: 307187]
- Stanley ER, Chitu V. CSF-1 receptor signaling in myeloid cells. *Cold Spring Harbor Perspectives in Biology*. 2014; 6:1–21.
- Stanley ER, Heard PM. Factors regulating macrophage production and growth. Purification and some properties of the colony stimulating factor from medium conditioned by mouse L cells. *The Journal of Biological Chemistry*. 1977; 252:4305–4312. [PubMed: 301140]
- Stefater JA 3rd, Ren S, Lang RA, Duffield JS. Metchnikoff's policemen: Macrophages in development, homeostasis and regeneration. *Trends in Molecular Medicine*. 2011; 17:743–752. [PubMed: 21890411]
- Sundquist KT, Cecchini MG, Marks SC Jr. Colony-stimulating factor-1 injections improve but do not cure skeletal sclerosis in osteopetrotic (op) mice. *Bone*. 1995; 16:39. [PubMed: 7742081]
- Suzu S, Ohtsuki T, Yanai N, Takatsu Z, Kawashima T, Takaku F, et al. Identification of a high molecular weight macrophage colony-stimulating factor as a glycosaminoglycan-containing species. *The Journal of Biological Chemistry*. 1992; 267:4345–4348. [PubMed: 1531650]
- Takeshita S, Namba N, Zhao JJ, Jiang Y, Genant HK, Silva MJ, et al. SHIP-deficient mice are severely osteoporotic due to increased numbers of hyperresorptive osteoclasts. *Nature Medicine*. 2002; 8:943–949.
- Takito J, Otsuka H, Yanagisawa N, Arai H, Shiga M, Inoue M, et al. Regulation of osteoclast multinucleation by the actin cytoskeleton signaling network. *Journal of Cellular Physiology*. 2015; 230:395–405. [PubMed: 25056912]
- Thompson, DW. On growth and form. Cambridge, UK: University Press; 1942.
- Ueki Y, Lin CY, Senoo M, Ebihara T, Agata N, Onji M, et al. Increased myeloid cell responses to M-CSF and RANKL cause bone loss and inflammation in SH3BP2 “cherubism” mice. *Cell*. 2007; 128:71–83. [PubMed: 17218256]
- Van Nguyen A, Pollard JW. Colony stimulating factor-1 is required to recruit macrophages into the mammary gland to facilitate mammary ductal outgrowth. *Developmental Biology*. 2002; 247:11–25. [PubMed: 12074549]
- Van Wesenbeeck L, Odgren PR, MacKay CA, D'Angelo M, Safadi FF, Popoff SN, et al. The osteopetrotic mutation toothless (tl) is a loss-of-function frameshift mutation in the rat *Csf1* gene: Evidence of a crucial role for CSF-1 in osteoclastogenesis and endochondral ossification. *Proceedings of the National Academy of Sciences of the United States of America*. 2002; 99:14303–14308. [PubMed: 12379742]
- Vérollet C, Gallois A, Dacquin R, Lastrucci C, Pandravadu SN, Ortega N, et al. Hck contributes to bone homeostasis by controlling the recruitment of osteoclast precursors. *The FASEB Journal*. 2013; 27:3608–3618. [PubMed: 23742809]
- Visvader J, Verma IM. Differential transcription of exon 1 of the human *c-fms* gene in placental trophoblasts and monocytes. *Molecular and Cellular Biology*. 1989; 9:1336–1341. [PubMed: 2524648]
- von Holst A, Sirko S, Faissner A. The unique 473HD-Chondroitinsulfate epitope is expressed by radial glia and involved in neural precursor cell proliferation. *The Journal of Neuroscience*. 2006; 26:4082–4094. [PubMed: 16611825]
- Wang Y, Berezovska O, Fedoroff S. Expression of colony stimulating factor-1 receptor (CSF-1R) by CNS neurons in mice. *Journal of Neuroscience Research*. 1999; 57:616–632. [PubMed: 10462686]

- Wang Y, Bugatti M, Ulland TK, Vermi W, Gilfillan S, Colonna M. Non-redundant roles of keratinocyte-derived IL-34 and neutrophil-derived CSF1 in Langerhans cell renewal in the steady state and during inflammation. *European Journal of Immunology*. 2016; 46:552–559. [PubMed: 26634935]
- Wang Y, Chang J, Yao B, Niu A, Kelly E, Breeggemann MC, et al. Proximal tubule-derived colony stimulating factor-1 mediates polarization of renal macrophages and dendritic cells, and recovery in acute kidney injury. *Kidney International*. 2015; 88:1274–1282. [PubMed: 26422503]
- Wang T, Kono T, Monte MM, Kuse H, Costa MM, Korenaga H, et al. Identification of IL-34 in teleost fish: Differential expression of rainbow trout IL-34, MCSF1 and MCSF2, ligands of the MCSF receptor. *Molecular Immunology*. 2013; 53:39–409.
- Wang Y, Lebowitz D, Sun C, Thang H, Grynblas MD, Glogauer M. Identifying the relative contributions of Rac1 and Rac2 to osteoclastogenesis. *Journal of Bone and Mineral Research*. 2008; 23:260–270. [PubMed: 17922611]
- Wang Y, Szretter KJ, Vermi W, Gilfillan S, Rossini C, Cella M, et al. IL-34 is a tissue-restricted ligand of CSF1R required for the development of Langerhans cells and microglia. *Nature Immunology*. 2012; 13:753–760. [PubMed: 22729249]
- Wehkamp J, Stange EF. Paneth cells and the innate immune response. *Current Opinion in Gastroenterology*. 2006; 22:644–650. [PubMed: 17053443]
- Wei S, Nandi S, Chitu V, Yeung YG, Yu W, Huang M, et al. Functional overlap but differential expression of CSF-1 and IL-34 in their CSF-1 receptor-mediated regulation of myeloid cells. *Journal of Leukocyte Biology*. 2010; 88:495–505. [PubMed: 20504948]
- Weilbaecher KN, Motyckova G, Huber WE, Takemoto CM, Hemesath TJ, Xu Y, et al. Linkage of M-CSF signaling to Mitf, TFE3, and the osteoclast defect in Mitf(mi/mi) mice. *Molecular Cell*. 2001; 8:749–758. [PubMed: 11684011]
- Weiner LM, Li W, Holmes M, Catalano RB, Dohnansky M, Padavic K, et al. Phase I trial of recombinant macrophage colony-stimulating factor and recombinant gamma-interferon: Toxicity, monocytosis, and clinical effects. *Cancer Research*. 1994; 54:4084–4090. [PubMed: 8033141]
- White GE, McNeill E, Channon KM, Greaves DR. Fractalkine promotes human monocyte survival via a reduction in oxidative stress. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2014; 34:2554–2562.
- Wiktor-Jedrzejczak W, Ahmed A, Szczylik C, Skelly RR. Hematological characterization of congenital osteopetrosis in *op/op* mouse. *The Journal of Experimental Medicine*. 1982; 156:1516–1527. [PubMed: 7130905]
- Wiktor-Jedrzejczak W, Bartocci A, Ferrante AW Jr, Ahmed-Ansari A, Sell KW, Pollard JW, et al. Total absence of colony-stimulating factor 1 in the macrophage-deficient osteopetrotic (*op/op*) mouse. *Proceedings of the National Academy of Sciences of the United States of America*. 1990; 87:4828–4832. [PubMed: 2191302]
- Woo KM, Kim HM, Ko JS. Macrophage colony-stimulating factor promotes the survival of osteoclast precursors by up-regulating Bcl-X(L). *Experimental & Molecular Medicine*. 2002; 34:340–346. [PubMed: 12526097]
- Yagi M, Miyamoto T, Sawatani Y, Iwamoto K, Hosogane N, Fujita N, et al. DC-STAMP is essential for cell-cell fusion in osteoclasts and foreign body giant cells. *The Journal of Experimental Medicine*. 2005; 202:345–351. [PubMed: 16061724]
- Yamane F, Nishikawa Y, Matsui K, Asakura M, Iwasaki E, Watanabe K, et al. CSF-1 receptor-mediated differentiation of a new type of monocytic cell with B cell-stimulating activity: Its selective dependence on IL-34. *Journal of Leukocyte Biology*. 2014; 95:19–31. [PubMed: 24052571]
- Yao C, Yao GQ, Sun BH, Zhang C, Tommasini SM, Insogna K. The transcription factor T-box3 regulates colony stimulating factor 1-dependent Jun dimerization protein 2 expression and plays an important role in osteoclastogenesis. *The Journal of Biological Chemistry*. 2014; 289:6775–6790. [PubMed: 24394418]
- Yeung YG, Jubinsky PT, Sengupta A, Yeung DCY, Stanley ER. Purification of the colony-stimulating factor 1 receptor and demonstration of its tyrosine kinase activity. *Proceedings of the National Academy of Sciences of the United States of America*. 1987; 84:1268–1271. [PubMed: 3029775]

- Yona S, Kim KW, Wolf Y, Mildner A, Varol D, Breker M, et al. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity*. 2013; 38:79–91. [PubMed: 23273845]
- Yoshida H, Hayashi S, Kunisada T, Ogawa M, Nishikawa S, Okamura H, et al. The murine mutation osteopetrosis is in the coding region of the macrophage colony stimulating factor gene. *Nature*. 1990; 345:442–444. [PubMed: 2188141]
- Yu W, Chen J, Xiong Y, Pixley FJ, Yeung YG, Stanley ER. Macrophage proliferation is regulated through CSF-1 receptor tyrosines 544, 559, and 807. *The Journal of Biological Chemistry*. 2012; 287:13694–13704. [PubMed: 22375015]
- Zhou P, Kitaura H, Teitelbaum SL, Krystal G, Ross FP, Takeshita S. SHIP1 negatively regulates proliferation of osteoclast precursors via Akt-dependent alterations in D-type cyclins and p27. *Journal of Immunology*. 2006; 177:8777–8784.
- Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, et al. Nomenclature of monocytes and dendritic cells in blood. *Blood*. 2010; 116:e74–e80. [PubMed: 20628149]
- Zou W, Reeve JL, Liu Y, Teitelbaum SL, Ross FP. DAP12 couples c-Fms activation to the osteoclast cytoskeleton by recruitment of Syk. *Molecular Cell*. 2008; 31:422–431. [PubMed: 18691974]
- Zou W, Reeve JL, Zhao H, Ross FP, Teitelbaum SL. Syk tyrosine 317 negatively regulates osteoclast function via the ubiquitin-protein isopeptide ligase activity of Cbl. *The Journal of Biological Chemistry*. 2009; 284:18833–18839. [PubMed: 19419964]

Fig. 1.

Regulation of mononuclear phagocyte development by CSF-1R. (A) Ontogenically distinct mouse macrophages are generated in three successive waves. (B) Contribution of EMP and HSC to tissue macrophage populations. *Red* and *blue arrows* indicate critical and partial contribution of CSF-1R signaling to development, respectively, *gray arrows* indicate possible contribution, *black arrows*, lack of requirement.

Fig. 2.

Developmental functions of tissue macrophages. **Bold letters** indicate processes shown to be dysregulated in *Csf1r^{-/-}* or *Csf1^{op/op}* mice. The drawings show details of the cellular and molecular interactions involved.

Fig. 3.

Defects in bone development associated with the absence of osteoclasts in *Csfr1*^{-/-} mice. *BM*, bone marrow; *CB*, cortical bone; *H*, hypertrophic chondrocyte region; *M*, matrix; *OB*, osteoblasts; *P*, proliferating chondrocyte region; *SO*, secondary ossification center; *TB*, trabecular bone. The *arrowheads* in the lower left panel point to canaliculi in the matrix. The *arrow* in the inset indicates an osteocyte process. *Panels republished with permission of John Wiley and Sons Inc., from Dai, X.-M., Zong, X.-H., Akhter, M. P., & Stanley, E. R. (2004). Osteoclast deficiency results in disorganized matrix, reduced mineralization, and abnormal osteoblast behavior in developing bone. Journal of Bone and Mineral Research, 19(9), 1441–1451; permission conveyed through Copyright Clearance Center, Inc.*

Fig. 4.
Regulation of OC differentiation by CSF-1 and RANKL.

Fig. 5. CSF-1R signaling for osteoclast precursor proliferation (A) and commitment (B), the upregulation of osteoclast-specific genes (C), preosteoclast fusion (D), bone resorption by mature osteoclasts (E), and osteoclast survival (F) is shown.

	Brain	Small intestine	Reproductive tract		
Source	Neurons	Intestinal epithelial cells	Granulosa cells	Uterine and oviduct epithelium	
Ligand	CSF-1 IL-34?	Cell surface CSF-1	CSF-1		
CSF-1R ⁺ Target cell	<p>Self-renewing neural stem cells</p> Neurons Purkinje cells	Stem cells Paneth cells	Oocytes	Preimplantation embryo (≥ Two-cell stage)	Trophoblastic cells
Effects	Neuronal differentiation and survival	Paneth cell maturation Gut innate immunity	Ovulation	Preimplantation development	Placental immunity

Fig. 6.
Direct regulation of nonmyeloid cell types by CSF-1R.

Fig. 7.

Roles of CSF-1R in development and adult physiology. The organism in which the relevant studies were performed is depicted.