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Metchnikoff's Policemen—Macrophages in Development, Homeostasis and Regeneration

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Abstract

Over the past decade, modern genetic tools have permitted scientists to study the function of myeloid lineage cells, including macrophages, as never before. Macrophages were first detected more than a century ago as cells that ingested bacteria and other microbes, but it is now known that their functional roles are far more numerous. In this review, we focus on the prevailing functions of macrophages beyond their role in innate immunity. We highlight examples of macrophages acting as regulators of development, tissue homeostasis, remodeling (the reorganization or renovation of existing tissues), and repair. We also detail how modern genetic tools have facilitated new insights into these mysterious cells.

An ever-expanding role for macrophages

Recent work has shed light on the diverse nature of myeloid cells. It is now recognized that subtypes of myeloid cells have varied developmental origins, such as microglia which are derived from the embryonic yolk sac and not replenished by blood-derived monocytes [1]. Tissue macrophages, however, are derived from hematopoietic stem cells, but their expansion can either be due to local proliferation or infiltration, depending on the stimulus [2]. While we often use the generic term “macrophage” in this review, it is quite clear that the location of the macrophage and the local environment affect gene expression and, therefore, cell phenotype.

After decades of research, it is now clear that macrophages do more than simply protect the host from foreign invaders. The known roles of myeloid lineage cells have been expanded such that innate immunity is now recognized as just one of a myriad of critical functions. This relatively new perspective on these ancient cells appears obvious in hindsight. Macrophages have evolutionary cousins in all species from invertebrates to mammals [3], and phagocytosis was a critical development in the course of evolution. This property, or some permutation of it, emerged in invertebrates before any semblance of innate immunity (Figure 1) [3]. In vertebrates, myeloid cells are found in nearly every tissue from the early

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stages of development, where they remain throughout the entire life of the organism. Furthermore, after injury or during disease, additional myeloid cells are recruited, even in the absence of pathogens, and disperse after repair or recovery.

Ilya Metchnikoff, the father of cellular immunology, believed in an expansive role for macrophages. He detailed the importance of phagocytic cells in clearing fungal infections from crustacea and bacterial infections from rabbits. Interestingly, he proposed that macrophages evolved first to regulate development (phagocytosing unwanted cells), and that these phagocytic traits set the stage for their evolution into effectors of innate immunity [4]. Beyond regulating development and maintaining order, Metchnikoff suggested that macrophages played a role during injury repair [5]. Specifically, he noted that in fish embryos injured by cauterization, the recruitment of macrophages to the injured tissue resembled their recruitment to sites of infection [5]. These broad-ranging, seminal studies set the stage for the next century of research into macrophage biology.

Historically, several additional insights shed light on the nature of macrophages. First, characterization of the mononuclear phagocyte system (MPS) revealed how macrophages move into areas of infection or disease. By the late 1960s, monocyte extravasation (see Glossary) was well-described [6]. Second, the discovery of “macrophage activation” detailed how macrophages increased the intensity of their response to a second infection following an initial infection. It was now clear that macrophages were more than simple bystanders performing phagocytic functions [7]. Furthermore, macrophages have been found to produce almost every known effector molecule including PDGFs (platelet-derived growth factors), IGFs (insulin-like growth factors), HGFs (hepatocyte growth factors), FGFs (fibroblast growth factors), TGFs (transforming growth factors), CSFs (colony stimulating factors), Wnt ligands, and many immune-related molecules. The coordinated release of such factors enables macrophages to dramatically affect the cellular milieu. In this review, we summarize work that has elucidated the mechanisms behind Metchnikoff’s original hypotheses.

Macrophages during development

Many studies have examined how macrophages or invertebrate phagocytes regulate developmental processes. Phagocytes certainly engulf dead cells, but they can also help decide which cells should apoptose [8]. More broadly, macrophages can also regulate developmental processes independent of apoptosis [9, 10]. In the following sections we discuss how macrophages can act in all three contexts (Figure 2).

Macrophages as Initiators of Apoptosis

In addition to performing the well-known clearance functions discussed in Box 1, macrophages can both initiate and protect cells from apoptosis. Events typically thought to occur downstream of apoptotic initiation, including the activation of DNA damage signaling pathways and the appearance of phagocytic “eat me” signals, may in fact be upstream initiators of programmed cell death [11–13]. Such a connection has been investigated using a range of model organisms.

Box 1

The traditional role of macrophages during development: simply phagocytes

In nearly all organisms, apoptosis is a critical component of normal development. Developmental apoptosis has been extensively studied in the nematode *C. elegans*, where exactly 131 cells of the 1,090 cells generated to form an adult worm undergo apoptosis

[99]. Similar apoptotic events have been characterized in the frog *Xenopus* [100] and in mammalian development [101]. In male mammals, for instance, mammary tissue is reduced by programmed cell death [102]. Obviously these dead cells must be subsequently removed. In lower organisms, removal is accomplished by adjacent cells that activate phagocytic gene programs [99]. In more complex invertebrates, such as *Drosophila*, and in vertebrates, professional phagocytes generally perform cell clearance. These professional phagocytes have a permanently enhanced capacity to perform phagocytic and digestive functions. The importance of developmental phagocytosis was made clear with studies of mice lacking functional macrophages. Interestingly, when macrophages were genetically ablated (via deletion of the critical macrophage transcription factor *PU.1*), other adjacent cells were shown to take the place of macrophages (semi-professional phagocytes) and engulf apoptotic cells [101]. This trans-differentiation occurred by the activation of genes that accomplish phagocytic and digestive functions [103]. Professional phagocytes are critical, though, because these macrophage-null animals have less efficient dead cell clearance and tissue remodeling is delayed [101].

In the nematode *Caenorhabditis elegans*, mutating components in the downstream clearance pathways (i.e. *ced-1*) results in diminished apoptosis and promotes cell survival [11, 12]. Developmental apoptosis of Purkinje cells in the mouse brain and neurons in the chick retina were shown to require microglia [13, 14]. Furthermore, during eye development, macrophages secrete Wnt7b to induce blood vessel regression [8, 15, 16]. Why would phagocytes be involved in inducing cell death? One possibility is to link phagocytosis and initiation of apoptosis to facilitate clearance of debris. A system where macrophages act as an obligatory participant may minimize secondary necrosis, which can occur during chronic inflammation or injury [17]. Therefore, compromised cells may only undergo apoptosis if the mechanisms for quick clearance are already at hand. Or, in tissues where phagocytes are not abundant, pre-apoptotic cells may send out phagocyte recruitment signals before initiating cell death [17]. So, from the nematode to the mouse, phagocyte presence may serve as an apoptotic checkpoint.

Macrophages as Regulators of Apoptosis-Independent Development

Macrophages also regulate development via mechanisms independent of apoptosis, which include tissue patterning, morphogenesis, and cell fate decisions.

Tissue Patterning

In higher organisms, blood vessel development provides an excellent example of patterning. Tissues must establish a blood vessel network of precise density, and in several contexts macrophages play an important role in vessel patterning.

One important tool to investigate the *in vivo* function of macrophages has been the *PU.1*^{-/-} strain of mice. These mice lack a transcription factor required for the differentiation of immune cells, including macrophages. *PU.1*^{-/-} mice exhibit altered hindbrain vasculature and abnormal retinal vessel remodeling [10]. Furthermore, *PU.1*^{-/-} mice display hyperproliferative and dilated dermal lymphatic vasculature [18]. *Csf1*^{op/op} are another useful mouse model. These mice lack the *csf1* gene and have a severe deficiency in mononuclear phagocytes [19]. These mice are named for their osteopetrotic phenotype and demonstrate severe skeletal abnormalities [19]. Like *PU.1*^{-/-} mice, these animals display abnormal vascular patterning in the hindbrain [10] and retina [10, 20].

Although there is still much to learn about how macrophages regulate vessel patterning, several recent studies have shed some light on the role of macrophages in this process. Data from the Ruhrberg group suggests that macrophages physically guide vessel anastomosis [10]. Additionally, macrophages are clearly sources of both pro- and anti-angiogenic factors, which can guide vessel network formation in many ways by, for example, degrading the extracellular matrix [21] or modifying the growth factor milieu [22]. In a recent report, myeloid cells were shown to use non-canonical Wnt signaling to fine-tune the retinal vascular plexus [23]. In this study, myeloid Wnts suppressed vessel branching by enhancing the secretion of Flt1, a naturally occurring inhibitor of vascular endothelial growth factor. While vascular patterning is just one example of tissue patterning, it is quite likely that macrophages, given their ubiquitous nature, play a similar role elsewhere.

Tissue Morphogenesis

There are several contexts in which macrophages, or cells of the myeloid lineage, have been shown to affect tissue morphogenesis. The most obvious example is bone development. Normal bone morphogenesis depends on osteoclasts, a cell derived from myeloid precursors, to remodel bone at the growth plate. Failure to remodel bone leads to osteopetrosis, a condition where the bone marrow cavities are malformed and obstructed. Both *PU.1^{-/-}* and *Csf1^{op/op}* mice lack osteoclasts and, therefore, have abnormal bone morphogenesis [19, 24].

Beyond osteoclasts, various myeloid cell types have been shown to regulate morphogenesis in other contexts. For many years, researchers have noted that many macrophages associate with developing mammary tissue [25]. Specifically, macrophages line the route of the future duct, migrating around and in front of the duct as it develops [26]. Investigation of the *Csf1^{op/op}* mice revealed abnormal mammary gland ductal morphogenesis [27]. When macrophages were ablated in developing mammary tissue, the mice had diminished ductal branching and shortened ductal length [9]. Although the precise mechanisms by which macrophages regulate ductal morphogenesis are unclear, preliminary work suggests that macrophages guide mammary duct outgrowth by remodeling the extracellular matrix, converting collagen 1 into collagen fibrils [26].

Other examples of abnormal tissue morphogenesis in macrophage-deficient mice indicate that macrophages play a role in the development of the pancreas [28], lung [29], and kidney [30]. Such effects are not limited to mice. In the frog *Xenopus laevis*, ablation of macrophages resulted in abnormal limb morphogenesis and death at metamorphosis [31]. Clearly there are a variety of morphogenesis phenotypes, and more work is required to understand macrophage function in these contexts.

Cell Fate Decisions

Beyond selecting which cells to eliminate, macrophages can help specify cell fate. In the developing pancreas, clusters of macrophages form a microenvironment in which islet cells develop [32]. When macrophages are absent from the pancreas, as in *Csf1^{op/op}* mice, there are 30–50% fewer insulin-producing β -cells present at birth [28, 33]. Increasing macrophages in pancreatic explants results in a parallel increase in functional β -cells [33].

In brain development, myeloid cells help regulate neuron survival and neurite process outgrowth [34], and loss of macrophages results in abnormal neural function [34]. Specifically, *Csf1^{op/op}* mice display abnormal brainstem auditory and visual evoked potentials, along with abnormal intracortical and cortical recordings [34, 35]. Macrophages are also required for normal hypothalamic-pituitary axis formation. In the brain, microglia respond to stimulation of their CSF1 receptor by regulating survival of inhibitory and excitatory neurons [36]. This function is important, as *Csf1^{op/op}* mice display abnormal

endocrine physiology [35, 37], an effect largely rescued by injection of Csf1 and activation of brain microglia. Interestingly, microglia are also important sources of nerve growth factor (NGF), a potent neuron growth and survival factor [37, 38], as well as other related neuron inducing factors [39]. The role of myeloid NGF is complex as myeloid NGF induced neuron apoptosis in the developing chick retina by activating the neurotrophin receptor p75 [14]. Additionally, very recent work indicates that microglia are essential mediators of brain development with an essential role in post-natal pruning of synapses [40].

In vivo studies have also correlated macrophage density with the extent of adiposity [41]. Mice with macrophage depletion are smaller and display diminished fat mass [24, 42]. One specific type of adipose tissue, brown fat, is completely absent during development from the mammary gland of *Csf1^{op/op}* mice [43]. In a separate context, macrophages have been linked to the development of myocytes, which is controlled by myeloid secretion of myocyte growth factors [44]. Combined, these results are especially intriguing given the recent observation that myoblasts and brown fat have the same developmental precursors, a fate decision that is controlled by a newly described transcriptional switch [45]. These examples, and the others above, implicate macrophages in the regulation of important cell fate decisions.

Macrophages during homeostasis

In terms of immune defense, the role of the macrophage in maintaining homeostasis is obvious. However, several recent papers have suggested that macrophages maintain homeostasis in other ways, including the regulation of leukocyte homeostasis, blood pressure physiology, reproductive functionality, and lipid metabolism.

Maintenance of hematopoietic cell levels

A major advance in our understanding of macrophage function was the identification of apoptotic cells [46] and, as discussed above, the observation that macrophages remove these cells by phagocytosis. Some cells in the body undergo apoptosis with great frequency, not only developmentally but also homeostatically and physiologically. For example several million neutrophils and erythrocytes are normally cleared from the circulation each day by the spleen and liver. Crucially, this clearance does not activate the macrophages that dispose of these cells. Similarly, in the thymus, spleen and other tissues with high levels of cell turnover, resident macrophages function in the clearance of dying cells without the release of pro-inflammatory factors. When macrophages were eliminated in the spleen and bone marrow, mice developed severe neutrophilia, splenomegaly, and a host of other issues [47]. Additionally, macrophages have been implicated as regulators of the hematopoietic stem cell (HSC) niche [48]. In this work, macrophages were shown to induce retention of HSCs by communicating with the nestin-positive niche, a process that was opposed by activation of the sympathetic nervous system. Without these important homeostatic functions for macrophages, the blood and other organs would quickly become overwhelmed with short-lived or underdeveloped cells.

Maintenance of blood pressure

Salt intake has been implicated as a cause of hypertension [49] but many physiologic mechanisms exist to diminish changes in blood pressure in response to elevated salt intake. One such mechanism is enhanced interstitial storage of Na⁺ [50, 51] and subsequent water drainage via the lymphatic system [52]. In response to high salt, rats transiently expand their lymphatic networks in a process that requires macrophages [52]. As sodium levels rise, macrophages respond to the enhanced interstitial pressure by secreting VEGF-C [52], a potent pro-lymphangiogenic molecule [53], which expands lymphatic capacity, drains fluid,

and results in a compensatory decrease in systemic blood pressure. This data is particularly interesting given the developmental studies implicating macrophages in the regulation of dermal lymphatic development [18, 53].

Additionally, macrophages have long been known to be a source of nitric oxide [54], a potent vasodilator and regulator of blood pressure. While the potential role for macrophage nitric oxide is expansive and certainly not limited to the regulation of blood pressure, it is clear that macrophages play an essential role in nitric oxide homeostasis [55].

Maintenance of reproductive functionality

The hypothalamic-pituitary axis is one of the most important regulators of reproduction. As discussed above, macrophages play a critical role in regulating hypothalamic-pituitary axis formation [35]. However macrophages also play an active role in reproductive homeostasis in both males and females. The best-documented example regards follicle rupture through the ovarian wall. Macrophages are recruited from the interstitial tissue to the theca layer immediately before ovulation [35, 56]. There, they are thought to regulate follicle rupture and egg release [56]. When macrophages are ablated, ovulation is significantly diminished; however, the mechanisms by which macrophages perform this function are unclear [36]. In the testis, macrophages comprise nearly 25% of the interstitial cell mass [57]. These macrophages establish close cell-cell contacts with Leydig cells [58, 59], the specialized testosterone-producing cells of the testis. Consistent with the suggested roles for macrophages in both female and male animals, female *Csf1^{op/op}* mice display abnormal ovulation patterns [60] and male *Csf1^{op/op}* mice have diminished testosterone levels and secondarily decreased libido and sperm count [61]. Most likely, macrophages play a combined role: regulating both the development of the neural hypothalamic-pituitary axis and day-to-day reproductive homeostasis.

Regulation of Lipid Metabolism

During states where lipid metabolism has gone awry, including obesity and atherosclerosis, new studies have suggested a regulatory role for macrophages. Atherosclerotic plaques are predominately composed of cholesterol-stuffed macrophages, also known as foam cells. Surprisingly, when macrophage activation was enhanced via injection of *Csf1*, atherosclerosis was diminished [62]. However, when atherosclerotic lesions were induced in *Csf1^{op/op}* animals, atherosclerotic lesion size was diminished [63]. In studies on whole-body lipid metabolism, macrophages have been correlated with expanding fat mass [41]. While the role of macrophages in disease will be discussed below, these observations do suggest that macrophages play a role in normal lipid homeostasis. Indeed, macrophages express receptors for lipids including low-density lipoprotein receptor (LDLR), LDLR-related protein, and scavenger receptor. Macrophages are therefore able to take up lipoproteins including β -very low density lipoproteins (β -VLDL) and chylomicron remnants [64].

Macrophages during repair and regeneration

Tissue regeneration occurs either in response to injury or as a result of tissue ablation. During injury (such as ischemic, mechanical, toxic, immune-mediated or infectious) repair occurs before regeneration. In contrast, after tissue ablation (such as partial hepatectomy or digit amputation), regeneration occurs without a repair phase. Here we address the role of macrophages in repair and regeneration.

Repair and regeneration of wounded tissues

The most well characterized example of macrophage involvement in repair and regeneration is in skin wounding. Ablation of macrophages from skin wounds was first reported in 1975

using anti-macrophage anti-serum [65]. These studies suggested that macrophages play a role in scarring, but also in regeneration of the injured skin. Over the past 10 years, the role of macrophages after injury has been further studied *in vivo* with the same tools used to study macrophages during development.

In *PU.1*^{-/-} mouse pups, skin wounds were found to heal normally but without scar formation [66]. In these animals, phagocytosis was unaffected as parenchymal cells took over the phagocytic role. Interestingly, dermal angiogenesis was abnormal, suggesting that during wound repair, macrophages have non-redundant roles in regulating scar formation and angiogenesis, but not phagocytosis. In contrast, ablation of macrophages in skin wounds of *CD11b-DTR* or *LysM-DTR* transgenic animals resulted in slower healing wounds characterized by delayed re-epithelialization, decreased collagen deposition, and reduced cell proliferation [67, 68]. These studies also showed that macrophage ablation resulted in abnormal wound vasculature, similar to the findings in *PU.1*^{-/-} mice. Additionally, new work has supported a role for macrophage osteopontin in regulating wound fibrosis [69]. When osteopontin levels were diminished, animals repaired their wounds faster and with less scarring. One possible explanation for the apparent discrepancies between model systems is that the *PU.1*^{-/-} studies were performed on very young mice. *In utero*, skin wounds heal without scarring, so perhaps young skin has greater intrinsic healing capacity than adult skin. In any event, wound macrophages play an important role during skin repair.

In both ischemic and toxic injury models of other organs, macrophage function has been likened to the skin wounding process [70–72]. Such examples of wound repair comprise three overlapping phases: inflammation, new tissue formation or proliferation, and remodeling. The consensus from ablation studies of liver, heart, kidney, pancreas, lung, brain, gut, and muscle is that macrophages play a key role in repair and regeneration when ablated throughout those phases [70–77]. In fact, emerging studies have implicated macrophages in the maintenance of stem cell niches in the colon and mammary glands [75, 78]. Without macrophages, mammary gland stem cells cannot reconstitute ectopic mammary glands.

Mechanisms of regulating repair and regeneration

While it is now accepted that macrophages are integral players in the repair and regeneration of many tissues (Figure 2), the mechanisms are less defined. Here we discuss several mechanisms, including efferocytosis and growth factor release.

Efferocytosis: phagocytosis of dying cells

As discussed above, macrophages can serve as a garbage disposal system for dying cells without the release of injurious or inflammatory factors. This property of macrophages is key to their role in regulating repair and regeneration.

As in development, it is clear that macrophages do not simply react to dead cells, but play an active role in regulating apoptosis. To date, more than 30 macrophage cell surface receptors have been implicated in recognizing apoptotic cells [79]. Many of these receptors have dual roles in activating macrophages and in initiating cell engulfment (Box 2). A number of key events occur in cells undergoing apoptosis, including shedding or modification of transmembrane receptors, oxidation of surface phospholipids, outer-membrane accumulation of phosphatidylserine and phosphatidylethanolamine [80]. These cell surface changes cause rapid opsonization by macrophage cell surface receptors. Several studies of single receptor deficiency or single opsonin deficiency have reported impaired clearance phenotypes [81–83]. While one consequence of aberrant recognition of dying cells is autoimmunity (Box 3), another is abnormal responses to tissue injury.

Box 2**The concept of macrophage activation**

Macrophage activation is a key component of initiating an efficient and robust immune response. The same principles apply when macrophages are involved in repair. In culture, a range of stimuli have been shown to activate cultured macrophages. Most notably, bacterial cell wall components serve as potent activators. Such factors include lipopolysaccharide, flagellin, and cytosine-guanine rich (CpG) microbial oligodeoxynucleotides, collectively known as pathogen-associated molecular patterns (PAMPs). PAMPs work by activating specific pattern recognition receptors, including but not limited to Toll-like receptors. By activating intracellular signaling pathways that include nuclear factor κ B and mitogen-activated protein kinase, macrophages secrete a broad range of proinflammatory cytokines including tumor necrosis factor, interleukin (IL)-1 β , IL-12, IL-18, IL-23, IL-6, and proinflammatory chemokines including macrophage inflammatory factor, MIP2, monocyte chemoattractant proteins (MCP), and CXCL1 (keratinocyte chemoattractant). And, as mentioned above, macrophages can generate reactive nitrogen and oxygen species including nitric oxide. This pattern of activation is broadly known as classical or M1 activation. In addition to foreign proteins, immune complexes (immunoglobulins, antigens, complement components, pentraxins, and other plasma proteins of the innate immune system) can activate macrophages resulting in a similar pattern of cytokine release. Evidence from many studies now indicates that molecules released from injured, damaged or stressed cells also have the capacity to activate macrophages using the same pattern recognition receptors. These injury molecules, broadly known as danger associated molecular patterns (DAMPs), may be central to regulating macrophage responses to tissue injury. In stark contrast, certain pathogens such as amoeba and schistosomes activate macrophages in a different manner. In response to these pathogens, macrophages generate high levels of transforming growth factor β (TGF β), IL-13, IL-10 and chemokines, including CCL17 and CCL22. This alternative pattern of activation is known as M2 activation. Certain DAMPs, such as adenosine, have now been reported to drive macrophage activation toward an M2 phenotype and such polarized macrophages promote reparative/regenerative functions. Importantly though, imposing classifications on macrophage activation states may be an over-simplification. In any event, the capacity of macrophages to engage in and perform reparative and regenerative functions likely depends on efficient macrophage activation.

Box 3**A role for macrophages in regulating autoimmunity?**

In several animal models with clearance receptor mutants, the animals appear to develop auto-immune phenotypes. Indeed, mice with deficiencies in complement (*c1q* knockouts) display significant autoimmune responses. Such phenotypes were thought to be directly due to abnormal presentation of self-antigens from the aberrantly cleared apoptotic cells. The delayed internalization of dying cells was proposed to cause a secondary necrotic degeneration of apoptotic cells, which in turn resulted in aberrant activation of antigen presenting cells (APC). This theory is probably untrue because the aging of apoptotic cells has not been convincingly demonstrated to result in distinct responses in macrophages. It is therefore more likely that macrophage dead-cell-recognition-machinery is anti-inflammatory, whereas recognition by other pathways is pro-inflammatory. Deletion of the anti-inflammatory pathway gives way to activating pathways that stimulate aberrant antigen presentation by APCs.

During wound repair, there is also a role for macrophage recognition of circulating complement factors. Upon injury to the kidney glomerulus, *c1q* deficient mice have exuberant inflammatory responses. C1q can function as an apoptotic cell opsonin. Several other non-complement opsonins, including milk fat globuli-8, have also been implicated in promoting silent clearance [83, 84]. During wounds, the opsonin Pentraxin-2 (PTX-2) directs dead cell clearance via Fcγ receptors that trigger IL-10 release and anti-inflammatory responses in tissue macrophages [85, 86]. Therapeutic administration of PTX-2 promotes anti-inflammatory responses in macrophages at sites of tissue injury, supporting the hypothesis that in severe injury anti-inflammatory pathways are overwhelmed [85]. By contrast, a number of studies suggest that opsonization of dying cells by the circulating complement proteins C3a or C4a promotes pro-inflammatory cytokine release by macrophages [87]. Most complement-mediated clearance, however, results in neutral responses in the macrophage. The pattern of receptor mediated recognition and clearance of dead cells at sites of tissue injury may dictate the reparative response.

In addition to opsonization, macrophage injury responses are affected by the intracellular handling of phagocytosed components. Failure to digest and recycle phagosomal contents can have profound implications for phagocytic capacity, and for the activation state of macrophages. Unfortunately, little is known about the intracellular processing of phagosomes. One macrophage protein central to phagosome processing, Gpnmb, has been implicated in normal tissue repair [88] as the absence of Gpnmb leads to phagosome accumulation that prevents normal tissue repair and regeneration [88].

Cytokine and Growth Factor release at sites of injury

Macrophages are increasingly recognized as mobile generators of cytokines and growth factors. Several macrophage factors have been associated with repair and regeneration. Macrophage HGF was shown to drive liver regeneration after resection [89, 90]. Macrophage PDGFs have been linked to regulation of fibrosis, wound repair and large vessel atheroma stabilization [91]. Macrophage TGFβ1 has been implicated in numerous pathological processes including fibrogenesis, but has also been linked to resolution of inflammation [92]. Recently, several studies have elucidated a role for macrophage Wnt ligands in the regenerative process, and have highlighted the Wnt pathway as a potential therapeutic avenue in tissue injury. The Wnt signaling pathway, which plays a vital role in development, is aberrantly activated in many cancers [93]. Recent studies have identified certain subpopulations of macrophages as important sources of Wnt ligands [23, 72]. In one series of studies, macrophage Wnt7b was shown to be critical for epithelial regeneration in response to injury [72]. In other studies, macrophage Wnts have been shown to affect blood vessel formation by regulating VEGF and Angiopoietin signaling in vascular endothelial cells [94]. Clearly, macrophages are capable of affecting the cytokine and growth factor milieu in response to injury.

Regulating vascularity in response to injury

Evidence from injury experiments indicates that myeloid cells have angiogenic and vascular repair functions [67, 76, 95]. Some studies have suggested a role for macrophages or monocytes in actually differentiating into endothelium, a phenomenon that appears to occur in culture systems, but probably not *in vivo* [96]. These ideas emanated from the suggestion that monocyte and endothelial cells shared a developmental precursor cell, an idea refuted by recent fate-mapping studies [97]. However, it is clear that myeloid cells do play a role in microvascular repair and vessel growth processes.

Many investigators have sought to identify a distinct subset of circulating monocytes that is dedicated to the angiogenic task, named endothelial progenitor cells (EPCs) or circulating

endothelial progenitors (CEPs). However, there is no consensus about defining markers, and it may be that monocytes simply respond to cues in the local environment to adopt angiogenic functions. A number of studies have implicated macrophage VEGFA164 in this process, but it is likely that a range of other macrophage cytokines (*e.g.* angiopoietin1 and IL-10) stimulate vascular repair and angiogenesis. Additionally, in mouse models of diabetic wound healing, macrophages appear to play an important role in lymphangiogenesis through the local release of VEGF-C and VEGF-D [98].

Conclusions

A host of recent findings have expanded our understanding of the function of myeloid lineage cells far beyond passive engulfer of dead debris. Here we have reviewed how macrophages regulate development, maintain homeostasis, and influence repair and regeneration. These emerging revelations should alter the way immunologists approach their studies. Indeed, many of the receptors, ligands, and pathways that immunologists study are used by immune cells to perform non-immune functions. Perhaps the entire definition of immunity and immune cells should be altered to reflect these emerging functions.

Additionally, non-immunologists tend to characterize inflammation as a detrimental side effect. The examples discussed in this review detail why this characterization is incorrect. In every context, biologists must now consider immune cell presence or infiltration as a biologically programmed component guiding development, homeostasis, repair, or regeneration. This point is especially prescient for developmental biologists where nearly every tissue contains tissue macrophages from very early on, an observation that is often ignored. Many model systems including zebrafish now allow for live imaging analysis of macrophages in response to wounding or infection. Such studies, coupled with genetic analysis, open up new possibilities in understanding non-immune macrophage functions.

Finally, this new perspective on macrophage function opens up a new avenue for potential clinical therapies. A patient's own macrophages can be easily isolated, manipulated, and re-injected. Because macrophages home to areas of pathology by design, this type of clinical intervention is quite reasonable. Clearly such therapies would rely on a detailed understanding of what macrophages normally do to affect homeostatic and disease-associated processes. For instance, novel treatments for hypertension could arise from our new understanding of how macrophages lower blood pressure [52]. Similar treatment strategies could be explored to address other diseases discussed in this review including cancer, diabetes, obesity, and atherosclerosis. In this light, more studies are certainly warranted to discover new ways in which macrophages function—they are present in nearly every tissue regardless of infection status. Understanding the mechanisms by which macrophages affect such a diverse array of biologic processes will open the door to a new understanding of both development and homeostasis and lay the groundwork for new therapeutic approaches.

Glossary

Phagocytosis	the absorption of extracellular particles into a cell, a process performed in higher organisms by professional phagocytes such as macrophages
Myeloid cells	a class of immune cells including macrophages, microglia, dendritic cells, and granulocytes (neutrophils, basophils, and eosinophils). These cells were classified together because they were thought to arise from the same precursor cell

Microglia	a specialized macrophage found in the central nervous system that is now recognized to have distinct embryonic origins from macrophages
Mononuclear phagocyte system (MPS)	a region of the immune system found predominately in the spleen and lymph nodes containing phagocytes such as macrophages that perform many functions including the phagocytosis of old blood cells
Monocyte extravasation	the process by which bone-marrow derived circulating monocytes move from the blood into the tissue
Anastomosis	the fusion of two distinct entities, often used when referring to blood vessels
Retinal vascular plexus	the network of blood vessels feeding the retina which consist of three interconnected parallel layers
Auditory and visual evoked potentials	chemical detection of neural activities that occur in response to audio of visual stimulation
Intracortical and cortical recordings	locations, either inside or on the surface of the cortex, where evoked potentials are detected
Atherosclerotic plaques	cholesterol rich deposits inside the wall of arteries that can obstruct blood flow or rupture and cause clot formation
Ischemic	an area that is deprived of necessary oxygen, usually by restricted blood-flow to the affected region
Hepatectomy	a procedure to remove the liver
CD11b-DTR transgenic mice	mice that have been genetically engineered to express the simian diphtheria toxin receptor (DTR) in CD11b-positive cells (macrophages and microglia). Since the endogenous mouse DTR is relatively insensitive to DT, injection of DT into these mice will preferentially destroy CD11b-positive cells
Liposomal clodronate	A chemical that is specifically delivered to phagocytic cells by the uptake of clodronate-loaded liposomes. Once engulfed by the phagocytic cell, the clodronate is released from the liposome carrier, accumulates, and induces apoptosis
Parenchymal cells	the non-stromal components of a tissue, namely the cells that make up the functional components of an organ
Opsonization	the process of coating or targeting an antigen that enhances phagocytosis of that antigen
Opsonin	the molecules that coat or target the antigen during opsonization, the most common of which is antibodies
Fcγ receptor	the phagocyte receptor that binds to the opsin and induces phagocytosis
IL-10	an interleukin that macrophages release to suppress or activate other adjacent immune cells

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Figure 1. Evolution of phagocytosis

The first eukaryotes were formed via endosymbiosis (essentially one bacteria phagocytosing another). Early multi-cellular organisms used phagocytosis to obtain nutrition. Later, these properties were coopted to form an innate immune defense. Eventually, designated phagocytes developed which were cells whose primary purpose was phagocytosis, as apposed to cells that developed the property when necessary.

Figure 2. The broad role of macrophages in development and during repair and regeneration Macrophages do much more than defend the host from foreign invaders. Macrophages are involved in a host of developmental processes that include determining cell fate (either by affecting what they become or by determining their survival), defining tissue morphogenesis (either directly or indirectly), and by patterning blood vessels. Importantly, many of the properties macrophages use during development are also at work during repair and regeneration. In this context, macrophages can become activated by a host of factors, and then assist in regeneration and/or perform standard immune functions.

Table 1

Myeloid cell function table

	Non-immune Myeloid Cell Function	Refs.
In Development	Initiating and/or responding to developmental apoptosis	[13, 14, 100, 101]
	Tissue patterning: regulation of blood vessel development	[10, 21, 22, 104]
	Tissue morphogenesis: bone development	[24, 105]
	Tissue morphogenesis: mammary duct development	[9, 25–27]
	Cell fate decisions: pancreatic beta-cell differentiation	[28, 32, 33]
	Neuron survival and circuit formation	[14, 34, 37–40]
	Adipose cell proliferation and survival	[24, 41–43]
In Homeostasis	Removal of short-lived hematopoietic cells	[46–48]
	Maintenance of blood pressure	[52]
	Nitric oxide homeostasis	[54, 55]
	Maintenance of reproductive function	[56–61]
	Lipid metabolism and atherosclerosis	[62–64]
In Repair and Regeneration	Repair of skin wounds: repair, regeneration, scar formation, fibrosis	[66–69]
	Role in muscle regeneration	[70, 71]
	Kidney repair and regeneration	[72]
	Liver injury and repair	[73, 89, 90]
	Regeneration of colon	[75, 76]
	Response to pancreatitis	[77]
	Vascular responses	[67, 74, 95]