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Macrophages in inflammation, repair and regeneration

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Abstract

Tissue injury triggers a complex series of cellular responses, starting from inflammation activated by tissue and cell damage and proceeding to healing. By clearing cell debris, activating and resolving inflammation and promoting fibrosis, macrophages play key roles in most, if not all, phases of the response to injury. Recent studies of the mechanisms underlying the initial inflammation and later tissue regeneration and repair revealed that macrophages bridge these processes in part by supporting and activating stem/progenitor cells, clearing damaged tissue, remodeling extracellular matrix to prepare scaffolding for regeneration and promoting angiogenesis. However, macrophages also have a central role in the development of pathology induced by failed resolution (e.g. chronic inflammation) and excessive scarring. In this review, we summarize the activities of macrophages in inflammation and healing in response to acute injury in tissues with differing regenerative capacities. While macrophages lead similar processes in response to tissue injury in these tissues, their priorities and the consequences of their activities differ among tissues. Moreover, the magnitude, nature and duration of injury also greatly affect cellular responses and healing processes. In particular, continuous injury and/or failed resolution of inflammation leads to chronic ailments in which macrophage activities may become detrimental.

Keywords: chronic inflammation, fibrosis, muscle, stem cell

REVIEW

Introduction

Healing processes following tissue injury can be broadly subdivided into regeneration and repair (Fig. 1) (1). Regeneration refers to the proliferation of cells and tissues to replace damaged and lost structures. Through complete regeneration, a lost or damaged tissue is completely reconstituted. By contrast, repair may restore some original structure, but the reconstitution is incomplete, and repair can cause structural derangement (1). Repair most often consists of a combination of regeneration and scar formation. The relative contributions of regeneration and scarring vary depending on the regenerative capacity of the tissue and the extent and nature of the injury (1). In tissues with high regenerative capacity, such as skeletal muscle and liver, complete functional regeneration of tissues can be obtained through regeneration of parenchymal cells (e.g., myocytes and hepatocytes). This entails differentiation of stem/progenitor cells and/or proliferation of existing parenchymal cells plus regeneration of the stroma (e.g. blood vessels through angiogenesis). In most tissues, however, complete restoration of intact tissues cannot be achieved, resulting in scar formation (2). Even in a tissue with high regenerative capacity, such as skin, where superficial wounds heal through regeneration of the epithelium, severe injuries heal through scar formation (1). In tissues with limited regeneration capacity, such as brain and heart, healing proceeds rapidly through processes of wound closure and fibrotic scarring at the expense of tissue structure and function (3, 4). The extent and nature of the injury (e.g. mild versus severe) greatly affect the consequences. In addition, persistent injury and/or failed resolution leads to chronic inflammation, which may result in fibrosis and tissue dysfunction (Fig. 1).

It will be apparent through the overview presented in the following sections that the sequence of events following an injury is to a substantial degree similar in all tissues, despite differences in the cells that form the tissues. If we consider skin wound healing in a typical injury model, the process entails three overlapping but distinct stages: (i) inflammation, (ii) cell proliferation and new tissue formation and (iii) remodeling and maturation (1, 2, 5). The inflammation phase is characterized by activation of the innate immune system, resulting

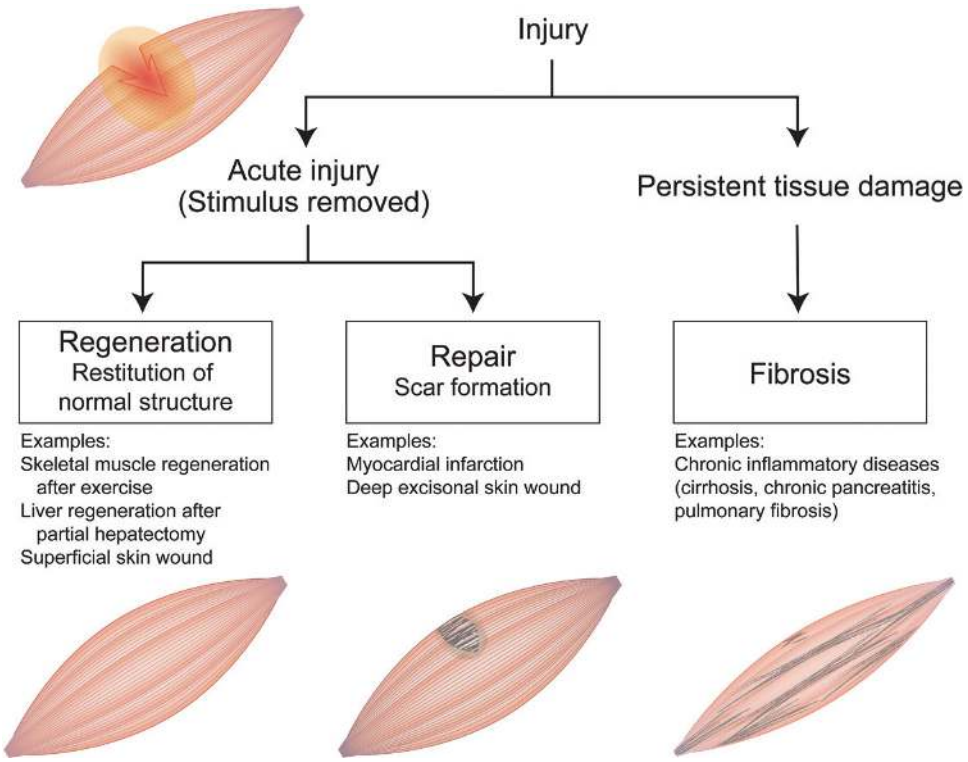

Fig. 1. Regeneration and repair after injury. Modified from a table in (1).

in an early influx of neutrophils followed by monocytes that may differentiate into macrophages. Macrophages and other immune cells not only clear debris and combat microbes, they also coordinate cellular processes that initiate the subsequent phase of new tissue formation, which will occur 2–10 days after the injury. During the tissue formation phase, granulation tissue composed of extracellular matrix (ECM) and newly formed blood vessels generated through angiogenesis fills the wound space. Proliferation and differentiation of parenchymal and stromal cells may then restore the integrity of the tissue. The last phase, remodeling and maturation, begins 2–3 weeks after injury and may last for years if scar tissue remains due to incomplete regeneration of the tissue. This phase is characterized by regression of blood vessels, resolution of inflammation and transformation of granulation tissue into scar tissue, within which ECM is remodeled from provisional ECM to a permanent collagenous matrix, leaving a mass that contains few cells and consists mostly of ECM.

Inflammation is a body's essential defense against damaged tissue and foreign bodies (6). Acute inflammation's primary goal is to eliminate injurious agents, such as microbes or dead cells. Once the injurious agents are eliminated, inflammation subsides. Although inflammatory processes may appear to have a damaging effect on tissues, which is especially evident in cases of chronic inflammation, inflammation is essentially protective and a prerequisite for repair and regeneration. If tissue integrity and homeostasis are restored, inflammation resolves during repair. However, if the wound healing responses are dysregulated or not properly resolved, inflammation can lead to the development of pathological fibrosis, which impairs normal tissue function and ultimately leads to organ failure (7).

Recent studies have shown that the cellular mechanisms and signaling pathways that control inflammation are interlinked with the mechanisms that control repair and regeneration. Indeed, it is inflammatory mechanisms that activate the processes of repair and regeneration. Immune cells, in particular, mediate processes extending from the initial inflammation to the healing and regeneration phases. Immune cells also mediate the restoration of normal tissue structure through communication with tissue-resident cells and function by clearing tissue debris, promoting angiogenesis, and supporting regeneration of parenchymal cells. Among immune cells, macrophages are a key cell type that not only perform clearing through phagocytosis, but also control such processes as angiogenesis and ECM remodeling, as well as inflammation. In fact, macrophages play important and diverse roles throughout most, if not all, stages of inflammation and healing, as well as the pathological remodeling that may contribute to disease processes.

Exhibiting highly plastic phenotypes and great diversity, macrophages are the major effecter cells mediating innate immunity. In addition to functioning in host defense, macrophages play important roles in tissue development, maintenance of tissue homeostasis and tissue regeneration (8–10). During the acute inflammation and subsequent healing processes triggered by injury, macrophages are essential for proper repair and recovery of homeostasis. However, under conditions of continuous insult due to genetic disorders (e.g. muscular dystrophy) or systemic metabolic dysregulation (e.g. obesity), their functions contribute to the pathological development of fibrosis, atherosclerosis, cancer and other chronic diseases. For that reason, studies of the

functions and regulatory mechanisms of macrophages are important for better understanding of both the protective and pathological roles of inflammation and regeneration.

In this review, we will discuss the mechanisms that underlie inflammation and regeneration/repair after tissue injury, focusing especially on macrophages as the cells executing and mediating diverse processes. We first present an overview of the activities of macrophages after injury and during regeneration of skeletal muscle, which is a well-studied model tissue with high regenerative potential. Macrophages are indispensable for skeletal muscle regeneration. We then discuss the functions of macrophages in tissues with differing regeneration potentials: liver, kidney and heart. Macrophages appear to perform similar tasks over the course of responses to tissue injury irrespective of tissue types; that is, clearing debris, remodeling ECM and promoting angiogenesis and scar formation. At the same time, however, the actions of macrophages can also greatly change depending on microenvironmental demands or signals. Consequently, their actions often may be tissue- and injury-specific. For instance, they promote proliferation and differentiation of stem/progenitor cells for regeneration in skeletal muscle, while they contribute to rapid healing with fibrosis in the heart. In this review, our focus is on sterile inflammation triggered by acute injury; we do not cover the functional properties of macrophages dealing with pathogens. In addition, we will discuss the mechanistic link and coordination between inflammation and repair/regeneration.

Macrophages in skeletal muscle regeneration

Satellite cells and muscle tissue-resident macrophages

Skeletal muscle is the dominant organ for locomotion, postural maintenance and energy metabolism in mammals. Skeletal muscle has a remarkable capacity for repair and regeneration in response to injury and disease. Although muscle-specific, resident muscle stem cells [also called satellite cells (SCs)] committed to the myogenic lineage play a crucial role in muscle regeneration by differentiating into replacement muscle fibers, the interplay between SCs and neighboring stromal cells, including immune cells, fibroblasts and vascular cells, is also necessary for proper tissue regeneration (11). Functional links between muscle regeneration and inflammation after muscle injury have been suggested for decades, and recent studies indicate there is tight coordination between inflammatory and regenerative processes. Macrophages play key roles in that coordination and are essential for recovery of the tissue integrity and function after muscle injury (12).

In the steady state, SCs are quiescent and located at the surface of muscle fibers, between the plasma membrane and the basal lamina surrounding muscle fibers (Fig. 2). SCs are activated by muscle damage, after which they undergo rapid proliferation for several days (Fig. 3). The descendants of the activated SCs, called myogenic precursor cells (MPCs) or myoblasts, undergo multiple rounds of division, differentiate and fuse together to form muscle fibers or fuse with existing muscle fibers (13, 14). Activated SCs also generate progeny that restore the pool of quiescent SCs. SCs and their progeny

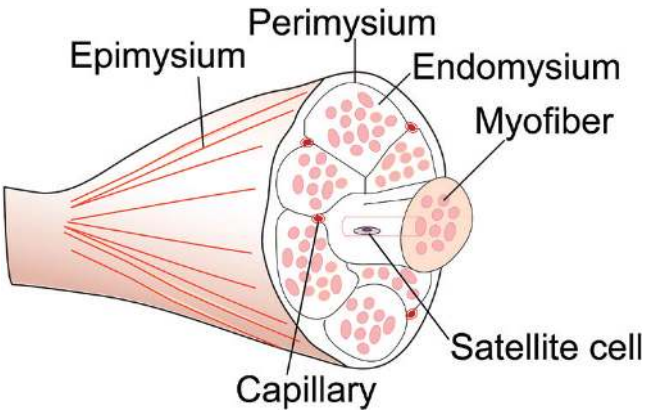

Fig. 2. Skeletal muscle anatomy. Quiescent muscle stem cells, SCs, are located between the plasma membrane and the basal lamina surrounding the myofiber. Tissue-resident macrophages are localized in the interstitial space within skeletal muscles, particularly in perimysium (the connective tissue surrounding muscle fascicles) and the epimysium (the connective tissue surrounding the whole muscle). Tissue-resident macrophages are often localized near capillaries.

are indispensable for muscle regeneration (15). For example, SC depletion blocks muscle regeneration (16), and impaired SC differentiation severely delays muscle regeneration and prolongs inflammation after muscle injury (17).

Tissue-resident macrophages are localized in the interstitial space between myofibers, particularly in the perimysium (the connective tissue surrounding muscle fascicles), epimysium (the connective tissue surrounding the whole muscle, Fig. 2) and perivascular space (21–24). Like SCs, resident macrophages are in a quiescent state in healthy muscle (15) but are rapidly activated by injury, including that induced by exercise. Although the number of resident macrophages is relatively small in healthy muscle (approximately one macrophage per five myofibers), their number can be rapidly increased by exercise (25). In addition, it has been suggested that resident macrophages interact with SCs and maintain an undifferentiated quiescent phenotype through direct communication or soluble factors in healthy muscle, but details of the mechanisms involved have not yet been elucidated.

Sequential process of muscle regeneration

While muscle inflammation is induced by acute and chronic damage such as physical injury or muscular dystrophy, it is also a common physiological response to exercise (26), which highlights the notion that the muscle inflammatory response is crucial for maintenance of muscle homeostasis. The most commonly used models of muscle injury, inflammation and repair include administration of myotoxic agents (cardiotoxin, notexin), chemicals (barium chloride) and physical procedures (freeze injury) in mice (27). While the spatiotemporal trajectories of regenerative processes differ among models, presumably due to differences in the magnitude of the injury and effects on immune cells (15, 27), these models similarly cause initial necrosis that is followed by inflammation and regeneration. Here, we mainly focus on results obtained with cardiotoxin-induced muscle injury because the inflammatory response has been best studied in that model.

Following the initial necrosis of myofibers, SCs are activated (Fig. 3). At the same time, immune cells, including neutrophils and macrophages, infiltrate the injured tissue, and necrotic myofibers are cleared through phagocytosis. The inflammatory response is coupled temporally and spatially to the initial stage of myogenesis, wherein activated SCs begin to proliferate and commit to differentiate into myocytes, which suggests a close link between myogenesis and the inflammatory response. Consistent with this linkage, the earliest stage of regeneration begins within the post-injury debris and inflammatory lesions, which are dominated by neutrophils and pro-inflammatory macrophages (15). During the next phase of muscle regeneration, small myofibers are formed through fusion of newly differentiated myocytes, while inflammation subsides. Angiogenesis is also activated (15), and the new myofibers grow in size and mature. Complete regeneration is attained within 1 month.

Immune cell accumulation within injured tissue

Neutrophils are the first non-resident cells to respond to skeletal muscle injury; they appear within 1–3 h after injury (Fig. 4) (18, 28). They are most likely recruited by resident macrophages, which secrete various chemokines, including CCL2 and CXCL1, as well as damage-associated molecular patterns (DAMPs) such as high-mobility group box 1 protein (HMGB1) (24, 29, 30). Neutrophil numbers reach a peak 12–24 h post-injury, after which they decline over a period of 3–4 days (28).

Depletion of neutrophils delays muscle regeneration following injury, demonstrating their contribution to muscle regeneration (31). In the absence of neutrophils, levels of necrotic debris are increased, suggesting that phagocytotic removal of debris by neutrophils is important for subsequent regeneration. In sharp contrast, in a contraction-induced muscle

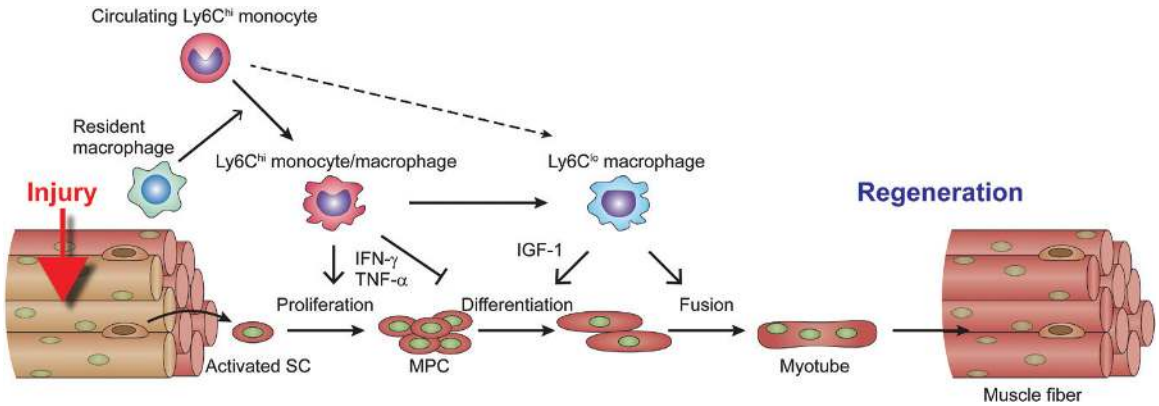

Fig. 3. Muscle regeneration and macrophages. Macrophages control proliferation, differentiation and maturation of myocytes during regeneration. The sequential changes in macrophage functionality are coordinated with the sequence of myocyte regeneration. Macrophages within injured muscle are derived from circulating Ly6C^{hi} monocytes. Early after injury (e.g. day 1 post-injury), Ly6C^{hi} cells, which may include monocytes, monocytes in the process of differentiating into macrophages and macrophages, predominate. After days 2–3, however, Ly6C^{lo} macrophages predominate. Lineage tracing studies indicate that Ly6C^{lo} macrophages are derived from early recruited Ly6C^{hi} cells (18, 19), but it is unclear whether Ly6C^{hi} cells that have differentiated into macrophages are converted to Ly6C^{lo} macrophages (20). It is possible that circulating Ly6C^{hi} monocytes directly differentiate into Ly6C^{lo} macrophages.

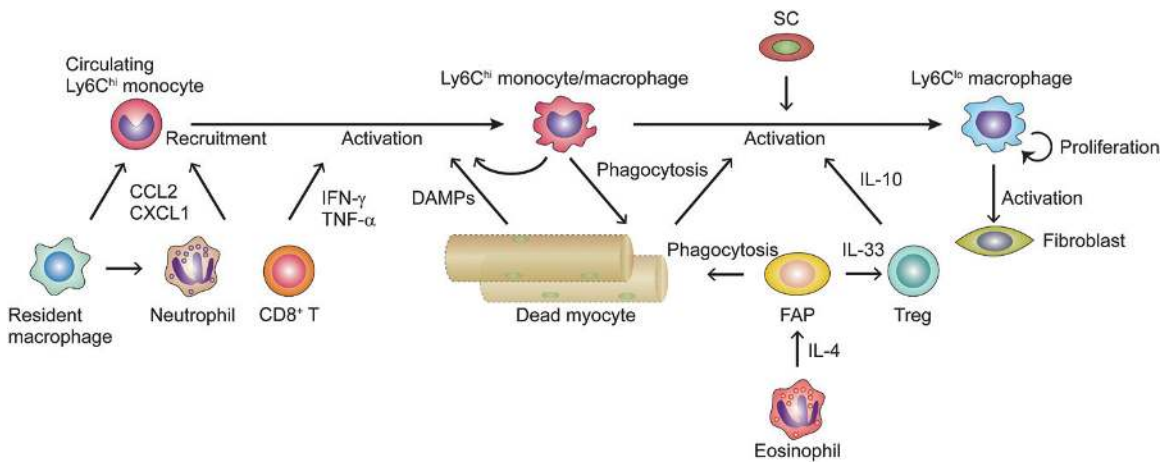

Fig. 4. Cell interactions during muscle regeneration. During muscle repair, macrophages interact with other immune cells, fibroblasts, FAPs and vascular endothelial cells in addition to the myocyte precursor cells differentiated from SCs. These reciprocal interactions coordinate inflammatory and repair processes.

injury model in CD18 (*Itgb2*)-deficient mice, which exhibit severely impaired neutrophil recruitment to injured muscle, muscle fiber injury and macrophage accumulation are reduced early after injury (3 days) and fiber regeneration is enhanced later (7 days) (32). This suggests a pathological effect of neutrophils. Together, these results suggest neutrophils contribute to a very early step toward regeneration by clearing debris and recruiting immune cells, such as monocytes, but they may also promote muscle damage (33, 34). It is also possible that neutrophil-induced muscle damage is required for preparation of the tissue for later regeneration, though this idea remains largely untested (33).

Following the onset of neutrophil invasion, circulating monocytes are recruited to the injured muscle tissue (Fig. 4). Of the two monocyte subpopulations (Ly6C^{hi} inflammatory monocytes and Ly6C^{lo} resident/patrolling monocytes) in the circulation, Ly6C^{hi} inflammatory monocytes enter the damaged muscle tissue, where they may differentiate into macrophages that change their phenotype and functionality over the course of inflammation and regeneration (Fig. 3) (18, 19). Ly6C^{lo} monocytes do not differentiate into macrophages within injured muscle (18, 19). Macrophage numbers continuously increase for 48 h after injury. The numbers then gradually decline, but they remain elevated throughout the processes of inflammation and regeneration (15). When the regeneration processes are complete, macrophage numbers return to homeostatic levels.

At least two macrophage subpopulations are involved (18, 35). Soon after injury, $CX_3CR1^{lo}Ly6C^{hi}$ (hereafter called Ly6C^{hi}) monocytes/macrophages derived from circulating Ly6C^{hi} inflammatory monocytes invade the injured tissue and reach peak numbers on day 1 or 2 after injury. As Ly6C^{hi} macrophages start to decline 2 days after injury, the numbers of $CX_3CR1^{hi}Ly6C^{lo}$ (Ly6C^{lo}) macrophages are increasing, and after day 3, they are the predominant macrophage population (18).

In mouse models where the accumulation of monocytes/macrophages within injured muscle is inhibited, muscle regeneration is impaired. The models include *Ccr2*$^{-/-}$ and *Ccl2*$^{-/-}$ mice (28, 36, 37), neutralization of granulocyte/macrophage colony-stimulating factor receptor (38), clodronate liposome-mediated monocyte/macrophage depletion (18, 39, 40) and depletion of CD11b^{+} monocytes/macrophages in CD11b-DTR (diphtheria toxin receptor) mice (41), muscle regeneration is impaired. This clearly demonstrates that monocytes/macrophages are essential for proper muscle regeneration following muscle injury.

Although myeloid cells dominate within injured muscle, lymphocytes also accumulate and play important roles. Conventional CD4^{+} T cells as well as Foxp3^{+}CD4^{+} regulatory T cells (T_{reg} cells) accumulate over a time-course similar to that of Ly6C^{lo} macrophages and reach peak numbers 4–5 days post-injury (42). The CD3^{+} T-cell fraction increases to ~35% of total CD45^{+} leukocytes by day 5 post-injury (42). Depletion of T_{reg} cells delays repair and prolongs inflammation (43). Muscle T_{reg} cells induce SC expansion in part through production of amphiregulin (42, 43). CD8^{+} T cells also accumulate within injured muscle and are thought to promote the accumulation of Ly6C^{hi} macrophages (44). In CD8^{+}-null (*Cd8a*$^{-/-}$) mice, SC proliferation is impaired and regeneration is delayed, demonstrating that CD8^{+} T cells are important members of the immune cell network regulating muscle regeneration.

Macrophage subtypes

The M1/M2 dichotomy of macrophages is a widely applied concept. However, that dichotomy was established based mainly on observations of cultured macrophages and applying the dichotomy to classify macrophages *in vivo* is not straightforward. In an *in vitro* setting, exposure to Toll-like receptor ligands or T_h1 cytokines, such as TNF-α and IFN-γ, polarizes macrophages into the pro-inflammatory M1 phenotype. M1 activation increases expression of pro-inflammatory cytokines and production of reactive oxygen species. By contrast, T_h2 cytokines such as IL-4 and IL-13 induce the M2 phenotype, which may have anti-inflammatory and wound healing functions (9).

Within injured muscle tissues, Ly6C^{hi} macrophages express higher levels of several pro-inflammatory cytokines, including TNF-α and IL-1β, while Ly6C^{lo} macrophages express higher levels of anti-inflammatory cytokines, including IL-10 and TGF-β (18). Accordingly, Ly6C^{hi} and Ly6C^{lo} macrophages are considered M1 and M2 type, respectively (15, 45). However, it is now clear that the M1/M2 dichotomy is not sufficient to describe the diverse phenotypes and functions of macrophages *in vivo* (46), where both M1 and M2 markers can be expressed simultaneously (47). Moreover, a recent transcriptomic study showed that the expression levels of most established M1 and M2 markers do not significantly differ in Ly6C^{hi} and Ly6C^{lo} macrophages in injured skeletal muscle (35, 48). Thus, the gene expression profiles of macrophages during skeletal muscle injury/repair are distinct from the *in vitro* M1 and M2 expression profiles. Consequently, the functions of muscle Ly6C^{hi} and Ly6C^{lo} macrophages cannot be deduced from the functional characteristics of *in vitro* M1 and M2 macrophages.

Studies of the signaling pathways active in macrophages within injured skeletal muscle also suggest that *in vivo* activation signals differ from *in vitro* M1/M2 stimuli. For instance, in regenerating muscle, STAT1, the major transcription factor downstream of IFN-γ receptor, is dispensable for expression of several IFN-γ-responsive genes identified *in vitro* (35). In addition, Ly6C^{lo} macrophages within regenerating muscle do not express higher levels of canonical M2 markers induced by IL-4 *in vitro* (35). Moreover, the absence of STAT6, the major downstream mediator of IL-4 signaling, does not affect muscle regeneration (49). Consistent with this, myeloid-specific deletion of *Il4ra* (α subunit of IL-4 receptor, which binds IL-4 and IL-13) does not affect muscle regeneration (50). This indicates the IL-4/IL-13 signal is dispensable for muscle regeneration and, presumably, for macrophage activation during regeneration. In addition, while previous studies have shown that hypoxia-inducible factors (HIFs) are important for M1/M2 gene expression *in vitro* (51), myeloid-specific deletion of *Hif1a* or *Hif2a* does not affect accumulation of Ly6C^{hi} and Ly6C^{lo} macrophages or regeneration of cardiotoxin-injected muscle (52). These results strongly suggest that *in vivo* macrophage activation stimuli and mechanisms are diverse and can differ from those seen *in vitro* with model stimuli.

Taking these limitations of the M1/M2 dichotomy in consideration, here we will distinguish macrophage subsets in the tissues mainly using surface marker phenotypes (e.g. Ly6C^{hi} and Ly6C^{lo}) when the marker expression patterns are well characterized. In tissues where surface marker patterns are not well documented, we will use M1 and M2 in accordance with the literature to distinguish pro-inflammatory cells from pro-resolution and/or profibrotic macrophages. That said, the consequences of the well-organized pro-inflammatory activity of M1 macrophages is to promote healing and regeneration. By contrast, continuous activation of M2 macrophages may impair tissue regeneration and function by promoting fibrosis. In an *in vivo* setting, therefore, the simple characterization of M1 as bad and M2 as good is inaccurate, to say the least. It is also worth noting that the elapsed time after a muscle injury has a much stronger impact on transcriptomes than the status of the Ly6C$^{hi/lo}$ subsets (35). The most significant differential changes were found between days 2 and 4 post-injury. This corresponds to the start of inflammation resolution, which indicates that both Ly6C^{hi} and Ly6C^{lo} macrophages change their functions during this transition from inflammation and resolution. It is, therefore, likely that the later functions of M1 (or Ly6C^{hi}) and M2 (or Ly6C^{lo}) macrophages differ from their earlier functions. In addition, because Ly6C^{lo} macrophages may arise from Ly6C^{hi} cells, the transition of the surface marker phenotype (e.g. Ly6C^{hi} to Ly6C^{lo}) may compromise clear identification of subpopulations. Collectively then, identification of subpopulations of macrophages can pose technical difficulties due in part to a lack of sufficient surface markers.

More importantly, a subpopulation identified with a set of surface markers may have different functions in different contexts (e.g. different tissues). As we will see in the following sections, over the course from injury to repair, macrophages often switch their phenotype from Ly6C^{hi} to Ly6C^{lo}. In many tissues, including skeletal muscle and renal tissues, Ly6C^{hi} macrophages generally promote inflammation, while Ly6C^{lo} macrophages promote fibrosis. Similarly, in the carbon tetrachloride (CCl_4)-mediated liver injury model, macrophages change their surface phenotype from Ly6C^{hi} to Ly6C^{lo} during the progression from injury to repair. However, although Ly6C^{hi} macrophages in liver produce pro-inflammatory cytokines like Ly6C^{hi} macrophages do in skeletal muscle, they also promote fibrosis (53, 54). Interestingly, Ly6C^{lo} macrophages promote matrix degradation and debris clearance, crucially contributing to scar resolution. As such, in the liver CCl_4 injury model, Ly6C^{hi} macrophages are pro-inflammatory and profibrotic, while Ly6C^{lo} macrophages are pro-resolution and antifibrotic. In addition, these liver macrophages express mixed M1/M2 marker genes. Clearly, a small number of surface markers is not sufficient to identify the various macrophage subsets and functions. These functional differences in Ly6C^{lo} and Ly6C^{hi} macrophages in different injury models highlight the functional diversity of macrophages with different microenvironments.

Sequential changes in macrophage function after muscle injury

Soon after injury, Ly6C^{hi} monocytes/macrophages derived from circulating Ly6C^{hi} inflammatory monocytes infiltrate the injured muscle area (Fig. 3) (18). These cells exhibit a non-dividing, F4/80^{lo}, pro-inflammatory phenotype and express pro-inflammatory cytokines such as IL-1β and TNF-α (18). Pro-inflammatory macrophages are localized near MPCs and stimulate them to migrate, proliferate and differentiate into myotubes, but inhibit their maturation and fusion (Fig. 3).

CCL2/MCP-1 is a key signaling molecule that recruits monocytes (Fig. 4). Deletion of *Ccl2* and its receptor gene *Ccr2* severely reduce accumulation of monocytes/macrophages in injured muscle tissues (18, 36, 37, 55). CCL2 is expressed by MPCs, injured fibers, resident macrophages and bone marrow-derived cells, particularly monocytes/macrophages (24, 28, 56). In addition, accumulation of Ly6C^{hi} macrophages is reduced in CD8^{+} T-cell-null mice (44). CD8^{+} T cells are situated adjacent to macrophages within injured muscle tissue and co-culture experiments indicate they activate production of CCL2 in neighboring macrophages, which suggests CD8^{+} T cells contribute to the accumulation of Ly6C^{hi} macrophages.

While pro-inflammatory Ly6C^{hi} macrophages mediate inflammation within injured muscle tissues (40), they are also crucial to later muscle regeneration. For instance, several models have shown that suppression of the initial accumulation of monocyte-derived macrophages impairs muscle regeneration and clearance of necrotic tissues (18, 28, 55, 57, 58). Accordingly, one important macrophage-mediated process at the inflammatory stage appears to be phagocytotic removal of dead cells and other debris (Fig. 4). However, macrophages' contributions to early processes that lead to muscle regeneration are not limited to phagocytosis.

Previous studies have shown that Ly6C^{hi} macrophages stimulate MPC proliferation and inhibit their differentiation and fusion *in vitro* (Fig. 3) (18, 56, 59). For example, *in vitro*-activated M1 pro-inflammatory macrophages promote expansion of MPCs, while M2-activated macrophages promote their differentiation and maturation (18, 60). *In vivo* observations also support the differential effects of Ly6C^{hi} and Ly6C^{lo} cells on MPCs. In an *in vivo* human muscle regeneration model, macrophages expressing M1 or M2 markers co-localized in regenerating areas 7 days after injury. Whereas macrophages expressing M1 markers, NOS2 and COX2, are preferentially associated with proliferating MPCs, and macrophages expressing the M2 marker CD206 are associated with differentiating myocytes (60). In addition, as will be discussed in the following section, cytokines and other mediators from Ly6C^{hi} and Ly6C^{lo} macrophages differentially affect MPCs. These findings support the notion that sequential changes in macrophage phenotype and function guide the progression of myocyte regeneration (Fig. 3).

After the peak of Ly6C^{hi} macrophage accumulation 1–2 days after injury, the Ly6C^{hi} macrophage numbers decline, and Ly6C^{lo} macrophages become the predominant macrophage subtype (18, 48). This transition of Ly6C^{hi} to Ly6C^{lo} cells coincides with the progression of biological processes from inflammation to resolution and regeneration (15). During this phase of the response to muscle injury, Ly6C^{lo} macrophages stimulate myogenic commitment of MPCs and promote the differentiation, maturation and fusion of muscle fibers. Ly6C^{lo} macrophages express higher levels of anti-inflammatory cytokines, such as IL-10, thereby contributing to the resolution of inflammation (18, 24).

Latex-labeling of circulating monocytes showed that while newly recruited cells exhibit a Ly6C^{hi}CX3CR1^{lo} surface phenotype, that changes to a Ly6C^{lo}CX3CR1^{hi} phenotype beginning 2 days post-injury (18). This suggests a phenotypic transition from pro-inflammatory Ly6C^{hi} cells to anti-inflammatory Ly6C^{lo} macrophages within muscle, though it is also possible that Ly6C^{hi} monocytes directly differentiate into Ly6C^{lo} macrophages. In contrast to Ly6C^{hi} cells, Ly6C^{lo} macrophages also proliferate *in situ*, and that proliferation contributes to a large increase in the number of Ly6C^{lo} macrophages (18). Because Ly6C^{hi} monocyte/macrophage recruitment appears to mainly occur early after injury (within 2–3 days after injury), it appears that Ly6C^{lo} macrophage proliferation contributes to the expansion of Ly6C^{lo} macrophages beginning around day 3 post-injury.

Coordinated transition from Ly6C^{hi} pro-inflammatory to Ly6C^{lo} pro-resolution/reparation macrophages is important for proper regeneration. Disruption of that transition by blocking early inflammation using IL-10 or anti-IFN-γ antibody, or late resolution due to treatment with anti-IL-10, compromises muscle regeneration (61, 62). Sequential transition of macrophage phenotype from M1 to M2 has also been observed during human muscle regeneration (59). These findings support the notion that proper temporal transition of macrophages is necessary for regeneration of damaged muscle (58). Once differentiation and fusion are complete, the number of macrophages declines to a very low level (18, 22).

In addition to newly recruited monocyte-derived macrophages, there are resident macrophages within skeletal muscle. The majority of these exhibit a F4/80^{+}Ly6C^{-}CX3CR1^{-} surface phenotype (24). After muscle injury, the resident macrophages express high levels of CCL2 and CXCL1 chemokines to recruit monocytes and neutrophils. TNF-α signaling appears to be particularly important for induction of CCL2 and CXCL1 (63). Selective depletion of resident macrophages greatly reduces the number of macrophages within injured muscle 24 h post-injury. Resident macrophages thus appear to contribute to the initial recruitment of neutrophils and monocytes to injured muscle tissues.

In several tissues, including the brain and liver, tissue-resident macrophages derive from embryonic progenitor cells and are maintained through local self-renewal independently of bone marrow contribution (64, 65). The origin of skeletal muscle resident macrophages remains unknown. Also unclear is the fate of resident macrophages after muscle injury. It may be that monocyte-derived cells replenish resident macrophages, as is observed in the heart (66), but this model needs to be tested.

Cytokine link between inflammation and regeneration

Cytokines are key mediators that act to coordinate the processes of inflammation and repair in inured muscle. Pro-inflammatory cytokines not only activate inflammation, they also initiate repair processes, which highlights the close interlink between inflammatory and repair/regeneration mechanisms. Dysregulation of cytokine signals disrupts this coordinated process and can lead to pathology, as will be discussed in later sections.

Ly6C^{hi} macrophages express such pro-inflammatory cytokines as IFN-γ, TNF-α, IL-1β and IL-6, which may promote inflammation and tissue injury (Fig. 3). However, they are also integral to regeneration by MPCs. For instance, IFN-γ is markedly increased in the injured tissue on days 1–5 post-injury (61) and plays a key role in the earliest stage of regeneration (15). IFN-γ may reinforce the pro-inflammatory macrophage phenotype (46) and directly regulate expression of genes that suppress muscle differentiation in MPCs and support MPC proliferation. Blocking IFN-γ signaling impairs macrophage accumulation and muscle regeneration (61). Inhibition of myogenesis by IFN-γ is mediated by major histocompatibility complex class II transactivator (CIITA) and Polycomb repressive complex 2 (PRC2), which epigenetically suppress myogenin-dependent muscle genes (67, 68).

Inhibition of TNF-α signaling also impairs muscle regeneration (69). TNF-α expression peaks 24 h post-injury in muscle tissues, and inflammatory cells are the primary source (69). Because TNF-α activates pro-inflammatory genes in macrophages (46, 63), it promotes the initial inflammatory response to muscle injury. However, TNF-α signaling also controls SC fate, in part by epigenetically repressing *Notch1* and *Pax7* expression. Notch signaling is required for maintenance of SCs in a quiescent state, and its attenuation leads to commitment of SCs to becoming MPCs (70–72). ADAMTS1, a metalloproteinase secreted from Ly6C^{hi} macrophages, inhibits Notch signaling by targeting NOTCH1 in SCs, leading to SC proliferation (73). Thus, Ly6C^{hi} macrophages suppress Notch signaling via TNF-α and ADAMTS1 production, which in turn activates SC proliferation.

Pax7 is a master regulator of SCs that controls their growth and proliferation while repressing genes important for muscle differentiation (74). Pax7 expression is repressed in SCs as they differentiate into myotubes (75). Consequently, TNF-α-mediated repression of *Notch1* and *Pax7* promotes SC activation and may prepare them for transition to differentiation (15). Activation of IFN-γ and TNF-α signaling thus drives pro-inflammatory macrophage activation but also promotes expansion of MPCs and prepares them for myogenic differentiation (15).

In the cardiotoxin injury model, IL-6 is abundantly expressed by infiltrating monocytes/macrophages from day 1 post-injury, and its expression continues up to day 7 (76, 77). Systemic deletion of *Il6* suppresses inflammation and impairs MPC proliferation and muscle regeneration. IL-6 is also produced by fibroadipogenic progenitors and SCs and may contribute to MPC proliferation and differentiation (78–80). Though these results are indicative of the pro-regenerative function of IL-6, inhibition of IL-6 receptor signaling using an antibody accelerates skeletal muscle regeneration and suppresses fibrosis after cardiotoxin injury (77). This highlights the context-dependent function of IL-6 in response to muscle injury.

Double *Il1a* and *Il1b* knockout delays muscle regeneration after cardiotoxin injury (81). Immune cell infiltration and local expression of IL-6 are suppressed, and Pax7^{+} MPC proliferation is delayed. Interestingly, *Il1* deletion from SCs suppresses their proliferation, and that effect is reversed by exogenous IL-1β, demonstrating that IL-1 expression in non-immune cells is also important for muscle repair.

As described above, phagocytotic Ly6C^{hi} macrophages accumulating early after injury are replaced by less-phagocytotic Ly6C^{lo} macrophages (82) beginning on day 2 post-injury and peaking on day 4–7 (15). This transition is coupled with transition from the inflammatory phase to the regenerative phase. Cytokine production also changes as regeneration proceeds. While the early inflammatory phase is characterized by expression of IFN-γ, TNF-α and CCL2, the later phases are characterized by increased expression of anti-inflammatory cytokines and growth factors, such as IL-10, TGF-β and IGF-1 (15).

IL-10 expression begins on day 1 post-injury and peaks around day 3 (83, 84). Deletion of *Il10* decreases expression of the M2 marker genes *Arg1* and *Cd163* on day 4 post-injury, which points to the involvement of IL-10 in the transition of macrophage phenotype and function (84). In addition, muscle regeneration is delayed by deletion of *Il10*. These results indicate that IL-10 is important for progression of the resolution and regeneration phases. Interestingly, skeletal muscle regeneration is also impaired when macrophages are prematurely skewed toward pro-resolution phenotypes through early treatment with IL-10 or genetic loss of mitogen-activated protein kinase phosphatase 1 (MKP-1), which restricts p38 MAPK activation (62). Apparently, premature initiation of the anti-inflammatory program can impair repair, which is indicative of the importance of the precise timing and coordination of the responses. Consistent with that idea, *ex vivo* experiments showed that IL-10 canceled the pro-proliferative effect of TNF-α on SCs when the cells were simultaneously treated with the two cytokines. Conversely, co-stimulation with IL-10 and TNF-α cancelled the differentiation-promoting effects of IL-10. These results demonstrate that timely, sequential expression of pro- and anti-inflammatory cytokines produced by differentially activated macrophages is essential for proper tissue healing and regeneration (62).

IGF-1 is abundantly expressed by Ly6C^{lo} macrophages within injured muscle, and its concentration peaks on day 3 post-injury (85). *In vitro*, IGF-1 promotes differentiation of SCs and, to a lesser degree, their proliferation (86). Local administration of IGF-1 to injured muscle in *Ccr2*$^{-/-}$ mice, where accumulation of both Ly6C^{hi} and Ly6C^{lo} macrophages and IGF-1 levels are greatly reduced, ameliorates the impaired muscle regeneration phenotype (85). This suggests that IGF-1 derived from Ly6C^{lo} macrophages is a crucial contributor to muscle regeneration. Similarly, muscle-specific overexpression of *Igf1* accelerates regeneration of cardiotoxin-injured muscle (87). Notably, in that *Igf1* overexpression model, levels of TNF-α and IL-1β are decreased, particularly on day 5 post-injury, which suggests an association between inflammation and repair that is modulated by IGF-1.

Regulation of macrophage phenotype transition

Transition from an environment dominated by IFN-γ and TNF-α to one rich in anti-inflammatory cytokines (e.g. IL-10 and TGF-β) and growth factors (e.g. IGF-1) may support macrophage functional transition (Table 1) (84, 88). The accumulation of T_{reg} cells coincides with the transition of the macrophage phenotype, suggesting T_{reg} cells may contribute to the transition via IL-10 and other mediators. Panduro *et al.* recently reported that early depletion of T_{reg} cells after injury promotes IFN-γ production in NK and effector T cells, which results in enhanced inflammatory activation of macrophages (89). In *mdx* mice, a mouse model of Duchenne muscular dystrophy in which the mice do not express dystrophin, T_{reg} cell depletion exacerbates muscle inflammation and modulates CD206 expression in Ly6C^{lo} macrophages (90). This suggests that T_{reg} cells regulate macrophage activity in skeletal muscle.

Table 1. Factors driving macrophage functional transition

Macrophage type	Inflammation	Resolution/repair
Microenvironment	DAMPs (30, 91, 92), fibrin (93), IFN-γ (61, 89), TNF-α (63)	IL-10 (84), IGF-1 (85, 87, 94), phagocytosis of dying cell (95)
Immune cell	Neutrophil (31), CD8$^+$ T (44), resident macrophage (63)	T_{reg} (90)
Cellular metabolism	Glycolysis (35)	OXPHOS (35)

Summary of the known environmental and cell-autonomous factors that control macrophage phenotypes after skeletal muscle injury. Note that many other environmental factors could be involved in the phenotypic transition within skeletal muscle. Moreover, in different tissues and different pathologies, the factors are very likely to be different.

IGF-1 is mainly expressed by monocytes/macrophages in injured muscle and myeloid-specific deletion of *Igf1* decreases numbers of CD206$^+$ macrophages, indicating its involvement in macrophage phenotype transition (85, 94). IL-1β expression by pro-inflammatory macrophages may also be important for later expression of IL-10 and TGF-β (62).

Phagocytosis of cell debris also greatly modulates macrophage gene expression (18). Engulfment of dead MPCs decreases production of TNF-α and increases production of TGF-β in cultured human monocyte-derived macrophages. Similarly, mouse bone marrow-derived macrophages switch their marker gene expression from M1 (NOS1, CCL3) to M2 (CD163, CD206 and TGF-β1) upon engulfment of apoptotic MPCs (95). AMP-activated protein kinase (AMPK) signaling is important for mediating the phenotype transition triggered by phagocytosis, and myeloid *Ampka1*$^{-/-}$ (α1 subunit of AMPK) suppresses expression of M2 marker genes in macrophages and impairs the shift from Ly6C^{hi} toward Ly6C^{lo} macrophages on days 2–3 after muscle injury (95). These results are consistent with the notion that phagocytosis of cell debris drives phenotypic switching from pro-inflammatory to pro-resolution.

In addition, recent studies have shown that phenotypic transition in macrophages coincides with marked changes in cellular metabolism. Metabolic reprogramming is reportedly crucial for activation of macrophages (96). Not only do metabolic pathways provide energy, they also provide precursors for biosynthesis of macromolecules essential for the tasks immune cells perform. Moreover, metabolic reprogramming supports the epigenetic changes that occur during differentiation and activation (97).

In vitro, M1 pro-inflammatory activation by lipopolysaccharide enhances glycolysis and suppresses oxidative phosphorylation (OXPHOS), while M2 activation by IL-4 activates OXPHOS (98, 99). Although glycolysis is less effective for

producing ATP than OXPHOS, it has been suggested that a switch toward glycolysis is important for rapid activation of ATP production and the generation of the biosynthetic intermediates required for inflammatory cytokine production (99). Because macrophages can proliferate within tissues, the activation of glycolysis may also be required for their proliferation *in vivo*. Other metabolic pathways, including a subset of the tricarboxylic acid (TCA) cycle, the pentose phosphate pathway and amino acid metabolism are also activated in M1 macrophages, and the metabolites from these pathways are important for increased production of reactive oxygen species, IL-1β and nitric oxide. As such, modulation of these cellular metabolic pathways is essential for execution of macrophage functions. And as M2 macrophages activate different metabolic pathways, differences in metabolic activation associate with differences in the functional activation of macrophages. Moreover, transcriptomic analyses after skeletal muscle injury indicate that genes associated with glycolysis are down-regulated during the period of transition from the inflammatory to the resolution phase (days 2–4 after cardiotoxin-mediated injury), while genes associated with OXPHOS and glutamine metabolism are up-regulated. This suggests that macrophages start to shift their metabolic programs before shifting their inflammatory status (35, 100).

In mouse and human macrophages, AMPK is a key hub that senses energy status and controls cellular metabolism (101, 102). AMPK is important for fatty acid oxidation (FAO), and it suppresses pro-inflammatory responses in macrophages *in vitro* (95, 103) and in obese adipose tissue and atherosclerosis (102, 104). Interestingly, *Ampka1* deletion impairs macrophage progression from the Ly6C^{hi} to Ly6C^{lo} phenotype after skeletal muscle injury (95). Moreover, AMPK-dependent activation of FAO induced by miR-33 inhibition is associated with up-regulation of M2 markers (105), while *Ampka1* deletion suppresses up-regulation of M2 markers in response to IL-4 or IL-10 in bone marrow-derived macrophages. This suggests that AMPK is important for anti-inflammatory M2-type activation and that AMPK-dependent changes in cellular metabolism contribute to phenotypic switching in macrophages. Consistent with that idea, myeloid *Ampka1* deletion in a cardiotoxin injury model leads to delayed muscle regeneration with decreased numbers of Ly6C^{lo} macrophages (95). In addition, although cellular metabolism responds to the microenvironment, it is also controlled in a cell autonomous manner via lipid metabolism, which affects macrophage activation states (106). Taken together, these findings highlight the close interlinkage between macrophage metabolism and function in inflammation and repair.

Interaction between macrophages and other cells

Fibro/adipogenic progenitors (FAPs) are a progenitor population that has bipotential to differentiate into both fibroblasts and adipocytes (79, 107). FAPs are activated upon muscle injury and support myogenic differentiation by producing IL-6 and IGF-1 (79). In a model of fatty degeneration, however, FAPs also gave rise to ectopic adipocytes within degenerating muscle (107) as well as fibroblasts that mediated fibrosis in *mdx* dystrophic mice (108). Interestingly, muscle damage results in rapid recruitment of eosinophils, which secrete IL-4 to activate FAP proliferation and inhibit their differentiation into adipocytes (50). IL-4 signaling thus promotes muscle regeneration via FAP activation (Fig. 4). In response to IL-4, FAPs proliferate and clear necrotic debris. Notably, in the same study, IL-4 signaling was dispensable for macrophage proliferation and muscle regeneration. The number of FAPs peaks 96 h after notexin-induced injury and then declines to pre-damage levels within 9 days (108). This rapid clearance of FAPs is induced by macrophages via TNF-α and is important for prevention of excessive fibrosis.

As discussed in the previous sections, macrophages greatly influence the behavior of SCs. The interaction between macrophages and SCs is reciprocal, and depletion of SCs delays phenotypic transition of Ly6C^{hi} to Ly6C^{lo} cells (48). SCs and MPCs also interact with endothelial cells and fibroblasts (109).

T_{reg} cells also contribute to muscle regeneration, in part by producing amphiregulin, which promotes MPC differentiation (43, 90). In *mdx* dystrophic mice, T_{reg} cell depletion exacerbates muscle inflammation and affects CD206 expression in Ly6C^{lo} macrophages (90), suggesting that their regulatory effects on macrophages in muscle are similar to those seen in other tissues.

Angiogenesis and fibrosis

Angiogenesis and vascular remodeling and maturation are essential for tissue regeneration (109). Inhibiting macrophage accumulation reduces angiogenesis (110, 111), demonstrating that macrophages contribute to the proper vascularization of regenerating muscle tissue. In addition, forced expression of peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α) in skeletal muscle induces angiogenesis. In this setting, PGC-1α promotes macrophage accumulation, which in turn activates angiogenesis (112).

After acute muscle injury, there is a transient increase in collagen deposition during the inflammatory phase, which is resolved later, during the resolution phase (113). In fact, efficient muscle repair requires the migration and proliferation of fibroblasts that produce new temporary ECM components. ECM is important for stabilization of the tissue, and acts as a scaffold for the new muscle fibers. SCs also utilize the basement membranes of pre-existing necrotic muscle fibers to ensure that new myofibers maintain similar positions. Accordingly, proper ECM production and remodeling are important for regeneration.

Macrophages promote fibrosis and its resolution. They also crucially contribute to ECM remodeling. Macrophages produce TGF-β1, which activates myofibroblasts that produce ECM (7). They may also promote fibrosis by influencing local immune cell activation toward type 2 inflammation. By contrast, macrophages may also produce matrix metalloproteinases (MMPs) and other degradative enzymes that affect ECM. Some MMP activity contributes to resolving fibrosis, while other activity appears to drive fibrosis (7, 114). Deletion of integrin-β3 (*Itgb3*) increases the CD206^{+} (M2-like) cell fraction among macrophages and enhances fibrosis after cardiotoxin-induced muscle injury (115). This suggests macrophages stimulate ECM production after acute muscle injury. In both acutely injured muscle and *mdx* dystrophic muscle, macrophages express TGF-β1 (Fig. 4) (116), which is a key

regulator of ECM production and fibrosis. Although these findings indicate macrophages are important for ECM production and remodeling during muscle regeneration, their precise roles are not yet well characterized.

Macrophages in muscle pathology

Inflammation and regeneration processes are tightly orchestrated, and interactions between MPCs, macrophages and other cells are spatiotemporally coordinated. The fact that exercise can trigger inflammation suggests that the adaptive response to acute injury has been under strong selective pressure, resulting in the evolution of an elaborate mechanism (15). In sharp contrast, the response to chronic damage is often insufficient to mediate structural or functional recovery. Under such conditions, spatiotemporal control of the activities of immune cells and other cells, such as fibroblasts, becomes disorganized. One example is muscular dystrophy, which is characterized by persistent inflammation and muscle wasting in which progressive fibrosis and tissue remodeling impair muscle function. In this pathological condition, chronic inflammation is responsible for secondary damage promoting muscle degeneration and fibrosis.

Although tightly regulated sequential macrophage activation is essential for muscle regeneration after acute muscle damage, the activity of macrophages may become detrimental under chronic inflammatory conditions. For instance, genetic deletion of *Ccr2* and pharmacologic inhibition of CCR2 in *mdx* dystrophic mice reduces recruitment of monocyte-derived Ly6C^{hi} macrophages, which is associated with suppression of inflammation and improvement of muscle function (117). This is indicative of the contribution made by macrophages to the persistent inflammation and pathology in this model. In addition, macrophages from *mdx* mice appear to promote fibrosis via TGF-β signaling (116), which in addition to activating fibroblasts, blocks TNF-induced apoptosis of FAPs, a source of fibroblasts (108). In *mdx* mice, chronic activation of TNF-α signaling also limits the regenerative potential of SCs by inhibiting Notch-1 signaling (118). Accordingly, blocking TNF-α signaling ameliorates muscle damage in *mdx* mice (119). Interestingly, a large subset of macrophages in *mdx* muscle express both *Tnf* and *Tgfb1*, indicating that when damage is chronic, macrophages adopt transcriptomes different from those observed after acute injury. These observations suggest that the ordered transition from a TNF-α-rich to a TGF-β-rich environment after acute muscle injury becomes disorganized under chronic damage conditions and contributes to unresolved inflammation and remodeling (108).

IL-6 appears to contribute to proper muscle repair by controlling inflammation and regeneration (76). Low levels of IL-6 promote SC activation and myotube regeneration. However, chronically elevated production of IL-6 promotes skeletal muscle wasting, which again points to the pathological impact of persistent inflammation. These findings demonstrate that in muscular diseases, dysregulated activation of macrophages and their altered functionality contribute to persistent inflammation, fibrosis, tissue remodeling and failed regeneration.

Macrophages in liver injury and regeneration

The liver has a remarkable capacity to regenerate in response to injury. Liver injury induces mature liver cells to proliferate to replace the damaged tissue (120, 121). However, under conditions in which hepatocyte proliferation is prevented or insufficient, such as chronic liver injury, a population of bipotent hepatic progenitor cells (HPCs) is activated to regenerate both cholangiocytes and hepatocytes. Macrophages promote both hepatocyte proliferation and HPC differentiation. After partial hepatectomy, macrophages are important for priming liver regeneration by secreting TNF-α and IL-6 (122). Indeed, macrophage depletion greatly compromises liver regeneration. In a mouse biliary injury model, macrophages that have engulfed hepatocyte debris express Wnt3a, which activates Wnt signaling in HPCs and promotes their hepatocytic differentiation (123).

In a mouse model of CCl_4-induced liver injury fibrosis is transient and spontaneously resolved after cessation of CCl_4 treatment (53). In that model, comparison of the effects of macrophage depletion during the period of CCl_4 treatment (i.e. injury phase) and the subsequent repair/resolution period showed the differential functions of macrophages at the different phases of the injury response. Early macrophage depletion during liver injury ameliorates fibrosis. By contrast, late macrophage depletion, during the repair period, results in a failure of resolution with persistent activation of the fibrotic response (53). During the injury phase, monocyte-derived Ly6C^{hi} macrophages predominate in the liver and promote fibrosis by supporting stellate cell activation (54, 124, 125). Kupffer cells may also exert profibrotic effects in certain contexts (126). During the recovery phase, Ly6C^{lo} macrophages become predominant and play a key role in resolution of fibrosis and repair through MMP production and phagocytotic clearance of debris (54, 127). Phagocytosis of hepatocyte debris induces a matrix-degrading phenotype with expression of MMPs in monocyte-derived macrophages (54). Moreover, stimulating phagocytosis *in vivo* reduces numbers of Ly6C^{hi} macrophages and increases those of Ly6C^{lo} macrophages, which is consistent with the idea that phagocytosis is important for phenotypic switching in monocyte-derived macrophages after liver injury.

Macrophages in acute kidney injury

Unlike with liver, resection of a kidney does not elicit organ regrowth, which is indicative of its limited regeneration capacity (128). However, the kidney does recover from various types of damage in part through regeneration of renal tubules (129). Differentiated tubular epithelial cells are thought to proliferate and repair damaged renal tubules, though the possibility that there is a progenitor population has not been excluded.

Acute kidney injury induced by ischemia/reperfusion (I/R) results in an initial accumulation of pro-inflammatory M1-type macrophages within the first 2 days. This is followed by predominant accumulation of M2-type macrophages, which proliferate *in situ* within the injured kidney (130). Inhibition of the initial accumulation of macrophages reduces tubular injury 24 h after I/R, suggesting pro-inflammatory macrophages promote renal injury at early times (130, 131). By

contrast, inhibition of the later increase in M2-type macrophages impairs tubular epithelial cell proliferation and delays recovery (130, 132). M2-type macrophages are also important for recovery in a mouse model of selective proximal tubule injury, which does not recruit monocyte-derived M1-type macrophages (132). During the kidney repair phase after I/R injury, macrophages express a Wnt ligand, Wnt7b, which stimulates epithelial cell proliferation and repair (133), and further suggests M2-type macrophages support tubular regeneration and repair.

Similarly, in a mouse unilateral ureteral obstruction (UUO) model, early accumulation of Ly6C^{hi} pro-inflammatory macrophages is followed by a predominant increase of Ly6C^{lo} M2-type macrophages (134, 135). In response to UUO, the transcription factor Krüppel-like factor 5 (KLF5) induces expression of the chemotactic proteins S100A8 and S100A9, which recruit inflammatory monocytes to the kidneys and promote their activation into Ly6C^{hi} macrophages (135). In *Klf5*$^{+/-}$ mice inhibition of early Ly6C^{hi} macrophage accumulation with a relative shift toward Ly6C^{lo} macrophages suppresses tissue damage, but promotes fibrosis, indicating that these two types of macrophages function differently in response to UUO. Indeed, while isolated Ly6C^{hi} macrophages promote tubular cell apoptosis, Ly6C^{lo} macrophages activate fibroblasts into myofibroblasts, which is suggestive of their role in fibrosis (135).

In both the I/R and UUO models, macrophage phenotypes change from early pro-inflammatory to later reparative and profibrotic ones. Inhibition of early pro-inflammatory macrophages improves renal function in these models, though it may be that the pro-inflammatory macrophages also contribute to processes leading to recovery, as was seen after skeletal muscle injury. While M2-type macrophages appear to be important for tubular epithelial cell proliferation and recovery, they may also promote fibrosis, in part by activating fibroblasts via TGF-β (135, 136). Consequently, dysregulated activation of macrophages likely leads to pathological tissue remodeling in chronic kidney disease.

Macrophages in myocardial infarction

Although proliferation of existing adult cardiomyocytes has been observed in mice and humans, the renewal rate is very limited so that meaningful regeneration of cardiomyocytes is unlikely to occur after pathological cardiomyocyte death, such as that induced by myocardial infarction (MI) (137). As discussed, in tissues with high regenerative capacity, such as skeletal muscle and liver, inflammation induced by tissue injury leads to repair through tissue regeneration. By contrast, because of the very limited regenerative capacity of the heart, MI inevitably results in tissue remodeling through a series of structural and functional changes, including scar formation in the infarct area, reactive hypertrophy of the remaining cardiomyocytes in the non-infarcted area and ventricular chamber dilation (138). Moreover, inflammation may be chronically activated in the non-infarcted area, leading to adverse cardiac remodeling and heart failure. As such, the inflammation and repair processes triggered by MI have potentially adverse consequences. On the other hand, the rapid replacement of dead tissue with scar tissue is critical for survival of the individual because of the continuous contraction of the heart and the lack of regenerative capacity. Accordingly, repair through scar formation is essentially adaptive in the heart.

Previous studies have shown that macrophages can be both protective and harmful after MI, and that the functions of macrophages differ during the different phases of the tissue response to myocardial injury as well as to the different types of cardiac injury. Moreover, the developmental origins differ among cardiac macrophages. In young mice, cardiac-resident macrophages derive from embryonic cells and are maintained through self-renewal (66, 139). Cardiac injury induces rapid accumulation of monocyte-derived macrophages. Aging also increases replacement of embryo-derived resident macrophages with monocyte-derived cells (140). Whether this transition from embryo-derived to monocyte-derived macrophages alters macrophage function and modulates cardiac homeostasis and/or pathological processes remains unclear, but the different origins of the macrophages almost certainly add a layer of complexity to their functional diversity.

Following MI, monocytes abundantly infiltrate the injured tissue and differentiate into macrophages (141). Because of the rapid recruitment of circulating monocytes, Ly6C^{hi} monocytes/macrophages predominate initially but then Ly6C^{lo} macrophages become predominant after day 5 (142). By contrast, resident Ly6C^{lo} macrophages within infarcted tissues disappear within 1 day via local death or exit from the infarct (143, 144). Blocking accumulation of monocyte-derived macrophages suppresses cell death among resident macrophages, which suggests Ly6C^{hi} monocytes/macrophages contribute to resident macrophage death (139). The recruited monocytes are also the major source of Ly6C^{lo} macrophages during the first 2 weeks after MI (143). Thereafter, local proliferation of macrophages appears to predominate. MI also increases macrophages within non-ischemic tissues through both accumulation of monocyte-derived macrophages and local proliferation (145).

Clodronate liposome-mediated depletion of monocytes/macrophages during the early phase of MI results in larger areas of debris and necrotic tissues. The early accumulation of Ly6C^{hi} monocyte-derived cells is thus important for clearance of debris (142). By contrast, depletion of macrophages after day 3 results in decreased fibrosis and angiogenesis, indicating that Ly6C^{lo} macrophages have profibrotic and pro-angiogenic functions. A separate study showed that early (4 h before MI), but not late (4 days after MI), macrophage depletion impairs left ventricular function and increases left ventricular dilation 7 days after MI, which points to the essential contribution made by early Ly6C^{hi} monocytes/macrophages to the proper adaptive response to MI.

Serial administration of clodronate liposome increases mortality after MI, presumably because of embolism formation from left ventricular thrombi (146). This serial macrophage depletion impairs debris clearance and scar formation. Left ventricular thrombi are formed presumably due to delayed re-endothelialization of the damaged left ventricular cavity, which highlights the importance of macrophages for repair of infarcted tissue. Similarly, it was observed in mice that loss of Reg3β, which is released by dedifferentiating

cardiomyocytes and mediates macrophage accumulation after MI, markedly reduces macrophage numbers and impairs healing and survival (147). In this model, the number of neutrophils expressing MMP-9 is increased, leading to left ventricular wall instability and rupture. Clearance of neutrophils by macrophages thus appears to be important for repair after MI. These observations demonstrate that macrophages are indispensable for clearance of necrotic debris as well as repair following MI.

As with macrophages in injured skeletal muscle, phagocytosis of dying cardiomyocytes appears to be important for the functional transition from pro-inflammatory to pro-resolution macrophages in the heart. A scavenger receptor, CD36, is important for phagocytosis of dying cells by monocyte-derived Ly6C^{hi} cells, and bone marrow-specific *Cd36* deletion increases both infarct size and the likelihood of cardiac rupture (148). After MI, Ly6C^{lo} macrophages that have engulfed dying cardiomyocytes express higher levels of MerTK and a transcription factor Nr4a1, which transactivates *Mertk* expression (148). MerTK is important for engulfment of dying cardiomyocytes and is predominantly expressed in Ly6C^{lo} macrophages beginning 3 days after MI (149). Deletion of *Mertk* severely impairs clearance of dead cardiomyocytes on days 5 and 7 after MI, which is associated with augmented tissue remodeling and functional deterioration by day 28. In addition, expression of pro-inflammatory cytokine genes, including *Tnf* and *Il6*, is increased at day 7 in *Mertk*$^{-/-}$ mice, while *Il10* expression is decreased. These results suggest that phagocytosis of dying cardiomyocytes by Ly6C^{hi} macrophages after MI triggers their differentiation/maturation into pro-resolution Ly6C^{lo} macrophages, that engulfment of dead cells by Ly6C^{lo} macrophages is important for resolution of inflammation, and that the proper transition of macrophage functions from pro-inflammatory to pro-resolution is crucial for resolution of inflammation and repair.

In addition to monocyte-derived macrophages, resident macrophages also contribute to early clearance of dying cardiomyocytes beginning 4 h after I/R injury, and phagocytosis of dead cardiomyocytes by resident Ly6C^{lo} macrophages increases expression of IL-10 and TGF-β (150). *Mertk* deletion also impairs clearance of dead cells and worsens cardiac injury in this model.

Macrophages also potentially mitigate MI injury by regulating fibrosis and angiogenesis (151). Deletion of *Trib1*, which encodes an adaptor protein involved in protein degradation, severely reduces M2-like macrophages in bone marrow, spleen, lung and adipose tissue (152). *Trib1* deficiency greatly suppresses accumulation of CD206^{+} M2-like macrophages, most of which are Ly6C^{lo}, 7 days after MI (153). *Trib1*$^{-/-}$ greatly reduces survival after MI, largely because of increased incidence of cardiac rupture, indicating that M2-like macrophages are important for fibroblast-mediated MI repair through scar formation. Ly6C^{lo} macrophages also promote angiogenesis which is important for healing after MI (154). In addition, macrophages support cardiomyocyte survival by producing and secreting myeloid-derived growth factor, which also has the potential to activate angiogenesis (155).

In addition to showing that macrophages are integral to healing after MI, clodronate liposome studies showed that inhibiting monocyte accumulation starting 1 week after MI improves left ventricular contractility and suppresses fibrosis in non-infarct tissues, which is indicative of the detrimental pro-remodeling function of macrophages (145). In a model of cardiomyocyte ablation wherein diphtheria toxin induces cell death among cardiomyocytes expressing DTR, the cardiac injury leads to recruitment of monocytes and monocyte-derived macrophages that have a robust pro-inflammatory phenotype (139). In this model, inhibition of monocyte influx into the injured heart decreases inflammation and enhances coronary angiogenesis, indicating monocyte-derived macrophages promote cardiac injury. In addition, while the initial replacement fibrosis (i.e. scar formation) is essential for preventing rupture of the ventricular wall after MI, reactive fibrosis activated within the infarct border zone and in the uninjured myocardium leads to ventricular remodeling that compromises cardiac function (156). These studies highlight the pathological activities of macrophages after MI. In particular, macrophages appear to crucially contribute to persistent inflammation and tissue remodeling in non-infarcted areas during the chronic phase after MI, which may lead to heart failure.

In sharp contrast to the adult heart, neonatal mouse heart can fully regenerate after apical resection or MI (157), but this robust regeneration capacity is lost by postnatal day 7 (P7). Depletion of monocytes/macrophages using clodronate liposomes following MI on P1 severely compromises regeneration of the myocardium and increases fibrotic scar formation (158). Comparison of the gene expression profiles of cardiac macrophages 3 days after MI on P1 and on P14 showed that the genes expressed in P1 macrophages were enriched in the gene ontology terms ‘angiogenesis’, ‘inflammation’, and ‘oxidative stress response’. In line with this, post-MI angiogenesis was suppressed in clodronate liposome-treated mice, which suggests P1 monocytes/macrophages support cardiac regeneration in part by promoting angiogenesis and neonatal cardiomyocyte proliferation (139, 158). The reduced expression of several angiogenic genes in P14 macrophages may indicate that the function of macrophages in hearts change between P1 and P14. Although it is likely that changes in the microenvironment, particularly the loss of regenerative capacity by cardiomyocytes, affects the differential gene expression on P1 versus P14, it is also possible that distinct macrophage subsets and/or lineages may contribute to the functional differences between P1 and P14 macrophages. In the diphtheria toxin-induced cardiomyocyte death model, cardiac injury increases only resident macrophages that exhibit reparative functionality in P1 to P7 mice, but it begins recruiting monocyte-derived macrophages on P14 (139). In this model, inhibition of monocyte recruitment to the injured heart preserves an embryonically derived macrophage subset, reduces inflammation, and enhances coronary angiogenesis in adult mice (139). Thus embryonically derived, resident macrophages have a reparative function, while monocyte-derived macrophages do not.

Conclusions

Through their diverse and changing functions, macrophages lead the complex tissue response to injury throughout the

course from inflammation to healing. They also play key pathological roles during chronic inflammation, which causes pathological remodeling and tissue dysfunction. Given the varied characteristics of different tissues (e.g. cell composition, regenerative capacity), the differences in their functional requirements (e.g. physical strain in skeletal muscle, incessant contraction of the heart, high metabolic activity in liver and kidney), and the differences in the nature of their likely injuries, it is perhaps unsurprising that macrophages exhibit a variety of functions and phenotypes during their responses to tissue injury. Nonetheless, it appears that acute injury triggers similar processes (e.g. inflammation, resolution and repair) in different tissues and that macrophages share at least some temporally regulated functions in all tissues.

In the early inflammatory phase triggered by tissue injury, monocytes and macrophages lead inflammation by expressing pro-inflammatory cytokines and interact with other pro-inflammatory immune cells. Macrophages are activated by various DAMPs, fibrin and pro-inflammatory cytokines (91–93). Hypoxic conditions may also affect macrophage activity (96). In addition to monocyte-derived macrophages, resident macrophages may contribute to the recruitment and activation of circulating immune cells.

Macrophages and monocytes are central players involved in the clearing of cell and tissue debris, which is prerequisite for successful healing. Clearance of neutrophils is also important to limit inflammation. In the skeletal muscle response to injury, pro-inflammatory cytokines promote stem/progenitor cell proliferation and control differentiation. Consequently, repair and regeneration processes are tightly linked to initial inflammatory processes. In other words, acute inflammation not only paves the way to healing through clearing and preparing the damaged tissue for repair and regeneration, it also guides the initial cellular response toward regeneration. The inflammation must then subside in coordination with activation of repair/regeneration processes. The observation that perturbation of that sequence, either by forced prolongation of inflammation or its premature resolution, is detrimental to skeletal muscle regeneration (61, 62) emphasizes the importance of temporal coordination of inflammation resolution with other ongoing cellular processes.

During this phase transition from inflammation to repair/regeneration, there are marked changes in macrophage functions and phenotypes. In many tissues, numbers of Ly6C^{hi} monocytes/macrophages decline and Ly6C^{lo} macrophages become predominant. This transition may also be recorded as a shift from M1 to M2 macrophages. These phenotypic changes are associated with dynamic changes in transcriptomes, which may occur regardless of the macrophage subtypes determined based on their surface markers (e.g., Ly6C^{lo}/Ly6C^{hi} and M1/M2), and may precede the transition of surface marker expression (35). The dynamic changes in macrophage function during inflammation and repair are thus much broader than can be deduced from the dichotomy of M1/M2 macrophage subsets.

The functional and phenotypic changes in macrophages appear to be driven by multiple factors, including both microenvironmental and endogenous cues (Table 1). For instance, phagocytosis of dying cells appears to be a key stimulus that induces anti-inflammatory and/or reparative functions in macrophages. Cytokines and other mediators also likely promote functional changes. Our knowledge is still limited to a relatively small number of mediators, and many additional active molecules are likely involved. In addition, in the later phase of the injury response, both monocyte-derived and resident macrophages may proliferate. This *in situ* proliferation may promote macrophage functional transition, though the mechanism linking cell proliferation to epigenetic regulation of macrophages remains poorly understood (159). The functional transition of macrophages also associates with changes in cellular metabolism, which can be affected by environmental cues as well as cell-autonomous mechanisms. While some of the mechanisms, including phagocytosis of dying cells, are commonly observed in different tissues, many signals, such as cytokines and growth factors, may be tissue- and/or injury-specific. Accordingly, macrophages are likely to have tissue- and/or injury-specific functions.

During the repair/regeneration phase, macrophages may contribute to healing in a variety of ways. For instance, they may suppress inflammation through expression of anti-inflammatory cytokines, such as IL-10. They may also control the expansion and differentiation of the stem/progenitor cells that regenerate tissues, as is seen in skeletal muscle. Or they may promote proliferation of existing parenchymal cells, as is seen in the liver and kidney. Even where macrophages do not directly support regeneration of parenchymal cells from stem/progenitor cells, they may support the survival of parenchymal cells via growth factors. In addition, they may indirectly support repair by parenchymal cells by promoting proliferation and activation of stromal cells, such as endothelial cells and fibroblasts, which build the microenvironment needed for repair and healing.

Macrophages have important regulatory functions in angiogenesis and ECM production. The stroma is where immune cells, vascular cells, and fibroblasts interact, and cellular processes occurring within the stroma are central to inflammation, repair and regeneration. For instance, formation of new blood vessels via angiogenesis is indispensable to tissue regeneration and repair (6). ECM synthesis and remodeling are also essential for formation of the scaffolding that supports regeneration. Moreover, ECM controls various aspects of growth, proliferation, movement, differentiation, and activation of the cells living within it (1). Indeed, damage to the ECM framework hinders regeneration and leads to scar formation. Properly organized ECM is thus essential for regeneration (1). During inflammation and healing after tissue injury, the ECM is remodeled through dynamic synthesis and degradation. After skeletal muscle injury, for example, transient ECM deposition occurs (113). This temporary ECM, which stabilizes the tissue and acts as a scaffold for new muscle fibers, is resolved during the progression of regeneration and disappears from regenerated tissue. Macrophages contribute to both the synthesis and degradation of ECM components, thereby controlling ECM dynamics. They express TGF-β and other mediators, which activate fibroblasts to produce ECM components, and they control the resolution of fibrosis, in part through expression of MMPs (15, 127). Conversely, ECM components may alter macrophage function and phenotype (160), which underscores the complex reciprocal interactions between macrophages and ECM, though the details of these interactions during repair and regeneration are not well understood.

Many of the inflammatory and reparative processes led by macrophages are commonly observed after injury in the different tissues discussed in this article. While it may appear that macrophages play a few variations on a common theme, there are also many significant differences. For instance, because of the very limited regenerative capacity of the adult heart, macrophages appear to have adjusted for rapid repair through scar formation (153). Similarly, neurons in the adult central nervous system have limited regenerative capacity. On the other hand, remyelination, the formation of myelin sheaths by myelin-forming oligodendrocytes newly differentiated from oligodendrocyte precursor cells, can robustly repair demyelination injury in young animals. Macrophages and microglia crucially contribute to remyelination, in part by controlling the proliferation and differentiation of oligodendrocyte precursor cells (161, 162). It is thus very likely that macrophages are highly tuned to the tasks necessary for repair throughout the array of different tissue structures found in complex organisms.

How then is macrophage tissue- and injury-specificity conferred? Recent studies have revealed the dynamic and flexible nature of the macrophage epigenome, which can be dynamically modulated by the microenvironment (163, 164). Each tissue macrophage has a distinct epigenome that appears to confer distinct functional properties and, upon injury, these macrophage epigenomes are dynamically modulated. Following acute injury, monocyte-derived macrophages enter the affected tissue and become predominant. Their epigenomes differ from those of tissue-resident macrophages when they initially enter. It is likely that a combination of epigenomic status and microenvironmental cues shapes their function. As discussed in this article, macrophages markedly change their function over the course of injury and repair. Such temporal dynamics are also likely driven by environmental cues as well as by cell-autonomous mechanisms. In that regard, location appears to be a key determinant of a macrophage's functions and tasks. For instance, pro-angiogenic macrophages are located in close proximity to blood vessels (165). Reciprocal interactions between macrophages and adjacent cells and/or ECM very likely shape a macrophage's characteristics. Accordingly, it is clear that the functional characteristics of macrophages are much more diverse than can be defined by small numbers of surface markers. To better understand this diversity, we will need to analyze the gene expression and localization of macrophages at the single cell level (166). The lineages of macrophages may also influence their function. In that regard, Satoh *et al.* recently identified a new population of monocytes/macrophages that are developmentally distinct from circulating Ly6C^{hi} monocytes and promote fibrosis (167). It is possible that careful lineage tracing may enable detection of additional developmental diversity in monocyte/macrophage lineages.

In this review we mainly focused on acute injury, which heals through well-coordinated cellular responses wherein macrophages with programs spatiotemporally tuned to repair are the central player. However, it is often the case that continued unresolved inflammation and repair progressively remodel tissue structure such that tissue function is impaired. This is particularly relevant to chronic non-communicable diseases, such as cardiovascular disease. In those settings, the actions of macrophages are often pathogenic. This review does not address those actions of macrophages in detail. However, by comparing the adaptive and reparative functions of macrophages in this review, we raise several related questions about the pathogenicity of macrophages. For instance, why does the behavior of macrophages become pathogenic? What drives the pathogenic activities and are they merely programmed responses? Clear answers to these questions are elusive. Given that macrophages are highly responsive to environmental cues, one might think that even within a pathogenic process, macrophages are just playing out their programs, which are essentially adaptive. But if a macrophage action, such as ECM production, continues too long and/or is activated too often, that activity may culminate in pathological consequences, such as fibrosis. Within a setting of disorganized interactions among many cells and ECM, it is conceivable that environmental cues given to macrophages are spatiotemporally more complex than those in coordinated repair processes. That said, many of the fundamental programs controlling macrophage activity may be shared in both physiological and pathological responses to tissue injury. There is clearly much to learn about the endogenous and exogenous regulatory programs of macrophage dynamics in response to injury, but such studies are opening up opportunities to therapeutically modulate macrophage function to promote regeneration and repair, to limit pathological remodeling, or to reverse tissue remodeling in chronic diseases.

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