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Monocyte/macrophage interactions with myogenic precursor cells during skeletal muscle regeneration

Marielle Saclier[1,2,3], Sylvain Cuvellier[1,2,3], Mélanie Magnan[1,2,3], Rémi Mounier[1,2,3] and Bénédicte Chazaud[1,2,3]

1 Institut Cochin, INSERM U1016, Paris, France
2 CNRS 8104, Paris, France
3 Université Paris Descartes, Paris, France

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Correspondence
B. Chazaud, Institut Cochin, INSERM U1016, 24 Rue du Faubourg Saint Jacques, Paris, France
Fax: 33-1 44 41 24 21
Tel: 33 1 44 41 24 63
E-mail: benedicte.chazaud@inserm.fr

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Adult skeletal muscle has the remarkable property of regenerating after damage, owing to satellite cells and myogenic precursor cells becoming committed to adult myogenesis to rebuild the muscle. This process is accompanied by the continuing presence of macrophages, from the phagocytosis of damaged myofibres to the full re-formation of new myofibres. In recent years, there has been huge progress in our understanding of the roles of macrophages during skeletal muscle regeneration, notably concerning their effects on myogenic precursor cells. Here, we review the most recent knowledge acquired on monocyte entry into damaged muscle, the various macrophage subpopulations, and their respective roles during the sequential phases of muscle repair. We also discuss the role of macrophages after exercise-induced muscle damage, notably in humans.

Introduction

Skeletal muscle, the most abundant tissue in humans, constitutes a highly adaptable and malleable tissue, responding to environmental and physiological demands. The high adaptability of skeletal muscle results from its well-known sensitivity to stimuli such as contractile activity (endurance exercise, electrical stimulation, and denervation), loading conditions (resistance training and microgravity), substrate supply (nutritional interventions), hormonal profile (thyroid hormone and testosterone), and environmental factors (hypoxia). Moreover, alteration of skeletal muscle plasticity is involved in the pathophysiology of various diseases, especially degenerative dystrophies [1]. Furthermore, adult skeletal muscle possesses the remarkable capacity to regenerate after injury, owing to the properties of a pool of cells, the satellite cells [2], that are capable of restoring muscle function throughout an individual's lifespan. Under steady-state conditions, muscle stem cells (satellite cells) are quiescent, and located between the plasma membrane and the basal lamina surrounding the myofibre. After an injury, satellite cells are activated, becoming myogenic precursor cells (MPCs), proliferate, migrate towards each other, differentiate, and finally fuse to form new myofibres. Inflammation is always associated with the process of muscle regeneration, although it is more or less pronounced, depending on the extent and type of damage. The presence of inflammatory cells, especially macrophages, was described in several experimental models more than 35 years ago. From the late 2000s, new tools, including transgenic

Abbreviations
COX, cyclooxygenase; DT, diphtheria toxin; DTR, diphtheria toxin receptor; EIMD, exercise-induced muscle damage; GFP, green fluorescent protein; IGF, insulin-like growth factor; IL, interleukin; MPC, myogenic precursor cell; NSAID, nonsteroidal anti-inflammatory drug; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor; WT, wild-type.

mouse strains and sorting of cell populations from the muscle, allowed a deeper analysis of the phenotypes and functions of immune cells during skeletal muscle repair, and the demonstration of important roles of macrophages in this process.

Are there any resident macrophages in normal adult steady-state muscle?

In normal adult skeletal muscle, macrophages are barely detectable. They have been observed in the interstitial space, and are more numerous in the perimysium (the connective tissue that surrounds muscle fascicles) and the epimysium (the connective tissue that surrounds the whole muscle, also called the fascia), where they are located near to the vessels and capillaries [3–5]. More recent calculations in humans have shown that macrophages are rare in the parenchyma (approximately one macrophage for five myofibres [6]). This was confirmed in mouse skeletal muscle, where the main location for resident macrophages is the epimysium [7]. Tangential sectioning of the whole muscle allows the cell wealth of this thin layer to be revealed. It is therefore possible to observe and compare the respective concentrations of resident macrophages in the epimysium and in the muscle parenchyma (Fig. 1).

Macrophages are essential for skeletal muscle regeneration

Macrophages are mainly derived from blood monocytes that have crossed the vessel endothelial barrier to reach the tissue. Macrophages have long been known to be associated with skeletal muscle regeneration. They appear in the damaged areas a few hours after injury in various models, such as toxin injection (lidocaine, barium chloride, notexin, and cardiotoxin) [8–10], partial excision [11], and eccentric exercise [12,13]. The first signal(s) causing the entry of monocytes into the injured muscle is (are) still unknown. Neutrophils are the first leukocytes to appear in the damaged areas, within the very first hours after injury, but only a few studies have explored their role. Systemic injection of antibodies against granulocytes impairs muscle regeneration and decreases the number of both neutrophils and macrophages in the damaged areas, although examination of leukocytes was not performed with specific antibodies [14]. Similarly, blocking of CD11b binding leads to inhibition of leukocyte entry a few hours after muscle injury [15]. Here, again, the numbers of neutrophils and macrophages were not assessed with specific antibodies, and the muscle tissue was not further analysed. In contrast, in the β_2-integrin CD18-deficient mouse, it has been shown that the recruitment of neutrophils (which express Ly6G) into the damaged muscle, but not that of macrophages (which express F4/80), is reduced, suggesting that neutrophils and macrophages are independently recruited into the injured muscle [16]. In this experiment, the number of injured myofibres was reduced in CD18-deficient mice as compared with wild-type (WT) mice at early time points after injury, and muscle regeneration parameters (kinetics of regenerating myofibres, cross-sectional area of the new fibres, and expression

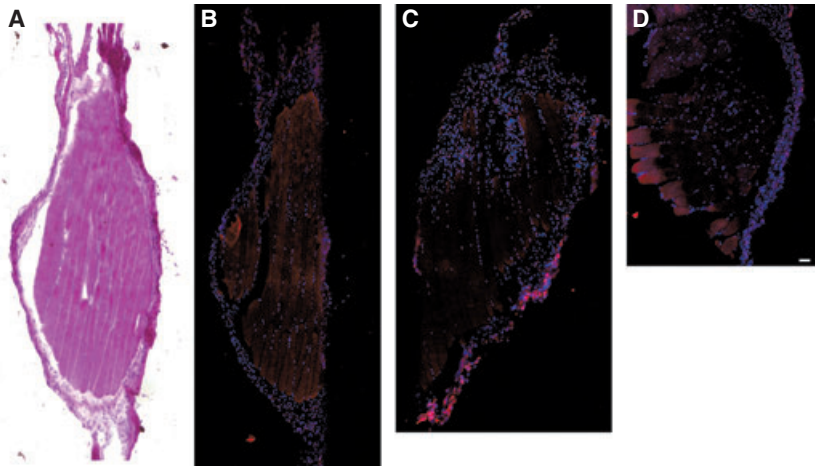

Fig. 1. Macrophage location in normal skeletal muscle. Tangential sections of normal adult muscle allow observation of the surrounding connective tissue of the muscle, the epimysium or fascia. In (A), haematoxylin and eosin staining shows the high cellularity of the fascia, surrounding the muscle parenchyma itself. (B) [same muscle as in (A)], (C) and (D) show examples of F4/80 immunostaining (red), which is specific for murine macrophages. Many cells residing in the epimysium are macrophages, whereas only scarce positive cells are observed between the myofibres. Blue, Hoechst. Bar: 50 μm.

of embryonic myosin heavy chain by regenerating myofibres) were improved [16], suggesting a protective effect of neutrophil depletion. Similarly, inhibition of monocyte/macrophage recruitment into the muscle (e.g. in CCR2-deficient or MCP-1-deficient mice [17–19], or in the presence of blocking antibodies against macrophage colony-stimulating factor receptor [20]; see below) does not alter neutrophil entry into muscle. The impairment of muscle regeneration in these conditions suggests that neutrophils play a marginal role in skeletal muscle repair. Resident cells may also participate in the recruitment of circulating monocytes into the damaged muscle. A recent study of the role of mast cells, which are immune sentinels in the tissues, has shown that the stimulation and inhibition of mast cell degranulation stimulate and block neutrophil entry into the muscle, respectively [21]. Unfortunately, the effect on monocytes/macrophages was not assessed in this work. Resident macrophages located in the fascia also participate in the recruitment of monocytes after muscle injury. In CD11b-diphtheria toxin (DT) receptor (DTR) mice, the human receptor for DT is expressed under the control of the CD11b promoter, making these cells sensitive to DT. In chimeric mice obtained after WT bone marrow transplantation into CD11b-DTR recipients, intravenous injection of DT induces ablation of resident fascia macrophages. In these conditions, recruitment of circulating monocytes into injured muscle is dramatically reduced [7]. MPCs have been also shown to attract monocytes *in vitro* through the secretion of a series of effectors [urokinase-type plasminogen activator, CX3CR1, CCL2, CCL22, and vascular endothelial growth factor (VEGF)] [22]. The study of the respective roles of mast cells, neutrophils and resident muscle cells in the recruitment of circulating monocytes requires further investigation to establish the kinetics of immune cell entry during the very first steps of the inflammatory response after muscle injury.

Neutrophils rapidly disappear from the regenerating muscle. In most of the regeneration models, the neutrophil number peaks at 24 h after injury, and then quickly drops; neutrophils are no longer detectable after 36–48 h. By contrast, the number of macrophages continuously increases during this period of time. Indeed, the macrophage number increases by eight-fold and 19-fold, respectively, at day 1 and day 3 after crush injury [5], and by 30–50-fold and 150–200-fold, respectively, at day 1 and days 3–4 following cardiotoxin injury, which is a more inflammatory model [18,23]. The macrophage number also increases by 30% 3 days after eccentric exercise in humans [6]. An intriguing observation is the persistence of macrophages during the whole process of skeletal muscle regeneration. Soon after injury, macrophages are first associated with the necrotic myofibres, where they phagocytose damaged myofibres and muscle debris. Once phagocytosis has ended, macrophages are still present in the regenerating areas, in even higher numbers, and are tightly associated with MPCs and young, regenerating myofibres. When differentiation and fusion are completed, the number of macrophages drops to a very low level [10,23]. Several studies have attempted to analyse the role of macrophages during skeletal muscle regeneration. To this end, several strategies were developed to block the entry of monocytes into the injured muscle.

Injection of antibodies against macrophage colony-stimulating factor receptor, which is expressed by circulating monocytes, impairs muscle regeneration while dramatically decreasing the number of macrophages present in the damaged areas [20]. Intravenous injection of clodronate-containing liposomes into WT mice, or of DT into CD11b-DTR mice, leads to a drastic decrease in the number of monocytes in the circulation (~ 90% of monocyte depletion is reached within 12 h, and it stays maximal for another 12 h, before the number returns to the normal value). Notably, although granulocytes, especially neutrophils, express CD11b, they are not targeted by DT in the CD11b-DTR model [24,25], providing a specific tool with which to study monocytes/macrophages. Partial monocyte depletion during the first 24 h after injury induces delay and impairment of skeletal muscle regeneration, characterized by the persistence of some necrotic myofibres and of inflammatory cells 9 days after injury, and the appearance of interstitial fat 14 days after injury [23,26]. Moreover, when the recruitment of circulating monocytes is totally prevented in the first 24 h after injury, muscle regeneration is totally inhibited, with the persistence of necrotic fibres until 7 days after injury [23]. By contrast, depletion of circulating monocytes from 2 days after injury does not alter muscle regeneration, showing that monocytes are recruited at once, at the time of injury [23]. Several studies have used various mouse strains and bone marrow transplantation experiments to demonstrate the requirement for macrophages for efficient muscle regeneration. Mice deficient in either the chemokine receptor CCR2 or its main ligand CCL2/MCP1 show impaired muscle regeneration, characterized by a decrease in the diameter of the new myofibres, a reduced number of capillaries, and fat accumulation. This is always associated with a dramatic decrease in macrophage infiltration into the muscle [17,18,27,28]. This effect is reversed by WT bone marrow transplantation into deficient recipients

[18,19,29,30], and CCR2$^{-/-}$ bone marrow transplantation into WT recipients phenocopies the total CCR2$^{-/-}$ phenotype [30]. These studies have shown that monocyte entry into the injured muscle mainly occurs through the CCR2–CCL2 axis. CXCL16, another chemokine, has also been shown to regulate monocyte/macrophage entry into the injured muscle [29]. Similarly, other molecular systems involved in cell migration, such as urokinase-type plasminogen activator [31–33] and β_3-integrin [34], are required for proper regeneration, as they regulate monocyte/macrophage entry into the damaged muscle. Altogether, these studies demonstrate that macrophages are indispensable for postinjury skeletal muscle regeneration.

Phenotypes of macrophages during skeletal muscle regeneration

From the very first analyses of inflammatory cells during skeletal muscle regeneration, various types of macrophage have been described. Pioneering work on rats has shown that macrophages expressing ED2$^+$ (the scavenger receptor CD163) are resident macrophages, and that they are not associated with phagocytosis of necrotic myofibres [3]. Soon after injury, ED1$^+$ (CD68) macrophages, which are not observed in normal muscle, infiltrate the damaged areas and become associated with phagocytosis of muscle debris. In contrast, ED2$^+$ macrophages are not found in these necrotic areas, but have been subsequently observed in regenerating areas, once phagocytosis has ended [10].

The heterogeneity of monocyte and macrophage populations has been investigated extensively. Jung *et al.* [35] described the CX3CR1$^{GFP/GFP}$ knock-in mouse, in which green fluorescent protein (GFP)$^+$ cells are mostly monocytes and their tissue descendants. Mouse monocytes comprise at least two phenotypically distinct subsets [36]. The main subset of Ly6C^{pos}(Gr1^{pos}) CX3CR1^{lo}CCR2^{hi}CD62L^{pos} cells produces high levels of tumor necrosis factor-α (TNF-α), interleukin (IL)-1, and nitric oxide. These cells have a short half-life during tissue damage, and migrates to inflamed tissues. The Ly6C^{neg}(Gr1^{neg})CX3CR1^{hi}CCR2$^{lo/neg}$CD62L^{neg} subset is smaller in size, and is found in inflamed and resting tissues [36]; on the basis of their high secretion of VEGF, Ly6C^{neg} monocytes are considered to be proangiogenic [37]. Both populations can infiltrate damaged tissues [38–40], where they differentiate into macrophages or dendritic cells and have different functions.

In the tissues, macrophages can adopt different phenotypes according to their environment [41,42], and they may switch from one phenotype to another, exhibiting high plasticity [43]. Several macrophage polarization profiles have been described, mainly from *in vitro* studies, each profile expressing a specific panel of markers, including cytokines, chemokines, growth factors, and cell surface antigens [44]. The existence of these phenotypes *in vivo* has been poorly assessed, and it is likely that macrophages exhibit a variety of phenotypes ranging between the four main activation states that have been described *in vitro* [42,44]. Two polarization states were first described, mirroring Th1–Th2 polarization. The classically activated (M1) macrophages, induced by bacterial moieties such as lipopolysaccharide or interferon-γ, secrete Th1 chemokines (CXCL9 and CXCL10) and proinflammatory cytokines (IL-1, TNF-α, IL-6, etc.). An alternatively activated (M2) macrophage phenotype was originally discovered as a response to the Th2 cytokine IL-4. M2 macrophages show high-level expression of scavenging mannose and galactose receptors, a phenotype of low IL-12/high IL-10 expression, and expression of the CCL17, CCL22 and CCL24 chemokines. These cells participate in polarized Th2 responses, help with parasite clearance, dampen inflammation, promote tissue remodelling and tumour progression, and have immunoregulatory functions. In an attempt to distinguish between various M2 macrophages found in either chronic inflammation or tissue repair, the M2 population has been subdivided into: M2a (after exposure to IL-4 or IL-13), associated with fibrogenesis and angiogenesis; M2b (triggered by immune complexes in combination with IL-1β or lipopolysaccharide); and M2c or anti-inflammatory/deactivated macrophages (triggered by IL-10 or glucocorticoids), associated with the resolution of inflammation and tissue repair [41]. The heterogeneity and plasticity of macrophage functional states indicate that typical M1 and M2 phenotypes are extremes of a spectrum encompassing a plethora of functional states [44]. Indeed, in *in vivo* pathophysiological conditions – which are characterized by a diversity and temporal evolution of activating signals – macrophages with intermediate or overlapping phenotypes have been observed.

The use of the CX3CR1$^{GFP/+}$ mouse helped in analysis of the recruitment of monocytes in regenerating skeletal muscle [23]. Normal muscle contains only CX3CR1^{hi}Ly6C^{neg} macrophages. Soon after toxic injury, only CX3CR1^{lo}Ly6C^{pos} (or F4/80^{pos}Ly6C^{pos}) monocytes infiltrate the injured muscle. This monocyte subset expresses CCR2 and, accordingly, it is dramatically reduced in CCR2-deficient mice, leading to defects in muscle regeneration [45]. One day after injury, this population declines, whereas the number of CX3CR1^{hi}/Ly6C^{neg} (or F4/80^{pos}Ly6C^{neg}) monocytes/macrophages greatly increases, partly because of the

capacity of this cell subset to proliferate. RT-PCR analyses showed that the first subset ($CX3CR1^{lo}$ $Ly6C^{pos}$) to invade regenerating muscle expresses higher levels of TNF-α and IL-1β, whereas the $CX3CR1^{hi}Ly6C^{neg}$ subset expresses high levels of IL-10 and transforming growth factor-β [23,46]. The sequential presence of an $F4/80^{pos}Ly6C^{pos}$ proinflammatory macrophage population and then of an $F4/80^{pos}Ly6C^{neg}$ anti-inflammatory macrophage population is later followed by the appearance of population of macrophages – probably coming from $F4/80^{pos}Ly6C^{neg}$ – in which both proinflammatory and anti-inflammatory markers are dampened at the very end of muscle repair [46].

This sequential presence of proinflammatory and then anti-inflammatory macrophages has been recently confirmed during muscle regeneration in humans. Injury was achieved by a series of voluntary eccentric contractions plus electrostimulation at the time of maximum contraction. This leads to a sequence of myofibre necrosis/regeneration throughout the muscle. Regenerating areas are well characterized by both CD56 labelling (which labels satellite cells, MPCs, and young, newly forming, myofibres in humans) and embryonic myosin heavy chain labelling (which characterizes young regenerating myofibres). Seven days after injury, macrophages expressing M1 markers [inducible NO synthase and cyclooxygenase (COX)-2] and M2 markers (arginase 1, CD206, and CD163) were observed in 80% of the regenerating areas, indicating that different subsets of macrophages are present at the same time in the same place within these regenerative areas. However, M1 and M2 macrophage markers were found to be differentially associated with regenerating areas containing or not containing myogenic cells positive for myogenin, a transcription factor associated with terminal myogenic differentiation. Interestingly, macrophages expressing M1 markers are preferentially associated with regenerative areas containing only proliferating MPCs, whereas macrophages expressing M2 markers are mainly associated with regenerative areas containing differentiating myogenic cells expressing myogenin [47].

Altogether, these findings show that injured skeletal muscle recruits $CCR2^{pos}LyC6^{pos}$ monocytes. The first steps of muscle regeneration, including phagocytosis of necrotic debris and expansion of myogenic cells, are associated with proinflammatory macrophages. Then, the late steps of regeneration, at the time of myogenic cell differentiation and myofibre formation, are associated with anti-inflammatory macrophages (Fig. 2). The sequential presence of macrophage subpopulations suggests different and sequential roles of macrophages in the myogenic process during muscle regeneration.

Trophic effects of macrophages on MPCs

As macrophages are present throughout the entire regeneration process, several studies have explored their role beyond phagocytosis, and particularly their effects on MPCs, which are the progeny of activated satellite cells. During muscle regeneration, MPCs proliferate and migrate, finally undergoing terminal myogenic differentiation, and fuse to form myotubes and new myofibres. A small subset of MPCs does not differentiate but returns to an undifferentiated state to replenish the satellite cell pool (self-renewal). *In vitro*, murine or human MPCs follow the same kinetics, with an expansion phase before migration and terminal differentiation and fusion into myotubes (or self-renewal).

The first *in vitro* analyses showed that macrophages and macrophage-conditioned medium stimulate MPC growth [22,48]. Macrophages are more efficient when they are in contact with MPCs [22]. It was further shown that these contacts mediate antiapoptotic signals [involving VCAM-1(CD106)/VLA-4(CD49d), ICAM-1(CD54)/LFA-1(CD11a), CX3CL1/CX3CR1, and PECAM-1(CD31)/PECAM-1(CD31)] on myoblasts, and even more so on myotubes [49]. Macrophages secrete mitogenic factors for MPCs, as they stimulate MPC proliferation [22,50,51]. Their effects on cell fusion have been debated since they were reported to either stimulate [48,52] or inhibit/delay [51] myogenic differentiation. Apart from the culture conditions, which may induce huge differences in the experimental outcomes, more recent studies have established that macrophages exert different activities on MPCs, depending on their inflammatory state. Proinflammatory M1 macrophages stimulate MPC proliferation and inhibit their fusion [23,47]. Conversely, M2 macrophages (both M2a and M2c) stimulate myogenesis by promoting MPC commitment into terminal differentiation and the formation of large myotubes [47]. Accordingly, *in vivo* depletion of intramuscular macrophages 5–6 days after injury leads to a decrease in the size of the newly formed myofibres [23], confirming the role of M2 macrophages in the late steps of myogenesis and regeneration. We also observed that M1 macrophages migrate more efficiently towards MPCs, confirming earlier studies showing that macrophages migrate towards muscle crush extract [53]. Conversely, in *in vitro* assays, MPCs

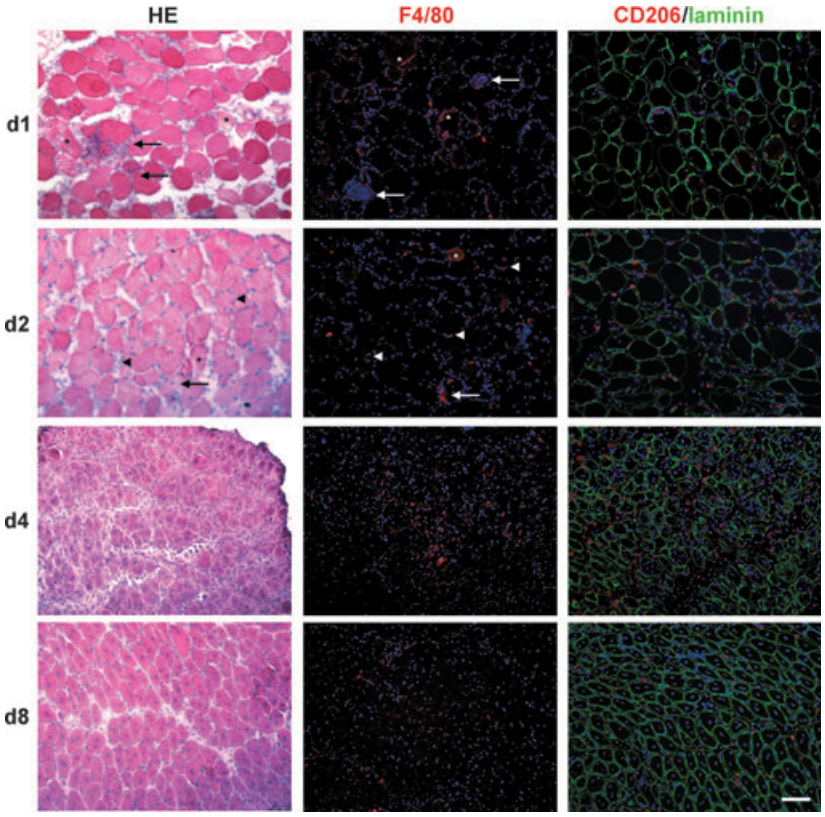

Fig. 2. Macrophages during the time course of skeletal muscle regeneration. Skeletal muscle was injured with cardiotoxin. Muscle was analysed on day 1, day 2, day 4 and day 8 after injury. The left panel shows haematoxylin and eosin (HE) staining; the middle panel shows F4/80 immunostaining (red). The right panel shows immunolabelling for laminin (green), a component of the basal lamina surrounding each myofibre, and CD206 (red), a marker of M2 macrophages (see text). On day 1, immune cells can be seen (black arrows), among which macrophages (white arrows) invade the damaged muscle. Few cells express CD206, and they are located under the basal lamina, indicative of phagocytosis of necrotic myofibres. On day 2, more macrophages are present. They are associated with necrotic myofibre phagocytosis (arrows), and are also present in the interstitium (arrowheads). CD206-expressing cells are mainly outside the basal lamina (excluding the possibility that they are myogenic cells, which have been shown to express CD206 *in vitro*). On day 4, a huge number of macrophages and $CD206^{pos}$ cells are observed. They are associated with new, growing, regenerating myofibres. On day 8 after injury, the muscle has almost completed the regeneration, and is composed of new, regenerated myofibres characterized by the central location of their nuclei. Only a few macrophages and $CD206^{pos}$ cells remain in the tissue. *Necrotic myofibres, which are sometimes nonspecifically immunostained. Blue, Hoechst. Bar: 50 μm.

migrate towards other MPCs (in order to fuse) or towards M2 macrophages, but much less towards M1 macrophages [47], indicating privileged interactions between macrophages and MPCs.

Macrophages are well known to secrete a variety of molecules, cytokines, and growth factors. Some of them have been identified as having effects on MPC proliferation, such as IL-6 [50,54] or TNF-α [55,56]. Accordingly, we have shown that M1 macrophages prevent myogenesis through the secretion of high levels of TNF-α, IL-6, and IL-1β, whereas M2 macrophages secrete low levels of TNF-α and transforming growth factor-β, which stimulate the formation of myotubes [47]. VEGF has been shown to stimulate MPC growth [47,57] and to promote engraftment of transplanted MPCs into skeletal muscle [58]. Granulocyte colony-stimulating factor, which is mainly secreted by M1 macrophages [59], stimulates MPC proliferation, and is essential for the muscle regeneration process [60]. MPCs express a wide range of chemokine receptors [61], whereas activated macrophages have been shown to secrete specific panels of chemokines and attractants [41,42]. It is likely that reciprocal attraction between macrophages and MPCs involves chemokines. For instance, macrophages express CXCL12 *in vivo* [62], whereas MPCs express its receptor, CXCR4. Accordingly, CXCL12 increases MPC migration, and silencing of CXCR4 in MPCs prevents their fusion [61]. An important regulator of muscle mass and recovery is insulin-like growth factor (IGF)-1 [63]. A

recent study performed in CCR2-deficient mice showed that macrophages are likely to be the main source of IGF-1 in the regenerating muscle [45]. In particular, the intramuscular Ly6C^{neg} macrophage subset, which presents an anti-inflammatory profile, expresses high levels of IGF-1, which promotes muscle regeneration [45] and dampens the inflammation [64]. Similarly, blocking macrophage-derived IGF-1 reduces their ability to protect muscle cells from atrophy [65]. These recent studies provide an initial list of molecular effectors secreted in a timely way by macrophages that are essential in the regulation of the sequential steps of myogenesis during muscle regeneration, including expansion of MPCs, migration, differentiation, and fusion into new, multinucleated structures.

Transition from proinflammatory to anti-inflammatory macrophages during skeletal muscle regeneration

It was shown by several groups that injured skeletal muscle recruits Ly6C^{pos} monocytes, whereas there is no evidence for the recruitment of Ly6C^{neg} monocytes at the time of injury or later. In a previous study, we analysed monocyte subset recruitment into injured muscle [23]. Circulating Ly6C^{pos} or Ly6C^{neg} monocyte subsets were specifically labelled in the circulation with fluorescent latex beads according to [66], as no genetic lineage tracing tool to follow monocyte/macrophage subsets is available. The appearance of fluorescent bead-containing macrophages in the muscle was then analysed 2–3 days after injury. Ly6C^{neg} monocytes were never found to be recruited into the injured muscle, whereas Ly6C^{pos} monocytes were recruited into the muscle and were found to be converted into Ly6C^{neg} cells in the tissue [23]. One cannot exclude the possibility that the phagocytosis of latex beads may alter the behaviour of the cells, although several controls have been performed [66,67]. However, the kinetics were analysed over very short periods of time (2–3 days). In addition, monocyte depletion from 2 days after injury does not alter skeletal muscle regeneration, indicating that no more monocytes are recruited from the blood to the injured muscle after day 2. Furthermore, in mouse deficient for cbl-b, a ubiquitin ligase involved in macrophage maturation, skeletal muscle regeneration is impaired, owing to increased expression of RANTES, despite an unchanged number of macrophages [68]. This also argues for a single wave of monocytes being recruited at the time of injury, followed by intratissue maturation of macrophages. Accordingly, macrophage size and F4/80 expression increase with time [23]. These results show that injured muscle recruits only Ly6C^{pos} monocytes, which are converted into Ly6C^{neg} monocytes within the muscle tissue during regeneration.

The cellular and molecular mechanisms responsible for the transition from proinflammatory to anti-inflammatory macrophages are poorly known. This transition must be precisely regulated. For instance, increasing M1 signalling leads to defects in muscle regeneration, as shown in mice defective for the CREB –Cebpb axis [69]. Several studies have shown that interference with the sequence of macrophage polarization states leads to a defect in muscle regeneration. In particular, it appears to be essential for the proinflammatory phase to occur before the switch towards the anti-inflammatory phase. Inhibiting interferon-γ alters macrophage function and impairs muscle regeneration [70]. Intramuscular injection of IL-10 at early time points after injury also impairs muscle regeneration, probably by preventing MPC proliferation [46]. Injection of antibody against IL-10 at late time points after injury alters muscle regeneration [46], a result confirmed in IL-10-deficient mice [71]. The macrophage phenotypic transition, which is crucial for proper muscle regeneration, is partly controlled by mitogen-activated protein kinase phosphatase-1. By restricting p38 mitogen-activated protein kinase activation (which leads to Akt activation), mitogen-activated protein kinase phosphatase-1 allows timely macrophage skewing, thereby permitting resolution of inflammation as tissue fully recovers [46].

Do macrophages participate in muscle regeneration occurring after exercise?

Most of the models of muscle regeneration involve rodents and use aggressive techniques. Injection of venom toxin is particularly useful, because it triggers a very reproducible time course of regenerative events. Moreover, this model is highly inflammatory, allowing for the investigation of immune cells. However, it is not physiologically relevant. The question remains of whether macrophages are involved in a physiological context, e.g. after exercise-induced muscle damage (EIMD). Most of the studies published so far have been performed in humans, and they usually rely on eccentric exercises, performed in one or two bouts. According to the topic of this review, we focus here only on studies describing inflammation at the cellular level. In almost all protocols, an increase in circulating leukocytes is observed, particularly an increase in neutrophil number and then in monocyte number, notably in the hours that follow the exercise, the monocyte number remaining high for 3–4 days [72–77]. Whereas

they are almost absent from normal untrained muscle, macrophages have constantly been detected in the muscle biopsies from 24 h after the exercise, their number reaching a plateau in 3–4 days [78–81]. Recently, an interesting investigation into the polymorphism of CCR2 and CCL2 has revealed the association of some single-nucleotide polymorphism with EIMD (blood levels of creatine kinase, which is a marker of muscle damage, soreness, and strength recovery) and strength gain, confirming the tight relationship between macrophages and muscle remodelling after exercise [82,83]. Finally, circulating leukocytes show similar transcriptomic signatures before and immediately at the end of a 30-min exercise, whereas their signature is completely changed 2 h later, when the inflammatory response starts to take place [84]. These studies indicate that macrophages participate in muscle recovery after damaging exercise.

These indications and evidence obtained from experimental data showing that macrophages are necessary for a good muscle regeneration question the use of inhibitors of inflammation to help muscle regeneration/recovery. EIMD includes delayed-onset muscle soreness, which is often treated with nonsteroidal anti-inflammatory drugs (NSAIDs). These are inhibitors of the activity of COX, some of them being specific for the COX2 member of the COX family. COX2 is important in macrophage functions. It participates both in the immune response, through the production of prostaglandin E_2, and in the resolution of inflammation. Indeed, PGE_2 at high concentrations feeds back to inhibit COX2 and 5-lipoxygenase (production of proinflammatory leukotrienes), while inducing 15-lipoxygenase (production of anti-inflammatory lipoxins), thus exhibiting anti-inflammatory effects [85–87]. Many studies have examined the effects of NSAIDs after EIMD. However, compilation of the data is extremely difficult, because of the extreme variation in the exercise model (time, intensity and duration, arm or leg, one or two bouts, etc.), the subjects (trained or not), the drug itself (there are a variety of NSAIDs, with different actions and side effects), the dose of the drug, the time point(s) at which the drug is administered, and, finally, the parameters that are evaluated (reviewed in [88,89]). An attempt to summarize the data follows. Three main types of NSAID effect on muscle are observed after exercise: (a) in both humans and rats, positive effects of NSAIDs on muscle force recovery and/or delayed-onset muscle soreness are reported – a lower extent of muscle damage is also observed, particularly when NSAIDs are given before the exercise [90–96]; (b) also, in various species (human, rabbit, and mouse), no effect of NSAIDs is observed, except for a decrease in soreness in human studies [97–101]; and (c) some studies, all performed in rodents, have shown a negative effect of NSAIDs and/or the inhibition of COX2 activity (by a specific inhibitor in COX2-deficient mice) during muscle regeneration. This is associated with a decrease in the number of immune cells, especially macrophages, leading to a decrease in the diameter of the new myofibres and to the establishment of fibrosis [102–105]. One study in humans reported better recovery a few days after exercise under NSAID treatment, although a deficit in the force of the treated patients was reported 1 month later, suggesting a negative effect in the long term despite a short-term benefit [106]. In the same way, a study analysed the effects of icing the muscle just after injury in rodents (a practice used in some sports), and showed a detrimental effect of icing on regeneration, with smaller fibres and more collagen deposition, associated with a delay in $ED1^+$ macrophage infiltration within the muscle [107].

NSAIDs have pleiotropic roles. They alter muscle protein synthesis, which is, of course, important for muscle recovery [89] and they also act on connective tissue cells [88]. Moreover, they have a detrimental effect on satellite cells themselves, as they inhibit satellite cell/MPC proliferation, as shown in human studies [108,109]. However, the animal data described above suggest that the inhibition of macrophage functions after injury is not beneficial for muscle regeneration. Further analyses, notably in humans, where injury models are less aggressive than in animals, and thus induce less damage, are required to assess the specific role of macrophages in EIMD. Moreover, knowledge on the timing of their involvement during the course of muscle regeneration will be of interest to enable efficient manipulation of the inflammatory compartment for the benefit of skeletal muscle recovery and homeostasis.

Conclusion

Our current knowledge on inflammation during postinjury skeletal muscle regeneration allows us to envisage inflammation as a beneficial – and not a detrimental – event. It also sheds new light on how the environment contributes to the regulation of myogenic cell fate/behaviour. Further studies are required to precisely identify the molecular mode of action of macrophages, particularly at the late stages of muscle regeneration, on myogenic cells, and also on fibrogenic and endothelial cells. Indeed, macrophages stimulate angiogenesis in various contexts [110], and they participate in fibrogenesis, although the respective properties of M1 and M2 macrophages in this process remain to

be established [111]. It is of particular importance to understand how macrophages are beneficial during muscle regeneration and how they are detrimental for the muscle tissue in degenerative myopathies characterized by chronic inflammation.

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