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Macrophages in injured skeletal muscle: a *perpetuum mobile* causing and limiting fibrosis, prompting or restricting resolution and regeneration

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Macrophages are present in regenerating skeletal muscles and participate in the repair process. This is due to a unique feature of macrophages, i.e., their ability to perceive signals heralding ongoing tissue injury and to broadcast the news to cells suited at regenerating the tissue such as stem and progenitor cells. Macrophages play a complex role in the skeletal muscle, probably conveying information on the pattern of healing which is appropriate to ensure an effective healing of the tissue, yielding novel functional fibers. Conversely, they are likely to be involved in limiting the efficacy of regeneration, with formation of fibrotic scars and fat replacement of the tissue when the original insult persists. In this review we consider the beneficial versus the detrimental actions of macrophages during the response to muscle injury, with attention to the available information on the molecular code macrophages rely on to guide, throughout the various phases of muscle healing, the function of conventional and unconventional stem cells. Decrypting this code would represent a major step forward toward the establishment of novel targeted therapies for muscle diseases.

Keywords: macrophages, skeletal muscle, innate immunity, wound healing, alternative activation

MUSCLE INJURY AND INFLAMMATION

Resident leukocytes in the healthy skeletal muscle are exceedingly rare. Thereafter, the skeletal muscle represents a microenvironment in which immunologic reactions depend on the characteristics of the noxious event and on the nature and the function of newly recruited immune cells (Wiendl et al., 2005). Muscle inflammation is a common physiologic response to exercise and the hallmark of acute and chronic damages such as strain injury or muscular dystrophies. Muscle inflammation has been felt to run a rather stereotypical course; recent data however indicate that when persistent triggers cause muscle damage, differences exist in the recruitment of the humoral innate immunity at the site of tissue injury. For example, activation of the complement cascade occurs and contributes to the disease in dysferlin-deficient mice, a model for the muscle wasting diseases referred to as dysferlinopathies, but not in *mdx* mice, a mouse model of Duchenne Muscle Dystrophy (Han et al., 2010).

The inflammation in acutely damaged muscle is characterized by a rapid and sequential invasion of leukocyte populations that persist while muscle repair, regeneration, and growth occur (Paulsen et al., 2010). Neutrophils represent the first leukocyte population in the damaged tissue. They were found in muscle early after exercise completion (Fielding et al., 1993) and infiltrate the tissue for as long as 5 days. Neutrophils release molecules that may contribute to the muscle membrane lysis that follows injury (Nguyen et al., 2005). The actual final effect of neutrophil recruitment in damaged skeletal muscle is however not completely elucidated and recent results suggest that infiltration by neutrophils *per se* may not be harmful but additional inflammatory stimuli are required to reveal their detrimental potential (Dumont et al., 2008). Some such stimuli may be directly generated as a consequence of myofiber lysis and ensuing release of endogenous inflammatory molecules or may be contributed by other recruited cells, such as platelets (Maugeri et al., 2009; Manfredi et al., 2010).

Interestingly, genetic disruption of the chemokine pathway related to inflammatory leukocyte recruitment reveals an apparent balance between the extent and the duration of tissue infiltration by leukocytes: absence of CXCL16 not only resulted in defective homing of macrophages and severely jeopardized tissue regeneration, but was associated with a persistent and important infiltration of the tissue by neutrophils, with unrestrained inflammation and eventual fibrosis (Zhang et al., 2009b). Conversely, a significant increase in macrophage accumulation and cell proliferation was observed in mice in which a transient neutropenia was induced at early times after injury (Godbout et al., 2010). In general, macrophages represent the dominant leukocyte population in the late phases of the homeostatic response to injury or in conditions in which the original inflammatory *noxa* persists. Their ability to perceive environmental cues and orchestrate the functional activities of other cells populations, such as immune cells or myogenic precursors (**Figure 1**), makes them an intriguing field of study in muscle biology.

MACROPHAGES: WHAT'S IN A NAME?

Macrophages were originally identified as phagocytic cells responsible for pathogen elimination and housekeeping functions in

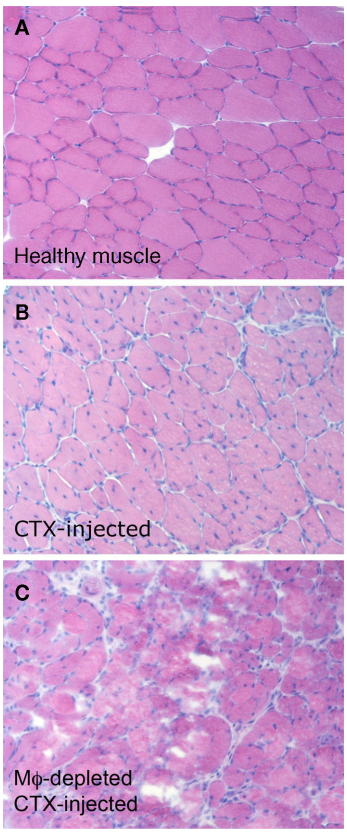

FIGURE 1 | Macrophages, recruited to the skeletal muscle after acute sterile injury, are necessary for effective tissue regeneration. Two months old C57BL/6 mice were treated i.m. with cardiotoxin (CTX). Mice were treated or not with clodronate-containing liposomes to deplete macrophages and sacrificed 15 days after CTX injection. *Tibialis anterior* and *quadriceps* muscles were collected. **(A)** Healthy muscles of untreated control mice. **(B)** The muscle of macrophage-competent mice undergoes effective and almost complete regeneration in 15 days: regenerating centronucleated fibers are evident throughout the section. **(C)** In the absence of macrophages, 15 days after acute sterile injury regenerating fibers are hardly evident. Degenerated fibers and cell remnants persist.

a wide range of organisms (Metchnikoff, 1905): they were thus included in the Mononuclear Phagocyte System, a population of cells, derived from bone marrow progenitors, that differentiate, enter the blood as monocytes and then the peripheral tissues to become resident macrophages or antigen presenting cells (Van Furth and Cohn, 1968).

Monocyte half-life in the blood is of about 1 day. This observation has fostered the concept that blood monocytes replenish macrophage or dendritic cell (DC) pools in peripheral tissues to maintain homeostasis (Ziegler-Heitbrock, 2000). This loop, by which tissues control the size and distribution of their macrophage populations, becomes evident when acute events, such as injury or infection occur.

Inflammation restricts the growth of invading microbes and guides, when the pathogen has been eliminated, its healing. Macrophages in particular represent an active link between innate and adaptive immunity, by regulating T lymphocyte activation and possibly shaping their polarization and function. The pioneering work on the role of T-cell-dependent protective autoimmunity in the healing of sterile spinal cord injuries makes this contention particularly cogent (Schwartz and Ziv, 2008; Shechter et al., 2009).

The role of macrophages is non-redundant. Depletion in the spleen of marginal zone macrophages, which interact with apoptotic material entering from the circulation, accelerated autoimmunity in mice genetically prone to systemic lupus erythematosus and caused significant mortality in wild-type mice repeatedly exposed to apoptotic cells (Mcgaha et al., 2011). Accumulation of apoptotic cell material *per se* triggers acceleration of systemic lupus erythematosus (Bondanza et al., 2003, 2004; Munoz et al., 2010). Macrophages recognize tags expressed by apoptotic cells: as a consequence they on one hand dispose of potentially reservoirs of autoantigens; on the other hand secrete regulatory cytokines that contribute to maintain self-tolerance (Manfredi et al., 2009; Elliott and Ravichandran, 2010). The clearance function of macrophages is crucial to limit the actual cross-presentation of apoptotic cell antigens and possibly to modify the cytokine environment in which the productive T-cell activation take place, thus favoring the establishment of protective, or at least not directly damaging, immune responses (Acharya et al., 2010; Elliott and Ravichandran, 2010; Brereton and Blander, 2011; Peng and Elkon, 2011).

Macrophages also support matrix remodeling and neoangiogenesis and have been implicated in conditions in which neoangiogenesis is potentially deleterious, including cancer (Qian and Pollard, 2010) but also non-neoplastic conditions, such as rheumatoid arthritis or endometriosis (Barrera et al., 2000; Bacci et al., 2009; Capobianco et al., 2010, 2011). All-together the data strongly support the ability of macrophages to perceive ongoing injury of various tissues, and to activate homeostatic programs that through the clearance of dying cells, the organization of neovessel generation, the regulation of the extracellular matrix remodeling and the activation of appropriate T lymphocyte responses leads to the effective healing.

Macrophage activation is clearly protective in the case of intense, short lasting injuries. In contrast, macrophage action sustaining regenerative and vascular responses can be deleterious in conditions such as persisting infectious diseases, chronic tissue damage, or event more notably tumors, in which the initial stimulus perceived by macrophages persists. Several excellent reviews have addressed the latter issue (e.g., see Biswas and Mantovani, 2010; Gordon and Martinez, 2010; Mantovani and Sica, 2010; Qian and Pollard, 2010; Squadrito and De Palma, 2011) and we will not discuss the issue further in this essay.

Dedicated pattern-recognition receptors (PRRs) are non-clonally expressed by most innate immune cells (Palm and Medzhitov, 2009). PRRs allow to indentify molecular structures shared by ample classes of microbes, referred to as pathogen-associated molecular patterns (PAMPs; Janeway, 1992). The activation of PRRs results in cascade of tightly coordinated events, including: (i) the production of cytokines and chemokines, which attract and activate leukocytes (Nathan, 2002) (ii) the activation of an acute phase response, with the production of conserved soluble PRRs, such as pentraxins (Manfredi et al., 2008) which tune leukocyte activation and limit their ability to damage the tissue; (iii) the migration of APC to draining lymph nodes, with

productive activation of naïve T lymphocytes. The expansion of antigen-specific T-cells is a key event in the establishment of an adaptive immunological response (Bevan, 2011).

Damage-associated molecular pattern (DAMP), an array of heterogeneous molecules that are released during cell and tissue necrosis, although non-microbial, share the ability to activate PRRs (**Table 1**). Ss a consequence DAMPs recognition elicits inflammation and prompts tissue regeneration and acquired T-cell-dependent immune responses even in the context of sterile injuries (Bianchi, 2007; Lotze et al., 2007; Rubartelli and Lotze, 2007; Urbonaviciute et al., 2008; Bianchi and Manfredi, 2009; Manfredi and Rovere-Querini, 2010; Maroso et al., 2010; Castiglioni et al., 2011; Liu et al., 2011b; Zhang et al., 2011). Macrophages undergo an extensive reprogramming of their functional properties in response to PAMPs (Nau et al., 2002; Martinon et al., 2010), but also to signals released directly from damaged tissues (London et al., 2011) and from lymphocytes (Tiemessen et al., 2007; Wong et al., 2010; Liu et al., 2011a).

Studies with various probes reveal a phenotype heterogeneity in macrophages that possibly reflects peculiar features and function of macrophages sub-populations within the microenvironment. In response to microenvironmental cues, macrophages in the tissue become potent effector cells integrated in a T helper (Th)-1 response, which kill microorganisms and tumor cells and produce copious amounts of cytokines. Microbial destruction is mediated at least partially by the production of reactive oxygen species (ROS) and nitric oxide (NO). The amount of NO produced is instrumental for the ability of macrophages to control the intracellular parasite *L. mexicana* (Mylonas et al., 2009). In humans, classically activated macrophages are important for resistance to mycobacteria and *Leishmania major* infection (Darrah et al., 2007). Macrophages may undergo an alternative activation pathway (referred to as "alternative activation") that endows them with the ability to tune inflammatory responses and adaptive immunity, scavenge debris, and promote angiogenesis, tissue remodeling, and repair (Mantovani et al., 2004).

Macrophages that infiltrate regenerating tissues in general belong to the second class of healing macrophages (Corna et al., 2010; Daley et al., 2010; O'brien et al., 2010; Schwartz, 2010; Brancato and Albina, 2011; Cairo et al., 2011; David and Kroner, 2011; Harel-Adar et al., 2011; Jaeschke, 2011; London et al., 2011; Wang and Harris, 2011), while unrestrained polarization toward a classically activated phenotype associates with defective healing and persistent inflammation (Sindrilaru et al., 2011). Healing (or alternatively activated) macrophages can be propagated *in vitro* by exposure to monocyte precursors to low concentrations of M-CSF in the presence of IL4, IL13, or IL10 (Mantovani et al., 2004). In contrast exposure to microbial components such as LPS in the presence of γIFN or to GM-CSF is an effective stimulus to elicit classically activated, inflammatory macrophages (**Figure 2**).

The dichotomy between inflammatory and tissue healing macrophages represents a "useful over-simplification" (Mantovani et al., 2009) of a sophisticated array of functions exerted by macrophage populations in injured tissues. The plasticity of macrophages in response to environmental cues has been characterized with particular attention in a model tissue, the skeletal muscle (Brunelli and Rovere-Querini, 2008; Chazaud et al., 2009; Tidball and Villalta, 2010).

Table 1 | Inflammatory sterile stimuli and associated diseases (Manfredi and Rovere-Querini, 2010; Rock et al., 2010, 2011; Zhang et al., 2010a; Castiglioni et al., 2011).

Molecule	Associated inflammatory conditions
Asbestos*	Asbestosis: lung inflammation and fibrosis. Mesothelioma and lung cancer
ATP, ADP, adenosine	Airway inflammation
Calcium pyrophosphate	Pseudogout: chronic inflammatory arthritis
Cholesterol crystal	Atherosclerosis: arterial inflammation and occlusion
DNA constituents	Systemic autoimmunity (SLE)
HMGB1	Sepsis, systemic autoimmunity (SLE), rheumatoid arthritis
Matrix constituents (hyaluronate, heparan sulfate, fibronectin, fibrinogen, elastin, and collagen derived peptide)	Idiopathic pulmonary fibrosis, COPD, nephritis, arthritis
Mitochondrial DNA	Trauma, systemic inflammatory response syndrome (SIRS)
Mitochondrial formyl peptides	Trauma, SIRS
Silica	Silicosis: lung Inflammation and fibrosis
Uric acid	Gout: chronic inflammatory arthritis

*via HMGB1 release? See (Yang et al., 2010).

A CASE FOR MACROPHAGES IN THE SKELETAL MUSCLE

Macrophages have been known for a long time to be associated with skeletal muscle injury (Robertson et al., 1993; Mclennan, 1996). Moreover they play a critical role in the pathogenesis and in the natural history of self sustaining muscle diseases, specifically including primary inflammatory myopathies (necrotizing autoimmune myositis, inclusion body myositis and polymyositis) and genetic diseases of the tissue (Duchenne and Becker muscular dystrophies; Dalakas, 2002; Villalta et al., 2009). *In vivo* studies have unequivocally shown that macrophages actually participate in the tissue repair process (St Pierre and Tidball, 1994; Mclennan, 1996; Chazaud et al., 2003; Warren et al., 2005; Summan et al., 2006; Arnold et al., 2007; Tidball and Wehling-Henricks, 2007; Segawa et al., 2008; Ruffell et al., 2009; Sun et al., 2009; Brigitte et al., 2010; Dumont and Frenette, 2010; Martinez et al., 2010; Vezzoli et al., 2010; Lu et al., 2011b). Macrophages, as discussed above, are professional scavengers of apoptotic cells and debris and produce a vast array of signals involved in matrix remodeling and neovessel formation. Data in various models of skeletal muscle injury, including hindlimb ischemia, freeze-injury, unloading/reloading sequences, and myotoxic agent injection indicate

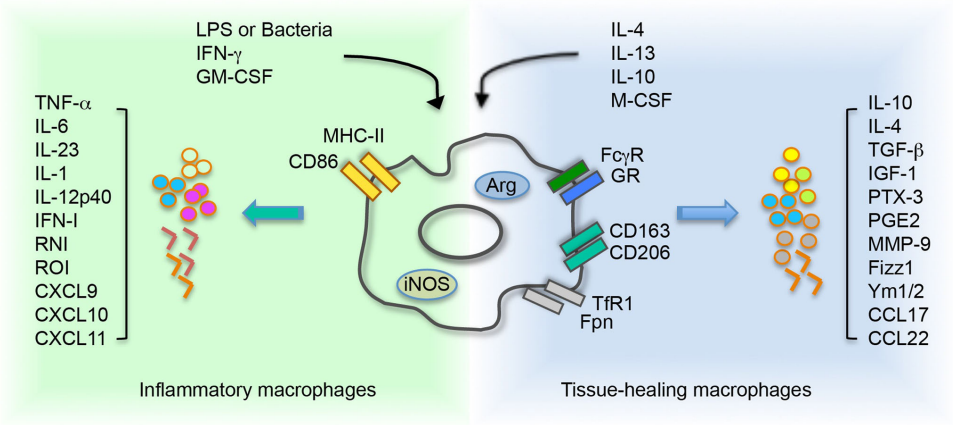

FIGURE 2 | Environment cues control and influence macrophage polarization. Inflammatory macrophage activation is driven by stimulation with LPS or bacteria, and/or IFN-γ or GM-CSF. These molecules induce iNOS activity on macrophages and consequent production of RNI. Treatment with IL4 and/or IL13, IL10 and/or M-CSF supports the activation of tissue healing macrophages. The cytokine network activates an arginase-dependent metabolism of arginine. In macrophages, acquisition of a specific polarization state determines a differential expression of receptors and secretion of molecules. LPS, lipopolysaccharide; IFN-γ, interferon-γ; GM-CSF, granulocyte macrophage-colony stimulating factor; M-CSF, macrophage-colony stimulating factor; MHCII, major histocompatibility complex class II; GR, galactose receptor; CD163, haptoglobin–hemoglobin complex receptor; CD206, mannose receptor; TfR1, transferrin receptor 1; Fpn, ferroportin; TGF-β, transforming growth factor β; IGF1, insulin like growth factor 1; PTX-3, pentraxin 3; PGE2, prostaglandin E2; MMP-9, matrix metalloproteinase 9; Fizz, resistin like molecule; Ym1/2, chitinase like protein 1/2; TNF-α, tumor necrosis factor α; IFN, interferon type I; RNI, reactive nitrogen intermediate; ROI, reactive oxygen intermediate.

that the recruitment of macrophages in the tissue occurs regardless of the characteristics of the original *noxa* (Tidball, 2002).

Arnold et al. (2007) identified a population of circulating monocytes selectively recruited in damaged muscle where they acquire a anti-inflammatory phenotype, correlated to tissue healing. Which are the signals involved in the functional polarization of macrophages in the injured/regenerating muscles? The phagocytosis of muscle cells debris is most likely to favor this transition (Arnold et al., 2007).

A macrophage population associated to the epimysial and perimysial connective tissue plays a crucial non-redundant role in monocyte attraction and activation in acutely injured skeletal muscle, providing signals that control their switch to tissue healing macrophages (Brigitte et al., 2010). Resident macrophages also attract in the injured skeletal muscle cells that express the CD11c integrin, a *bona fid*e marker of myeloid DCs. These cells are endowed with antigen presenting capacity and with the ability to migrate from the muscle into draining lymph nodes (Brigitte et al., 2010). They represent attractive candidates to link the response to injury in the tissue to the local activation and recruitment of T lymphocytes, a hallmark of persistent skeletal muscle inflammation.

Activated myogenic precursors also generate chemoattractive signals for inflammatory cells, and their ability to recruit them at the site of muscle injury is further upregulated by the interaction with macrophages (Chazaud et al., 2003). After injury, myogenic precursors, injured fibers, resident macrophages, and recruited monocytes are a source of CCL2/MCP1 (Chazaud et al., 2003; Brigitte et al., 2010; Lu et al., 2011a). Indeed, severe impairments in skeletal muscle regeneration occur in mice defective of the chemokine-CC-motif receptor 2-deficient ($CCR2^{-/-}$), which is activated by CCL2/MCP1 (Warren et al., 2005). CCR2 expression on bone marrow derived cells is essential for robust macrophage recruitment after acute sterile injury and muscle regeneration. Surprisingly, injured muscle of lethally irradiated mice transplanted with CCR2-deficient bone marrow cells contain, despite impaired muscle regeneration, increased numbers of myogenic progenitor cells (Sun et al., 2009), suggesting that macrophages are required for precursor cells to fuse and yield effective myofiber formation. Drastic reduction of macrophage recruitment in injured muscle of $CCR2^{-/-}$ mice associates to a dramatically reduced expression of insulin like growth factor 1 (IGF1), a central regulator of muscle regeneration (Lu et al., 2011b). This observation suggests that macrophages regulate muscle healing through IGF1. Indeed, in $CCR2^{-/-}$ mice, local IGF1 injection at least partially makes up for the lack of recruited macrophages (Lu et al., 2011b).

In vitro, IGF1 elicits in muscle cells a biphasic response, first stimulating cell proliferation and subsequently enhancing myogenic differentiation (Rosenthal and Cheng, 1995), a sequence of events that could be teleologically suited to sustain the repair of damaged tissue. *In vivo*, expression of a muscle specific transgene encoding a locally acting isoform of IGF1 prompts hypertrophy and regeneration in senescent skeletal muscle (Musaro et al., 2001).

THE MUSCLE MICROENVIRONMENT AND THE INFLAMMATORY RESPONSE

The muscle environment and the mechanisms through which it modulates regeneration have attracted much attention in the recent years (Paylor et al., 2011). Several studies have investigated in particular whether similar events occur during the regeneration of adult skeletal muscle and during embryogenesis (Charge and Rudnicki, 2004). The microenvironment in which the two events occur is strikingly diverse: specifically muscle development occurs without any substantial contribution by infiltrating cells,

which are instead present in regenerating muscle at concentrations that exceed 100,000 inflammatory cells/mm^3 of tissue (Wehling et al., 2001; Paylor et al., 2011). Recent evidences suggest that a model in which myogenesis occurs independently of the activation of inflammatory pathway may be far too simplistic: for example, myoblasts of mouse embryos and regenerating myocytes in injured adult mouse skeletal muscle, but not mature myocytes, express the receptor for granulocyte colony stimulating factor (G-CSF). Moreover, the C-CSF/G-CSF receptor pathway is crucial for skeletal myocyte development and regeneration (Hara et al., 2011).

Although several molecules have been identified not to be dispensable for muscle regeneration, the overall array of signals macrophages deliver in the tissue and the hierarchy among them is far from being elucidated. At early stages after acute injury macrophages mostly secrete inflammatory molecules, including CCL2/MCP1 and TNFα, which may favor tissue wasting *via* activation of the FoxO transcription factor (Sandri et al., 2004; Zhao et al., 2007). Simultaneously, they dispose of apoptotic cells and fiber remnants: apoptotic cell clearance has been shown in other systems to trigger the release of cytokines involved in the termination of the inflammatory response and in immune regulation, such as TGFβ and IL10 (Huynh et al., 2002; Zhang et al., 2011; see also below).

At later stages macrophages actively sustain fiber reconstitution (Summan et al., 2006; Arnold et al., 2007; Shireman et al., 2007). At this stage they mainly secrete cytokines that may play a trophic function, such as IGF1 (Musaro et al., 2001; Summan et al., 2006; Pelosi et al., 2007) or IL10 (Strle et al., 2007, 2008; Tidball and Villalta, 2010).

In *mdx* mice, a well-accepted model for human Duchenne's muscular dystrophy, the expression of IL10 modulates macrophage activation and reduces the membrane damage: in this system, IL10 has been proposed to deactivate the inflammatory profile of macrophages infiltrating damaged muscle at the early, acute stage of muscle disease, promoting a switch toward an alternative activation profile (Villalta et al., 2010). At later phases however the persistence of alternatively activated macrophages in conditions in which the tissue can not heal may actually play a deleterious role: for example the sustained production in *mdx* mice of TGFβ may be involved in the fibrotic substitution of the myofibers, a hallmark of the advanced phases of muscular dystrophy: the cytokine indeed is associated to fibroblast activation and proliferation, leading to sustained collagen production and eventually to fibrosis (Vidal et al., 2008).

An exclusive population of progenitors of both fibroblasts and adipocytes has been recently clearly identified in skeletal muscle (Joe et al., 2010; Uezumi et al., 2010), referred to as fibro/adipogenic progenitors (FAP). These cells remain in a quiescent state in normal conditions, undergo a dramatic but transient proliferation in response to injury, contextually delivering trophic signals for proliferating myogenic precursors. Fibrotic scar substitution and accumulation of lipid filled adipocytes is a feature of conditions of the failed regeneration of the skeletal muscle, like it occurs dramatically in muscle dystrophies but at some extent also during physiological aging: when myogenic precursors fail to replace the damaged tissue, FAP would according to recent models (Rodeheffer, 2010; Paylor et al., 2011) take over, differentiating into adipocytes and possibly fibroblasts, thus ensuring the structural continuity of the tissue.

Various stem cell populations, including mesenchymal stem cells (MSC) and neural precursor cells (NPC), regulate the leukocyte fate, through mechanisms involving cell–cell contact and/or various soluble factor. MSC inhibit proliferation of various immune cells, including T, B, and NK lymphocytes (Groh et al., 2005; Krampera et al., 2006; Sotiropoulou et al., 2006; Spaggiari et al., 2006) and specifically influence affect DCs function through the release of IL6, (Djouad et al., 2007), of the Notch ligand Jagged-2, which induces the generation of regulatory DCs (Zhang et al., 2009a) and of prostaglandin E2 (Spaggiari et al., 2009), while NPC restrict the activation of DCs via a BMP-4-dependent-mechanism (Pluchino et al., 2009). MSC also reprogramming macrophages toward an alternatively activated profile (Ohtaki et al., 2008; Kim and Hematti, 2009), which is instrumental in a model of skin wound healing for effective wound repair (Zhang et al., 2010b). The possible cross-talk between FAPs and other precursors of mesenchymal origin and inflammatory cells infiltrating injured skeletal muscle, although demonstrated in other model tissues (Stappenbeck and Miyoshi, 2009; Zhang et al., 2010b; Ehninger and Trumpp, 2011), has not to the best of our knowledge been directly investigated so far.

MACROPHAGES AND MYOGENIC PRECURSORS

In the absence of macrophages injured muscle fail to regenerate (**Figure 1**; see also above), even if the number and function of the cell populations with myogenic potential in the tissue, the eventual effectors of muscle healing that repair or replace injured or dead fibers (Mauro, 1961), are not directly affected. The results indicate that macrophages actively "license" myogenic precursors to carry out their program, i.e., to proliferate, differentiate, and fuse and thus to regenerate the tissue. This is not an isolated feature of the skeletal muscle: macrophages for example sustain the stem cell survival in various tissues, including the skin and the bone marrow, a limiting step for their regeneration (Tothova and Gilliland, 2007; Blanpain and Fuchs, 2009; Discher et al., 2009; Gurumurthy et al., 2010).

It is still not clear at which level(s) macrophages actually specifically act. For example, myogenic progenitor cells were significantly increased in injured muscle of mice with CCR2-defective bone marrow cells even if muscle regeneration is severely affected (Sun et al., 2009). Satellite cells are considered the resident "stem-like" cells in skeletal muscle and are responsible for muscle growth and regeneration in postnatal life (Holterman and Rudnicki, 2005). In response to muscle injuries, quiescent satellite cells undergo activation, proliferate, and fuse with each other or with damaged fibers (Kuang et al., 2008); conversely, some precursors undergo self-renewal, and thus maintain the integrity of the quiescent satellite cell pool (Zammit, 2008; Kang and Krauss, 2010). A pathway strictly associated to the control of neoangiogenesis, which comprises the interaction between angiopoietin 1 (Ang1) and its receptor Tie-2 and the downstream activation of the ERK1/2 kinase, has been in elegant studies implicated in the ability of satellite cells to re-enter the stem cell niche (Abou-Khalil et al., 2009; Mounier et al., 2011), thus suggesting that endothelial cells and possibly other non-muscle cells, are involved in the maintenance of the

satellite cell niche. The observation that even single transplanted satellite cells both differentiate and self-renew after transplantation *in vivo* (Sacco et al., 2008) provides a formal demonstration that satellite cells are indeed endowed with stem cell properties. Telomeres length in muscle cells and the control of muscle stem cell regenerative capacity represents, in particular in the setting of muscular dystrophies, a particular attractive target for the action of inflammatory molecules (Sacco et al., 2010).

Despite the concentrated effort of several groups, our actual insight on the role of the microenvironment in determining the overall outcome of the tissue response to injury is still fragmentary. Recent studies specifically highlight the importance of mechanical factors, such as tissue rigidity/elasticity in regulating the fate of muscle stem cells (Gilbert et al., 2010), revealing important caveats that apply to the *in vitro* systems that are commonly used. Other environmental influences are possibly as relevant: for example, we have recently observed that regeneration after an acute sterile injury of the skeletal muscle is accompanied by the substantial generation of ROS production, which is counterbalanced and rapidly overcome by the generation of antioxidant moieties. Mitochondria are initially responsible for ROS formation while at later time points, non-mitochondrial sources are involved. Both regenerating fibers and macrophages express high levels of free thiols and antioxidant enzymes, such as superoxide dismutase 1 (SOD1) and thioredoxin (Vezzoli et al., 2011). The well-characterized role of a reduced environment in maintaining the extracellular function of DAMP molecules (Lotze et al., 2007; Rubartelli and Lotze, 2007; Carta et al., 2009; Rubartelli and Sitia, 2009), either directly released by damaged fibers of actively secreted by infiltrating macrophages suggests that the antioxidant response directly contributes in the acutely injured tissue to homeostasis.

Preliminary data from our laboratory indicate that macrophages also influence other cells with myogenic potential, such as mesoangioblasts. Mesoangioblasts are vessel-associated progenitors that ameliorate defective muscle structure and function in dystrophic mice and dogs (Sampaolesi et al., 2003, 2006; Tedesco et al., 2010). A clonal analysis of embryonic explanted organ rudiments led to the positive identification of a "mesoangioblast" cell population in the embryonic dorsal aorta (De Angelis et al., 1999) and later studies implicated pericytes associated with microvascular walls in the human skeletal muscle as their human counterpart (Dellavalle et al., 2007). Mesoangioblasts are endowed with the ability to cross the vessel wall, a feature missing in satellite cell-derived myogenic precursors (Dellavalle et al., 2007): when injected into the blood, mesoangioblasts are indeed able to migrate outside the vessel toward injured and inflamed tissues, including the dystrophic muscle (Sampaolesi et al., 2003), possibly following chemotactic signals generated by activated innate cells, macrophages in particular (Lolmede et al., 2009). These features allow a systemic delivery of mesoangioblasts, thus overcoming migration-related problems described for other stem cell populations. In the recent years various tools were investigated to improve mesoangioblast migration toward damaged muscle and their local differentiation to optimize future cell therapy protocols for muscular dystrophies (Tedesco et al., 2010).

Macrophages are important to recruit and locally activate mesoangioblasts, committing them to myogenic differentiation. Conversely, we have observed that mesoangioblasts regulate gene expression of *in vitro* bone marrow derived macrophages (Bosurgi et al., unpublished results). Genes associated to macrophage scavenger function, phagocytic activity, chemokines release, and response to cytokines, which are regulated at the expression level, are all targets of mesoangioblast action. Their regulation is selective, since several other genes associated to macrophage housekeeping functions, to the iron metabolism and to the redox control are unaffected. We are actively verifying the possibility that mesoangioblasts prime the macrophages function toward a regulatory activity and this finely tuned cross-talk regulates the outcome of tissue remodeling.

CONCLUSION

In summary, myogenic precursors derived from satellite cells and other muscle stem cells are the final effectors of muscle regeneration. Substantial evidence indicates that they need licensing by accessory cells, in particular by inflammatory cells such as macrophages. Other mesenchymal precursors expand when muscle regeneration fails, leading to the eventual fibrotic scar and fat replacement of the tissue. The latter event is again possibly dependent on the inflammatory environment of the tissue. The efforts of the next years are likely to break the code by which the immune response controls the regeneration of the skeletal muscle, thus leading to the development of effective targeted therapies for genetic defects of the tissue and for the most common causes of physiological muscle wasting and sarcopenia.

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