Sca-1 influences the innate immune response during skeletal muscle regeneration

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Long KK, Pavlath GK, Montano M. Sca-1 influences the innate immune response during skeletal muscle regeneration. Am J Physiol Cell Physiol 300: C287–C294, 2011. First published December 1, 2010; doi:10.1152/ajpcell.00319.2010.—Efficient muscle regeneration requires the clearance of dead and dying tissue via phagocytosis before remodeling. We have previously shown that mice lacking stem cell antigen-1 (Sca-1) display a defect in skeletal muscle regeneration characterized by increased fibrosis and decreased turnover of the extracellular matrix. In the present study we demonstrate that Sca-1−/− mice have a defect in their capacity to recruit soluble IgM, and subsequently C3 complement, to damaged muscle. We hypothesize that this defect in recruitment delays or decreases phagocytosis by macrophages, contributing to the previously observed fibrotic phenotype of these mice. As the primary source of soluble IgM is peritoneal B-1a cells, which are a subset of self-renewing B cells, we analyzed this cell population and observed a significant reduction in B-1a cells in Sca-1−/− animals. Interestingly, these mice are protected from ischemia–reperfusion injury, an acute inflammatory reaction also mediated by IgM and C3 complement that has been linked to a deficit in B-1a cells in previous studies. Collectively, these data reveal a novel role for Sca-1 in innate immunity during muscle regeneration and indicate that further elucidation of immuno-myogenic processes will help to better understand and promote muscle regeneration.

B-1a cell; complement C3; IgM; regeneration; fibrosis; ischemia

THE INITIAL CLEARANCE of necrotic and damaged tissue from injured muscle is an essential component of muscle regeneration; mouse strains with reduced kinetics of immune infiltration display inefficient muscle regeneration characterized by persistence of necrotic tissue and increased fibrosis compared with mouse strains with robust infiltration (34). Skeletal muscle regeneration proceeds through three interrelated and overlapping phases: 1) initial tissue necrosis, inflammatory infiltration, and macrophage mediated clearance; 2) localized muscle precursor cell proliferation, differentiation, and fusion; and 3) extracellular matrix (ECM) remodeling and wound resolution (21, 44). Upon injury, chemoattractants produced by damaged muscle recruit macrophages that recognize and clear debris and necrotic tissue, as well as promote muscle precursor cell proliferation and prevent apoptosis of myogenic cells (1, 9, 12, 34). The initial inflammatory milieu has been related to the efficiency of muscle repair (47).

An early step in the clearance of necrotic and apoptotic cells involves the activation of the classical complement pathway (33, 37). The complement cascade is an innate immune process that consists of multiple soluble proteins that assemble on the surface of target cells, resulting in a cytolytic response (42). Although the complement cascade can be activated through three pathways (i.e., classical, alternative, and mannose-binding lectin), each with unique features in their activation and amplification, all three pathways converge on the C3 component, ultimately leading to the formation of a cytolytic pore or membrane attack complex (MAC) (43, 49). During pathogen clearance, complement activation is triggered by the formation of the antibody:antigen complex, and deficiency in the complement component C3 results in an increased susceptibility to bacterial infection (26, 32).

Damaged tissue, including apoptotic and necrotic cells, display altered cell surface expression patterns that are recognized by natural IgM. These antibodies are produced mainly by the nonconventional B cell subset B-1a cells, and binding results in complement deposition, uptake, and phagocytosis by macrophages (33, 36–38). B-1a cells (previously referred to as Ly-1/CD5+ B cells) can be distinguished from conventional B cells (B2) by their developmental origin, surface marker expression, function, and capacity for self-renewal (24). In the adult mouse B-1a cells are located primarily in the peritoneal cavity; they are derived from progenitors present in the fetal omentum and fetal liver but are largely absent from adult bone marrow (18, 24, 28).

Natural IgM antibodies produced by B-1a cells are present before exogenous antigenic stimulation. The repertoire of natural IgM antibodies, however, is fairly restricted and includes antibodies that recognize multiple conserved structures, such as nucleic acids, heat shock proteins, and phospholipids (3, 17). Soluble natural IgM can function as autoantibodies through recognition of self-antigens expressed on the surface of dying cells (25, 37). These antibodies provide an innate immune response that is present before adaptive immunity and have been implicated in mediating early defense against infection, as well as prevention of autoimmunity and immunosurveillance against tumors (14, 30). Mice deficient in soluble IgM display profound deficits in complement deposition and phagocytosis of apoptotic cells (38). The importance of IgM-mediated complement activation in tissue regeneration has been demonstrated using mice deficient of the complement component C3; these mice display profound deficits in liver regeneration characterized by fibrosis due to an inability to clear damaged tissue (31).

Stem cell antigen-1 (Sca-1) is a member of the Ly-6 family of small (12–15 kDa) GPI-linked proteins (48) initially identified in activated T-cells (54). Sca-1 has since been detected in multiple tissues, including multiple cells of hematopoietic lineages, cells of the mammary gland, liver, prostate, heart, and skeletal muscle (20). Sca-1 is required for the self-renewal of hematopoietic and mesenchymal stem cells, as well as for T cell development and function (2, 7, 22, 46, 51). We previously demonstrated that Sca-1−/− mice display inefficient muscle
regeneration characterized by an increased fibrogenic phenotype (23). Sca-1<sup>−/−</sup> mice display 3.5- and 2.2-fold increases in fibrotic index 7 and 14 days postinjury, respectively, and damaged muscle is characterized by increased collagen and fibronectin. Interestingly, loss of Sca-1 in a mouse model of muscular dystrophy (mdx) increases the severity of fibrosis already present in this model, demonstrating that Sca-1 regulates fibrosis in both acute and chronic models of muscle injury (23).

Sca-1 is highly expressed in multiple populations of the immune system (20), and the innate immune response plays a critical role in normal muscle regeneration (1, 11, 12). Because Sca-1 is expressed extensively in immune cells, we were interested in determining whether defects in innate immune function contribute to the muscle regeneration phenotype observed in Sca-1<sup>−/−</sup> muscle. Here we present evidence that the loss of Sca-1 affects the recruitment of IgM and complement C3 to injured muscle. We also show that Sca-1<sup>−/−</sup> mice display significantly decreased peritoneal B-1a cell numbers. We further show that Sca-1<sup>−/−</sup> mice fail to mount a normal response to ischemia-reperfusion injury, consistent with a defect in innate immune-mediated recognition of necrotic tissue damage. Our observations reveal a novel role for Sca-1 in the modulation of B-1a cell number and B-1a-driven deposition of IgM and complement in regenerating tissue. These data help to further define the interplay between muscle and the innate immune effectors during skeletal muscle regeneration to include not only macrophages but also B-1a cells. The data presented here provide a novel therapeutic strategy in the management of fibrosis through modulation of the IgM/complement axis during the muscle regenerative process.

MATERIALS AND METHODS

Animals and hindlimb ischemia. Sca-1<sup>−/−</sup> mice backcrossed 10 generations to the Balb/c background were provided by W. Stanford (45). Control age- and sex-matched Balb/c mice were purchased from Charles River Laboratories. Adult mice between the ages of 8–12 wk were used for all experiments. Muscle regeneration of the gastrocnemius muscle was induced by injection of 40 μl of 1.2% BaCl2 in PBS as described (35), and samples were collected at the indicated times postinjury. Hindlimb ischemia was induced by femoral ligation as described previously (13), and muscles were collected 7 days postligation. All animals were handled in accordance with the institutional guidelines of Boston Medical Center and Emory University.

Antibodies. The following antibodies and their appropriate isotype controls were purchased from BD Biosciences: CD5-FITC (53-7.3), IgM-PE (11-26c.2a), IgM-APC (II/41), B220-PerCPCy5.5 (RA3-6B2), and CD19-PECy7 (1D3). Anti-C3 was purchased from Abcam, and FITC donkey-anti-rat IgG was from Jackson ImmunoResearch.

Preparation of muscle lysates. Regenerating gastrocnemius muscles were collected at the indicated time points and homogenized in 1 ml RIPA buffer (25 mM Tris, pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, and 0.1% SDS) with complete mini-protease inhibitor tablets added (Roche). The contralateral leg from each mouse was collected as an un.injected control. Homogenates were centrifuged at 21,000 g for 15 min to pellet insoluble matter. Protein concentrations were determined using BCA reagent (Pierce).

Serum collection. Whole blood was collected by cardiac puncture, placed into Microtainer serum separator tubes (BD Biosciences), and allowed to clot. Samples were centrifuged at 5,000 g for 20 min to remove red blood cells.

Determination of IgM, IgG, and C3 complement levels. IgM and C3 levels were determined by ELISA. The IgM ELISA was purchased from Assay Designs, and the C3 ELISA was from Immunology Consultants Laboratory. Levels of IgG were analyzed using the Easy Titer IgG Assay Kit from Thermo Scientific. Serum and muscle homogenates were diluted according to the kit manufacturer before use. Results from muscle homogenates were normalized to total protein concentration.

Collection of muscles and morphometric measurements. Muscles were collected from Balb/c (n = 4) and Sca-1<sup>−/−</sup> (n = 6) mice 7 days after ligation of the femoral artery using standard dissection techniques and immediately frozen. Serial 10-μm sections were collected along the length of the muscle and stained with hematoxylin and eosin. Images were obtained using a Nikon SMZ1000 microscope equipped with a video camera and SPOT imaging software version 4.6.1.38. ImageJ software (version 1.42q) was used to quantify the amount of injury observed in ischemic muscles. Briefly, the total area of each section, as well as the area of injured regions, was determined, and the percentage of the total area injured was calculated. Injured muscle was defined as the presence of fibrotic tissue, centrally located (regenerating) myonuclei, and the absence of intact myofibers with peripheral myonuclei.

Immunofluorescence. Muscle sections were rehydrated in PBS, blocked in PBS with 5% donkey serum, and incubated with an anti-C3 antibody (Abcam) followed by detection with a FITC conjugated donkey-anti-rat secondary antibody (Jackson). Images were collected using an Olympus BX41 fluorescent microscope (Olympus Optical) equipped with a DP71 CCD camera (Olympus) and DP controller/DP manager software (Olympus). Representative images are shown.

Isolation and cytometric analysis of peritoneal cells. Peritoneal cells were isolated from Balb/c and Sca-1<sup>−/−</sup> mice as described (39). Briefly, 5 ml cold PBS with 5% FBS were injected into the peritoneal cavity of each mouse. After the peritoneum was gently massaged to dislodge any attached cells, the accumulated liquid was removed from the cavity with a clean syringe. The cells were washed once in buffer (PBS with 0.5% BSA, 0.2 mM EDTA) before being stained. To quantify the number of B-1a cells, cells were first incubated with Fc-receptor block (BD Biosciences) for 10 min, washed, and then resuspended in fresh buffer. Cells were stained with the indicated antibodies or the appropriate isotype controls and analyzed on an LSRII using FACSDiva software (BD Biosciences). B-1a cells were identified based on the surface phenotype IgM<sub>high</sub>IgD<sub>low</sub> B220<sup>+</sup>CD5<sup>+</sup>. Ten thousand cells were analyzed for each sample. Analyses were performed using FlowJo version 9.0.1 (TreeStar).

Statistics and image assembly. Student’s t-test was used to determine the significance between two groups. Two-way ANOVA was used to determine the significance between multiple groups. A confidence level of P < 0.05 was accepted for statistical significance. Analyses were conducted using GraphPad Prism 4.0 and STATA for Macintosh. Images were assembled using Adobe Photoshop and Illustrator CS and were not modified other than uniform adjustments to size, color levels, brightness, and contrast.

RESULTS

Sca-1<sup>−/−</sup> mice display reduced serum IgM and an inability to recruit IgM to sites of muscle damage. Clearance of damaged tissue by macrophages is critical to muscle regeneration, and an inability to remove apoptotic and necrotic cell can lead to fibrosis (34). Given the extensive expression of Sca-1 cells of the innate immune system, as well as the fibrogenic phenotype observed in Sca-1<sup>−/−</sup> muscle, we examined whether Sca-1 affects removal of debris from damaged tissue. Natural IgM antibodies play a critical role in the clearance of necrotic and apoptotic cells, and an inability to clear this debris promotes development of autoimmune and inflammatory diseases (3, 36). IgM binds to autoantigens presented upon tissue damage (such as phosphatidylcholine), resulting in reduced...
complement recruitment and decreased clearance of debris by macrophages (14). Whereas IgM is required for clearance of necrotic and apoptotic cells in other organs (e.g., liver, peritoneum), to our knowledge a role for IgM recruitment in the removal of damaged tissue from muscle during regeneration has not been demonstrated (30, 31, 36). We therefore examined levels of soluble IgM in extracts generated from wild-type (WT) and Sca-1−/− muscle 5 days postinjury to determine whether loss of Sca-1 influences IgM recruitment to damaged muscle (Fig. 1).

Extracts from the contralateral legs of each mouse were also examined as an uninjured control. While baseline levels of IgM were lower in uninjured Sca-1−/− when compared with WT, this difference was not significant (Fig. 1A). As shown in Fig. 1A, in muscles from WT mice the level of IgM increased sixfold when compared with the contralateral control leg in response to injury (Fig. 1A). Surprisingly, however, IgM levels were significantly reduced in injured muscle isolated from Sca-1−/− mice as compared with WT mice. To determine whether this blunted response in IgM recruitment was muscle specific or systemic, we measured soluble IgM levels in the serum from mice of both genotypes. Consistent with the more profound deficit in muscle, serum from Sca-1−/− mice displayed a twofold decrease in soluble IgM relative to WT (Fig. 1B). These data indicate that, while IgM is recruited to injured muscle in the WT mouse, this response is severely attenuated in Sca-1−/− mice. These data suggest that a minimum threshold level of soluble IgM antibodies that recognize and promote debris clearance are absent in Sca-1−/− mice.

To determine whether Sca-1−/− mice display decreases in other immunoglobulin isotypes, we also determined levels of IgG in normal and regenerating muscle, as well as in serum. Unlike the results observed with IgM, levels of IgG did not increase in regenerating muscle, and no differences in muscle IgG (Fig. 1C) or serum IgG (Fig. 1D) were detected between genotypes at either timepoint.

Sca-1−/− mice display reduced complement C3 recruitment to regenerating muscle. We hypothesized that the reduced intramuscular IgM observed in Sca-1−/− mice during regeneration would result in an inability to recruit complement, since previous studies have shown that IgM binds to autoantigens on necrotic cells, leading to deposition of complement and subsequent recruitment of phagocytic macrophages (25, 33, 36). Complement deposition is critical for removal of damaged tissue, as mice deficient for C3 display reduced hepatic regeneration characterized by fibrosis and an inability to clear damaged parenchyma (31). We therefore measured levels of the complement protein C3, which has a central role in the complement cascade and is critical for phagocytosis of apoptotic and necrotic cells in vitro and in vivo (33, 36).

As shown in Fig. 2A, whereas regenerating muscle from WT animals displayed a 2.7-fold significant increase in the amount of C3 present relative to control in response to injury, there was a significant decline in C3 detected in regenerating Sca-1−/− muscle (Fig. 2A). This attenuated C3 deposition was likely due to the reduced IgM levels in Sca-1−/− and not to reduced systemic C3 levels, since serum C3 levels in WT and Sca-1−/− mice did not differ (Fig. 2B). These data suggest that an inability to recruit IgM to damaged muscle leads to a reduction in complement deposition and downstream events including the clearance of dead tissue by macrophages, thereby contrib-

![Fig. 1. Stem cell antigen-1 (Sca-1)−/− mice have a reduced capacity to recruit IgM to regenerating muscle. A: gastrocnemius muscles were collected from wild-type (WT) and Sca-1−/− muscle 5 days after BaCl2 injection. Muscles from the contralateral (C) leg of each mouse were collected as an uninjured control. Muscles were homogenized, and the amount of IgM was measured by ELISA. n = 4 for each genotype; *P = 0.0009 for injury effect and P = 0.0019 for genotype effect. WT, open bars; Sca-1−/−, solid bars. B: blood was collected from WT and Sca-1−/− mice (4 per genotype) via cardiac puncture and allowed to clot. Red blood cells were removed by centrifugation. Levels of IgM were determined by ELISA. *P = 0.003. Muscle (C) and serum (D) samples used in A and B were analyzed for levels IgG using the Easy-Titer IgG kit (Thermo Scientific). Muscle samples were normalized to protein concentration.](http://ajpcell.physiology.org/doi/10.1152/ajpcell.00252.2010)
Fig. 2. Sca-1/−/− mice have a reduced capacity to recruit complement C3 to regenerating muscle. A: gastrocnemius muscles from WT (n = 4) and Sca-1/−/− (n = 3) mice were collected 5 days after BaCl2 injection. Muscles from the contralateral leg were collected as an uninjured control. Muscles were homogenized and levels of C3 determined by ELISA. Samples were normalized to protein concentration. *P = 0.0009 for injury effect on C3 levels and P = 0.01 for genotype effect on C3 levels. WT, open bars; Sca-1/−/−, solid bars. B: for serum analysis, blood was collected by cardiac puncture and allowed to clot. Red blood cells were removed by centrifugation, and C3 concentration determined by ELISA. WT, n = 4. Sca-1/−/−, n = 3.

Fig. 3. Sca-1/−/− mice have fewer B-1a cells than WT. Peritoneal cells (PerC) were isolated from WT (n = 4) and Sca-1/−/− mice (n = 3). Cells were immunostained, and the number of B-1a cells (IgM^high IgD^low B220^low CD5^low) was determined for each mouse. A: representative flow plots are shown to demonstrate the gating strategy used. Cells were first gated on IgM^high cells, followed by selection of the IgD^low population, and finally gated on the B220^low CD5^low population. B: representative flow plots of fluorescence minus one (FMO) controls are shown to demonstrate specificity of antibody staining. C: quantitation of the percentages of B-1a cells relative to total peritoneal cell number. *P = 0.001.

Fig. 2. Sca-1/−/− mice have a reduced capacity to recruit complement C3 to regenerating muscle. A: gastrocnemius muscles from WT (n = 4) and Sca-1/−/− (n = 3) mice were collected 5 days after BaCl2 injection. Muscles from the contralateral leg were collected as an uninjured control. Muscles were homogenized and levels of C3 determined by ELISA. Samples were normalized to protein concentration. *P = 0.0009 for injury effect on C3 levels and P = 0.01 for genotype effect on C3 levels. WT, open bars; Sca-1/−/−, solid bars. B: for serum analysis, blood was collected by cardiac puncture and allowed to clot. Red blood cells were removed by centrifugation, and C3 concentration determined by ELISA. WT, n = 4. Sca-1/−/−, n = 3.

Sca-1/−/− mice have reduced numbers of B-1a cells. The majority of natural IgM is produced by a subset of B cells termed B-1a cells, which, in the adult mouse, are located primarily in the peritoneal cavity (24, 28). B-1a cell progenitors are present in the fetal omentum and fetal liver but are absent in adult bone marrow (18). Interestingly, in contrast to conventional B2 cells, the maintenance of B-1a cells throughout life is achieved via self-renewal (19, 27, 29). The B-1a subset is defined by a characteristic cell surface expression pattern; i.e., IgM^high IgD^low B220^low CD5^low (53).
To investigate the possibility that the reduced serum IgM and C3 levels present in injured muscle from Sca-1−/− mice result from altered B-1a cell numbers, peritoneal cells were isolated from WT and Sca-1−/− mice, and the percentages of B-1a cells were determined by flow cytometry using the cell surface phenotype described above. The gating strategy used to identify B-1a cells is shown in (Fig. 3, A and B). Strikingly, while 7.8% of peritoneal cells isolated from WT mice were B-1a cells, only 2.4% of cells isolated from the peritoneum of Sca-1−/− mice displayed the B-1a phenotype (Fig. 3C). This was a specific deficit, since there was no observed difference in CD45+ cells or a difference in the total number of B cells, defined by CD19 expression (data not shown). These data suggest that the reduced levels of circulating IgM and the reduced muscle levels of IgM and C3 in the Sca-1−/− mice may be an outcome of fewer B-1a cells. Alternatively, there may be an absence of B-1a cells producing IgM that recognize autoantigens that are presented in this injury model, similar to what has been suggested by Zhang and colleagues (56).

**Sca-1−/− mice are protected from ischemia-reperfusion injury.** Ischemia-reperfusion injury is an acute inflammatory response, wherein tissue damage occurs upon the return of blood supply to tissue after a period of ischemia-induced hypoxia. Although ischemia itself results in limited tissue damage, the majority of injury occurs upon restoration of blood flow (10). Multiple studies suggest that periods of ischemia lead to expression of conserved self-antigens, which are then recognized by IgM and complement. In this scenario, IgM and complement recruitment result in the formation of a MAC, with release of multiple inflammatory mediators and ultimately direct cell lysis (15, 40, 50, 55). In contrast with the normal clearance of debris and necrotic cells in tissue regeneration, during reperfusion injury, the tissue damage is actually caused by the innate immune response such that mice lacking either IgM or the complement component C3 are protected from damage (55).

Because we observed reduced circulating IgM levels, a blunted IgM and C3 recruitment in the Sca-1−/− mouse during regeneration and given the previous observation that mice lacking IgM or C3 are protected from reperfusion injury, we hypothesized that Sca-1−/− muscle would also be protected. Therefore, we induced ischemia in the hindlimbs of WT and Sca-1−/− mice using femoral ligation, a standard model of reperfusion injury (13) and then assessed damage in gastrocnemius and soleus muscles 7 days postligation in hematoxylin-and eosin-stained sections. Of the four WT mice examined, all

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**Fig. 4.** Sca-1−/− mice are protected from reperfusion injury. Reperfusion injury was induced in WT (n = 4) and Sca-1−/− (n = 6) mice by femoral ligation. Gastrocnemius and soleus muscles were collected 7 days after ligation, sectioned, and stained with hematoxylin and eosin. A–C: representative images of WT muscle following reperfusion injury. Low magnification (A) image is shown to demonstrate the wide extent of damage. Bar in A = 150 μm. Bar in B = 60 μm. D and E: representative images from one Sca-1−/− mouse displaying moderate reperfusion injury. The low magnification image (D) shows the limited extent of the damage across the muscle. The higher magnification images show an area of injury (E) and one with no detectable damage (F). G–I: low (G) and high (H, I) magnification images of a Sca-1−/− muscle displaying no detectable damage following reperfusion injury. J–L: immunofluorescence demonstrating reduced C3 deposition in Sca-1 ischemic tissue. Sections from WT (K) and Sca-1−/− (L) ischemic muscle immunostained with antibody to C3, isotype control (J).
had significant injury evident throughout the entire muscle (Fig. 4, A–C, Fig. 5, A and B). The damaged tissue was characterized by extensive fibrosis and the widespread presence of both necrotic and regenerating (centrally nucleated) myofibers. In contrast, Sca-1−/− muscles displayed surprisingly reduced damage; 4 of the 6 muscles examined had almost no identifiable injury (Fig. 4, G–I), whereas 2 of the 6 had only modest localized regions of damage (Fig. 4, D–F). When quantified, ~60% of WT muscle sections displayed significant injury (defined as areas of fibrosis, centrally nucleated myofibers, or degenerating myofibers), while on average only 9% of the Sca-1−/− sections were injured (Fig. 5A). The percentage of tissue injury for each individual mouse is shown in Fig. 5B. We next investigated whether the protection from reperfusion injury demonstrated by Sca-1−/− mice correlates with decreased C3 deposition. Sections from WT and Sca-1−/− ischemic muscle were immunostained with an antibody to C3. As shown in Fig. 4, WT muscle displays significant deposition of C3 (Fig. 4K), while drastically reduced in muscle derived from Sca-1−/− mice (Fig. 4L). These data further support the finding that Sca-1, through its effects on B-1a cell numbers and circulating IgM and C3 levels, plays a critical role in muscle physiology and in the activation of innate immune mechanisms during injury.

**DISCUSSION**

In this report we demonstrate that IgM and C3 complement are recruited to damaged tissue during normal skeletal muscle regeneration. Recruitment of IgM and complement is essential for the recognition and clearance of dead cells by phagocytic macrophages, promoting efficient regeneration in other tissues (33, 36–38). Interestingly, during reperfusion following ischemia, the IgM/complement system instead has a detrimental effect on tissue. Reperfusion injury results in the expression of self-antigens that recruit IgM/complement, leading to formation of the MAC and cell lysis (15, 40, 50, 55). We also show that these processes are influenced by Sca-1, since recruitment of IgM and C3 complement does not occur in injured muscle from Sca-1−/− mice. These mice display reduced levels of serum IgM and blunted recruitment of IgM and C3 to sites of muscle damage. During acute muscle injury the inability to recruit IgM results in a lack of complement deposition that likely leads to decreased phagocytosis by macrophages and lack of debris clearance, thereby promoting the fibrogenic phenotype previously reported in these mice (23). Interestingly, this same inability to recruit IgM/C3 protects Sca-1−/− mice from reperfusion injury. We were also able to identify a reduction in the number of peritoneal B-1a cells in the Sca-1−/− mice. As these cells produce the majority of soluble IgM (24), we suggest that their reduced numbers account for the reduction in circulating IgM and predispose Sca-1−/− mice to a dysregulated response to injury-induced regeneration and ischemia-induced damage.

The role of Sca-1 in the IgM/complement system via its effects on B-1a cell numbers may explain several other phenotypes associated with the Sca-1−/− mouse. For example, mice lacking the C3 component of complement display defects in hematopoietic stem cell (HSC) homing to the bone marrow (52), and a similar HSC homing defect has been observed in Sca-1−/− mice (8). The homing deficiency of the C3−/− mice has been attributed to decreased secretion of matrix metalloproteinase-9 (MMP9) by HSCs; MMP9 facilitates migration of HSCs through the endothelial barrier as well as promoting interaction of HSCs with other cells in the bone marrow (52). Interestingly, we previously identified decreased MMP activity in skeletal muscle isolated from Sca-1−/− mice, which we hypothesized is a contributing factor to the reduced extracellular matrix turnover and increased fibrosis observed in regenerating muscle of these mice (23). Whereas the potential contribution of IgM to the function of C3 in HSC homing was beyond the scope of this study, we speculate that IgM involvement in the regulation of HSC homing by C3 may explain the convergence of these phenotypes observed in the C3−/− and Sca-1−/− mice. Similarly, we would anticipate that muscle regeneration in the C3−/− mouse would be hindered by lack of complement deposition and debris clearance, resulting in a fibrotic phenotype similar to that observed in the Sca-1−/− mouse. Other studies demonstrating that C3−/− mice exhibit fibrosis and other defects during liver regeneration help to underscore the role of innate immune effector mechanisms in tissue regeneration (31).
Natural IgM antibodies are present before antigen exposure and are able to bind pathogens without undergoing antibody rearrangement, thus providing an innate defense that precedes adaptive immunity (3). IgM binding results in complement deposition on the foreign organism resulting in MAC pore formation and subsequent initiation of the adaptive immune response (30). Indeed, mice deficient for IgM are extremely susceptible to systemic bacterial infections (5). Given the observed defect in IgM in the Sca-1−/− mice, it will be of interest to determine whether loss of Sca-1 expression increases the susceptibility to bacterial infections. Additionally, IgM plays a protective role in the development of autoimmune diseases, as mice lacking IgM are more likely to develop systemic lupus erythematosus (SLE) (6). This protective effect of IgM is thought to result from the ability of IgM to clear apoptotic and necrotic cells, which would otherwise provide a source of autoantigens and promote the development of autoimmunity (30). It is unknown whether Sca-1−/− mice may be susceptible to SLE or other autoimmune disorders. However, based on our observation that Sca-1−/− mice are protected from ischemia-reperfusion injury, we speculate that these mice may also be prone to autoimmune disorders.

Notably, although the Sca-1−/− mice only display a 50% reduction in the level of serum IgM, there was a profound inability to recruit IgM to the site of muscle damage, in contrast with the robust recruitment observed in normal mouse controls. We speculate that a threshold of circulating IgM may be required for infiltration of IgM to sites of muscle damage, and the levels present in Sca-1−/− serum fall below this threshold. Similar to Sca-1−/− mice, mice lacking complement receptor 2 (CR2) are protected from ischemia-reperfusion injury and have a 30–40% decrease in B-1a cell numbers, although they have normal levels of soluble IgM. This phenotype suggests that CR2−/− mice may be deficient in their IgM repertoire, lacking antibodies capable of recognizing self-antigens presented during reperfusion injury (16, 41). Indeed, experiments with clonal B-1a cells have identified an IgM antibody capable of initiating reperfusion injury (56). It would therefore be of interest to determine whether Sca-1−/− mice have a restricted IgM repertoire. However, since the amount of fibrosis in regenerating Sca-1−/− muscle does decrease over time (23), this indicates that non-IgM dependent mechanisms of tissue remodeling and clearance exist.

We also demonstrate that mice lacking Sca-1 have reduced numbers of B-1a cells, which are the primary producers of soluble IgM. How Sca-1 affects B-1a cell numbers is unclear. B-1a cells are derived from progenitors present in the fetal omentum and fetal liver, and differentiation of these cells ceases at 3–6 wk of age (18, 27, 29). Sca-1 has been implicated in the self-renewal of hematopoietic and other stem cell populations (20), so perhaps Sca-1 also regulates the self-renewal of B-1a cells or their progenitor population. Interestingly, Boes et al. (4) have demonstrated that mice deficient in soluble IgM have enhanced numbers of B-1a cells, suggesting that IgM itself is important in regulating B-1a cells differentiation and/or maintenance. As these mice are engineered to completely lack soluble IgM, in contrast to Sca-1−/− mice in which IgM levels are reduced, a direct comparison is difficult. However, the results from Boes et al. raise the intriguing possibility for a feedback mechanism that links the number of B-1a cells to the levels of soluble IgM. Although Sca-1−/− mice have reduced soluble IgM, they do not exhibit increased B-1a cell numbers, suggesting that this feedback mechanism may have additional complexity. An additional possibility is that the subset of IgM antibodies that mediate this feedback are preserved in Sca-1−/− mice.

The data presented here suggest a novel role for Sca-1 in the response to autoantigens exposed during injury. We show that Sca-1−/− mice have reduced numbers of B-1a cells and low levels of circulating IgM and IgM recruited to sites of injury. Based on our results we suggest a model wherein the reduction in B-1a cell numbers results in decreased soluble IgM. This reduction in IgM in turn limits the ability to recruit complement to sites of muscle damage, leading to decreased clearance of dead cells and tissue and thereby promoting a muscle fibrogenic phenotype present in these mice.

In addition to acute injury response, fibrosis is an undesirable complication in many chronic myopathies, including muscular dystrophies (21). Future studies will be needed to determine whether a control of timing and dosage of the IgM/complement pathway may provide a broad tool for minimizing fibrosis during these compromised muscle regeneration diseases.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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