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Allograft Inflammatory Factor-1 Is Expressed By Macrophages in Injured Skeletal Muscle and Abrogates Proliferation and Differentiation of Satellite Cells

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Abstract. Secretion of regulatory peptides by macrophages in injured skeletal muscle constitutes a pivotal determinator of tissue homeostasis. We analyzed expression of a novel Ca^{2+}- binding peptide expressed by activated macrophages, the allograft inflammatory factor-1 (AIF-1), in rat devascularized skeletal muscle. AIF-1 expression was observed in 94% of all macrophages at the site of the injury 48 hours postdevascularization. The physiological function of AIF-1 in injured skeletal muscle was analyzed using a rat in-vitro model of satellite cell proliferation and differentiation. Addition of AIF-1 to the culture medium resulted in a concentration-dependent and reversible reduction of the total number of cells expressing M-cadherin ($p \leq 0.0001$), a mediator of the differentiation process of skeletal muscle cells, the proliferation associated PCNA ($p \leq 0.0001$), and the initiator of muscle differentiation myogenin ($p \leq 0.0001$). These results provide convincing evidence that activated AIF-1 expressing macrophages constitute the predominant cell type in skeletal muscle 48 hours postinjury, and that AIF-1 regulates reduced proliferation, differentiation, and activation of satellite cells.

Key Words: Allograft inflammatory factor-1; Cell culture; Immune response; Macrophages; M-cadherin; Myogenin; Proliferating cell nuclear antigen.

INTRODUCTION

Skeletal muscle regeneration is a sequential process that involves the immigration of a diverse range of cell populations to the site of injury (1). Following the damage of myofibers, release of soluble cytokines is thought to attract leucocytes and macrophages to initiate the regeneration process (2). Within 48 hours (h), macrophages constitute the predominant cell type at the site of the lesion (3, 4). While the role of macrophages in clearing necrotic debris is well documented, evidence accumulates that their pathophysiological function extends to the differential regulation of key determinators of tissue homeostasis and immune recognition. This is noteworthy because skeletal muscle regeneration is dominated by the outgrowth of cytokine-sensitive myogenic precursors, satellite cells, which adhere to the basal lamina of the sarcolemma. Following injury, satellite cells undergo mitosis and fuse to form small precursor myotubes which then fuse with myofibers, become reinnervated, and consequently acquire the characteristics of mature fibers.

Only single factors that control satellite cell homeostasis have been described, and recent results provide convincing evidence that macrophages are a prime source of cytokine delivery in injured skeletal muscle. Both detrimental and beneficial effects of macrophage-derived soluble factors on myoblast differentiation and proliferation have been reported. Transforming growth factor-β, platelet-derived growth factor, fibroblast growth factor, and epidermal growth factor all inhibit terminal differentiation of myoblasts (5–8). Conversely, unknown factors secreted by macrophages have been described to contribute to satellite cell proliferation (9, 10) and myoblast proliferation (11).

The biological characterization of soluble factors expressed in macrophages might help to explain the complex skeletal muscle regeneration process. A candidate peptide expressed by activated macrophages capable of infiltrating a wide variety of lesions is the allograft inflammatory factor-1 (AIF-1). AIF-1 is a 17-kd IFN-γ (interferon gamma) inducible Ca^{2+}-binding EF-hand peptide. EF-hand family proteins share a common amino acid motif capable of binding intracellular free Ca^{2+}, consecutively changing conformation, and eliciting distinct downstream signaling pathways. Several proteins that share identical amino acid sequences with AIF-1 include Iba-1 (ionized Ca^{2+}-binding adaptor molecule-1) and MRF-1 (microglia response factor-1) that have been localized to activated macrophages infiltrating degenerating and inflamed tissues.

We used immunocytochemistry to study expression of AIF-1 in injured rat skeletal muscle in vivo. Double labeling experiments with antibodies directed against monocytes/macrophages (ED1) characterized the nature and the functional status of AIF-1^+ against the rat major histocompatibility complex class II molecule (OX6) cells.

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The physiological role of AIF-1 was analyzed using a rat in-vitro model of satellite cell proliferation and differentiation with satellite cells attached to myofibers. A biologically active recombinant AIF-1 peptide preparation and irrelevant control peptides were added to the cell culture medium. Cell cultures were then analyzed using fluorescence labeling experiments with antibodies directed against M-cadherin, proliferating cell nuclear antigen (PCNA), and myogenin.

MATERIALS AND METHODS

Production and Purification of Recombinant AIF-1 and Control Peptide

A synthetic gene, *toolbox121*, encoding AIF-1 was subcloned into a pET (Angewandte Gentechnologie Systeme, Heidelberg, Germany) expression vector. Recombinant protein was produced in BL21(DE3) *E. coli* cells (AGS, Heidelberg, Germany) by induction with IPTG for 4 h. Recombinant AIF-1 was purified by standard nickel-chelate chromatography. Protein concentration was determined by the Bradford assay with bovine serum albumin as a standard (BioRad, Munich, Germany). A recombinant inactive control peptide, toolbox102, was prepared in parallel as described above (12, 13).

Hybridoma Cell Lines and Monoclonal Antibodies Against Recombinant AIF-1

Balb/c mice were repeatedly immunized with 50–100 μg of recombinant AIF-1 and hybridoma cell lines were established. Supernatants were screened by ELISA, Western blotting, and blocking experiments (13). AIF-1 immunohistochemistry has been described (14).

Devascularization and Preparation of Tissue Sections

In order to evaluate AIF-1 expression in injured skeletal muscle, we induced ischemia in the M. soleus muscles of 16-week-old male Lewis rats. Tendons and blood vessels of the connective tissues were tied. Postsurgical treatment included supplementation of the drinking water with buprenorphine (Temgesic). Two days after devascularization, skeletal muscles were removed, fixed in 4% buffered formaldehyde, pH 7.4, dehydrated in an ascending alcohol sequence, and finally embedded in paraffin. Sections of 5-μm thickness were mounted on sialinized slides, deparaffinized in a descending alcohol sequence, transferred to distilled water, and stained by immunocytochemistry.

Immunocytochemistry

Prior to immunocytochemistry, the slices were irradiated in a microwave oven 7 times for 5 min in citrate buffer (2.1 g sodium citrate/l, pH 6.0) and incubated in nonspecific porcine serum 1:10 in TBS pH 7.5 (90 % Tris balanced salt solution containing 0.025 M Tris, 0.15 M NaCl) for 15 min. The primary mouse anti-AIF-1 antibody was added to the sections overnight at 4°C (13). Antibody binding was detected by biotinylated rabbit anti-mouse IgG $F(ab)_2$ antibody fragment (Dako, Hamburg, Germany) and AB-complex (Dako), both at a dilution of 1:400 in TBS for 30 min. The reaction was visualized with diaminobenzidine (Fluka, Buchs, Switzerland) and counterstained with hematoxylin. Double labeling experiments were used to provide a quantitative basis of the cellular origin of AIF-1 expression. Slices were pretreated as described above. Differentiating antibodies directed against ED1 (monocytes) or OX6 (MHC-class II, all Serotec, Oxford, UK) were added to the sections at a dilution of 1:100 in TBS-BSA (10% bovine serum albumin in TBS). Visualization was achieved by rabbit anti-mouse IgG (Dako) diluted at 1:20 in TBS for 30 min, APAAP complex (Dako) diluted 1:80 in TBS for 30 min and Fast Blue BB salt chromogen. To avoid antibody cross-reactivity in double labeling experiments, slices were irradiated in a microwave for 20 min in citrate buffer (15). Complete inhibition of alkaline phosphatase function was achieved as previously described (16). Then, AIF-1 was immunolabeled as described above. Ten high power (400×) magnification fields were evaluated in devascularized skeletal muscle preparations each in ED1/AIF-1 and OX6/AIF-1 double labeled slices. Double labeled cells were counted using an eyepiece grid and compared with the number of ED1 single labeled cells for each double labeling procedure.

Isolation and Culture of Muscle Fibers

Single isolated fibers with attached satellite cells were prepared from flexor digitorum brevis muscles of about 7-week-old male Wistar rats as described before (17, 18). Connective tissue was removed and the muscles were incubated for 3 h at 37°C with occasional agitation in 0,2% collagenase (Boehringer, Mannheim, Germany) resuspended in preparation medium containing MEM (minimal essential medium), 10% horse serum (PAN Systems, Aidenbach, Germany) and 1% antibiotic-antimycotic mixture (Sigma, Deisenhofen, Germany). The fibers were teased and triturated 4 times with a wide mouth pipette, followed by sedimentation at 1g. The final sediment was aliquoted and plated on slides coated with isotonic poly-L-lysin (Sigma) and Vitrogen 100 (Collagen GmbH, Ismaning, Germany) as described (18). Then plates were incubated for 30 min at 37°C, 5% CO_2. Cultures then received cultivation medium containing MEM, 20% controlled process serum replacement-type 1 (Sigma), 1% horse serum (Pan-Systems), and 1% antibiotic antimycotic solution (Sigma). In experiments with AIF-1, the cytokine was added to the culture medium at the indicated concentrations. Culture medium and cytokines were replaced daily.

Fixation

Cultures were fixed at days 0 to 4 after plating as described previously (19). Briefly, cultures were immersed in 1% buffered formaldehyde, pH 7.4 for 2 min, washed in ice cold PBS, and consecutively incubated in methanol at −20°C for 10 min. Then the cultures were washed in 0.1% Triton X-100/PBS and stored in 0.1% sodium acid/PBS.

Immunofluorescence Labeling

Analysis was performed using indirect fluorescence labeling experiments with rabbit-anti-M-cadherin (20) mouse-anti-PCNA (proliferating cell nuclear antigen, Boehringer) and mouse anti-myogenin (clone F5D, Pharmingen, San Diego,

CA). Visualization was achieved by adding FITC conjugated goat anti-mouse (Dianova, Hamburg, Germany) or goat anti-rabbit TRITC (Dianova). Nuclei were counterstained with DAPI (Boehringer).

Controls and Evaluation

In immunocytochemistry, controls included labeling experiments with primary antibodies with irrelevant specificity (anti-glial fibrillary acidic protein, Boehringer) and experiments lacking the primary antibody. Further, blocking experiments confirmed the specificity of AIF-1 immunocytochemistry. Complete blockage of immunoreactivity was achieved after incubating the anti-AIF-1 antibody with recombinant AIF-1 peptide as described (14). Untreated satellite cell culture data has been described before (21). Labeling of satellite cells was evaluated in 230–437 muscle fibers per labeling experiment. All experiments were repeated 3 times using 3 different skeletal muscle cell preparations. Only round or oval-shaped cells located in the morphological equivalent of the basal lamina and plasmalemma attached to the myofibers were evaluated. Controls included labeling experiments lacking primary antibody and blocking experiments with AIF-1 peptide. Functional controls included heat-inactivated AIF-1 and an irrelevant recombinant peptide preparation, toolbox102 (12). For each day in culture and labeling procedure, the mean number of labeled cells/fiber was compared by the Mann-Whitney *U* test.

RESULTS

In Vivo Immunocytochemistry

We analyzed expression of AIF-1 in normal and injured soleus muscle of 16-week-old male Lewis rats using a previously described and characterized monoclonal antibody (13). In normal rat soleus muscle, no AIF-1 expressing cells were observed in the perimysial connective tissues or in the vasculature (Fig. 1A). In injured rat soleus muscle, 72% of all cells expressed AIF-1 48 h post-devascularization (Fig. 1B). Double labeling experiments revealed the cellular origin of AIF-1 expressing cells. Ninety-four percent of all ED1$^+$ macrophages at the site of the injury coexpressed AIF-1 (Fig. 1C). Only single ED1$^+$ macrophages did not express AIF-1. Five percent of all macrophages coexpressed OX6 (data not shown).

Cultivation of Satellite Cells and Expression of M-Cadherin, PCNA, and Myogenin

Myofibers with attached satellite cells were removed and subsequently cultivated. Satellite cells adhered to the basal lamina and plasmalemma of the myofiber and had a round or oval shape. Immediately after and at days 1–4 following cultivation, expression of M-cadherin, PCNA (Fig. 2A) and myogenin (Fig. 2B) analyzed by fluorescence visualization techniques were used to characterize proliferation and differentiation in satellite cells.

In standard cultivation conditions, the number of M-cadherin$^+$ satellite cells/fiber (Fig. 3A) at time 0, 0.5 $\pm$ 0.04 (mean $\pm$ SEM) increased to 1.3 $\pm$ 0.06 at day 1, 1.6 $\pm$ 0.07 at day 2, and 1.5 $\pm$ 0.07 at day 3, and consequently decreased to 0.8 $\pm$ 0.05 at day 4. In parallel, the number of PCNA$^+$ satellite cells/fiber (Fig. 3B) was 0.04 $\pm$ 0.02 at time 0, increased to 2.2 $\pm$ 0.11 at day 1, and decreased to 1.3 $\pm$ 0.06 at day 2, 0.59 $\pm$ 0.05 at day 3, and 0.13 $\pm$ 0.04 at day 4. The number of myogenin$^+$ cells (Fig. 3C) was 0.1 $\pm$ 0.02 at time 0, and 0.09 $\pm$ 0.03 at day 1. At day 2, there was a considerable increase to 2.74 $\pm$ 0.16, followed by a decrease to 1.66 $\pm$ 0.11 at day 3, and 0.34 $\pm$ 0.05 at day 4.

AIF-1 Exerts Concentration-Dependent Effects On Satellite Cells

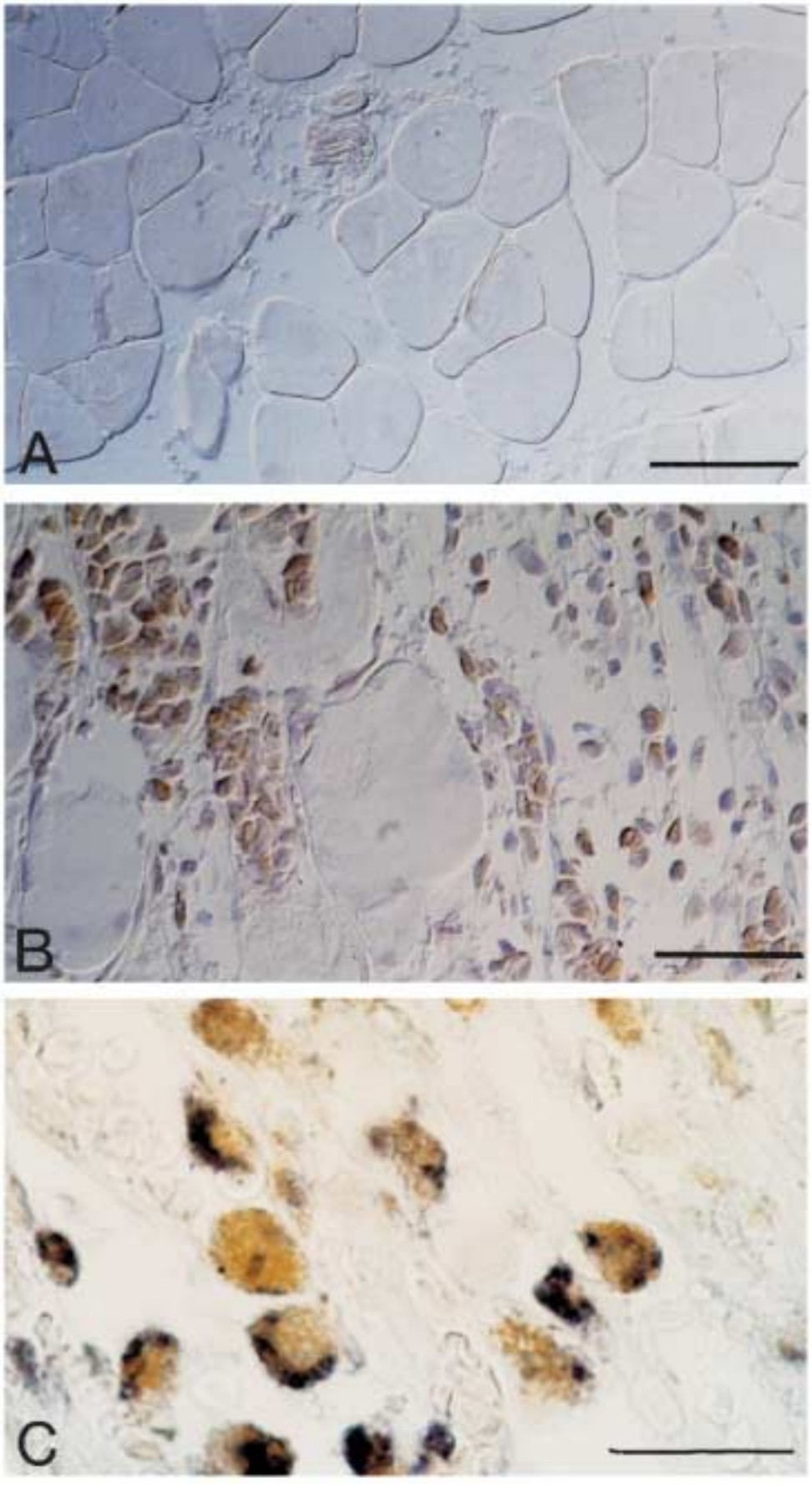
In order to determine whether AIF-1 exerts biological effects on satellite cells, we counted the number of cells expressing M-cadherin and PCNA at AIF-1 concentrations ranging from 0 to 3,750 ng/ml (Fig. 4A). We observed the strongest reduction of satellite cells expressing M-cadherin and PCNA at a concentration of 750-ng AIF-1/ml medium. Addition of up to 3,750 ng/ml did not further reduce the number of M-cadherin and PCNA expressing cells. Thus, all other experiments were conducted with 750 ng/ml AIF-1.

AIF-1 Reduces the Number of M-Cadherin$^+$ Satellite Cells

Addition of AIF-1 abolished the increase of M-cadherin$^+$ satellite cells significantly ($p \leq 0.0001$) at days 1, 2, and 3 compared with untreated cultures (Fig. 3A). The mean number of M-cadherin$^+$ cells remained almost unaltered with 0.44 $\pm$ 0.05 (mean $\pm$ SEM) at day 1, 0.56 $\pm$ 0.07 at day 2, 0.63 $\pm$ 0.07 at day 3, and 0.54 $\pm$ 0.06 at day 4. Supplementation of cell cultures with control peptide preparations restored the effects observed in untreated cultures. Supplementation of the culture medium with heat inactivated AIF-1 and the control peptide toolbox102 resulted in a constant increase in the number of satellite cells at cultivation days 1, 2, and 3.

AIF-1 Reduces the Number of PCNA$^+$ Satellite Cells

When satellite cell preparations were treated with AIF-1, we observed no increase in the mean number of PCNA$^+$ cells/fiber as observed in the untreated cultures with 0.15 $\pm$ 0.05 (mean $\pm$ SEM) at day 1, 0.08 $\pm$ 0.04 at day 2, and 0.06 $\pm$ 0.04 at day 3 (Fig. 3B). The difference in the number of PCNA$^+$ cells between AIF-1 treated and untreated cultures was highly significant at days 1, 2, and 3 ($p \leq 0.0001$). Addition of heat-inactivated AIF-1 and control peptide preparations to the culture medium resulted in a similar proliferative response as observed in untreated cultures. Following supplementation with heat inactivated AIF-1, we observed an increase of the mean number of PCNA$^+$ cells/fiber to 1.86 $\pm$ 0.26 at day 1, and consequently, a gradual decrease to 0.43 $\pm$ 0.15 at day 2, and 0.24 $\pm$ 0.12 at day 3. Following


A
B
C

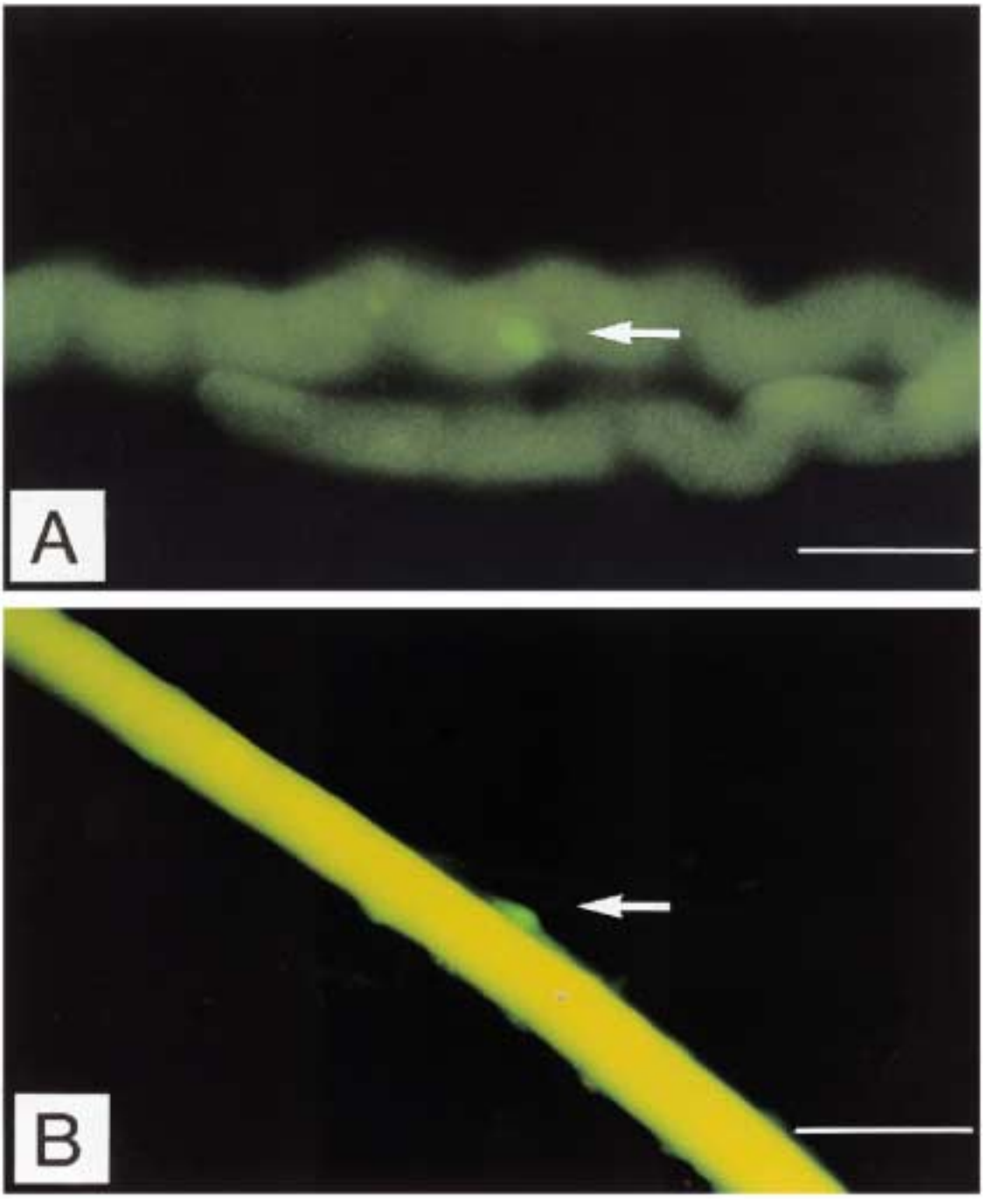


Fig. 2. Immunofluorescence labeling experiments were used to detect M-cadherin, PCNA (A), and myogenin (B) expressing satellite cells (arrows). Scale bars: A, B = 50 μm.

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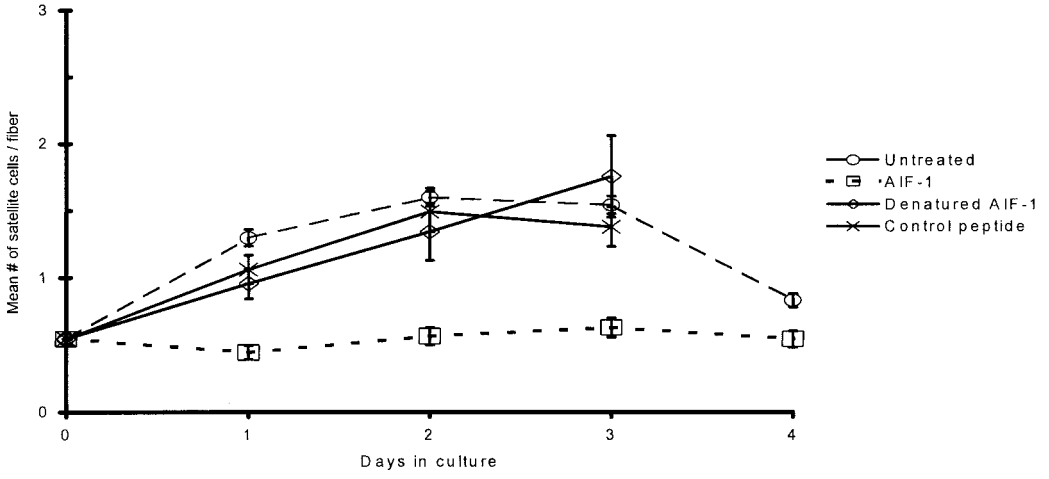
Fig. 1. In normal muscle, there were no single AIF-1 expressing cells in the perimysial matrix or in the vasculature (A). No counterstain was applied. In injured rat skeletal muscle preparations, AIF-1 (brown) expression was observed in 72% of all cells (B). Sections were counterstained by hematoxylin (blue). Double labeling immunocytochemistry confirmed the cellular origin of AIF-1 expressing cells (brown). Ninety-four percent of $ED1^+$ macrophages (dark blue) coexpressed AIF-1 (brown, arrows) (C). No counterstain was applied here. Scale bars: A = 100 μm, B = 50 μm, C = 25 μm.

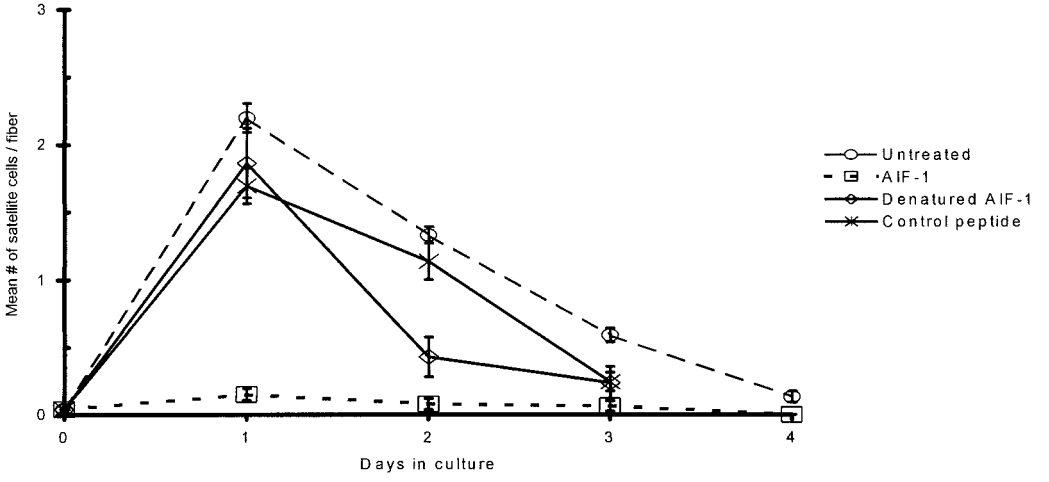
M-Cadherin

A



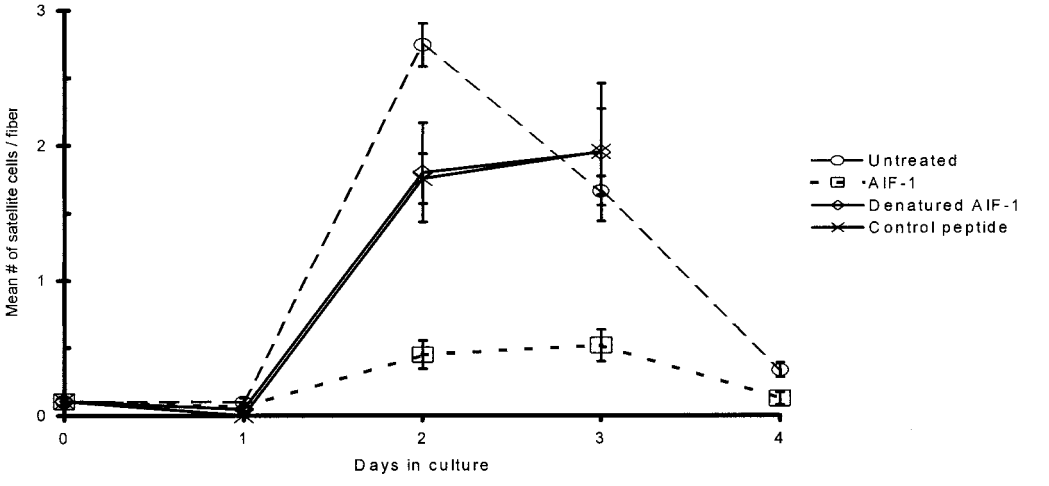
PCNA

B



Myogenin

C



addition of the control peptide toolbox102 preparation, we again detected restoration of the number of PCNA$^+$ cells/fiber as described in untreated cultures.

AIF-1 Reduces the Number of Myogenin$^+$ Satellite Cells

Following supplementation of the cell culture medium with AIF-1, there was a significantly ($p \leq 0.0001$) reduced increase in the mean number of myogenin$^+$ satellite cells/fiber at days 2 and 3 compared with untreated control preparations (Fig. 3C). The mean number of myogenin$^+$ satellite cells/fiber remained almost unaltered with 0.063 ± 0.030 (mean $\pm$ SEM) at cultivation day 1, 0.447 ± 0.105 at cultivation day 2, 0.518 ± 0.118 at cultivation day 3, and 0.125 ± 0.047 at day 4. Addition of heat inactivated AIF-1 and the control peptide resulted, again, in the reconstitution of the effects observed in the untreated culture. At day 1, the number of myogenin$^+$ cells decreased slightly and consecutively increased gradually at days 2 and day 3. Addition of control peptide to the culture medium resulted in a gradual increase of the mean number of myogenin$^+$ cells satellite cells/fiber over the cultivation period.

Abrogation of M-Cadherin$^+$, PCNA$^+$ and Myogenin$^+$ Satellite Cells by AIF-1 Is Reversible

In order to analyze whether the reduction of M-cadherin$^+$, PCNA$^+$, and myogenin$^+$ cells induced by AIF-1 is reversible, we added AIF-1 at time 0 but not at days 1–3, resulting in a 24-h incubation period. Culture medium without AIF-1 was added to the cultures at days 1–3. Although the number of M-cadherin$^+$, PCNA$^+$, and myogenin$^+$ satellite cells was reduced at day 1, we observed a significant ($p < 0.005$) reconstitution of the number of M-cadherin$^+$, PCNA$^+$, and myogenin$^+$ cells at days 2–4 (Fig. 4B). Consequently, at days 2–4, the number of M-cadherin$^+$, PCNA$^+$, and myogenin$^+$ cells did not significantly ($p = 0.19$) differ as compared with untreated cultures.

DISCUSSION

In vivo immunolocalization of AIF-1 revealed its expression in 94% of ED1$^+$ macrophages at the site of the injury 48 h postdevascularization. Physiological significance of this finding was revealed by cell culture experiments of muscle fibers containing satellite cells. Addition of AIF-1 to the culture medium resulted in a concentration-dependent and reversible reduction of the total number of cells expressing M-cadherin ($p \leq 0.0001$), PCNA ($p \leq 0.0001$), and the transcriptional activator myogenin ($p \leq 0.0001$) compared with controls.

The Allograft Inflammatory Factor-1 (AIF-1)

The *AIF-1* gene is located within the HLA class III genomic region (22) and has originally been cloned from activated macrophages of human (GenBank accession #U49392) and rat (GenBank accession #U17919) atherosclerotic allogenic heart grafts undergoing chronic transplant rejection (23). Using online database research, we found that AIF-1 is identical with Iba-1 (ionized Ca^{2+}—binding adaptor molecule-1) of both rat (DNA DataBank of Japan accession #D82069) and human (DNA DataBank of Japan accession #D86438) origin (24) and MRF-1 (microglia response factor-1, DNA DataBank of Japan accession #AB000818) of rat origin (25). Another peptide, IRT-1 (interferon-responsive transcript-1) expressed in activated human endothelial cells, shares extensive amino acid homology with AIF-1 (26). AIF-1, MRF-1, and Iba-1 are probably identical, while IRT-1 is an evolutionary conserved, functionally related variant.

Distinct functional properties of AIF-1 are still unresolved, but recent reports suggest AIF-1 expression as a modulator of the immune response during macrophage activation (23). AIF-1 is a 17 kd IFN-γ inducible Ca^{2+}-binding EF-hand peptide. In skeletal muscle, Ca^{2+} ions directly affect regulation of the myosin-actin interaction through the action of tropomyosin and troponin on thin filaments (27). EF-hand proteins on the other hand interact with Ca^{2+} in order to regulate not only contractility (28) but also a wide range of cellular processes, such as nucleotide metabolism, cell cycle control, differentiation, and signal transduction by oscillating shifts in compartment-bound Ca^{2+} (29). Furthermore, the transcription of a number of eukaryotic genes through transcription factors interacting with calcium response elements is induced by a rise in intracellular Ca^{2+} concentration (30). In the monocytic lineage of cells, the members of the EF-hand S-100 supergene-family S100A8 (MRP-8), S100A9 (MRP-14), and S100A12 are prominently expressed during activation. Consequently, their involvement has been suggested in a wide range of Ca^{2+}- dependent cellular processes (31). Although formal proof is lacking, these data provide convincing evidence that AIF-1 interferes with complex regulatory functions in skeletal muscle degeneration and regeneration.

AIF-1 Is Expressed by Macrophages in Injured Muscle

Expression of AIF-1 and homologous peptides has been described in a wide variety of hemopoietic and dendritic cell populations in different organs, including lymphatic tissues, reproductive organs, and brain. Accumulation of AIF-1 expressing cells has been associated with

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Fig. 3. The number of satellite cells expressing M-cadherin (A), PCNA (B) and myogenin (C) was significantly reduced in AIF-1 treated cultures compared with untreated control preparations, denatured AIF-1, and control peptide supplementation.

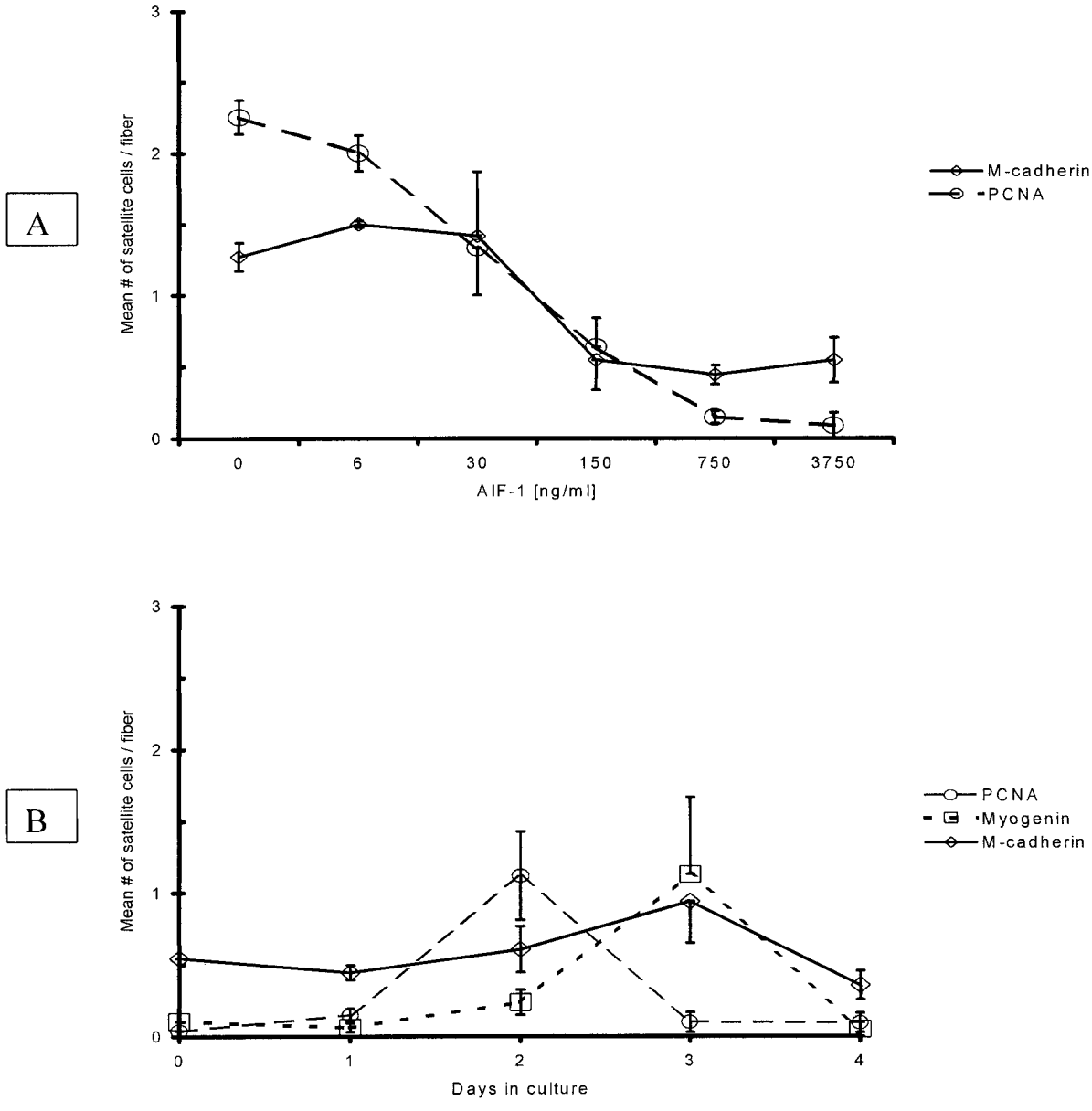

Fig. 4. AIF-1 abrogates the number of PCNA and M-Cadherin expressing satellite cells in a concentration-dependent manner (A). A partial reconstitution of the number of M-cadherin$^+$, PCNA$^+$, and myogenin$^+$ cells was observed at days 2–4 when AIF-1 was added at time 0 only, but not on the following days (B).

degeneration, inflammation, autoimmune disease, and neoplasia (13, 14). Induction of AIF-1 expression was observed following stimulation with IFN-γ (22) and in microglia following apoptotic degeneration of cerebellar granule neurons (25). Therefore, it was proposed that AIF-1 might play a role in the restructuring of a wide range of tissues.

This is the first description of AIF-1 expression in skeletal muscle injury. Accumulation of AIF-1$^+$ macrophages in injured rat skeletal muscle 48 h postdevascularization indicates that AIF-1 might play an equally significant role in the complex process of tissue remodeling following tissue damage including the differential regulation of the immune system and pathophysiological degeneration. However, it remains to be established whether the accumulation of AIF-1 expressing macrophages is limited to a certain period of time following skeletal muscle injury. This is of considerable interest because skeletal muscle degeneration and regeneration is a highly organized sequential process (32). One of the earliest events that accompanies skeletal muscle damage is the accumulation of leukocytes (3). After 2 days, macrophages are the predominant cell type (4). Previous experiments have shown that macrophages play a crucial role in the removal of necrotic muscle and chemotactically attract leucocytes to the site of injury (2). In addition, macrophages can exert

differential mitotic activity on myogenic precursor cells (5–11). These data provide convincing evidence that accumulation of macrophage subpopulations influences the complex skeletal muscle regeneration process by the sequential expression of soluble factors. The detailed biological function of AIF-1 at distinct moments in this process therefore would provide further insight into the sequential course of skeletal muscle regeneration.

AIF-1 Inhibits Differentiation of Satellite Cells

M-cadherin, a member of the multigene cadherin family of calcium-dependent intercellular adhesion molecules, mediates myoblast interaction during the terminal differentiation process of skeletal muscle cells. Its pattern of expression, both in vivo and in cell culture, implies that M-cadherin is important for skeletal muscle development, in particular the fusion of myoblasts into myotubes (33). M-cadherin is found in embryonic somitic and nonsomitic striated muscles, in committed but not in migratory precursor cells (34), and in quiescent satellite cells (35). Little is known about interaction of M-cadherin with other factors that influence muscle cell development. M-cadherin binds in a calcium-dependent manner and depletion of divalent cations inhibits myoblast fusion. In the absence of divalent cations, M-cadherin binding to cell-cell contacts was inhibited (36). Therefore, it is tempting to speculate that soluble AIF-1 secreted by macrophages infiltrating injured skeletal muscle causes the downregulation of M-cadherin and concomitantly reduced differentiation of myoblasts to adult muscle cells. Depletion of AIF-1 expressing macrophages from regenerating skeletal muscle or antagonization of AIF-1 expression might therefore constitute a promising strategy to accelerate skeletal muscle regeneration. Taken together, M-cadherin is thought to play a role in the differentiation process of satellite cells. The reduction of M-cadherin$^+$ cells by AIF-1 therefore suggests an important role of AIF-1 in prolonging the process of skeletal muscle regeneration.

AIF-1 Inhibits Proliferation of Satellite Cells

Although there is evidence that AIF-1 plays an important role in inflammation and regeneration, only limited data is available so far to provide a detailed biological understanding. A possible link between skeletal muscle cell proliferation and AIF-1 induced inhibition of satellite cell proliferation has been revealed recently. The novel peptide interferon responsive transcript (IRT-1), a basic protein that contains a leucine zipper motif, has been described to inhibit proliferation in vascular smooth muscle cells following IFN-γ treatment, but not serum deprivation, IL-1β (interleukin-1β), PDGF (platelet-derived growth factor), TGF-β (transforming growth factor type beta), or bFGF (basic fibroblast growth factor) supplementation (26). Using data mining techniques, we have compared IRT-1 and AIF-1 cDNA sequences and detected considerable homology between AIF-1 and IRT-1. Although downstream signaling and concise biological effects that lead to expression of AIF-1 or IRT-1 remain to be established, our findings provide substantial evidence for the idea that AIF-1 reduces the proliferation of skeletal muscle cells.

The Number of Myogenin$^+$ Satellite Cells is Concomitantly Decreased by AIF-1

Myogenin belongs to a family of transcriptional activators with the capacity to initiate muscle differentiation in many nonmuscle cell types. These factors, which include MyoD1, myogenin, myf-5, and MRF4, share homologies with each other and belong to a superfamily of Myc-related proteins. Expression of these regulatory proteins results in auto-activation and cross-activation of other members of the family and in the transcriptional activation of several markers of terminal differentiation. Sequence analysis revealed a conserved basic domain in each protein that is required for binding to specific DNA sequences of the E-box type and for myogenic activation. A conserved helix-loop-helix (HLH) domain allows homo- and heterodimerization of these muscle-specific proteins with each other and with certain ubiquitously expressed proteins, such as the E2A gene products (37).

Reduction of myogenin expression, in parallel with the reduction of proliferation in satellite cells, has been suggested for a wide range of growth factors, including fibroblast growth factor-6, insulin-like growth factors, and PDGF-BB. Furthermore, close associations between differentiation and proliferation were described (5, 38, 39). Following inhibition of the proliferation by AIF-1, concomitant inhibition of myogenin expression in satellite cells can therefore be regarded as a sequential event in the disruption of the proliferation associated signalling cascade. However, recently published results reported the concomitant upregulation of myogenin following an increase of the extracellular Ca^{2+} concentration (40). This might indicate another mechanism for the interaction between AIF-1 and myogenin. Accumulation of AIF-1 expressing macrophages in injured skeletal muscle might accordingly cause a concomitant decrease of myogenin expression followed by a reduction of myogenic activation. Accordingly, antagonization of AIF-1 expression or depletion of AIF-1 expressing macrophages from injured skeletal muscle might constitute a novel strategy to improve regeneration following injury. Our observation that AIF-1 reduces the number of myogenin$^+$ cells in injured skeletal muscle, therefore, provides further insight into the sequential events of AIF-1 mediated inhibition of satellite cell proliferation.

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