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Expression of $\alpha 7\beta 1$ Integrin Splicing Variants during Skeletal Muscle Regeneration

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Integrin $\alpha 7\beta 1$ is a laminin receptor, both subunits of which have alternatively spliced, developmentally regulated variants. In skeletal muscle $\beta 1$ has two major splice variants of the intracellular domain ($\beta 1A$ and $\beta 1D$). $\alpha 7X1$ and $\alpha 7X2$ represent variants of the $\alpha 7$ ectodomain, whereas $\alpha 7A$ and $\alpha 7B$ are variants of the intracellular domain. Previously we showed that during early regeneration after transection injury of muscle $\alpha 7$ integrin mediates dynamic adhesion of myofibers along their lateral aspects to the extracellular matrix. Stable attachment of myofibers to the extracellular matrix occurs during the third week after injury, when new myotendinous junctions develop at the ends of the regenerating myofibers. Now we have analyzed the relative expression of $\beta 1A/\beta 1D$ and $\alpha 7A/\alpha 7B$ and $\alpha 7X1/\alpha 7X2$ isoforms during regeneration for 2 to 56 days after transection of rat soleus muscle using reverse transcriptase-polymerase chain reaction and immunohistochemistry. During early regeneration $\beta 1A$ was the predominant isoform in both the muscle and scar tissue. Expression of muscle-specific $\beta 1D$ was detected in regenerating myofibers from day 4 onwards, ie, when myogenic mitotic activity began to decrease, and it became more abundant with the progression of regeneration. $\alpha 7B$ isoform predominated on day 2. Thereafter, the relative expression of $\alpha 7A$ transcripts increased until day 7 with the concomitant appearance of $\alpha 7A$ immunoreactivity on regenerating myofibers. Finally, $\alpha 7B$ again became the predominant variant in highly regenerated myofibers. Similarly as in the controls, $\alpha 7X1$ and $\alpha 7X2$ isoforms were both expressed throughout the regeneration with a peak in $\alpha 7X1$ expression on day 4 coinciding with the dynamic adhesion stage. The results suggest that during regeneration of skeletal muscle the splicing of $\beta 1$ and $\alpha 7$ integrin subunits is regulated according to functional requirements. $\alpha 7A$ and $\alpha 7X1$ appear to have a specific role during the dynamic phase of adhesion, whereas $\alpha 7B$, $\alpha 7X2$, and $\beta 1D$ predominate during stable adhesion. *(Am J Pathol 2002, 161:1023–1031)*

Integrins are a family of transmembrane receptor molecules, which participate in vital biological processes such as embryonic development, cell differentiation, maintenance of tissue integrity, and cell-extracellular matrix (ECM) interactions in general.[1–4] Integrins do not only physically link cytoskeleton to the ECM, but they also have an important role in transducing mechanical and chemical signals into the cells. Integrins are heterodimers composed of two subunits, α and β, that both consist of extracellular, transmembrane, and cytoplasmic domains. The β subunits are believed to target integrins to sites where cells adhere to the ECM and for interaction with the cytoskeleton, whereas α subunits participate in the determination of the specificity of the ligand binding and signaling.[5–8] At least 18 α and 8 β subunits have been described in mammals.[9]

The $\beta 1$ integrin subunit is widely expressed in different cells, which adhere to the ECM.[3] It can associate with several α subunits. The predominant dimer in skeletal muscle is integrin $\alpha 7\beta 1$, which binds to muscle associated laminins.[10–13] The cytoplasmic domain of the $\beta 1$ subunit is associated with the cytoskeletal actin via several molecules located subsarcolemmally, such as α-actinin, talin, vinculin, paxillin, and tensin.[14–17] In skeletal muscle the $\alpha 7\beta 1$ integrin adhesion complex connects the contractile proteins of myofibers to the ECM. They are concentrated at myotendinous junctions (MTJ), where firm myofiber-tendon attachments are formed allowing transformation of the force created by muscle contraction into movement.

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The β1 integrin subunit has five isoforms with alternatively spliced cytoplasmic domains.[9,18–24] The β1A isoform is present in many tissues, whereas β1D is a muscle-specific variant and the predominant β1 isoform in striated muscle.[22–24] β1A is abundantly expressed in proliferating myogenic precursor cells, but during myodifferentiation it is replaced by the β1D isoform. The onset of β1D expression coincides with the time of myoblast withdrawal from the cell cycle. In mature skeletal muscle β1A is expressed only at a low level, if at all, whereas the predominant β1D becomes concentrated in the sarcolemma of MTJs, neuromuscular junctions (NMJs), and costameres.[22,24,25]

The α7 subunit has at least five isoforms, three with alternatively spliced cytoplasmic domains (A, B, and C) and two with alternatively spliced extracellular domains (X1 and X2).[11,26–28] The α7A and α7C isoforms appear to be restricted to skeletal muscle in contrast to α7B, which is also expressed in nonmuscle tissues.[10] In skeletal muscle α7B is expressed in proliferating, mobile myogenic precursor cells. Its expression is diminished during differentiation *in vitro*, but α7B is still detected in adult myofibers with restricted localization to NMJs and MTJs.[29,30] Expression of the α7A and α7C isoforms begins during terminal myogenic differentiation of precursor cells simultaneously with the expression of myogenin.[27,30] The α7A isoform is also localized to the NMJs and MTJs, whereas α7C is present extrajunctionally. The α7X1 and α7X2 isoforms are both found in myogenic cells.[11,28] In replicating myoblasts and myotubes the X1 isoform predominates or the X1 and X2 isoforms are expressed in approximately equal amounts, but in adult skeletal muscle X2 is the dominant isoform.[10,11,28]

Alternatively spliced isoforms of both α and β subunits differ in their signaling activity, in their specificity and affinity for ligands and interaction with cytoskeleton.[9] The expression of these isoforms appears to be developmentally regulated and obviously these functional differences can provide the variation in regulatory mechanisms needed to meet the requirements of the different biomechanical adhesion situations during muscle development. The cellular events during regeneration of skeletal muscle have been traditionally described to recapitulate those occurring during development, although the requirements for biomechanical adhesion in regenerating mature muscle do differ from those in immature developing muscle. In this study we have analyzed, whether this recapitulation also extends to the expression of β1A and β1D, α7A and α7B, and α7X1 and α7X2 splicing variants at different phases of regeneration after a shearing type of skeletal muscle injury induced in rat by transection of the soleus muscle.

Materials and Methods

Muscle Injury and Tissue Preparation

Fifty adult male Sprague-Dawley rats were used in this study. The average age at the time of injury was 12 weeks. The animals were housed in cages and fed with commercial pellets and water *ad libitum*. The research protocol was accepted by the ethical committee for animal experiments of the University of Tampere.

Under anesthesia, the animals were unilaterally injured by a complete transection of soleus muscle. The uninjured contralateral muscle served as the control. The injury method has been described in detail in our previous study.[31] Animals were divided into 10 groups. These were sacrificed 2, 3, 4, 5, 7, 10, 14, 21, 28, and 56 days postoperatively with an overdose of carbon dioxide. Three animals from each group were used for morphological analysis and two were used for isolation of RNA.

Histology and Immunohistochemistry

Soleus muscles were collected and frozen in isopentane cooled with liquid nitrogen. Frozen samples were cut into 5-μm longitudinal sections and stained with hematoxylin and eosin for structural analysis. For immunohistochemical studies the following antibodies were used: mouse monoclonal antibody H36 to the α7 integrin subunit (which recognizes a determinant on the extracellular domain of all isoforms[12]), rabbit polyclonal antibodies to the α7A integrin (antibody α7CDA2), α7B integrin (α7CDB2), which recognize the respective A and B cytoplasmic domains,[27,30] mouse monoclonal antibodies to the β1 integrin subunit (clone HMβ1-1; Pharmingen, San Diego, CA), the β1D integrin isoform (clone 2B1) and desmin (clone ZSD1; Zymed, San Francisco, CA). The bound antibodies were visualized using appropriate avidin-biotin peroxidase kit (Vectastain; Vector Laboratories, Burlingame, CA) with diaminobenzidine as the chromogen and hematoxylin as the counterstain.

RNA Purification and Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

For RNA analyses muscle tissue ~2 mm proximally and distally to the injury site was dissected from each animal and the two samples from each time point were pooled. Total RNA was obtained using the guanidium thiocyanate/CsCl method.[32] Reverse transcription and subsequent PCR were performed with 1.0 μg of total RNA using the Gene Amp PCR kit (Perkin-Elmer, Roche Molecular Systems, Inc., Branchburg, NJ). Synthesized cDNAs were amplified by PCR in a Perkin Elmer DNA Thermal Cycler. The primers used in the amplification of the β1A and β1D variants of the β1 cytoplasmic domain were NZ1 (5′-TTGTGGAGACTCCAGACTGTCCTACT-3′) and PE6 (5′-TCATTTTCCCTCATACTTCGGATT-3′) (designed from Argraves et al[18] and Holers et al[33]). NZ1 and PE6 oligonucleotide primers flank the region where β1A and β1D splicing variants differ from each other, ie, β1D contains a specific 81-bp insertion compared to β1A.

The primers for the amplification of the α7A and α7B variants of the α7 cytoplasmic domain were 3154 (5′-GTTGTGGAAGGAGTCCC-3′) and 3155 (5′-GTCTTC-CCGAGGGATC-TT-3′) (designed from Collo et al[26]). α7A contains a segment resulting from alternative splicing of a 113-bp sequence in the mRNA that is not present in α7B. The primers for the amplification of the α7X1 variant of the

extracellular domain were 5′-CTATCCTTGCGCAGAATGAC-3′ and 5′-GCCAGGGTGGAGCTCTG-3′, and the primers for α7X2 were 5′-CTATCCTTG-CGCAGAATGAC-3′ and 5′-GTGACCAACATTGATAGCTC-3′ (designed from Ziober et al[28]) (CyberGene AB, Huddinge, Sweden).

The cycle parameters for the amplification of the β1A and β1D mRNAs were: denaturation at 94°C for 2 minutes, annealing at 55°C for 1.5 minutes, extension at 72°C for 3 minutes for 40 cycles with a 5-minute final elongation at 75°C. The corresponding parameters for α7A and α7B were: denaturation at 94°C for 1 minute, annealing at 56°C for 1 minute, extension at 72°C for 2 minutes for 40 cycles with a 7-minute final elongation at 72°C. The parameters for X1 and X2 were: denaturation at 94°C for 1 minute, annealing at 67°C for 1 minute, extension at 72°C for 2 minutes for 40 cycles with a 7-minute final elongation at 72°C. PCR products were analyzed on 1.5 (X1/X2) or 2% agarose gel stained with ethidium bromide, using a 100-bp ladder as a standard. The intensity of the bands was quantified using Microcomputer Imaging Device version M4 (Imaging Research Inc., Brock University, St. Catharines, Ontario, Canada) and the relative proportion of the intensity of each splicing variant was expressed as a percentage of the total intensity of both bands at each time point. PCR reactions were controlled in identical conditions but with different cycle numbers (15 to 40). The accumulation of PCR products was even and the number of cycles did not affect the ratio of bands.

Results

General Course of the Regeneration Process

The histopathological regeneration process followed the same time sequence and pattern as has previously been described in this type of muscle-shearing injury.[31,34] The stumps of the transected muscle retract and a gap is formed between them. The transected myofibers die back (are necrotized) over a distance of 1 to 2 mm within their breached basement membrane (BM; Figure 1A). By the earliest time point in this study (day 2), myogenic precursor (satellite) cells had already proliferated for a day and begun to fuse to form myotubes. By day 5 myotubes had filled the original ruptured BM cylinders and started to penetrate into the connective tissue scar between the regenerating muscle stumps (Figure 1B). At approximately day 14 cross-striation was already visible in the sarcoplasm, indicating differentiation into myofibers, although many myonuclei were still centrally located. At the same time the ends of regenerating myofibers began to attach firmly to the scar by newly formed MTJs. By day 21 most regenerating myofibers had acquired their final mature form with well-organized cross-striation, peripherally located myonuclei, and distinct new MTJs. From days 21 to 56 the scar between the stumps contracted and diminished in size, whereby the stumps were brought closer to each other, but they did not fuse and remained attached to the scar by MTJs until the end of the observation period (Figure 1C).

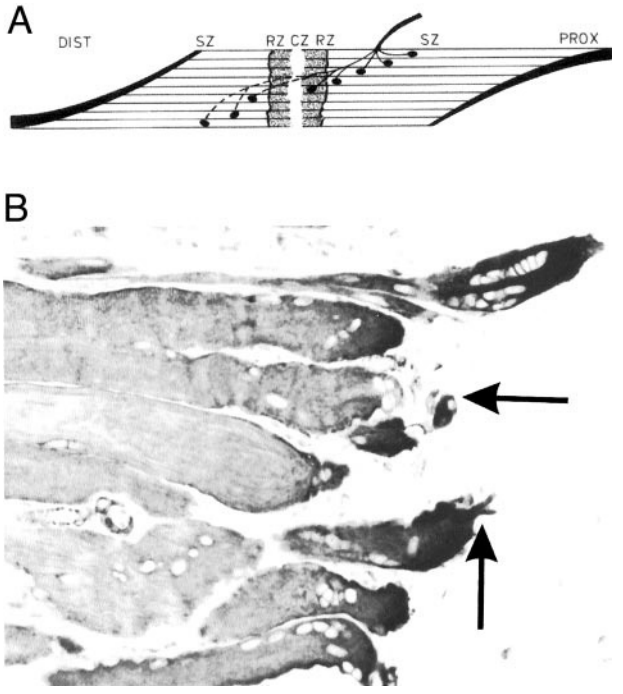

Figure 1. A: A schematic picture of the shearing type muscle injury. Disrupted myofibers contract and a gap (central zone = CZ) is formed between the stumps. The transected myofibers die back (become necrotized) over a distance of 1 to 2 mm, in which zone (RZ) regeneration within the original breached BM occurs. SZ = survival zone. **B:** The original BM cylinders of the RZ are filled by desmin-immunopositive myotubes that have begun to penetrate into the scar between the stumps (**arrows**) (day 5 after injury). **B:** Desmin immunoperoxidase staining with hematoxylin counterstain. Original magnification, ×205.

Differential Expression of the β1 and α7 Splice Variants during Regeneration

β1A and β1D Cytoplasmic Domain Isoforms

During myogenesis β1A isoform is expressed in proliferating myogenic precursor cells later followed by β1D isoform in differentiating cells. In our model of regeneration, two RT-PCR products corresponding to the β1A (264 bp) and β1D (345 bp) isoform transcripts were detected at each time point and in the control muscle (Figure 2A). During days 2 to 21 after the injury the relative levels of β1D transcript were only 0 to 11% of the total β1 RNA (β1A plus β1D). Correspondingly the levels of β1A transcript varied from 100 to 89% (Figure 2B). After day 21 the relative amount of β1D mRNA isoform increased to the level of 51% by day 56, which was only slightly lower than the corresponding value of 65% in control muscle.

Because the mRNA measurements may be influenced by the mRNAs from inflammatory cells we re-evaluated the β1D expression by immunohistochemistry. In immunohistochemical preparations the surviving parts of the transected fibers stained with the β1D-specific antibody throughout the regeneration process with accentuation at the MTJs (Figure 2C). Corresponding to the low relative level of β1D mRNA the myotubes in the regenerating zone were immunonegative for β1D before day 4 of regeneration (Figure 2D). On day 4 weak β1D immunore-

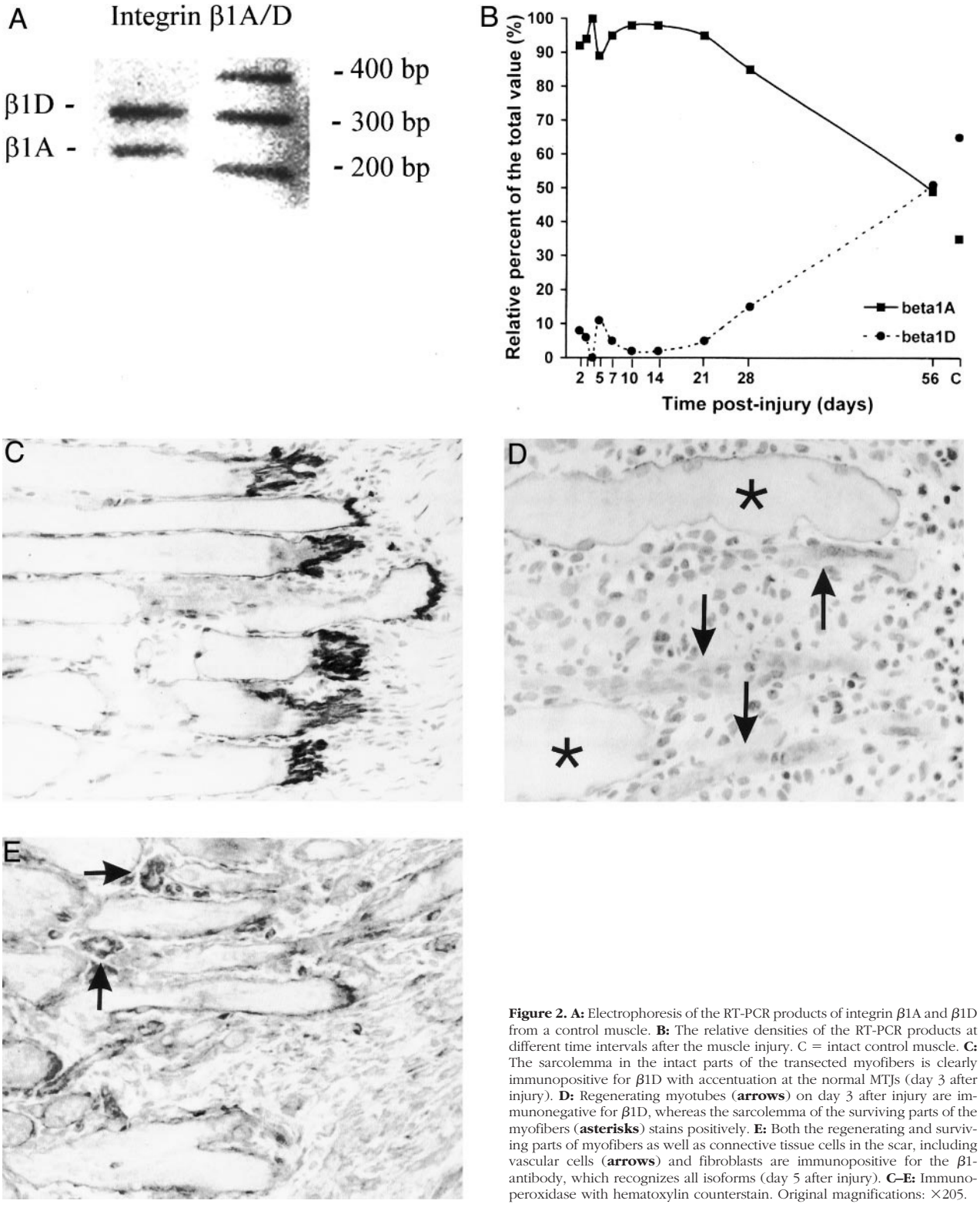

Figure 2. A: Electrophoresis of the RT-PCR products of integrin β1A and β1D from a control muscle. **B:** The relative densities of the RT-PCR products at different time intervals after the muscle injury. C = intact control muscle. **C:** The sarcolemma in the intact parts of the transected myofibers is clearly immunopositive for β1D with accentuation at the normal MTJs (day 3 after injury). **D:** Regenerating myotubes (**arrows**) on day 3 after injury are immunonegative for β1D, whereas the sarcolemma of the surviving parts of the myofibers (**asterisks**) stains positively. **E:** Both the regenerating and surviving parts of myofibers as well as connective tissue cells in the scar, including vascular cells (**arrows**) and fibroblasts are immunopositive for the β1-antibody, which recognizes all isoforms (day 5 after injury). **C–E:** Immunoperoxidase with hematoxylin counterstain. Original magnifications: ×205.

activity was detected in the cytoplasm and in the sarcolemma it appeared as small patches. Cytoplasmic β1D immunoreactivity gradually increased until day 7 in the distal parts of the regenerating fibers and simultaneously the sarcolemmal staining gradually became more homogeneous and intense. From day 10 onwards the cytoplasmic β1D immunoreactivity decreased and had practically disappeared by day 21. From day 14 onwards the sarcolemma of the regenerated myofibers was homogeneously β1D-immunopositive with the strongest staining at the ends of the fibers, where new MTJs are formed (Figure 2, E and F). No β1D was detected in connective

tissue cells in the scar between the stumps at any time interval.

On the other hand, the β1 antibody that recognizes all isoforms of β1 stained the sarcolemma of the regenerating parts already on day 3 (Figure 2E). Besides, with this antibody connective tissue cells, fibroblasts, and vascular cells in the interposed scar were also clearly β1-positive. The relative proportion of β1 immunoreactivity in the scar in comparison to that in the muscle decreased, because the scar contracted and diminished in size and cellularity. The immunoreactivity in individual fibroblasts/myofibroblasts also appeared to decrease.

α7A and α7B Cytoplasmic Domain Isoforms

During myogenesis *in vitro* α7B is the sole isoform in proliferating cells. In contrast, α7A is detected on terminal myogenic differentiation. Bands corresponding to RT-PCR products from both α7A (283 bp) and α7B (170 bp) cytoplasmic domain transcripts were detected at each time point during regeneration and in the control muscle (Figure 3, A and B). The relative level of α7A transcript increased from day 2 to day 4 from 15 to 96% of the total of α7A plus α7B. After day 4 the proportion of α7A transcript gradually decreased and that of α7B increased until day 56 to the values 8% of α7A and 92% of α7B, which closely correspond to the proportions of α7A and α7B transcripts in the control tissue.

Immunohistochemical staining disclosed strong α7B immunoreactivity in the sarcolemma of the intact parts of myofibers with accentuation in the MTJs, whereas the same structures were negative for α7A (Figure 3, C and D). On day 2 the small myotubes were immunonegative for α7A, but on day 3 immunoreactivity was clearly discernible in the sarcoplasm of the regenerating parts (Figure 3E). This localization persisted until day 28, although with decline in the sarcoplasm and accentuation in the sarcolemma. Immunoreactivity of α7B in the sarcolemma of the regenerating myofibers could be discerned on day 7. Thereafter it persisted and increased with time. On day 56 the immunolocalization in the regeneration zone corresponded to that in the control situation: there was no reactivity for α7A in the sarcolemma, but strong reactivity for α7B with clear accentuation at the new MTJs (Figure 3F).

α7X1 and α7X2 Extracellular Domain Isoforms

Both α7X1 and α7X2 are present in the early stages of myogenesis, but in normal adult skeletal muscle the X2 isoform has been reported to be the only extracellular domain isoform. Because of the minimal difference in the size of the X1 and X2 bands these PCR reactions were performed in parallel. Two bands corresponding to the α7X1 (220 bp) and α7X2 (200 bp) extracellular domain isoforms were detected at each time point after injury and in the control muscle (Figure 4A). Some PCR reactions for X1 produced another somewhat larger band, but the correct band could be identified on the basis of its size. The relative level of α7X1 transcript compared to total α7X1 plus α7X2 increased from 51% on day 2 to 72% on day 4 after injury. It gradually decreased thereafter to 38% on day 10 (Figure 4B). From day 10 to day 56 the proportion of α7X1 transcript was relatively constant. On day 56 the proportions of α7X1 and α7X2 were 39% and 61%, respectively. In the control muscle the corresponding values for α7X1 and α7X2 were 40% and 60%, respectively.

Discussion

General Aspects

During development myogenic precursor cells migrate from somites to sites where they proliferate, fuse into myotubes, and differentiate into mature myofibers. These fibers firmly attach to the ECM of tendons forming MTJs to implement muscle function as weight-bearing and motion-producing tissue. This process requires closely regulated cell-ECM communication. Integrins serve a major role as transmembrane mediators between cytoskeletal and ECM proteins in functional muscle. Because the requirements for the cell-ECM interactions at different stages of the myogenic differentiation vary considerably, the functions of sarcolemmal integrin molecules must vary accordingly. This is reflected in the alternative splicing of mRNA for the α7 and β1 integrin subunits during development.[9–11]

The regeneration process that takes place after a shearing type of muscle injury has similarities with the formation of skeletal muscle during development: precursor cells proliferate, migrate, fuse, and finally the regenerated myofibers become firmly reattached to the ECM.[35–37] However, regenerating myofibers are exposed to greater physical stress than developing myogenic cells. This additional force most likely modifies the interaction between myofibers and the ECM. Proliferation in regenerating myofibers continues until approximately day 5 after injury. Thereafter the regenerating ends of the injured myofibers emerge from the original ruptured basal lamina cylinders and penetrate into the scar between the stumps. Therefore, the growing ends of these fibers are not yet firmly attached to the ECM, but myofibers reinforce their dynamic adhesion mediated by α7β1 integrin along their lateral aspects, where adhesion mediated by dystrophin and associated molecules normally prevails.[38] This reinforced lateral adhesion most likely reduces the risk of rerupture of the muscle and allows use of the muscle before the repair process is completed. However, the firm and stable attachment does not occur until new MTJs with abundant α7β1 integrin develop at the ends of these stumps during the third week (days 14 to 21) after the injury, whereas dystrophin at the ends is not normalized until approximately day 56 after injury suggesting a subordinate role for dystrophin in mechanical adhesion.[31,34,38] It is likely that alternative splicing of the mRNA of the α7 and β1 integrin subunits underlies these different cell-ECM interactions during regeneration.

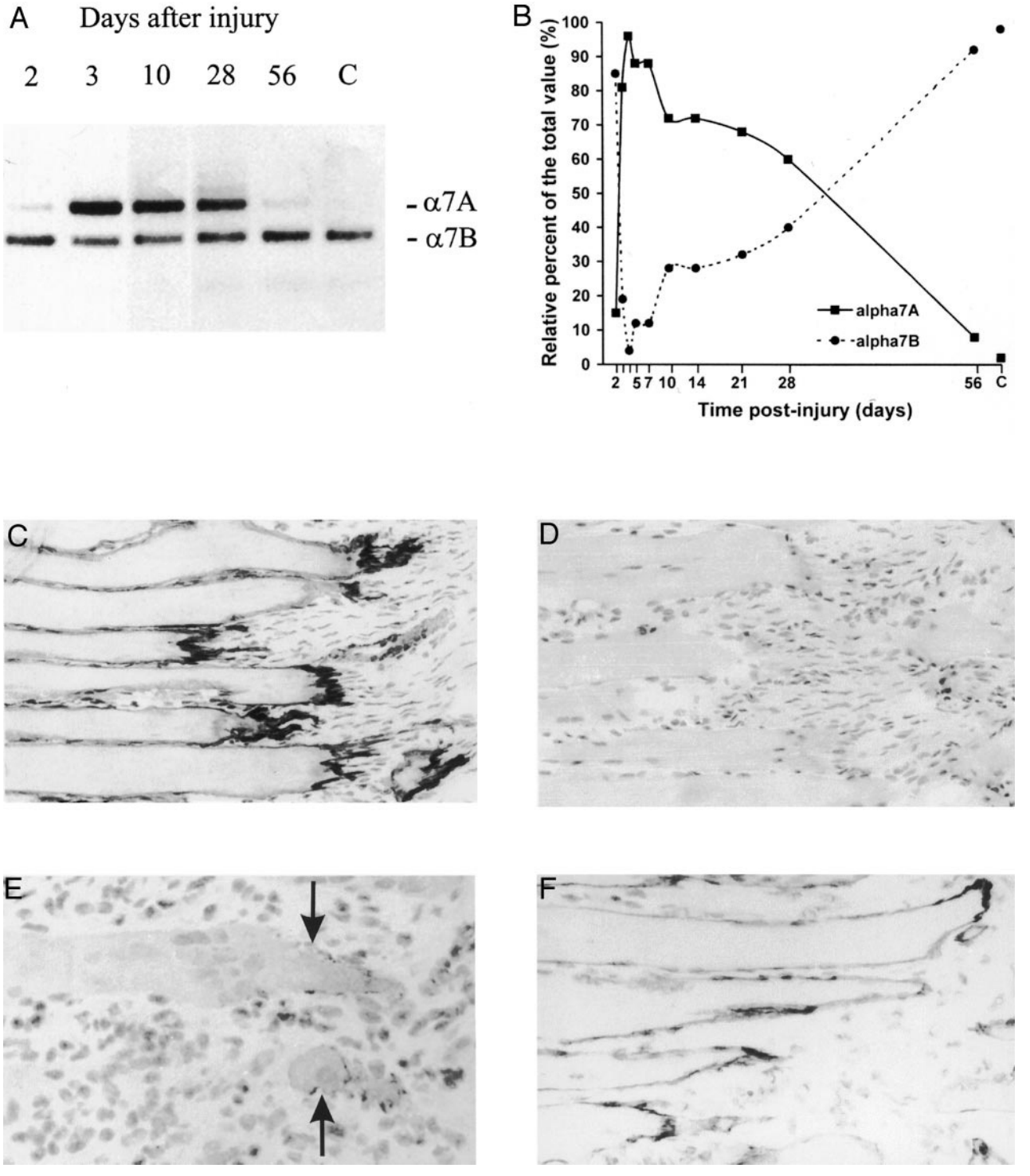

Figure 3. A: Electrophoresis of the RT-PCR products of α7A and α7B isoforms. **B:** The relative densities of the isoform products at different time intervals after the muscle injury. C = intact control muscle. **C:** The sarcolemma in the intact parts of the transected myofibers is strongly immunopositive for α7B with accentuation at the MTJs. **D:** In an adjacent section both the sarcolemma and MTJs are immunonegative for α7A. **E:** The sarcoplasm and the sarcolemma in a patchy manner in the regenerating part (**arrow**) on day 5 after injury stain positively with α7A antibody, but the surviving part remains negative. **F:** Both the sarcolemma and the new MTJs attaching the ends of the regenerated myofibers to the interposed scar are immunopositive for α7B. Day 56 after injury. **C–F:** Immunoperoxidase staining with hematoxylin counterstain. Original magnifications: ×195.

Expression of β1 Isoforms

In our study the mRNA measurements indicated that the expression of the nontissue-specific β1A isoform dominated until ~28 days, whereafter the ratio of β1A and the muscle-specific β1D isoforms corresponded to that in the control muscle. However, immunohistochemical staining demonstrated that cytoplasmic β1D was detectable in

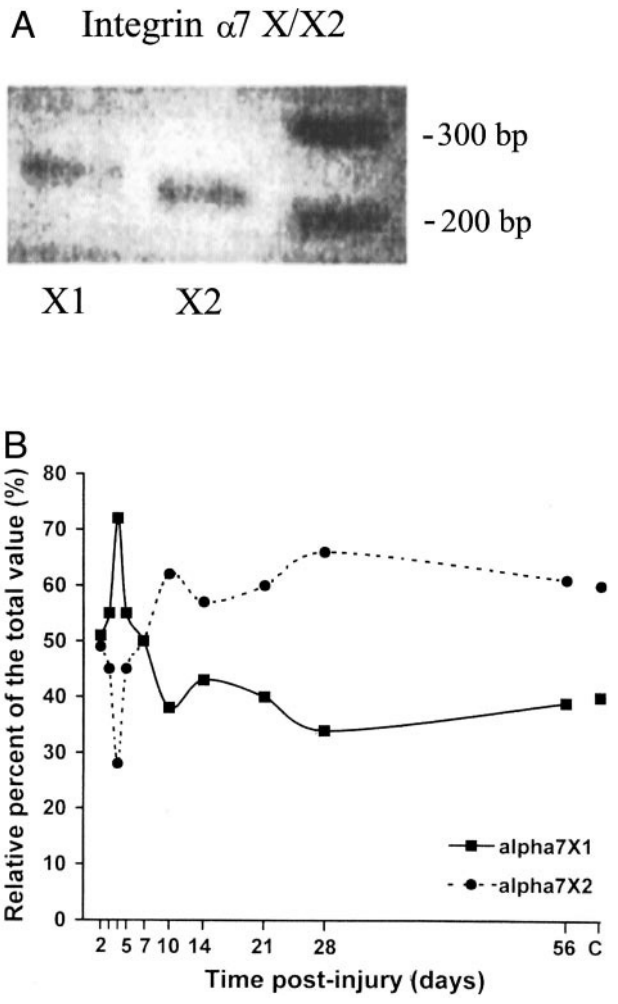

Figure 4. A: Electrophoresis of the RT-PCR products of α7X1 and α7X2 isoforms from a control muscle. **B:** The relative densities of the isoform products at different time intervals after the muscle injury.

myotubes already on day 4 and patches of sarcolemmal β1D soon thereafter, and furthermore, the sarcolemmal staining became more intense with formation of the new MTJs by approximately day 14.

Studies of myogenesis *in vitro* have demonstrated that the early expression of the β1A isoform, present in many different tissues, is down-regulated on differentiation and this is paralleled by up-regulation of the expression of the muscle-specific β1D isoform.[22,24,25] This switch occurs when myoblasts withdraw from the cell cycle, and is consistent with the reported growth inhibitory properties of the β1D isoform.[39] In our previous studies mitotic activity during regeneration ceases approximately day 5 after injury, when the old basal lamina cylinder is filled by regenerating myotubes (see Figure 1, A and B).[40] Thus, on the basis of our PCR results the β1A to β1D transcriptional switch appeared to take place considerably later during far advanced myodifferentiation. However, cytoplasmic β1D was immunohistochemically detectable in myotubes already on day 4 and in the sarcolemma soon thereafter, whereas the connective tissue cells in the scar between the stumps were immunopositive with the antibody recognizing all β1 isoforms. Thus, the relative predominance of the β1A expression is most likely because of the abundance of β1A mRNA in the fibroblasts/myofibroblasts and vascular cells in the scar, which at the early stages forms the major component in the tissue sampled for RT-PCR analysis. Therefore, the β1A to β1D switch in myofibers could not be detected in the PCR until at the later stages, when the scar diminished in size and the proportion of regenerating myofibers and the relative amount of muscle-specific β1D increased in the tissue sample.

The time period of low β1D expression with up-regulated β1A in regenerating myotubes represents the dynamic adhesion stage.[38] This stage in the regeneration of muscle may correspond to the situation in most nonmuscle cells in which the affinity of the interactions of integrins with ECM ligands and cytoskeletal proteins is relatively low, although obviously sufficient for the anchorage and traction needed.[3,15,16] In contrast, in mature, fully functional skeletal muscle the tensile forces transmitted from the cytoskeleton to the ECM by integrins are remarkably great and this transmission is implemented at specialized structures, the MTJs. Accordingly, the most marked up-regulation of β1D expression appeared to occur parallel to the formation of new MTJs as a sign of a firm adhesion to the ECM.[31,38] This is consistent with the results of Belkin and colleagues[41] who showed that β1D integrin interacts more strongly with the actin cytoskeleton than β1A and infers an important role for α7β1D in forming extremely stable and strong associations with the cytoskeleton that are required during muscle contraction. Furthermore, through inside-out signaling the specific structure of the β1D cytoplasmic domain has been shown to activate the ligand binding of the extracellular domain of the β1D integrin subunit.[41]

Expression of α7A and α7B Isoforms

At the time of active satellite cell proliferation on day 2 after muscle injury, the ratio of α7A/α7B transcripts was similar to that reported in replicating myoblasts during early *in vitro* myodifferentiation, ie, α7B was the predominant isoform.[26,27,28] However, thereafter the ratio during the *in vivo* regeneration deviates from the developmental pattern. The relative level of α7B decreased on day 3, whereas the period of active replication is over after day 5. The α7A isoform remained predominant during the active growth of the regenerating myofibers from days 3 to 14 until their firm attachment to ECM, although the relative level of α7B expression gradually increased with a fairly similar timetable as the β1D isoform. Finally at the late stage of regeneration, α7B became the predominant isoform similarly as it is in the control muscles.[10] This pattern of isoform expression was also verified immunohistochemically with the α7A- and α7B-specific antibodies. Thus, α7A may have a specific role in regenerating muscle during the dynamic adhesion stage, whereas in mature skeletal muscle it appears to have a minor role. Thus, α7Bβ1D integrin known to be localized to both MTJs and NMJs appears to be the integrin variant that contributes most to the firm adhesion of myofibers to ECM.[10]

In our study the samples for mRNA purification were taken from the midbelly of soleus, where the NMJs are located and where the abundant new MTJs are formed in the regenerating muscle. The α7B cytoplasmic domain has been shown to contain several motifs that present a rich potential for participating in the interaction between α7B and cytoskeleton. There is for example a potential actin-binding sequence as well as regions that may be involved in transduction of signals initiated outside the cell.[27] In the same study by Song and colleagues[27] the α7B cytoplasmic domain was shown to undergo a change in conformation in response to binding laminin, which may modulate physiological responses in the myofibers.

Expression of α7X1 and α7X2 Isoforms

In our study the α7X1 isoform was relatively more abundant during the early regeneration process, whereas α7X2 was the dominant isoform during the late repair process and in the control muscles. This timing during the repair process is consistent with the expression pattern reported during skeletal muscle development.[10,11,28] It supports the suggested importance of the α7X1 isoform during dynamic adhesion situations related to muscle development (motility, fusion, remodeling, repair, and matrix assembly).[42] It also conforms with a recent study, in which α7X1β1 was suggested to be a physiological receptor for laminins 8 and 10, which laminins are expressed in developing skeletal muscle[7] and during the recovery of muscle injury.[43] In contrast, the α7X2 isoform underlies more stable adhesion functions, eg, in MTJs, NMJs, and costameres.[42]

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