Increased Susceptibility to Complement Attack due to Down-Regulation of Decay-Accelerating Factor/CD55 in Dysferlin-Deficient Muscular Dystrophy

Katrin Wenzel, Joanna Zabojszcza, Miriam Carl, Semjon Taubert, Antje Lass, Claire L. Harris, Mengfatt Ho, Herbert Schulz, Oliver Hummel, Norbert Hubner, Karl Josef Osterziel and Simone Spuler

*J Immunol* 2005; 175:6219-6225; doi: 10.4049/jimmunol.175.9.6219

http://www.jimmunol.org/content/175/9/6219

---

**Supplementary Material**  http://www.jimmunol.org/content/suppl/2005/10/19/175.9.6219.DC1

**References**  This article cites 63 articles, 27 of which you can access for free at: http://www.jimmunol.org/content/175/9/6219.full#ref-list-1

**Subscription**  Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**  Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**  Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Increased Susceptibility to Complement Attack due to Down-Regulation of Decay-Accelerating Factor/CD55 in Dysferlin-Deficient Muscular Dystrophy\textsuperscript{1,2}

Katrin Wenzel,† Joanna Zabojszcza,* Miriam Carl,* Semjon Taubert,* Antje Lass,* Claire L. Harris,§ Mengfatt Ho,§ Herbert Schulz,¶ Oliver Hummel,¶ Norbert Hubner,¶ Karl Josef Osterziel,¶ and Simone Spuler\textsuperscript{3*}

Dysferlin is expressed in skeletal and cardiac muscles. However, dysferlin deficiency results in skeletal muscle weakness, but spares the heart. We compared intraindividual mRNA expression profiles of cardiac and skeletal muscle in dysferlin-deficient SJL/J mice and found down-regulation of the complement inhibitor, decay-accelerating factor/CD55, in skeletal muscle only. This finding was confirmed on mRNA and protein levels in two additional dysferlin-deficient mouse strains, A/J mice and Dysf\textsuperscript{−/−} mice, as well as in patients with dysferlin-deficient muscular dystrophy. In vitro, the absence of CD55 led to an increased susceptibility of human myotubes to complement attack. Evidence is provided that decay-accelerating factor/CD55 is regulated via the myostatin-SMAD pathway. In conclusion, a novel mechanism of muscle fiber injury in dysferlin-deficient muscular dystrophy is demonstrated, possibly opening therapeutic avenues in this to date untreatable disorder. \textit{The Journal of Immunology,} 2005, 175: 6219–6225.

\textsuperscript{1} Myology Research Group, Department of Neurology, Charité University Hospital, Berlin, Germany; \textsuperscript{2} Department of Cardiology, Franz-Volhard Clinic, Helios Clinic, Berlin, Germany; \textsuperscript{3} Complement Biology Group, Department of Medical Biochemistry and Immunology, University of Wales College of Medicine, Cardiff, United Kingdom; \textsuperscript{4} Division of Medical Sciences, National Cancer Center, Singapore, Singapore; and \textsuperscript{5} Max Delbrück Center for Molecular Medicine, Berlin, Germany

Received for publication July 14, 2005. Accepted for publication August 18, 2005.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

\textsuperscript{†} This work was supported by research grants from the Humboldt University (to S.S. and K.-J.O.).

\textsuperscript{‡} The mutations are submitted to the Leiden Muscular Dystrophy Database (http://dmd.nl/). The MIAME-compliant microarray data are available at (www.ncbi.nlm.nih.gov) under accession no. GSE2507.

\textsuperscript{*} Address correspondence and reprint requests to Dr. Simone Spuler, Myology Research Group, Department of Neurology, Charité University Hospital, Augustenburger Platz 1, 13353 Berlin, Germany. E-mail address: simone.spuler@charite.de

\textsuperscript{§} Abbreviations used in this paper: LGMD2B, limb girdle muscular dystrophy 2B; CARP, cardiac ankyrin repeat protein; DAF, decay-accelerating factor; EGR1, early growth response 1; MAC, membrane attack complex; PI, propidium iodide.

Copyright © 2005 by The American Association of Immunologists, Inc.
decay-accelerating factor (DAF/C5d5), membrane cofactor protein (CD46), and C5d5 (15).

In this study we show that murine and human dysferlin-deficient muscle fibers lack the complement inhibitory factor, C5d5/DAF. As a consequence, dysferlin-deficient nonnecrotic muscle cells express C5b9-MAC and are more susceptible to complement-mediated lysis in vitro.

Materials and Methods

Mice

Female SJL/J mice and C57BL/6 mice were purchased from Charles River Laboratories. The microarray experiments were performed in mice 32–34 wk of age. At this age, SJL/J mice showed marked histological signs of muscular dystrophy. Lymphomas were not detected. Muscle sections for immunohistochemistry were obtained from SJL/J mice at 12, 16, 20, 28, and 32 wk of age. For each age group, three mice were examined. Muscle sections from A/J and D dysf–/– mice were obtained at 16 wk of age. All experiments were approved by local committees.

Total RNA preparation

RNA was extracted from mouse right quadriceps muscle, the left and right ventricles of mouse heart, and human skeletal muscle using TRIzol reagent (Invitrogen Life Technologies). Total RNA was treated by deoxyribonuclease I (Invitrogen Life Technologies) and was purified using the RNeasy Mini Kit (Qiagen).

Microarray experiments

Nonpoored microarray experiments were performed with cRNA prepared from quadriceps muscles and left ventricles of five SJL/J and five C57BL/6 mice using GeneChip Murine Genome U74Av2 (Affymetrix). Arrays were hybridized with 16 Y yield Transcription Kit (Ambion) and was labeled with biotin-11-CTP and Synthesis System (Roche). cRNA was produced by MEGAscript High crograms of RNA was transcribed in double-stranded cDNA using a cDNA

Microarray data analysis

The resulting signals were processed using Affymetrix MicroArray Suite 5.0 software (MASS.0) with a target intensity of 200. After standard data quality checks, we used the MASS.0 expression signal values of each data set for statistical analysis. Probe sets showing an absent call throughout all comparison groups were removed. A Nalimov test with a threshold of p < 0.001 was used to exclude outliers. Student’s t test (unpaired, two-tailed assumed unequal variance) was used to check the differences between two selected experimental groups.

Quantitative real-time RT-PCR (TaqMan)

cDNA was synthesized from 5 µg of total RNA using PowerScript reverse transcriptase (BD Clontech) and an oligo(dT)18 primer. Real-time PCR experiments were performed using TaqMan chemistry on ABI PRISM 7700 Sequence Detection System (Applied Biosystems). Each reaction was performed in a singleplex format and contained TaqMan Universal PCR Master Mix (Applied Biosystems), 900 nM forward and reverse primers, and 200 nM TaqMan probe (BioTez). An annealing/extension temperature of 58°C and 40 cycles were used. Primer/probe sets were designed using Primer Express 1.5 software (Applied Biosystems; Table I). For every sample, three independent runs in triplicate were performed, and the relative change in gene expression was quantified by the comparative threshold cycle method (16). Unpaired two-tail unequal variance t test with a significance threshold of p < 0.05 was used to compare the individual changes in threshold cycle values of the control and experimental group.

Patients

Patients were followed in the Neuromuscular Unit of Department of Neurology, Charité University Hospital (Berlin, Germany). Genomic sequencing of DYSF was performed if LGMD2B was suspected clinically and by immunohistochemistry and/or Western blotting. Patients included in this study gave their written informed consent. All studies were performed according to Declaration of Helsinki principles.

Table II. mRNA expression of regulatory proteins of the complement system in SJL/J mice

<table>
<thead>
<tr>
<th>Gene Product</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Probe</th>
<th>Ref. Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBGd</td>
<td>GCAAGTTCCTGAAACTTCG</td>
<td>TCCCTGGACTTACAGG AA</td>
<td>FAM-TGACCACCTTTGCTGAGCAAGCTATT - TAMRA</td>
<td>NM_013551</td>
</tr>
<tr>
<td>DAF1</td>
<td>GTACGAGACCTCCTCTGACG</td>
<td>GCTGACAGACTGATAGCTGGAGC</td>
<td>FAM-TGGACATCGATTCTGGAACGTATT - TAMRA</td>
<td>NM_001016</td>
</tr>
<tr>
<td>DAF2</td>
<td>AGCACCACTCTGACGTGG</td>
<td>CTTGACGATGGCTTTGCTTCC</td>
<td>FAM-CAGAACACACACAAGAACATGGTTGGTCAATT - TAMRA</td>
<td>NM_007827</td>
</tr>
<tr>
<td>Myostatin</td>
<td>AGTTCGAGACCTCCTCTGACG</td>
<td>GCTGACAGACTGATAGCTGGAGC</td>
<td>FAM-TGGACATCGATTCTGGAACGTATT - TAMRA</td>
<td>NM_013551</td>
</tr>
<tr>
<td>CARP</td>
<td>GTACGAGACCTCCTCTGACG</td>
<td>GCTGACAGACTGATAGCTGGAGC</td>
<td>FAM-TGGACATCGATTCTGGAACGTATT - TAMRA</td>
<td>NM_013551</td>
</tr>
<tr>
<td>SMAD2</td>
<td>CTTGACGATGGCTTTGCTTCC</td>
<td>CTTGACGATGGCTTTGCTTCC</td>
<td>FAM-CAGAACACACACAAGAACATGGTTGGTCAATT - TAMRA</td>
<td>NM_013551</td>
</tr>
<tr>
<td>SMAD3</td>
<td>GGCCTTCCTGACGTGGCTTCC</td>
<td>CTTGACGATGGCTTTGCTTCC</td>
<td>FAM-CAGAACACACACAAGAACATGGTTGGTCAATT - TAMRA</td>
<td>NM_013551</td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β2-MG</td>
<td>ACTGAAAAAGTAGTGTATGCTGCTTCC</td>
<td>CATTTCCACCTCTTCTGATGCTCT</td>
<td>FAM-TGGACATCGATTCTGGAACGTATT - TAMRA</td>
<td>NM_004048</td>
</tr>
<tr>
<td>DAF/CD55</td>
<td>AGATATCTTCTTCGCACTGCA</td>
<td>GCTGACAAGAGCACGCTGTATTGAGGG</td>
<td>FAM-TGGACATCGATTCTGGAACGTATT - TAMRA</td>
<td>NM_000574</td>
</tr>
<tr>
<td>Myostatin</td>
<td>GCTTCTAATCTGGCAGAC</td>
<td>GCTTCTAATCTGGCAGAC</td>
<td>FAM-TGGACATCGATTCTGGAACGTATT - TAMRA</td>
<td>NM_003529</td>
</tr>
<tr>
<td>Egr1</td>
<td>TCTTACGTTTGTGCTGTTTCC</td>
<td>CTTGACGATGGCTTTGCTTCC</td>
<td>FAM-CAGAACACACACAAGAACATGGTTGGTCAATT - TAMRA</td>
<td>NM_001964</td>
</tr>
<tr>
<td>CARP</td>
<td>GTATGTTTCTGCTGTACCAG</td>
<td>GCTGACAGACTGATAGCTGGAGC</td>
<td>FAM-TGGACATCGATTCTGGAACGTATT - TAMRA</td>
<td>NM_014391</td>
</tr>
<tr>
<td>SMAD2</td>
<td>TGGACGATGCTGATGCTGCTTCC</td>
<td>TGGACGATGCTGATGCTGCTTCC</td>
<td>FAM-CAGAACACACACAAGAACATGGTTGGTCAATT - TAMRA</td>
<td>NM_05901</td>
</tr>
<tr>
<td>SMAD3</td>
<td>GGCCTTCCTGACGTGGCTTCC</td>
<td>GGCCTTCCTGACGTGGCTTCC</td>
<td>FAM-CAGAACACACACAAGAACATGGTTGGTCAATT - TAMRA</td>
<td>NM_05902</td>
</tr>
<tr>
<td>SMAD4</td>
<td>GGCCTTCCTGACGTGGCTTCC</td>
<td>GGCCTTCCTGACGTGGCTTCC</td>
<td>FAM-CAGAACACACACAAGAACATGGTTGGTCAATT - TAMRA</td>
<td>NM_058539</td>
</tr>
</tbody>
</table>

Table I. Primer and probe sequences used for TaqMan RT-PCR

<table>
<thead>
<tr>
<th>Gene Product</th>
<th>Ref. Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>NM_003077</td>
</tr>
<tr>
<td>DAF</td>
<td>NM_000574</td>
</tr>
<tr>
<td>DAF</td>
<td>NM_013551</td>
</tr>
<tr>
<td>Myostatin</td>
<td>NM_013551</td>
</tr>
<tr>
<td>Myostatin</td>
<td>NM_000574</td>
</tr>
<tr>
<td>Egr1</td>
<td>NM_001964</td>
</tr>
<tr>
<td>CARP</td>
<td>NM_014391</td>
</tr>
<tr>
<td>SMAD2</td>
<td>NM_05901</td>
</tr>
<tr>
<td>SMAD3</td>
<td>NM_05902</td>
</tr>
<tr>
<td>SMAD4</td>
<td>NM_058539</td>
</tr>
</tbody>
</table>

a LV. Left ventricle; NC, not significantly changed.
Immunohistochemistry

Murine DAF was detected with polyclonal rat anti-mouse Ab (MDI) (17); human CD55 was detected with SM1141PS (Acris Antibodies). Anti-human C5b9 mAb (DakoCytomation) was applied for MAC detection. Anti-phospho-MADR2 mAb against phosphorylated SMAD2 was obtained from EMD Biosciences. Double-immunofluorescent staining for SMAD protein (FITC) and nuclear membrane protein lamin A/C (Novocastra; Cy3) were examined using a two-photon microscope (Leica).

Complement attack assay

Myoblast/myotube cultures and complement attack assays were performed according to published protocols (18, 19). Myoblasts were grown in SMG-Medium (Promo Cell) supplemented with Promo Cell Supplement Mix, gentamicin (40 μg/ml; Invitrogen Life Technologies), 2 mM glutamine, and 10% FCS. Myoblasts were transferred on 96-well plates and grown to near confluence. Differentiation into myotubes was induced with DMEM containing 2% heat-inactivated horse serum. For complement attack assays, wells were incubated in sextuplicate for 30 min with normal human serum diluted 1/5 and 1/20 in Veronal buffer (Hollborn & Sohne) containing 1% BSA. Half the wells were preincubated with anti-human CD55 mAb (5 μg/ml). Propidium iodide (PI; 0.5 μg/ml) was added to assess killing. The total number of myotubes was compared with PI-positive cells using a fluorescent tissue culture microscope (Leica) and by FACS analysis.

Results

DAF/CD55 is down-regulated in dysferlin-deficient mice

We used the GeneChip Murine Genome U74Av2 array to compare the gene expression profiles of skeletal and cardiac muscles of SJL/J mice with dysferlin deficiency to those of C57BL/6 control mice. Analysis of gene expression in the nonpooled skeletal muscle of SJL/J vs control mice revealed 291 differentially expressed genes at a threshold of $p < 0.001$ (see Supplementary Table I online).

DAF1 was 5-fold down-regulated in skeletal muscle of SJL/J compared with skeletal muscle of C57/BL6 mice, with a significance of $p = 0.0000009$. In contrast, in left cardiac ventricle, we found a mild 1.5-fold up-regulation. Similar results were observed with DAF2 (8.2-fold down-regulation in skeletal muscle, $p = 0.0027$; 4-fold up-regulation in heart). Therefore, analyzed individually, these two complement inhibitory factors, corresponding to human CD55, are significantly differentially expressed in skeletal muscle and heart. CD59, another well-described inhibitor of complement activation, was not differentially expressed (Table II). There was no significant difference in the expression of complement receptor 1, complement component factor H, or factor H-like 1 in skeletal muscles of dysferlin-deficient and control mice (Table II). The differential expression of DAF1 and DAF2 was validated by TaqMan RT-PCR and revealed a 4-fold down-regulation of DAF1 ($p = 0.005$) and a 2-fold down-regulation of DAF2 ($p = 0.003$; Fig. 1). The mild up-regulation of cardiac DAF was confirmed independently for the left and right ventricles (not shown).

In accordance with the results obtained by microarray and Taq-Man RT-PCR, the DAF/CD55 protein was absent by immunohistochemical staining of SJL/J quadriceps muscle, but was readily detectable on the sarcolemma of C57/BL6 control muscle (Fig. 2, A and D). A decrease in DAF/CD55 was found in SJL/J mice of all age groups (12, 16, 20, 28, and 32 wk; at least two mice per age group were tested), indicating that CD55 down-regulation is not merely a consequence of age and progressive dystrophic changes in muscle. Skeletal muscle tissues obtained from two additional dysferlin-deficient mouse strains (A/J and Dysf$^{-/-}$) at 16 wk of age also revealed the absence of DAF/CD55 (Fig. 2, B and C). Protein expression of DAF/CD55 in SJL/J myocardial tissue was not different from that in C57/BL6 control mice (Fig. 2, E and F).
DAF/CD55 is down-regulated in LGMD2B patients

We next studied skeletal muscle from four patients with dysferlin-deficient muscular dystrophy (LGMD2B). The diagnosis of LGMD2B was confirmed by the absence of dysferlin in immunohistochemical staining, in Western blot analysis, and by genomic sequencing of DYSF (Table III). All patients had reduced sarcomeral CD55 expression compared with normal skeletal muscle (Fig. 2, G and H). The degree of down-regulation of CD55 varied between patients, from trace staining to complete absence. Staining for CD46 and CD59, two other complement inhibitory molecules, was normal in all patients and controls (not shown).

The expression of DAF/CD55 in human skeletal muscle was also analyzed at the RNA level by TaqMan analysis. Compared with four control specimens from healthy individuals, DAF/CD55 mRNA in LGMD2B was 2.1-fold reduced (Fig. 1, E and F).

Dysferlin-deficient human myotubes are susceptible to complement attack

Functionally, the absence of CD55 should lead to an increased sensitivity against complement-mediated lysis. Human myotube cultures obtained from normal (n = 2) and dysferlin-deficient human skeletal muscle (n = 3; at least two independent experiments per patient) were established and exposed to complement-mediated lysis. Lysed and dead cells were identified by PI uptake. Normal human myotubes were resistant to complement-mediated lysis (Fig. 3, A and B). This effect could be partially inhibited by pre-incubation with anti-CD55 Ab (Fig. 3C). On the contrary, myoblasts and myotubes obtained from patients with dysferlin deficiency were highly susceptible to complement attack (Fig. 3, A and D). The percentage of lysed cells was not altered by the addition of anti-CD55 mAb (Fig. 3E).

In immunohistochemistry, the presence of the C5b9 MAC on the surface of nonnecrotic muscle fibers was demonstrated in four of four muscle specimens obtained from dysferlin-deficient patients (Fig. 3, F and G).

DAF/CD55, myostatin, and SMAD

To elucidate possible regulatory mechanisms of DAF/CD55 in dysferlin deficiency, we concluded that if DAF/CD55 down-regulation in dysferlin deficiency only plays a role in skeletal muscle, but not in heart, there should be genes that 1) are differentially expressed in dysferlin-deficient skeletal muscle and cardiac tissue and 2) regulate DAF/CD55. Indeed, within the microarray data obtained from dysferlin-deficient SJL/J mice, a small group of differentially expressed and potentially regulatory genes was identified: myostatin, SMAD2, SMAD3, SMAD4, cardiac ankyrin repeat protein (CARP), and early growth response 1 (EGR1). Therefore, the expression of these genes was quantified in skeletal and cardiac tissues by TaqMan RT-PCR in SJL/J mice and also in skeletal muscle from patients with dysferlin-deficient muscular dystrophy. In both mice and patients, compared with controls, myostatin, SMAD3, and SMAD4 were significantly down-regulated in skeletal muscle (Fig. 4A). In the heart, SMAD and myostatin were not differentially expressed in SJL/J and C57BL/6 mice (not shown). On the protein level, because of the availability of Abs, only phosphorylated SMAD2 was investigated, and it could be shown to also be markedly reduced in LGMD2B (Fig. 4B) compared with normal controls (Fig. 4C). CARP and EGR1 were strikingly up-regulated in skeletal muscle (Fig. 4A) and were reduced in heart (down-regulation of 2.5- and 4-fold in left and right ventricles, respectively).

To investigate whether any of these identified, differentially expressed, regulatory genes might influence DAF/CD55 expression, the DAF/CD55 promoter sequence (20) was analyzed for transcription factor binding sites using the MATInspector program (Genomatix) (21). This analysis revealed a binding site for the SMAD complex, GTCTggacct (22–24), indicating that SMAD might influence DAF/CD55 expression. Among the 291 differentially expressed genes in skeletal muscle, we could not identify other SMAD-regulated genes that are known to have significance for muscular dystrophies.

Discussion

The objective of our study was the investigation of differential pathways in cardiac and skeletal muscles that protect one tissue
and render the other susceptible to damage within a single organ-

ism. This approach might help to identify relevant pathways and
possible candidates for therapeutic intervention. Dysferlin-defi-
cient muscular dystrophy appeared to be a suitable model for this
approach, because dysferlin is expressed in skeletal and cardiac
muscles, but, clinically, the heart is thought to be unaffected in
LGMD2B (25, 26). We demonstrate down-regulation of DAF/CD55 on
mRNA and protein levels in dysferlin-deficient mice and
LGMD2B patients, leading to activation of the MAC of the com-
plement cascade on skeletal muscle cells. In vitro, dysferlin-defi-
cient human myotubes are highly susceptible to complement at-
tack, whereas normal human myoblasts/myotubes are not (19).
The underlying mechanism appears to be a lack of myostatin, lead-
ing to down-regulation of SMAD proteins, with a negative effect
on DAF/CD55 expression.

DAF/CD55 inhibits complement activation by interfering with
C3 and C5 in the classical and alternative pathway (27). It is absent
in paroxysmal nocturnal hemoglobinuria (28, 29), contributing to
an increased sensitivity of RBC and platelets to complement attack
(30). Transgenic expression of DAF/CD55 is used in xenotrans-
plantation to prevent complement-mediated hyperacute rejection
(31). Interestingly, DAF/CD55 also suppresses T cell immunity,
either locally or systemically (32). It is well known that some
muscular dystrophies, such as dysferlin-deficient muscular dystro-
phy, studied in this report, or facioscapulohumeral muscular dys-
trophy, might exhibit inflammatory changes in muscle that may
cause histological confusion with primary inflammatory muscle
diseases such as polymyositis (33–36). A local lack of DAF/CD55
may contribute to these changes.

Several possibilities exist about the mechanism of DAF/CD55
down-regulation in dysferlin deficiency. DAF/CD55 is a GPI-
anchored protein (37). The absence of sarcolemmal CD55/DAF
might therefore be secondary to GPI anchor deficiency. This is
well documented in paroxysmal nocturnal hemoglobinuria (38, 39)
and was also proposed in diabetic retinopathy in humans and rats
(40), but in dysferlin deficiency, down-regulation of DAF/CD55 in
skeletal muscle does not seem to be dependent on the GPI anchor-
ing of DAF protein; DAF/CD55 is reduced not only on the protein
level but also, strikingly, on the mRNA level. The fact that DAF/CD55
down-regulation could be demonstrated in SJL/J mice of all age groups between 12 and 34
wk excludes the possibility that this phenomenon is merely a con-
sequence of muscle degeneration and regeneration. Other muscular

---

**FIGURE 3.** Complement lysis assay and binding of C5b9-MAC to nonnecrotic muscle
cells. A, Quantification of PI uptake of myotubes
after exposure to complement (ratio of PI-posi-
tive cells after exposure to complement to Ver-
onal buffer control), n, number of wells counted.
Normal human (B and C) and dysferlin-deficient
(D and E) human myoblasts after exposure to comple-
ment (B and D) and after preincubation
with anti-CD55 Ab and subsequent exposure to
complement (C and E). F and G, Serial sections
of quadriceps muscle in LGMD2B (patient 1),
demonstrating dystrophic changes with increase
in connective tissue and pathological variation in
fiber size (Gomori-TriChrome stain). There was
sarcolemmal expression of C5b9-MAC on non-
necrotic muscle fibers. Staining was performed
with anti-C5b9 mAb and Cy3-labeled donkey
anti-mouse Ab.
dystrophies and inflammatory myopathies with prominent necrotic and regenerating changes do not display the C5b9 MAC on the surface of nonnecrotic muscle fibers (10).

The regulation of DAF/CD55 is not fully understood. Several cytokines, such as TNF-α (44, 45), IFN-γ (44), TGF-β (46), vascular endothelial growth factor (47) and basic fibroblast growth factor (48), induce DAF/CD55. Furthermore, CD55 is up-regulated by PGE2 on colon cancer cells (49). We therefore analyzed the promoter sequence of CD55 (20) for putative transcription factor binding sites that are differentially expressed in skeletal and cardiac muscles. Interestingly, the promoter sequence of human CD55 contains a SMAD-binding element. Phosphorylated SMAD2 and -3 proteins combine with SMAD4 and translocate into the nucleus as a result of activation of the activin II receptor by myostatin (22–24). In this investigation, SMAD2, -3, and -4 are shown to be markedly down-regulated in dysferlin deficiency, and the hypothetical influence on CD55 transcription is intriguing. Addi-
tional studies are in progress to identify the exact mechanism by which dysferlin, myostatin, SMAD, and CD55 interact.

Myostatin, or growth and differentiation factor 8, is a member of the TGF-β superfamily (50) and a negative regulator of muscle mass (reviewed in Ref. 51). Its effect on muscle cells includes induction of cell arrest (52, 53) and inhibition of differentiation of muscle progenitors into myotubes (54). It is not resolved whether myostatin predominantly acts as an autocrine or a paracrine factor. In myogenic cell lines, myostatin appears to function in an autocrine fashion (55). The role of endogenous myostatin in muscular dystrophies has not been investigated. However, mutations in the gene encoding myostatin lead to a marked increase in muscle mass (50, 56), demonstrated in myostatin knockout mice, in cattle, and in a single pediatric patient (57–59). The muscle hypertrophy has raised some hope about the possible suitability of myostatin as a therapeutic target in muscular dystrophies (60).

This study identifies down-regulation of CD55 and subsequent deposition of MAC on nonnecrotic muscle cells as functionally relevant factors of muscle cell injury in dysferlin deficiency. It is tempting to speculate on possible therapeutic options for this to date untreatable disorder. For example, eculizumab, a humanized chimeric Ab against C5, was found to be effective in paroxysmal nocturnal hemoglobinuria (61). More unspecifically, i.v. Igs have been shown to interfere with complement factors C3a and C5a (62, 63) and might inhibit complement activation on muscle cells in dysferlin deficiency.

Disclosures
The authors have no financial conflict of interest.