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Human Septic Myopathy: Induction of Cyclooxygenase, Heme Oxygenase and Activation of the Ubiquitin Proteolytic Pathway

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Background: **Skeletal muscle failure and wasting are manifestations of sepsis in humans that leads to serious and prolonged complications. The authors investigated the role of the major proinflammatory and antiinflammatory pathways, namely the inducible isoforms cyclooxygenase (COX-2) and heme oxygenase (HO-1), and the ubiquitin proteolytic pathway in skeletal muscle of septic patients.**

Methods: **Protein expression was detected by Western blot techniques. Muscle biopsies were taken from two muscle groups, rectus abdominis and vastus lateralis, of septic and control patients.**

Results: **The study showed an increase in COX-2 and HO-1 proteins expression and an activation of the proteolytic ubiquitin pathway with a parallel increase in free ubiquitin and ubiquitinated proteins in skeletal muscle of septic but not of control patients. In addition, those patients who would die from septic shock expressed more COX-2 and HO-1 proteins in muscle biopsies than did those patients who would survive.**

Conclusions: **This study showed a marked involvement of local proinflammatory and antiinflammatory pathways and, more importantly, demonstrated the existence of an active ubiquitin proteolytic pathway in skeletal muscle of septic patients. Activation of ubiquitin pathway could be involved in sepsis-related muscle catabolism and wasting.**

SEPSIS is the systemic inflammatory response associated with an infectious insult. Despite improved therapy and better understanding of the mechanisms underlying the pathogenesis of sepsis, the incidence of vital organ failure and the consequent mortality rate remain high. Sepsis induces severe and persistent alterations in skeletal muscle. There are characterized by both muscle wasting and a prolonged decrease in muscular force that may last more than a year.[1–6] Muscle dysfunction, which occurs in 40–70% of septic patients,[7,8] may have a negative clinical impact by compromising recovery. Indeed, sepsis-related respiratory muscle failure delayed weaning from mechanical ventilation[9] and prolonged intensive care unit and hospital stay[6] by predisposing to complications such as pulmonary infection and thromboembolic disease. Pathophysiology underlying sepsis-induced muscular failure is still poorly understood.[10] Several proinflammatory pathways are implicated, including proinflammatory cytokines,[11] cyclooxygenases,[12] reactive oxygen species,[13] and the nitric oxide pathways, which are primarily described in animal models.[14,15] Although increased peroxynitrite production related to the overexpression of inducible nitric oxide synthase was recently associated with skeletal muscle dysfunction in septic patients,[5] the role of other major proinflammatory pathways such as the inducible isoform of cyclooxygenase (COX-2)[16] and antiinflammatory pathways, mostly heme oxygenase (HO), remains to be explored in humans.

We recently showed that peroxynitrite-induced protein nitration may lead to protein inactivation in the rectus abdominis of septic patients.[17] Although protein nitration in combination with impaired mitochondrial function[18] may explain the reduction in muscular force, it does not completely explain the frequently seen muscle wasting. The latter may be related to degradation of contractile proteins and then stimulation of catabolic pathways, such as the ubiquitin pathway[19,20] (additional information regarding this is available on the ANESTHESIOLOGY website at http://www.anesthesiology.org). Indeed, among cellular proteolytic pathways, the ubiquitin-proteasome pathway is specifically implicated in the breakdown of abnormal proteins, including peroxynitrite-induced nitrated proteins.[21,22]

Accordingly, the aim of the current study was to investigate cellular expression of constitutive and inducible isoforms of COX and HO and, more importantly, the ubiquitin proteolytic pathway in skeletal muscle biopsies taken from both septic and control patients.

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Materials and Methods

Patients

Two groups of patients were included in the study. One group (table 1) (n = 24, septic group) consisted of patients with sepsis as defined by Bone *et al.*[23] and at

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Table 1. Characteristics of Septic Patients

	Gender	Age (yr)	Site of muscle biopsy	Cause of infection	Bacteria	SAPS II	LOD Score	Length of immobilization before biopsy (days)	Outcome (length of stay in ICU) (days)
1	M	22	Rectus abdominis	Mediastinitis	*Staphylococcus epidermidis, Lactobacillus paracasei*	37	5	0	Alive (22)
2	M	60	Rectus abdominis	Mediastinitis	*Staphylococcus aureus*	23	2	0	Alive (3)
3	M	57	Rectus abdominis	Mediastinitis	*Staphylococcus aureus*	56	9	0	Alive (27)
4	M	41	Rectus abdominis	Mediastinitis	*Streptococcus anginosus, Bacteroides capillosus*	58	8	0	Alive (30)
5	M	62	Rectus abdominis	Mediastinitis	unknown	72	12	6	Alive (25)
6	F	79	Rectus abdominis	Peritonitis	*Escherichia coli, Bacteroides fragilis*	84	11	5	Alive (98)
7	M	66	Rectus abdominis	Colitis	unknown	71	12	0	Alive (18)
8	F	86	Rectus abdominis	Peritonitis	*Escherichia coli, Pseudomonas aeruginosa Streptococcus*	71	11	1	Died (2)
9	M	55	Rectus abdominis	Peritonitis	unknown	30	1	0	Alive (5)
10	F	75	Rectus abdominis	Peritonitis	*Morganella morganii*	49	4	10	Alive (3)
11	M	82	Rectus abdominis	Peritonitis	*Enterobacter cloacae*	66	4	1	Alive (7)
12	F	84	Rectus abdominis	Peritonitis	*Enterobacter cloacae, Clostridium perfringens*	66	4	15	Died (41)
13	F	42	Rectus abdominis	Peritonitis	*Enterobacter cloacae, Proteus*	37	3	0	Alive (56)
14	F	78	Rectus abdominis	Urinary tract infection	*Escherichia coli, Staphylococcus aureus*	65	7	18	Died (6)
15	M	87	Rectus abdominis	Central venous catheter infection	*Escherichia coli,*	44	6	5	Died (12)
16	F	62	Rectus abdominis	Meningitidis	*Staphylococcus aureus*	83	10	5	Died (4)
17	M	77	Vastus lateralis	Urinary tract infection	*Escherichia coli*	54	7	1	Died (55)
18	M	68	Vastus lateralis	Peritonitis	unknown	97	8	0	Died (3)
19	M	72	Vastus lateralis	Peritonitis	unknown	45	9	0	Died (1)
20	F	83	Vastus lateralis	Peritonitis	*Streptococcus pneumoniae*	53	6	0	Died (8)
21	F	74	Vastus lateralis	Gangrenous gall bladder	*Escherichia coli*	65	8	0	Died (4)
22	M	45	Vastus lateralis	Pneumonia	unknown	45	8	14	Died (3)
23	M	39	Vastus lateralis	Meningococcal sepsis	*Streptococcus meningitidis*	67	9	0	Alive (9)
24	F	31	Vastus lateralis	Necrotizing Fasciitis	*Streptococcus*	51	10	0	Alive (40)

Patients with rectus abdominis (n = 16) and vastus lateralis (n = 8) biopsy showed no differences in age, Simplified Acute Physiology Score II (SAPS II) and Logistic Organ Dysfunction (LOD) score. Median/mean values for all 24 septic patients are presented in the results section.

ICU = intensive care unit.

least one of the following signs of organ dysfunction: systolic blood pressure <90 mm Hg despite adequate fluid replacement or need for vasopressors to maintain blood pressure; oliguria (urine output $<0.5\ ml \cdot kg^{-1} \cdot h^{-1}$); metabolic acidosis (pH <7.3 or lactate >2 mmol/l); Pao_2/Fio_2 ratio <200 mm Hg; platelet count <100,000/mm^3; or altered mental status. The other group (n = 10, control group) consisted of patients undergoing elective laparotomy or hip replacement for nonseptic conditions. Patients with cancer, chronic inflammatory processes, chronic heart failure or patient receiving glucocorticoids drugs were excluded from study as these may alter expression of nitric oxide synthase, COX, and HO proteins.[24,25] The duration of immobilization was defined as the time during which patients were kept in bed until the day of biopsy.

To assess the severity of the clinical conditions, the Simplified Acute Physiology Score II[26] and the Logistic Organ Dysfunction Score[27,28] were calculated for each patient at the time of biopsy. In living patients, muscle weakness was evaluated when the patient recovered from sepsis and was defined as decrease of muscular force.

The study was performed with the approval of the local boards governing research on human subjects at

our institution (Comité Consultatif pour la Protection des Personnes dans la Recherche Biomédicale, Hôpital Saint-Louis, Paris, France, and Ethics Committee, University College of London Hospitals, London, UK). All patients or theirs relatives gave informed written consent.

Muscle Biopsies and Preparations:

A biopsy specimen was obtained from either the rectus abdominis or vastus lateralis muscle within 24 h after the onset of sepsis and mechanical ventilation. The former was obtained during the initial phase of the laparotomy: after skin incision and dissection through the subcutaneous fat, the anterior sheet of the rectus muscle was opened with scissors and a biopsy specimen obtained. Sampling was not performed if the muscle was in direct contact with the site of infection, thus avoiding tissue where protein expression may be directly affected by local inflammation. Biopsies from vastus lateralis were taken percutaneously from septic patients in the intensive care unit, using Henckel Tilley forceps *via* a small incision through the skin with blunt dissection through subcutaneous tissue and muscle capsule.[18] For control group, the biopsies were obtained during the initial phase of either laparotomy (rectus abdominis) or hip surgery (vastus lateralis). Both control and septic patients received paralytic agents only during surgery.

Tissue samples were immediately frozen into liquid nitrogen and stored at −80°C for <6 months before subsequent measurement of protein expression. The various assays and the reading of immunostained biopsies were performed in a blinded fashion.

COX and HO Detection in Muscle

Detection of Constitutive and Inducible Isoforms of COX (Respectively COX-1 and COX-2) Protein by Western Blot. Tissue samples were homogenized in a Triton lysis buffer. Proteins in the tissue homogenates were separated by electrophoresis on 7.5% sodium dodecyl sulfate polyacrylamide gel in a electrophoretic buffer. They were transferred overnight at 4°C to a nitrocellulose membrane (Bio-Rad Laboratories, Marnes la Coquette, France). Membranes were first blocked at room temperature with 5% nonfat dry milk, bovine serum albumin 1% in a Tris-buffered saline Tween; they were then incubated with a COX-1 monoclonal antibody (Oxford Biomedical Research/Euromedex, Mundolsheim, France) at a 1:1000 dilution for COX-1 protein and with a COX-2 monoclonal antibody (Oxford Biomedical Research/Euromedex) at a 1:200 dilution for COX-2. A lysate of human umbilical vascular endothelial cells stimulated with 4-Phorbol-12-Myristate-13-Acetate was used as a positive control for detection of COX-2 protein. Antibody detection was performed with a chemiluminescence substrate using the Amersham ECL kit (Amersham Biosciences, Freiburg Germany). After detection, membranes were stained with Red Ponceau solution and the total amount of proteins was assessed. Protein expression was analyzed with a Las 1000-plus system (Raytest, Fugifilm, St Quentin en Yvelines, France) and protein quantification was obtained with Image Gauge Ver 3,4 software (Fugifilm).

Detection of COX-1 and COX-2 by Immunohistochemistry. Muscle samples obtained from rectus abdominis were frozen quickly in liquid nitrogen and embedded in paraffin. Five-micron sections were mounted on poly-L-lysine-coated microscope slides. The sections were rinsed in phosphate-buffered saline and incubated in blocking buffer. Slides were then incubated with a COX-2 rabbit polyclonal antibody (L320 R6, kindly donated by J. Maclouf, Ph.D. and H. Habib, Ph.D., Inserm U.348, Hopital Lariboisière, Paris, France) and a COX-1 rabbit polyclonal antibody (L6 R3, donated by J. Maclouf and H. Habib) at the antibody concentration of 1:100 for both proteins. After washing in phosphate-buffered saline, slides were exposed to a biotinylated antirabbit 5% human serum (BioRad) diluted to 1:100. Localization of COX-1 and COX-2 was revealed with streptavidin fluoresceine rabbit antibody (BioRad) at 1:50 dilution. The fluorescence of the slides was examined by a microscope Leica. Specificity of immunostaining was evaluated by omission of the primary antibody and by using pooled nonimmune antirabbit immunoglobulin G instead of the primary antibody at equivalent concentration, and processed as above.

Detection of HO-1 and HO-2 Protein by Western Blot. Proteins were separated by electrophoresis on 12% sodium dodecyl sulfate polyacrylamide gel. The membranes were incubated with a mouse HO-1 monoclonal antibody (Stressgen/Tebu SA, Le Perray en Yvelines, France) used at a 1:2000 dilution for HO-1 protein and with a rabbit HO-2 polyclonal antibody (Stressgen/Tebu SA) used at a 1:1000 dilution for HO-2. HO-1 peptide SPT-896 (Stressgen/Tebu SA) was used as a positive control for HO-1.

Detection of Ubiquitin Activity

Ubiquitin activity was performed by Western blot in tissue samples homogenized in sodium dodecyl sulfate instead of Triton. Proteins were separated by electrophoresis on 12% sodium dodecyl sulfate polyacrylamide gel and membranes were incubated with a mouse ubiquitin monoclonal antibody (Santa-Cruz Biotechnologies/Tebu SA) used at a 1:100 dilution. This allowed quantification and comparison of the expression of free ubiquitin protein in tissue samples of control and septic patients. The positive control of free ubiquitin protein was full length ubiquitin of human origin produced in *Escherichia Coli* as 35 kD when tagged to its fusion protein (Santa Cruz Biotechnology/Tebu SA).

When ubiquitin is active, it binds to altered proteins to initiate their breakdown. To evaluate the activity of ubiquitin pathway in our samples, we quantified the concen-

tration of ubiquitinated proteins (as previously described)[29,30] by Western blot. Values were standardized with the Ponceau S intensity.

Materials and Reagents

Sodium dodecyl sulfate, glycerol, 2-mercaptoethanol, bromophenol blue, and the electrophoretic system were obtained from Bio-Rad (Marnes la Coquette, France). Chemiluminescence substrates were obtained from Amersham Biosciences (Freiburg, Germany). All other chemicals were purchased from Sigma-Aldrich (St Quentin Fallavier, France).

Statistical Analysis

Values are given as median (IQR) except age, Simplified Acute Physiology Score II, and Logistic Organ Dysfunction Score (mean ± SD). All results obtained from rectus abdominis and vastus lateralis biopsies were similar in control patients and are thus pooled. Differences were analyzed by the Mann-Whitney *U*-test. The relation between free ubiquitin protein expression and ubiquitinated proteins expression was assessed by linear regression analysis. Statistical significance was accepted at $P < 0.05$.

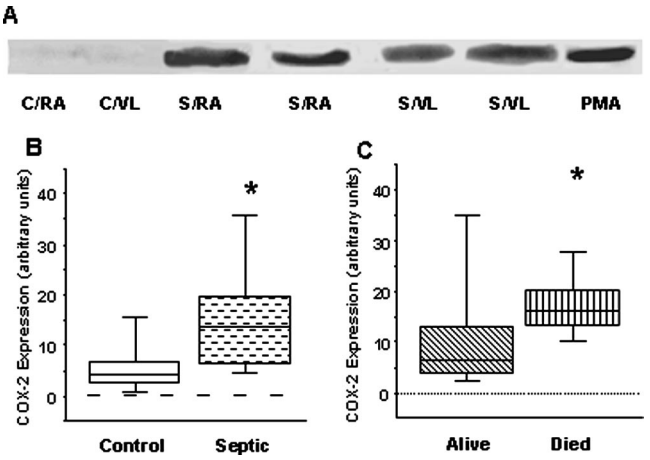

Fig. 1. (*A*) Representative Western blot analysis of COX-2 protein from respectively rectus abdominis muscle and vastus lateralis muscle in control patients (C/RA and C/VL, respectively) and in septic patients (S/RA and S/LT, respectively). A main band with an estimated molecular weight of 72 kD corresponding to COX-2 was present in samples from both S/RA and S/VL and human umbilical vascular endothelial cells stimulated by 4-Phorbol-12-Myristate-13-Acetate (PMA). (*B*) Muscle COX-2 expression in control (combined C/RA and C/VL, n = 10) and septic (S/RA [n = 16] and S/LT [n = 8]) patients; *$P < 0.02$. (*C*) Muscle COX-2 expression in patients who will survive and who will die; *$P < 0.006$.

Results

Characteristics of the septic patients are given in table 1. Age was similar between the control (n = 10) and septic (n = 24) groups (mean ± SD, 54 ± 12 yr *versus* 64 ± 19 yr, respectively $P = 0.13$). Organ injury was reflected by a higher Simplified Acute Physiology Score II in septic compared with control patients (59 ± 18 *versus* 11 ± 4, $P < 0.0001$), by a high Logistic Organ Dysfunction Score (7 ± 3), and by a prolonged duration of ventilatory support (13 ± 14 days) in the septic group. Eleven patients died within 14 days in septic group; none died in the control group. The median value of the length of immobilization in septic patients was 0 days (extreme values, 0–18 days). Four of the 13 septic patients who survived developed muscle weakness.

Detection of COX and HO Proteins

COX Proteins. Muscular homogenates expressed more COX-2 protein, the inducible isoform of COX, as detected by Western blot, in septic compared with control patients (fig. 1A). Quantification of COX-2 protein expression confirmed the Western blot results and showed a median value of 13.4 (3.5–39.8) arbitrary units in septic patients *versus* 4.0 (0–17.7) arbitrary units in control patients ($P < 0.005$ *versus* control, fig. 1B). Figure 1C also shows that expression of COX-2 in muscle is higher in patients that will die (16.2 [6.1–36.1] arbitrary units) *versus* those who will survive (6.3 [0.0–39.8] arbitrary units, $P < 0.006$). No difference in COX-1 expression was observed between septic and control groups; the median values were 20.8 (0–63.0) arbitrary units and 20.0 (9.3–48.8) arbitrary units, respectively ($P = 0.44$).

Histologic analysis showed no inflammatory infiltrate and an absence of tissue edema in the muscles of both septic and control patients (fig. 2). It also showed increased COX-2 staining, reproducibly observed in the sarcolemma of skeletal muscle myocytes of all septic patients expressing COX-2 protein as shown by Western blot. By contrast, a very low level of COX-2 staining was found in control patients. Figure 2 also shows similar COX-1 staining in the control and septic patients, mostly localized to the sarcolemma.

HO Proteins. Western Blot analysis (fig. 3A) revealed that the expression of inducible HO-1 protein was greater in muscle homogenates taken from septic patients compared with that taken from control patients. This was confirmed by quantification that showed a median value of HO-1 expression of 13.4 (0–60.0) arbitrary units in rectus abdominis muscle of septic patients *versus* 0.4 (0–18.0) arbitrary units in control patients ($P < 0.01$, fig. 3B). Figure 3C also shows that expression of HO-1 in muscle is higher in patients that will die (22.4 [8.7–60.0] arbitrary units) *versus* those who will survive (3.9 [0.0–19.9] arbitrary units) ($P < 0.006$). Patients who did not develop muscle weakness tended to express more HO-1 than patients who develop muscle weakness: 12.0 (1.0–1.8) arbitrary units and 1.1 (0–2.2) arbitrary units ($P = 0.07$), respectively. No difference in

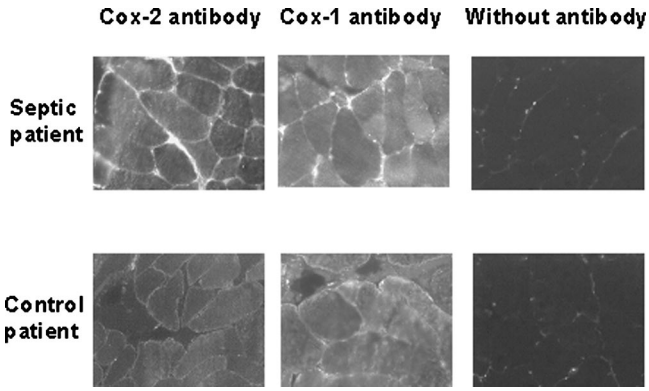

Fig. 2. Photomicrographs illustrating the use of immunochemistry to identify the presence and localization of COX-2 and COX-1 protein in control and septic muscle. Increased COX-2 staining was reproducibly observed in skeletal muscle myocytes of all septic patients expressing COX-2 protein in Western blots. No COX-2 staining was found in control patients. A similar degree of COX-1 staining was present in control and septic patients. When present, both COX-2 and COX-1 proteins were mostly localized to the cytoplasmic membrane of studied myocytes. COX-1 or COX-2 staining was not observed when the primary antibody was omitted. No tissue section showed positive immunostaining with nonimmune serum (data not shown).

HO-2 expression was detected between control and septic groups (data not shown).

Ubiquitin Proteolytic Activity

Higher concentrations of both free ubiquitin and ubiquitinated proteins were detected in muscle homogenates of septic patients compared with control patients (figs. 4 and 5). Quantification of free ubiquitin protein

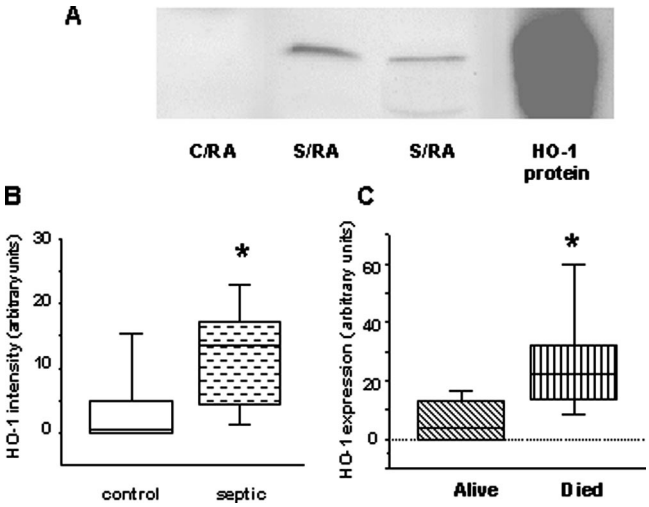

Fig. 3. (*A*) Representative Western blot analysis of HO1 protein from rectus abdominis muscle in control (C/RA) and septic (S/RA) patients. A main band with an estimated molecular weight of 32 kD corresponding to HO-1 was present in muscles from septic patients and in HO-1 peptide SPT-896 (Stressgen/Tebu SA) (used as positive control). (*B*) Muscle HO-1 expression in control (n = 10) and septic patients (S/RA; n = 16); * $P < 0.01$. (*C*) Muscle HO-1 expression in patients who will survive and in patients who will die;* $P < 0.006$.

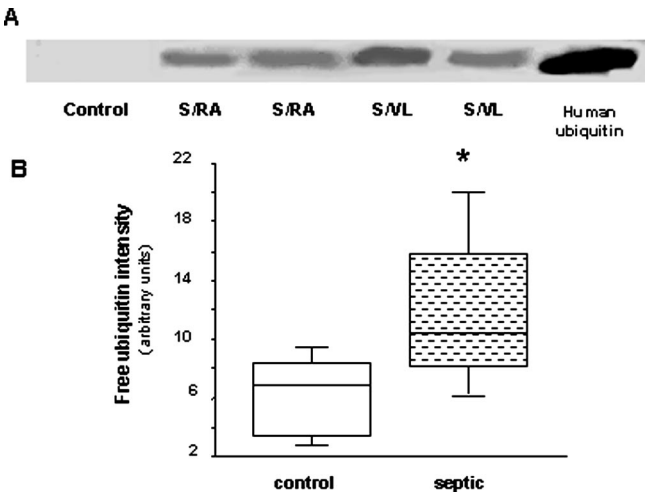

Fig. 4. (*A*) Representative Western blot analysis of free ubiquitin protein from rectus abdominis (S/RA) and vastus lateralis (S/VT) muscle in septic patients and in control patients (control). A main band was present in samples from S/RA and S/VT. (*B*) Muscle free ubiquitin expression quantified in control (n = 6) and septic (n = 16) patients. Because no difference was observed between RA and VT, these data were combined in both control and septic patients. * $P < 0.02$.

expression confirmed Western blot results and showed a median value of 10.5 (6.1–20.6) arbitrary units in septic patients *versus* 6.9 (2.7–9.7) arbitrary units in muscle biopsies of control patients ($P < 0.02$, fig. 4B). This supports a higher ubiquitin proteolytic activity that affected several proteins in human sepsis. Indeed, figure 5A shows that ubiquitinated proteins are more marked in septic than in control patients with several bands

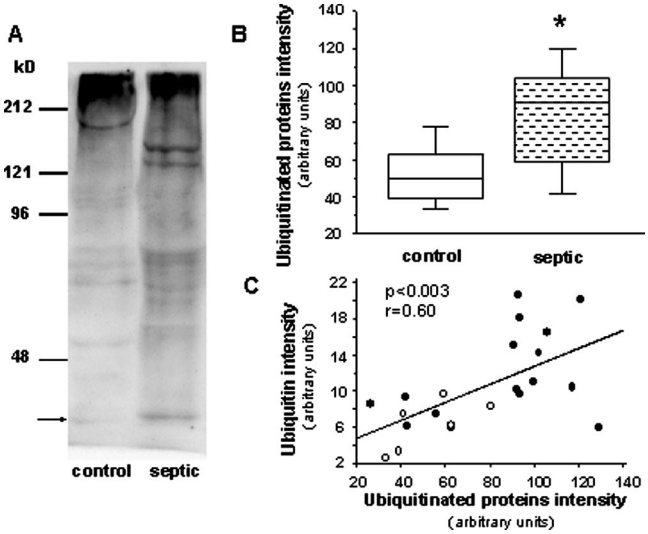

Fig. 5. (*A*) Western blot analysis of rectus abdominis muscle ubiquitinated proteins from one control and one septic patient. Positions of molecular weights are indicated on the left side of the gel. The arrow shows free ubiquitin. (*B*) Muscle ubiquitinated protein expression quantified in control (n = 6) and in septic (n = 16) patients; * $P < 0.02$. (*C*) Relationship between the intensity of muscular ubiquitin expression and the intensity of ubiquitinated proteins in septic (●) and control (○) patients, r = 0.60, $P < 0.003$.

having molecular mass of 160, 130, and 60 kD, which were consistently detected in septic patients but lacking in control patients. By contrast, one ubiquitinated protein with an apparent molecular mass of 180 kD was present in control patients but was not found in septic patients. No difference in ubiquitin activity was detected between muscle homogenates from either thigh or abdominal muscles (data not shown). Quantification of all muscle ubiquitinated proteins showed that the concentration was greater in septic compared with control patients: 92.8 (26.5–128.9) arbitrary units *versus* 49.8 (32.8–79.9) arbitrary units, respectively ($P < 0.02$, fig. 5B) and that the concentration of ubiquitinated proteins paralleled the expression of ubiquitin protein ($r = 0.60$, $P < 0.003$) (fig. 5C).

Expression of free ubiquitin and ubiquitinated proteins were similar between patients immobilized >24 h compared to those <24 h (data not shown) and between patients that died <14 days *versus* patients that survived.

Discussion

The current study shows the overexpression of two other major inducible enzymes involved in the inflammatory cascade (the proinflammatory COX-2 and the antiinflammatory HO-1) and stimulation of an active proteolytic ubiquitin-proteasome pathway, in anatomically different muscles of septic patients.

The immune reaction to microbial invasion is increasingly seen as a local proinflammatory response limited by protective systemic antiinflammatory responses.[31,32] Sepsis-induced nitric oxide synthase overexpression has been repeatedly described as a major player in the proinflammatory reaction to microbial invasion in mammals. Indeed, we and others have previously identified overexpression and increased activity of nitric oxide synthase in septic patients in various tissues including vascular wall,[33] left ventricular wall,[34] and skeletal muscle.[5] The current study further shows overexpression of another highly potent proinflammatory protein, COX-2, in muscle biopsies of rectus abdominis and vastus lateralis of septic, but not control, patients. COX-2 was mostly located in sarcolemma. COX-2 end products have been implicated in the skeletal muscle[12,35–37] and myocardial contractile failure in septic animals.[38] Accordingly, our findings strongly suggest a role of COX-2 in human sepsis although its specific effects on the inflammatory cascade and skeletal muscle contractile dysfunction remain to be explored.

In addition to these proinflammatory pathways, we also demonstrate the existence of a tissue-based antiinflammatory pathway, namely overexpression of HO-1, in our septic patients. Skeletal muscle overexpression of HO-1 that is accompanied by increased HO activity has been shown to play a protective role in the muscle of lipopolysaccharide-treated rats.[13] Our study suggests that HO-1 could have a protective role in skeletal muscle taken from septic patients because HO-1 expression tended to be greater in patients who did not develop muscle weakness. Although the exact role of HO-1 remains to be explored, one may suggest that it limits the local consequences of the proinflammatory effects of nitric oxide synthase and COX-2, including peroxynitrite production and protein nitration/inactivation.

We recently showed that muscle failure is at least partly related to nitric oxide synthase–induced peroxynitrite production in septic patients.[5] Peroxynitrite, a powerful oxidant, nitrates tyrosine residues into 3-nitrotyrosine, which likely alters protein function.[17] It is, however, unknown whether those nitrated proteins are degraded and what pathway is involved in protein breakdown in sepsis. More globally, mechanisms of sepsis-related muscle wasting are unknown[10] and might be related to a stimulation of catabolic pathways. Of these pathways, the ubiquitin-proteasome pathway[39] was described to be more specifically implicated in the breakdown of abnormal proteins, including peroxynitrite-induced nitrated proteins.[21,22] Indeed SIN-1, a peroxynitrite donor, has been shown to stimulate the proteasome pathway, leading to an increased rate of protein degradation, including myosin degradation.[21] Furthermore, inhibition of the proteasome pathway leads to an increased cellular content of 3-nitrotyrosine and ubiquitinated proteins.[40] In septic mammals, ubiquitin-proteasome activity is increased in rat skeletal muscle,[19,41,42] whereas both ubiquitin mRNA and myofibrillar proteolysis are increased in septic patients.[20] Our current study extends these previous studies by showing, for the first time, increased expression of an active ubiquitin protein in the skeletal muscles of septic patients. Indeed, our study shows a parallel increase in free ubiquitin expression and in ubiquitinated proteins in various muscles of septic compared with control patients. This suggests that the newly expressed ubiquitin protein binds to various "abnormal" proteins with different molecular mass (the ubiquitinated proteins) to activate the proteasome proteolytic pathway leading to muscle wasting. Detection of ubiquitinated proteins suggests that ubiquitin-conjugating enzymes and ligases[42–44] are also active in human septic muscle. Detection of ubiquitinated proteins could indicate an accumulation of proteins, suggesting an inhibition of proteasome activity. Studies have shown that expression of proteasome components are increased and that its proteolytic activity is also increased during sepsis in muscle.[41] We found that expression of free ubiquitin and ubiquitinated proteins, in the early phase of sepsis, was similar between patients who develop muscle weakness and patients who do not. Although we did not measure a marker of myofibrillar catabolism, this suggests that muscle catabolism is one of the pathways leading to muscle weakness in septic patients.

Others factors, such as immobilization, are known to

be involved in the stimulation of the ubiquitin proteolytic pathway.[41] Nevertheless, in our study, this factor does not seem to influence the ubiquitin pathway because no difference in the expression of free ubiquitin and ubiquitinated proteins was found between immobilized and nonimmobilized septic patients.

Proinflammatory pathway activation and its consequences on muscle function have been primarily studied in the rectus abdominis in septic patients.[5,16] However, these biopsies, taken from patients with peritonitis or postcardiac surgical mediastinitis, may have been contaminated by the infected area. The current study extends previous work by showing that COX-2, HO-1, and ubiquitin activity are similarly overexpressed in rectus abdominis and in muscles distant from the infected area, namely the vastus lateralis. Accordingly, our data suggest that the protein overexpression and contractile failure previously observed in abdominal wall muscle[5] is a generalized phenomenon in septic patients and is related to circulating diffusible factors rather than local factors.

In summary, our study demonstrated a marked involvement of local proinflammatory and antiinflammatory pathways and of an active ubiquitin proteolytic pathway in skeletal muscle of septic patients. Involvement of ubiquitin pathway in other sepsis-induced organ dysfunction, including myocardial dysfunction, remains to be investigated in septic patients.

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References

1. Orellana R, O'Connor P, Nguyen H, Bush J, Suryawan A, Thivierge M, Fiorotto M, Davis T: Endotoxemia reduces skeletal muscle protein synthesis in neonates. Am J Physiol Endocrinol Metab 2002; 283:E909–16
2. Burke JF, Pontopiddan H, Welch CE: High output respiratory failure: An important cause of death ascribed to peritonitis or ileus. Ann Surg 1963: 581–94
3. Cohen CA, Zagelbaum G, Gross D, Roussos C, Macklem PT: Clinical manifestations of inspiratory muscle fatigue. Am J Med 1982; 73:308–16
4. Coakley JH, Nagendrau K, Hanavar M, Hinds CJ: Preliminary observations on the neuromuscular abnormalities in patients with organ failure and sepsis. Intensive Care Med 1993; 19:323–8
5. Lanone S, Mebazaa A, Heymes C, Danialou G, Henin D, Pederoso JJ, Panis Y, Zedda C, Billiar T, Payen D, Aubier M, Boczkwoski J: Muscular contractile failure in septic patients: Role of the inducible nitric oxide synthase pathway. Am J Respir Crit Care Med 2000; 162:2308–15
6. Herridge M, Cheung A, Tansey C, Matte-Martyn A, Diaz-Granados N, Al-Saidi F, Cooper A, Guest C, Mazer C, Mehta S, Stewart T, Barr A, Cook D, Slutsky A: One-year outcomes in survivors of the acute respiratory distress syndrome. N Engl J Med 2003; 348:683–93
7. Tennila A, Salmi T, Pettila V, Roine RO, Varpula T, Takkunen O: Early signs of critical illness of polyneuropathy in ICU patients with systemic inflammatory response syndrome or sepsis. Crit Care Med 2000; 26:1360–3
8. Bolton CF: Evidence of neuromuscular dysfunction in the early stage of the systemic inflammatory response. Intensive Care Med 2000; 26:1179–80
9. Maher J, Rutledge F, Remtulla H, Parkes A, Bernardi L, Bolton CF: Neuromuscular disorders associated with failure to wean from the ventilator. Intensive Care Med 1995; 21:737–43
10. Hudson LD, Lee CM: Neuromuscular sequelae of critical illness. N Engl J Med 2003; 348:745–7
11. Wilcox PG, Wakai Y, Walley KR, Cooper DJ, Road J: Tumor necrosis factor alpha decreases in vivo diaphragm contractility in dogs. Am J Respir Crit Care Med 1994; 150:1368–73
12. Boczkwoski J, Dureuil B, Pariente R, Aubier M: Preventive effects of indomethacin on diaphragmatic contractile alterations in endotoxemic rats. Am Rev Respir Dis 1990; 142:193–8
13. Taillé C, Foresti R, Lanone S, Zedda C, Green C, Aubier M, Motterlini R, Boczkwoski J: Protective role of heme oxygenases against endotoxin-induced diaphragmatic dysfunction in rats. Am J Respir Crit Care Med 2001; 163:753–61
14. Boczkwoski J, Lanone S, Ungureanu-Longrois D, Danialou G, Fournier T, Aubier M: Induction of diaphragmatic nitric oxide synthase after endotoxin administration in rats. J Clin Invest 1996; 98:1550–9
15. El Dwairi Q, Comtois A, Guo Y, Hussain SN: Endotoxin-induced skeletal muscle contractile dysfunction: Contribution of nitric oxide synthases. Am J Physiol 1998; 274:C770–9
16. Ejima K, Layne MD, Carvajal IM, Kritek PA, Baron RM, Chen YH, vom Saal J, Levy BD, Yet SF, Perrella MA: Cyclooxygenase-2 deficient mice are resistant to endotoxin-induced inflammation and death. FASEB J 2003; 17:1325–7
17. Lanone S, Manivet P, Callebert J, Launay J, Payen D, Aubier M, Boczkwoski J, Mebazaa A: Inductible nitric oxide synthase (NOS2) expressed in septic patients is nitrated on selected tyrosine residues: implications for enzymic activity. Biochem J 2002; 366:399–404
18. Brealey D, Brand M, Hargreaves I, Heales S, Land J, Smolenski R, Davies N, Cooper C, Singer M: Association between mitochondrial dysfunction and severity and outcome of septic shock. Lancet 2002; 360:219–23
19. Tiao G, Fagan JM, Samuels N, James H, Hudson K, Lieberman M, Fisher JE, Hasselgren PO: Sepsis stimulates nonlysosomal, energy-dependent proteolysis and increases ubiquitin mRNA levels in rat skeletal muscle. J Clin Invest 1994; 94:2255–64
20. Tiao G, Hobler S, Wang JJ, Meyer TA, Luchette FA, Fisher JE Hasselgren PO: Sepsis is associated with increased mRNAs of the ubiquitin-proteasome proteolytic pathway in human skeletal muscle. J Clin Invest 1997; 99:163–8
21. Grune T, Blasig IE, Sitte N, Roloff B, Haseloff R, Davies KJ: Peroxynitrite increases the degradation of aconitase and other cellular proteins by proteasome. J Biol Chem 1998; 273:10857–62
22. Souza JM, Choi I, Chen Q, Weisse M, Daikhin E, Yudkoff M, Obin M, Ara J, Horwitz J, Ischiropoulos H: Proteolytic degradation of tyrosine nitrated proteins. Arch Biochem Biophys 2000; 380:360–6
23. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knauss WA, Schein RMH, Sibbald WJ: Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Chest 1992; 101:1644–55
24. Weber CM, Eke BC, Maines MD: Corticosterone regulates heme oxygenase-2 and NO synthase transcription and protein expression in rat brain. J Neurochem 1994; 63:953–62
25. Rouet-Benzineb P, Eddahibi S, Raffestin B, Laplace M, Depend S, Adnot S, Crozatier B: Induction of cardiac nitric oxide synthase 2 in rats exposed to chronic hypoxia. J Moll Cell Cardiol 1999; 31:1697–708
26. Le Gall JR, Lemeshow S, Saulnier F: A new simplified acute physiology score (SAPS II) based on the European /North American multicenter trial study. JAMA 1993; 270:2957–63
27. Le Gall JR, Klar J, Lemeshow S, Saulnier F, Alberti C, Artigas A, Teres D: The Logistic Organ Dysfunction System: A new way to assess organ dysfunction in the intensive care unit. ICU Scoring Group. JAMA 1996; 276:802–10
28. Meitnitz PG, Lary T, Valentin A, Steltzer H, Krenn GG, Le Gall JR: Evaluation of the logistic organ dysfunction system for the assessment of organ dysfunction and mortality in critically ill patients. Intensive Care Med 2001; 6:992–8
29. Tiao G, Fagan J, Roegner V, Lieberman M, Wang JJ, Fischer JE, Hasselgren PO: Energy-ubiquitin-dependent muscle proteolysis during sepsis in rats is regulated by glucocorticoids. J Clin Invest 1996; 97:339–48
30. Sultan KR, Dittrich BT, Leisner E, Paul N, Pette D: Fiber type-specific expression of major proteolytic systems in fast- to slow-transforming rabbit muscle. Am J Physiol Cell Physiol 2001; 280:C239–47
31. Munford RS, Pugin J: The crucial role of systemic responses in the innate (non-adaptative) host response. J Endotoxin Res 2001; 7:327–32
32. Munford RS, Pugin J: Normal responses to injury prevent systemic inflammation and can be immunosuppressive. Am J Resp Crit Care Med 2001; 163: 316–21
33. Stoclet JC, Martinez MC, Ohlmann P, Chasserot S, Schott C, Kleschyov AL, Schneider F, Andriantsitohaina R: Induction of nitric oxide synthase and dual effects of nitric oxide and cyclooxygenase products in regulation of arterial contraction in human septic shock. Circulation 1999; 100:107–12
34. Thoenes M, Forstermann U, Tracey WR, Bleese NM, Nussler AK, Scholz H, Stein B: Expression of inducible nitric oxide synthase in failing and non-failing human heart. J Mol Cell Cardiol 1996; 28:165–9
35. Hussain SN: Respiratory muscle dysfunction in sepsis. Mol Cell Biochem 1998; 179:125–34
36. Wilcox P, Milliken C, Bressler B: High-dose tumor necrosis factor alpha produces an impairment of hamster diaphragm contractility: Attenuation with a prostaglandin inhibitor. Am J Resp Crit Care Med 1996; 153:1611–5
37. Murphy TD, Gibson RL, Standaert TA, Woodrum DE: Diaphragmatic failure during group B streptococcal sepsis in piglets: The role of thromboxane A2. J Appl Physiol 1995; 78:491–8

38. Mebazaa A, De Keulenaer G, Paqueron X, Andries L, Ratajczak P, Lanone S, Frelin C, Longrois D, Payen D, Brutsaert D, Sys S: Activation of cardiac endothelium as a compensatory component in endotoxin-induced cardiomyopathy: Role of endothelin, prostaglandins and nitric oxide. Circulation 2001; 104:3137–44

39. Ciechanover A: The ubiquitin-proteasome proteolytic pathway. Cell 1994; 79:13–21

40. Lee MH, Hyun DH, Jenner P, Halliwell B: Effect of proteasome inhibition on cellular oxidative damage, antioxidant defences and nitric oxide production. J Neurochem 2001; 78:32–41

41. Hobler S, Williams A, Fisher D, Wang JJ, Sun X, Fisher JE, Monaco J, Hasselgren PO: Activity and expression of 20S proteasome are increased in skeletal muscle during sepsis. Am J Physiol 1999; 277:R434–40

42. Hobler SC, Wang JJ, Williams A, Melandri F, Sun X, Fisher JE, Hasselgren PO: Sepsis is associated with increased ubiquitin-conjugating enzyme E214k mRNA in skeletal muscle. Am J Physiol 1999; 276:R468–73

43. Chai J, Wu Y, Sheng ZZ: Role of ubiquitin-proteasome pathway in skeletal muscle wasting in rats with endotoxemia. Crit Care Med 2003; 31:1802–7

44. Wray CJ, Mammen JM, Hershko DD, Hasselgren PO: Sepsis upregulates the gene expression of multiple ubiquitin ligases in skeletal muscle. Int J Biochem Cell Biol 2003; 35:698–705