Muscle Wasting and IL-6

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Abstract

Muscle wasting is a common feature of systemic inflammation and malignant diseases. The primary cause is accelerated proteolysis. Protein degradation was found to be accelerated by IL-6 with activation of intracellular proteases in C2C12 myotubes. IL-6 transgenic mice displayed extensive muscle wasting together with an increase of proteolytic pathways. The increase in activities and mRNA levels of cathepsins (B and L) and the poly-ubiquitin (Ub) mRNA level could be inhibited by treatment with IL-6 receptor antibody (MR16-1), which inhibits the binding of IL-6 to the receptor. Muscle wasting and an increase in the serum IL-6 level were observed with Colon-26 adenocarcinoma. This muscle wasting and increases in the activity and mRNA level of cathepsin L and the poly-Ub mRNA level could be inhibited by MR16-1 treatment via inhibiting the action of IL-6. IL-6 production and atrophy of muscle fibers were induced by intramuscular injection of turpentine oil. The injected muscle displayed increased activities and mRNA levels of cathepsins (B,L). The treatment with MR16-1 suppressed the increase of cathepsin activities.

IL-6 is considered to be a proteolysis-inducing factor due to its modulation of muscle proteolytic systems. IL-6 plays a pivotal role in the muscle wasting observed in systemic inflammation, cancer cachexia and inflammed muscle.

Key words: muscle wasting, IL-6, IL-6 transgenic mouse, IL-6 receptor antibody, proteolytic system, turpentine oil.

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In muscle, the rate of protein synthesis and breakdown is balanced. An increase in degradation and a decrease in synthesis can cause loss of muscle mass, and extensive muscle wasting is observed in cancer cachexia, sepsis and acidosis. A major factor of muscle wasting under these catabolic conditions is an increase in protein degradation. Studies have been done to elucidate the factors which induce muscle proteolysis. Clows [5, 6] identified a proteolysis-inducing activity in serum from septic patients and found that the cleaved products of IL-1 may be candidates for the proteolysis-inducing factor (PIF). These substances have not yet been purified, so that their molecular and biochemical nature remains unclear. TNF has long been considered to be a PIF since recognized as a cachectin. Rats treated with recombinant TNF-α showed a significant decrease in skeletal muscle protein and an increase in muscle protein degradation [11, 18]. In humans, administration of rTNF-α caused an increase in nitrogen efflux from the skeletal muscle [46]. In AH30-bearing rats, anti-TNF antibody treatment prevented protein degeradation [7]. However, *in vitro* studies have not proven a direct effect of TNF-α or IL-1 on muscle proteolysis [15, 25, 30].

One strong PIF candidate is IL-6. Goodman et al. [17] reported that recombinant human IL-6 (rhIL-6) administration to rats increased amino acid release from the muscle, and Strassman [37, 38] demonstrated that IL-6 could induce cancer cachexia and muscle wasting, though the *in vitro* effect was not examined. To be recognized as a PIF, the following criteria need to be met : (1) direct proteolytic activity is confirmed in an *in vitro* system, (2) intracellular proteolytic systems are modulated *in vitro* and *in vivo*, (3) the proteolytic effect can be blocked by treatment with a specific antibody *in vivo* and *in vitro*, and (4) the level in circulation increases in clinical situations inducing muscle wasting, such as in sepsis and cancer cachexia. In the case of IL-6, its serum level has been reported to increase in sepsis

and cancer cachexia, suggesting it to be a potential PIF candidate [3, 21, 23, 33].

In Vitro Assay System for Muscle Proteolysis and IL-6 Effects

Muscle culture systems have been employed to evaluate the effects of various agents on protein breakdown and synthesis in muscles. An increase in protein breakdown has been demonstrated by using the affected muscles harvested from animals undergoing various treatments, such as sepsis, fasting, acidosis and denervation. However, no agent has been yet found to induce protein breakdown (revealed by an increase of released amino acids from the incubated muscle) when added to culture of normal muscle tissue [15]. The central muscle core becomes too hypoxic during the experiment and the duration of muscle incubation is so limited that any increase in protein breakdown is barely reflected as an increase in amino acid release from the cultured muscle. The half-life of myosin, a representative long-lived structural protein in the muscle, is approximately 27 hours [20]. The maximum incubation period of 2 hours for a muscle culture system would be too short to observe changes caused by the addition of agents. Also, contamination of the cultured muscles by blood-borne cells, such as macrophages, granulocytes and monocytes, may modulate the response of myocytes. Therefore, it is preferable to adopt a cell culture system of myotubes as a model of the muscle to elucidate the direct action of biologically active agents. The activity of myotubes can be maintained for more than 48 hours, which would enable the estimation of protein degradation by evaluating protein half-life over longer intervals.

C2C12myoblasts fuse to form multinucleated myotubes and express muscle specific proteins during differentiation. C2C12myotubes contain proteolytic systems as does the mature muscle [8, 9]. Examinations of proteolytic systems are important, because agents which can induce protein degradation (shortening of protein half-life) must theoretically have some effect on proteolytic systems. The half-lives of long-lived proteins were measured by the method of Gulve et al. [20] with some modifications [10]. Long-lived proteins constitute most of the proteins of the cells and have an average half-life of 25 hours under our culture conditions. In contrast, short-lived proteins constitute only a small fraction of the total proteins (less than 1% in liver) and have an average half-life of less than 60 minutes. We tested various inflammatory cytokines considered to have PIF activity. As shown in Table 1, only IL-6 shortened half-lives of long-lived proteins. In contrast, IL-1α and β had no effect, and TNF-α prolonged the half-lives.

In the next step, the effect of IL-6 on proteolytic systems was examined. The intracellular proteolytic process is composed of lysosomal and non-lysosomal pathways, in which intracellular proteases are directly responsible for protein degradation. Non-lysosomal proteolysis occurs via the Ub-proteasomes pathway. Abnormal (mutant or damaged) proteins, short-lived proteins (regulatory and rate limiting proteins, cell-cycle related proteins), and long-lived proteins (contractile proteins et al.) are selectively degraded by this system. Proteasomes, multicatalytic ATP-dependent protease

Table 1. Effect of inflammatory cytokines on half-life of long-lived proteins in C2C12 myotubes.

Cytokines	Concentrations in medium	t1/2 (h) (1% FBS)		% change	(0.5 mg/ml BSA)		% change
				(%)			(%)
control		25.60 ± 1.87	(n = 15)	100	21.36 ± 3.25	(n = 6)	100
IL-6	10 U/ml	24.96 ± 1.29	(n = 6)	98	19.09 ± 2.87*	(n = 6)	89
	100 U/ ml	23.79 ± 1.55*	(n = 6)	93	20.96 ± 3.84	(n = 6)	98
TNF-A	10 U/ml	25.95 ± 1.03	(n = 6)	101	24.05 ± 4.51	(n = 3)	113
	100 U/ml	26.85 ± 0.84	(n = 6)	105	22.66 ± 1.90	(n = 3)	106
	1000 U/ml	28.27 ± 0.45*	(n = 6)	110	23.23 ± 0.98	(n = 3)	109
IL-1α	10 U/ml	26.80 ± 2.05	(n = 3)	105	N.D.		
	100 U/ml	26.46 ± 0.30	(n = 3)	103	N.D.		
	1000 U/ml	27.28 ± 1.13	(n = 3)	107	N.D.		
IL-1β	10 U/ml	26.20 ± 0.88	(n = 3)	102	N.D.		
	100 U/ml	26.77 ± 1.44	(n = 3)	105	N.D.		
	1000 U/ml	26.58 ± 0.86	(n = 3)	104	N.D.		

C2C12 myotubes grown on 12-well dishes were used for the measurement of protein breakdown in the presence of the indicated amounts of cytokines. Breakdown of long-lived proteins was measured in the presence of 1% FBS or 0.5 mg/ml BSA. Data for long-lived proteins are expressed as mean ± SD. The statistical analysis was done using the Mann-Whitney test. * $P < 0.05$ vs. control. N.D., not determined.

complexes, occur in two forms, 20S (the latent form) and 26S (the active form, which is a complex of 20S proteasomes and 19S regulators) [29]. Ub-conjugated proteins are recognized and bound by 19S regulators, which release the Ub chain and catalyze the entry of the proteins into 20S proteasomes. Degradation occurs in an ATP-dependent manner in 26S proteasomes, which contain multiple proteolytic sites within its two central rings. The activation of proteasomes may occur via interconversion of the latent form to the active form in association with the action of regulatory components. The 20S and 26S proteasomes can be separated from each other by their molecular weight and proteolytic activity with or without the presence of activators.

Extracellular endocytosed proteins, such as plasma proteins, hormones and lipoproteins, and membrane proteins are completely degraded within lysosomes containing cathepsins (B, H, L and D) and other hydrolases. Some cytosolic proteins are degraded by being engulfed in autophagic vacuoles that fuse with lysosomes. Cathepsins B and L are the main agents of lysosomal degradation in the muscle and their contribution to accelerated muscle proteolysis in catabolic conditions has been controversial.

As shown in Fig. 1, IL-6 dose-dependently modulated the proteolytic properties of proteasomes. The addition of 100 units/ml of IL-6 increased the activity of 26S proteasomes by about 30% and decreased the activity of 20S proteasomes by 12%. Interconversion of proteasomes from the latent to the active form occurred with IL-6 treatment. Northern blot analysis disclosed that the level of mRNA encoding subunits of 20S proteasomes (C2,C8) and subunit of 26S proteasomes (S4) were elevated. By treatment with the same amount of IL-6, the activities of cathepsins B and B+L were increased by 53.5% and 21.7%, respectively, and the levels of mRNAs encoding cathepsins B and L were elevated by 57.8% and 80.8%, respectively. TNF could inhibit the activities of proteasomes (20S and 26S) and cathepsins (B and B+L), which agreed with the finding that it prolonged the half-lives of long-lived proteins in myotubes. It is now evident that IL-6 directly induces proteolysis via activating proteolytic systems in myotubes. However, extrapolation from the results of the *in vitro* model to the *in vivo* effect has limited validity, since it is not known to what extent myotubes resemble myocytes in an adult individual. In this *in vitro* system, reduction of the half-life of proteins may be relevant to enhanced proteolysis, and the degradation of the proteins as a whole could be evaluated but not the specific degradation of myofibrillar proteins. Therefore, the next step was to confirm the effect of IL-6 on muscle wasting in an *in vivo* system.

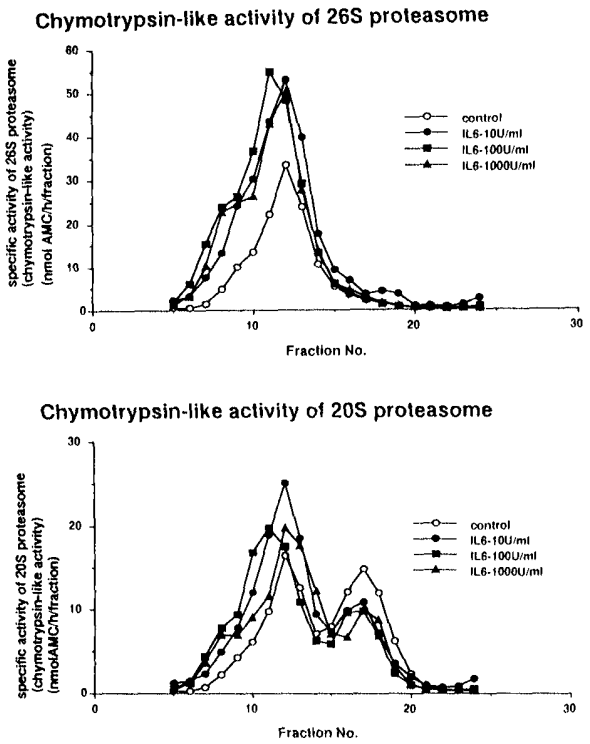

Figure 1. Modulation of proteolytic properties of 20S and 26S proteasomes by the treatment with IL-6. C2C12 myotubes maintained in the fusion medium (DMEM+1%FBS) were incubated for 48 hours in the presence of various amounts of IL-6. The chymotrypsin-like activity of proteasomes was measured using Suc-Leu-Leu-Val-Tyr-MCA as a substrate after fractionation using a 10-40% (v/v) glycerol gradient. In the absence of 1% SDS, the activity of 26S proteasomes (the active form) was identified at the fraction 8-10 (upper pane1). In the presence of 1% SDS, the activities of both 26S and 20S (the latent form) proteasomes were measured at the fractions 8-10 (26S) and 13-15 (20S), respectively (lower panel). IL-6 dose-dependently increased the activity of 26S proteasomes and decreased the activity of 20S proteasomes.

IL-6 Transgenic Mouse and Muscle Wasting

C57BL/6JLd-IL-6 transgenic mice were produced by microinjection of the 3.3-KbpSphI-XhoI fragment (L^d-6) containing human IL-6cDNA fused with the H-$2L^d$ promoter into the pronucleus of fertilized egg from C57BL/6J mice [39]. IL-6 transgenic mice developed IgG1 plasmacytosis, hepatosplenomegaly and muscle weight loss, despite the overall body weights not being different from those of the control mice. Changes in proteolytic pathways took place in the muscle of the IL-6 transgenic mice [43, 44]. As shown in Table 2, the

activities of cathepsins B and B+L increased by 20 and 6.2 times, respectively. The mRNA levels of cathepsins B and L also increased by 2.8 and 2.6 times, respectively. Immunohistochemical study revealed strong positive staining with a fine granular appearance of both cathepsins in the atrophying myofibers in these transgenic mice. The mRNA levels of mono- and poly-Ubs increased to 193 and 169%, respectively, while no difference was found for the mRNA levels of 20S and 26S proteasomes. The mRNA level of muscle specific calpain, calpain 3, was reduced 50%. A recent paper reported that mutations in the proteolytic enzyme calpain 3 causes limb-girdle muscle dystrophy type 2A [34]. Though the physiological role of calpain 3 protein has been unknown, a counter-regulatory role of calpain 3 in muscle proteolysis was implicated. All these changes could be completely abolished by treatment with IL-6 receptor antibody (MR16-1). MR16-1 prevents the binding of human IL-6 to the mouse IL-6 receptor, thus blocking the association of a non-ligand-binding 130-KD signal transducing molecule, gp 130 [41]. Oncostatin M, leukemia inhibitory factor and ciliary neutropic factor, whose receptor complexes share the signal-transducing component gp 130, may also activate lysosomal cathepsins, increase the mRNA levels of Ubs and induce muscle proteolysis.

As reported by Goodman [17], repeated administration of rIL-6 to rats increased the release of amino acids from isolated muscles. We examined the effects of intraperitoneal injection of rhIL-6 at a 4-hour interval to increase the activity of cathepsins and cause muscle atrophy [12]. However, we could not find any increase in the activity of cathepsins nor changes in muscle weight. Injected rhIL-6 disappears rapidly from circulation with a plasma half-life of 3 minutes and is predominantly distributed in the liver (about 80%), kidney and intestine [4]. Injected IL-6 has little effect on the metabolism of muscles. Soluble IL-6 receptor (IL-6R) enhances the action of IL-6 because a complex of IL-6 and soluble IL-6R is associated with the membrane-anchored signal transducer, gp 130 [40]. In the IL-6 transgenic mouse, the production of soluble IL-6R increases at the age of 10 weeks together with an increase in serum IL-6. Soluble IL-6R may be needed to exert the proteolytic effect of IL-

Table 2. Changes in proteolytic pathways in IL-6 transgenic mouse and effects of anti-murine-IL-6 receptor antibody (MR 16-1).

	control mice	IL-6 transgenic mice	IL-6 transgenic mice treated with MR16-1
Body weight	20.5 ± 1.2	21.7 ± 2.7	19.7 ± 1.2
Muscle weight	119.3 ± 9.1	91.0 ± 15.2	117.3 ± 10.8
Lysosomal cathepsin			
Cathepsin B activity (n mol AMC /h/mg protein)	3.3 ± 0.4	66.6 ± 30.6	4.1 ± 0.6
Cathepsin B+L activity (n mol AMC /h/mg protein)	67.3 ± 5.9	419.3 ± 171	58.0 ± 14.9
Cathepsin B mRNA	100%	277%	120%
Cathepsin L mRNA	100%	257%	170%
Ubiquitin-proteasomes			
mono-ubiquitin mRNA	100%	193%	92.5%
poly-ubiquitin mRNA	100%	169%	142%
20S subunits			
C2 mRNA	100%	ND	ND
C8 mRNA	100%	ND	ND
26S subunits			
S4 mRNA	100%	ND	ND
S7 mRNA	100%	ND	ND
Calpain			
m Calpain mRNA	100%	ND	ND
Calpain 3 mRNA	100%	50%	ND

*P < 0.05, § P < 0.0 1 (vs. control and MR 16-1 treated mice). ND: not different from the level in control mice.
IL-6 transgenic mice were randomized to receive PBS or MR 1 6-1 with 2 mg/body intravenously once at 5 week old and 100 mg/body intraperitoneally twice weekly from 6 to 14 wk old. At 16 wk old the body weights and gastrocnemius muscle weights were measured. The activities of cathepsins (B, B+L) and mRNA levels of cathepsines (B, L) ubiquitins (mono, poly), subunits of proteasomes and calpains were measured in the gastrocnemins muscle. As controls, normal liltermate C57BL/6J mice were used.

6. In another experiment, we observed that the mRNA levels of cathepsins (B,L) and poly-Ub in mouse muscle increased after a single injection of rhIL-6 (unpublished data). Thus, chronic elevation of IL-6 as found in the IL-6 transgenic mouse or coupling to soluble IL-6R may be needed to increase the enzymatic activity of lysosomal cathepsin.

The functional importance of lysosomal proteolysis in muscle wasting has been questioned. In the muscle culture system, inhibition of cathepsin activity does not affect overall protein degradation or myofibrillar proteolysis in fasting [48], denervation atrophy [14], and sepsis [22]. The contribution of cathepsins to muscle proteolysis in the IL-6 transgenic mouse is uncertain, though it is clear that IL-6 activates the cathepsin system and mediates muscle atrophy.

Calpains have not been considered to play an important role in muscle degradation in denervation atrophy [14], fasting [48], and tumor bearing rats [2, 42]. Since they are committed to limited proteolysis of targeted proteins, they have been proposed to have regulatory rather than degradative roles. We could not observe any change in mRNA level of m calpain in the muscle of the IL-6 transgenic mouse, and the contibution of m calpain was thus considered limited. However, further investigation would be necessary to elucidate the role of calpain 3 in muscle proteolysis.

Muscle Wasting in Cancer Cachexia and the Role of IL-6

Cancer cachexia is a characteristic syndrome observed in cancer patients. Alteration in the metabolism of proteins and amino acids occurs because of the effects of both starvation and chemical mediators derived from cancer tissues. In the muscle, protein synthesis decreases and protein degradation increases. To study this phenomenon, murine colon-26 adenocarcinoma (C-26), an undifferentiated carcinoma, was transplanted into syngenic animals. Mice inoculated with the C-26 tumor undergo marked cachexia associated with weight loss of skeletal muscles and adipose tissues. The role of IL-6 in cancer cachexia was examined by using anti-IL-6 receptor antibody (MR16-1) [13].

As shown in Table 3, the skeletal muscle underwent severe atrophy in C-26 tumor-bearing mice but treatment with MR16-1 partially prevented weight loss of the gastrocnemius muscle. Proteolytic pathways in the muscle were modulated in cancer-bearing mice. The mRNA levels of cathepsins B and L increased to 236% and 826% of those in the pair fed control, respectively, and the activity of cathepsin L, which significantly increased in C-26-bearing mice, decreased on treatment with MR16-1. IL-6 mediates the activation of the lysosomal proteolytic system, especially cathepsin L, and lysosomal proteolysis increases in wasting muscles in cancer cachexia. The contribution of lysosomal proteolysis to the bulk of muscle protein degradation in the tumor-bearing state has been questioned. Baracos et al. [2] demonstrated that the inhibition of lysosomal functions causes only a minor reduction in muscle proteolysis, and Llovera et al. [28] observed that the ratio of lysosomal proteolysis to total proteolysis is not higher than that in normal rats. When the Ub-proteasomes pathway was examined, the mRNA levels of poly-Ub and proteasomes in the muscle of C-26 bearing mice increased significantly, indicating that the cytosolic Ub-proteasomes system may play an important role. Ub and proteasomes have been reported to share a close functional relationship and their mRNA levels increase in the tumor-bearing state in a coordinated manner [2, 42]. Since the treatment with MR16-1 inhibited the change in poly-Ub mRNA in C-26 tumor-bearing mice, the mRNA of poly-Ub is likely to be regulated directly by IL-6, while other factors may be involved in the regulation of mRNA of the proteasomes.

Another feature of cancer cachexia is loss of epididymal fat weight. Administration of MR16-1 neither inhibited this loss nor influenced the serum triglyceride level. IL-6 does not contribute to the decrease of fat weight, though at a high concentration of IL-6, it can reduce lipoprotein lipase activity in adipose tissue [19]. Soda et al. [36] also found that administration of rhIL-6 to mice did not induce any decrease of epididymal fat weight.

Denerative Myopathy and IL-6

Intramuscular injection of certain agents, such as bupivacaine and plasmocid, causes acute myofiber necrosis and consequently induces muscle atrophy, as observed in muscle dystrophic diseases [32]. Activation of the proteolytic systems in muscle is known to be involved in the pathogenesis of degenerative myopathies [16, 26, 27, 35]. At a very early stage of plasmocid-induced myopathy, myofibers were stained with anti-cathepsin L antibody in the absence of the infiltration of macrophages [27]. In Duchenne muscular dystrophy, positive staining for cathepsins was observed in the intramyofibral portion of atrophic fibers in the presence of marked infiltration of macrophages [27]. Thus, lysosomal cathepsins may play an important role in autophagocytosis and heterophagocytosis of the muscle fiber under these pathologic conditions.

We noticed that the pattern of immunohistochemical staining of cathepsins in the muscle of IL-6 transgenic mice shared many similarities with those in the early stage of plasmocid-induced myopathy [27] and in distal myopathy with rimmed vacuoles [24]. We, thus, hypothesized that IL-6 may be involved in the pathogenesis of muscle degeneration induced by injection of plasmocid or inflammatory agents via activation of intramyofibral lyosomal proteinase. The inflammatory agent, turpentine oil, may induce muscle

Table 3. Changes in proteolytic pathways in C-26 bearing mice and effects of anti-murine-IL-6-receptor antibody (MR16-1).

	Pair-fed control	C-26 bearing mice	C-26 bearing mice treated with MR16-1
Body weight (g)			
Day 17	24.1 ± 0.21	19.2 ± 0.86*	20.4 ± 0.56*
Tumor weight (mg)	815 ± 83.5	962 ± 132.2	
Gastrocnemius muscle (mg)	130 ± 2.6	89 ± 6.3*	109 ± 2.9§
Epididymal fat (mg)	294 ± 28	46 ± 20*	45 ± 10*
Lysosomal Cathepsin			
Cathepsin B activity (n mol AMC /h/mg protein)	4.9 ± 0.2	5.7 ± 0.4	4.2 ± 0.4
Cathepsin B+L activity (n mol AMC /h/mg protein)	49.7 ± 2.0	59.5 ± 2.9*	44.9 ± 2.9§
Cathepsin B mRNA	100 ± 2	236 ± 38*	206 ± 30
Cathepsin L mRNA	100 ± 9	826 ± 105*	482 ± 148§
Ubiquitin-proteasomes			
poly-ubiquitin mRNA	100 ± 10	269 ± 23*	148 ± 30§
20S subunits			
C2 mRNA	100 ± 9	201 ± 25*	155 ± 32
C8 mRNA	100 ± 9	247 ± 47*	176 ± 39
26S subunits			
S4 mRNA	100 ± 7	167 ± 23*	156 ± 19*
S7 mRNA	100 ± 17	244 ± 50*	252 ± 38*
Calpain			
Calpain 3 mRNA	100 ± 13	130 ± 10	122 ± 10

* $p < 0.05$, vs.control, § $p < 0.05$, vs. C-26 bearing mice.

The pair-fed control mice were sham operated on day 0 and were injected subcutaneously with 0.5 mg of rat IgG every other day from day 4 to day 16. In the C-26 bearing and MR16-1 treated C-26 bearing mice, a small incision was made on the back, and mice were inoculated subcutaneously with 8 mg of C-26 tumor on day 0. They were given rat IgG or MR16-1, respectively, with the same schedule as for the control. On day 17, body weights and gastrocnemius muscle weights were measured. The activities of cathepsins (B,B+L) and mRNA levels of ubiquitin, subunits of proteasomes and calpain 3 were measured in the gastrocnemius muscle.

degeneration and atrophy as does bupivacaine or plasmocid. We therefore examined the potential role of IL-6 in the process of muscle degradation in turpentine oil-induced muscle degeneration [45].

In turpentine oil-induced muscle degeneration, the activities of cathepsins B and B+L in the muscle began to rise at 12 hours after injection, when infiltration of macrophages had not yet become evident. The activities markedly increased after 1 day, together with massive infiltration of macrophages, and atrophy of myofibers was observed. These macrophages showed strong staining for cathepsins. Strong expression of IL-6 mRNA was found at 12 hours in the muscle injected with turpentine oil, and serum IL-6 increased, remaining high until day 3. IL-6 in the injected muscle may induce activation of lysosomal cathepsins and consequently enhance autodigestion of myofibrils. These degenerated myofibers are ultimately phagocytosed by the infiltrating macrophages. The involvement of IL-6 in the activation of cathepsins was confirmed by an experiment using rat anti-murine IL-6 receptor antibody, MR16-1, as shown in Table 4. The MR16-1 treatment significantly suppressed the activities of cathepsins B and B+L at day 3 after the injection of turpentine oil. However, the levels in the injected muscle treated with MR16-1 were still significantly higher than those in the opposite muscle. Inhibition of the action of IL-6 by MR16-1 did not completely inhibit the increase of cathepsin activities. Consequently, the autodigestion of myofibrils could not be abolished and infiltration of macrophages was not blocked. There may be other factors responsible for inducing the activities of cathepsins and the myofibral autodigestion. When the IL-6-deficient mice received intramuscular injection of turpentine oil, the activities of cathepsins (B, B+L) in the injected muscle were as high as in the control mice (unpublished observation). Some compensatory mechanisms for lysosomal cathepsins may be operating in the IL-6 deficient mice. Thus, IL-6 is not solely responsible for the up-regulation of lysosomal cathepsins.

Table 4. Effect of anti-murine IL-6 receptor antibody (MR16-1) on cathepsin activities in the gastrocnemius muscle after turpentine oil injection.

Gastrocnemius Muscles	Cathepsins	Treatments MR16-1	Rat IgG
		(n mol AMC /h / mg protein)	
Turpentine oil - injected site	B	23.09 ± 5.64*#	58.77 ± 15.53#
	B+L	232.72 ± 79.94*#	403.00 ± 103.58#
Opposite site	B	3.89 ± 1.49	4.10 ± 1.34
	B+L	12.67 ± 5.80	13.29 ± 4.52

* $p < 0.05$, MR 1 6-1 vs. rat IgG, # $P < 0.01$, turpentine oil -injected site vs. opposite site.
Mice received intraperitoneal injection of either rat IgG (1 mg) or MR 16-1 (1 mg) 30 minutes before the injection of turpenine oil into right gastrocnemius muscle. Three days after the turpentine oil injection, both muscles were harvested for analysis of cathepsin B and B + L activities. In this model, mRNA level of IL-6 was highest at 12 hours in the turpentine oil-injected muscle and decreased at day 1. In contrast, the level was undetectable in the opposite site at all time points. Serum IL-6 levels were 1213 ± 118 pg/ml and 421 ± 219 pg/ml at 12 hours and day 3, respectively in the turpentine oil-injcted rats. In the saline injected rats, the levels were 48.3 ± 24.6 pg/ml and 20.7 ± 10.9 pg/ml at 12 hours and day 3, respectively. The mRNA levels of cathepsins B and L were highest at day 3 in the turpentine oil-injected muscle, while the levels in the opposite muscle slightly increased from day 1 to day 5. For assessment of muscle atrophy, the sizes of type 1 and 2 myofibers in the cross section of muscle were measured. The sizes of both types of myofibers in the turpentine oil-injected muscle sequentially decreased after injection and the differences in size compared to the opposite muscle became significant after day 3.

Summary

IL-6 can induce protein degradation in myotubes *in vitro* and in matured muscles *in vivo* and is responsible for muscle wasting associated with sepsis, cancer cachexia and inflammed muscle. The action of IL-6 is mediated by potentiation of the intracellular proteolytic pathways. Lysosomal cathepsins, especially cathepsin L, are transcriptionally up-regulated by IL-6 and their enzymatic activities are augmented. Ubiquitin is transcriptionally up-regulated and interconversion of proteasomes from the latent to the active form may be mediated by the transcriptional activation of proteasomes.

The mechanism of up-regulation of proteolytic machinery by IL-6 is not known, though it is speculated that nuclear factors activated by IL-6, such as acute phase response factor [47] and NF-IL6 [31], may regulate the transcription of genes of cathepsins(B, L), ubiquitins and proteasomes. Since these nuclear factors are activated not only by IL-6 but also by other mediators, IL-6 is not a single regulator of proteolytic machinery. Nevertheless, an understanding of the interrelationship between IL-6 and intracellular proteolytic pathways may help clarify the biological activities of IL-6, which regulates pleiotrophic functions of cells and tissues [1]. Some functions of IL-6 may be ascribed to the activation of proteolytic pathways. For instance, IL-6 stimulates osteoclast formation and bone resorption, resulting in hypercalcemia. The proteolytic system activated by IL-6 may be involved in stimulation of bone resorption.

The important role of IL-6 in muscle wasting opens the possibility of therapeutic control of extensive muscle degradation. The IL-6 receptor antibody would be clinically useful for preventing excess muscle wasting under catabolic conditions if nutrients are externally availble. Proteolytic pathways are thought to be involved in the pathophysiological actions of IL-6.

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