Transforming growth factor-β inhibition of proteasomal activity: a potential mechanism of growth arrest

Laura Tadlock, Yoko Yamagiwa, James Hawker, Carla Marienfeld, and Tushar Patel. Transforming growth factor-β inhibition of proteasomal activity: a potential mechanism of growth arrest. Am J Physiol Cell Physiol 285: C277–C285, 2003. First published March 19, 2003; 10.1152/ajpcell.00550.2002.—Although the proteasome plays a critical role in the controlled degradation of proteins involved in cell cycle control, the direct modulation of proteasomal function by growth regulatory signaling has not yet been demonstrated. We assessed the effect of transforming growth factor (TGF)-β, a potent inhibitor of cell growth, on proteasomal function. TGF-β selectively decreased hydrolysis of the proteasomal substrate Cbz-Leu-Leu-Leu-7-amido-4-methyl-coumarin (z-LLL-AMC) in a concentration-dependent manner but did not inhibit hydrolysis of other substrates Suc-Leu-Leu-Val-Tyr-AMC (suc-LLVY-AMC) or Cbz-Leu-Leu-Glu-AMC (z-LL-LE-AMC). An increase in intracellular oxidative injury occurred during incubation with TGF-β. Furthermore, in vitro hydrolysis of z-LLL-AMC, but not suc-LLVY-AMC, was decreased by hydrogen peroxide. TGF-β did not increase cellular expression of heat shock protein (HSP)90, a potent inhibitor of z-LLL-AMC hydrolysis in vitro. The physiological relevance of TGF-β inhibition of proteasomal activity was studied by assessing the role of z-LLL-AMC hydrolysis on cyclin-dependent kinase inhibitor expression and cell growth. TGF-β increased expression of p27KIP1 but did not alter expression of p21WAF1 or p16INK4A. The peptide aldehyde Cbz-Leu-Leu-ucinal (LLL-CHO or MG132) potently inhibited z-LLL-AMC hydrolysis in cell extracts as well as increasing p27KIP1 and decreasing cell proliferation. Thus growth inhibition by TGF-β decreases a specific proteasomal activity via an HSP90-independent mechanism that may involve oxidative inactivation or modulation of proteasomal subunit composition and results in altered cellular expression of key cell cycle regulatory proteins such as p27KIP1.

THE PROTEASOME is a multicatalytic complex present in all eukaryotic cells, and it constitutes the major extralysosomal proteolytic system involved in ubiquitin-dependent intracellular protein degradation (8). Known substrates of the proteasome include short-lived proteins with important cell cycle regulatory functions as well as transcriptional regulators, oncoproteins, tumor suppressors, and enzymes. The essential role of the proteasome in intracellular protein degradation and the necessity for controlled degradation of cell cycle regulatory molecules place the proteasome at the center of cell growth control. Thus modulation of proteasomal activity represents a potential mechanism for the regulation of cell cycle activity.

Transforming growth factor (TGF)-β is a potent inhibitor of cell growth and is a member of a superfamily of dimeric polypeptide growth factors that are distinguished from most other cytokines by their ability to limit cell growth (17, 26). TGF-β has diverse effects in addition to regulation of cell growth and participates in morphogenesis and differentiation (18). The varied cellular functions of this pleiotropic cytokine underlie its critical role in pathophysiological processes such as carcinogenesis. TGF-β receptor molecules are expressed ubiquitously, and growth inhibition has been shown to occur in a broad range of epithelial, endothelial, and hematopoietic cells (19).

TGF-β inhibits cell division by triggering a program of cyclin-dependent kinase (cdk) inhibitory responses that culminate in G1 cell cycle arrest. In epithelial cells from the skin, lung, and breast, TGF-β rapidly elevates expression of p15INK4B (34). p15INK4B binds to and inhibits cdk4 and cdk6 as well as displacing the p27KIP1 protein from these complexes (30, 43). In proliferating cells, p27KIP1 remains bound to cdk4 and cdk6 complexes, but when mobilized by TGF-β, p27KIP1 binds to and inhibits cdk2 (29). Prevention or inhibition of G1 cyclin-cdk activation by TGF-β inhibits the phosphorylation of the retinoblastoma protein (14). In addition, E2F activity is also impaired by TGF-β (35). In keratinocytes, colon and ovarian epithelial cells, TGF-β additionally elevates the expression of the p27KIP1-related inhibitor p21WAF1/CIP1, and in mammary epithelial cells, it represses the cdk-activating phosphatase cdc25A. Furthermore, in many cell types, TGF-β inhibits c-myc expression, which may play a pivotal role in the loss of G1 cyclins, downregulation of Cdc25A, or induction of the cdk inhibitor p15INK4B (4). Thus growth inhibition by TGF-β involves cellular expression of multiple cell cycle regulatory molecules.
Proteasomal degradation controls the cellular expression of many key cell cycle regulatory molecules including p27KIP1, which participates in TGF-β growth inhibition (23, 27). The degradation of a diverse range of proteins is facilitated by the presence of multiple catalytic activities within the proteasome with the capability for the endoproteolytic cleavage of peptide bonds on the carboxyl side of acidic, basic, and hydrophobic residues of proteins (32). The activities can be differentially regulated with alterations in biological significance. Differential regulation of these activities can occur by changes in proteasomal subunit composition and may have biological significance (1, 11). To determine the role of dysregulated proteasomal function during cell growth inhibition by TGF-β, we first characterized the effect of TGF-β on various proteasome-associated hydrolytic activities. TGF-β specifically inhibited a hydrolytic activity characterized and quantitated by cleavage of the peptide 7-amido-4-methyl-coumarin (AMC) substrate Cbz-Leu-Leu-Leu-AMC (z-LLL-AMC). We next assessed putative cellular mechanisms involved in the modulation of z-LLL-AMC activity by determining the roles of oxidative stress as well as heat shock protein (HSP90, an endogenous inhibitor of z-LLL-AMC hydrolysis). Finally, to address the physiological relevance of TGF-β inhibition of proteasomal activity, we assessed the role of z-LLL-AMC hydrolysis on cell growth and the expression of cell cycle regulators.

EXPERIMENTAL PROCEDURES

Cell lines. The human biliary epithelial H69 and Mz-ChA-1 cell lines were used for our studies. H69 cells were obtained and used as previously described (24). Mz-ChA-1 cells were kindly provided by Dr. J. G. Fitz (University of Colorado Health Sciences Center, Denver, CO) and were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum. Mz-ChA-1 cells express very low levels of Smad4 mRNA and are resistant to TGF-β growth inhibition. The responsiveness of Mz-ChA-1 cells to TGF-β can be restored by transfection with Smad4 (44).

Sedimentation velocity analysis. Mz-ChA-1 cells were grown to 50–100% confluence in 100 × 20-mm plates. The cells were then washed with cold phosphate-buffered saline (PBS) and lysed with 1 ml of a buffer containing 10 mM HEPES, 100 mM KCl, 5 mM MgCl₂, and 0.1% Triton X-100 (pH 7.2), transferred to 12 × 75-mm borosilicate tubes, and homogenized by 20 strokes with a Tissue Tearor (Biospec, Bartlesville, OK). The homogenate was centrifuged at 21,000 g for 20 min at 4 °C with a Microfuge R centrifuge (Beckman Instruments, Torrance, CA) using a 1 ml centrifuge tube with a 3 ml Beckman LS-70M Ultracentrifuge with a SW41Ti rotor (Beckman Instruments), the gradient was separated into 12 fractions of 1 ml each. Protein content was assessed by the Bradford assay, and proteasomal activity was assessed in each fraction at 37 °C for 30 min.

Measurement of cytosolic proteasomal activity. Cells were incubated in 10% fetal bovine serum with or without TGF-β for 24 h. The cells were then washed with PBS and lysed with 1 ml of a hypotonic buffer containing 25 mM HEPES, 5 mM MgCl₂, 1 mM EGTA, and freshly added 0.5 mM PMSF, 2 μg/ml pepstatin, and 2 μg/ml leupeptin. Cells were then homogenized by 20 strokes with a Tissue Tearor. The homogenate was centrifuged at 21,000 g for 45 min at 4 °C with a Microfuge R centrifuge. The protein content in the supernatant cytosolic fraction was measured with the Bradford reagent. Protease activity was assayed by adding 50 μl of cytosolic protein (containing 40–75 μg protein) to 0.5 ml of buffer containing 25 mM HEPES, pH 7.5, 10 mM dithiothreitol, 0.1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 0.5 mM PMSF, 2 μl/ml aprotinin, and 20 μM fluorescent substrate. The substrates used to assay for proteasomal activity were z-LLL-AMC, Suc-Leu-Leu-Val-Tyr-AMC (suc-LLVY-AMC), and Cbz-Leu-Leu-Glu-AMC (z-LLL-AMC). After incubation at 37 °C for 30 min, fluorescence was measured every 5 min for 30 min with a fluorometer (TD700; Turner Designs, Mountain View, CA) using excitation and emission wavelengths of 360 and 480 nm, respectively. Hydrolysis of all three substrates was linear for up to 1 h, and a shift in its absorbance of 0.1 absorbance units was used for the determination of the amount of cytosolic extract used. For experiments involving HSP90, 20 μM substrate in enzyme buffer was added to 10 μg of cytosolic protein in a final volume of 200 μl in 96-well plates. Fluorescence was monitored with a CytoFluor 4000 fluorescence plate reader (PerSeptive Biosystems, Foster City, CA) using excitation and emission wavelengths of 360 and 460 nm, respectively. With each experiment, standard curves were generated with AMC and proteasomal activity was expressed as picomoles of AMC per milligram of protein per minute.

Plasmids and transfection. Plasmids for Smad4 were obtained from Dr. A. Roberts (National Cancer Institute, Bethesda, MD). Mz-ChA-1 cells were transiently transfected with a Perfect Lipid transfection kit (Invitrogen, Carlsbad, CA). Cells were seeded in 24-well plates, grown in serum-containing medium to 40–60% confluence, and then incubated for 4 h in serum-free medium containing plasmid DNA and lipid in a ratio of 6:1. The medium was then replaced with serum-containing medium. Cells were used 24 h after transfection. Cells were cotransfected with cytomegalovirus (CMV)-β-galactosidase (β-gal) to normalize for transfection efficiency, and β-gal activity was assessed in cell lysates with chlorophenol red-β-d-galactopyranoside monosodium (CPRG) and measurement of absorbance at 575 nm.

Quantitation of reactive oxygen species generation. Quantitation of intracellular generation of reactive oxygen species was performed with the oxidant-sensitive fluorescent probe dihydroethidium. This membrane-permeant probe is oxidized within the cell to ethidium, resulting in a marked red shift in its fluorescence spectra. Moreover, the oxidized ethidium remains within the cell because it intercalates with DNA. The fluorescence of ethidium bound to DNA can then be used to quantitate the generation of reactive oxygen species in intact cells (42). H69 cells were incubated in 96-well plates (1,000 cells/well) in the presence of 20 μM dihydroethidium in Krebs-Ringers-HEPES buffer for 30 min at 37 °C. Fluorescence was quantitated with excitation and emission wavelengths of 530 and 620 nm, respectively, and a fluorescence multiplate reader (CytoFluor 4000).

Immunoblot analysis. Confluent cells in culture were trypsinized and sonicated for 20 s at 4 °C (sonic dismembrator; Fisher Scientific, Pittsburgh, PA) in a lysis buffer containing 50 mM Tris base, 2 mM EDTA, 100 mM NaCl, 1% NP-40, and one mini protease inhibitor cocktail tablet in 25 ml. Protein content was determined by the Bradford assay.
Protein samples were separated on 4–12% gradient polyacrylamide gels (Novex, San Diego, CA) under reducing conditions and electroblotted to positively charged 0.45-μm nitrocellulose membrane (Millipore, Bedford, CA). The membranes were soaked for 5 min in transfer buffer (13.4 mM Tris, pH 8.3, 20% methanol, 108 mM glycine). Blots were preblocked in 20 mM Tris, 150 mM NaCl, 0.1% Tween 20, 5% nonfat dry milk for 3 h at room temperature. Membrane-bound cdk inhibitors p16 INK4A, p27 KIP1, and p21 WAF1/CIP1 were detected by incubating the membrane overnight at 4°C with the respective monoclonal mouse anti-human primary antibody, used at a 1:500 dilution. The primary antibodies were diluted in a solution containing 20 mM Tris, 150 mM NaCl, 0.1% Tween 20, and 5% nonfat dry milk. The membrane was washed twice with 20 mM Tris, 150 mM NaCl, and 0.1% Tween 20 (TTBS) and then incubated with the secondary antibody, a polyclonal goat anti-mouse immunoglobulin-peroxidase conjugate (Zymed, San Francisco, CA) at a 1:1,000 dilution for 60 min at 4°C. The secondary antibody was diluted in TTBS buffer. For all immunoblots, membranes were washed twice with 20 mM Tris, 150 mM NaCl, and 0.1% Tween 20 with a size >700 kDa, suggesting that the proteasomal complex was the major cellular source of these hydrolytic activities.

We then assessed the effect of TGF-β on hydrolysis of these substrates. Biliary epithelial H69 cells were incubated with 10, 1, 0.1, or 0 ng/ml TGF-β for 24 h. Substrate hydrolysis was then fluorometrically assessed in cytosolic extracts. Basal activity was 1.12, 8.33, and 14.52 nmol AMC·mg protein⁻¹·min⁻¹ for suc-LLVY-AMC, z-LLL-AMC, and z-LLE-AMC, respectively. Incubation with TGF-β did not significantly alter the hydrolysis of suc-LLVY-AMC, and a modest increase in z-LLL-AMC was noted only at high concentrations of TGF-β (Fig. 1, A and B). Unexpectedly, TGF-β markedly decreased z-LLE-AMC hydrolysis in a concentration- and time-dependent manner (Fig. 1, C and D). Incubation with the proteasome inhibitor lactacystin (10 μM) for 24 h decreased z-LLL-AMC hydrolysis under basal conditions to 18.7 ± 9.2% of controls and during incubation with 10 ng/ml TGF-β to 8.4 ± 3.2% of controls. The inhibitory effect of TGF-β on z-LLL-AMC hydrolysis was completely abolished by preincubation with the protein synthesis inhibitor cycloheximide (100 μg/ml) for 1 h, indicating that TGF-β inhibition of z-LLL-AMC hydrolysis involves de novo protein synthesis.

Biliary epithelial Mz-ChA-1 cells have defective TGF-β-mediated intracellular signaling that can be restored by Smad4 (44). Inhibition of z-LLL-AMC activity was not observed in Mz-ChA-1 cells. However, partial inhibition of z-LLL-AMC hydrolysis was observed in cells transiently transfected with Smad4 (Fig. 2). In combination, these studies indicate that TGF-β transcriptionally and selectively modulates a proteasome-associated hydrolytic activity with a substrate specificity different from that of suc-LLVY-AMC, a classic substrate used to assay proteasomal chymotrypsin-like activity (22).

TGF-β increases cellular oxidative stress. TGF-β has been shown to stimulate cellular production of hydrogen peroxide and to induce intracellular oxidative stress in several different cell types (6, 7, 21, 31, 39, 40). Furthermore, inhibition of z-LLL-AMC activity was described in response to metal-catalyzed oxidative stress (5). Thus we assessed the potential role of alterations in intracellular oxidative stress on proteasomal function. Cellar extracts were incubated with hydrogen peroxide for varying periods of time to increase intracellular oxidative stress (Fig. 3A). Incubation with hydrogen peroxide decreased z-LLL-AMC hydrolysis in...
a time-dependent manner. Likewise, we observed an increase in intracellular reactive oxygen intermediate (ROI) formation during incubation with TGF-β (Fig. 3B). Preincubation of cells with the antioxidant U-83836E (100 μM) prevented the loss of z-LLL-AMC activity in response to TGF-β (Fig. 3C). This concentration of U-83836E was chosen on the basis of preliminary studies showing effective reduction in ROI and lipid peroxidation and lack of significant cytotoxicity (Ref. 25 and unpublished data). These findings suggest that cellular oxidative stress may lead to inactivation of z-LLL-AMC hydrolytic activity in response to TGF-β. A potential mechanism of TGF-β modulation of proteasomal function may thus involve oxidative inactivation of specific proteasomal subunits mediating the z-LLL-AMC hydrolysis.

**Inhibition of proteasomal hydrolysis by HSP90.** Intracellular oxidative stress may increase expression of heat shock proteins. HSP90 has been shown to inhibit proteasomal z-LLL-AMC cleavage (41). Thus we tested the possibility that the effects of TGF-β may be mediated via an HSP90-dependent mechanism. Hydrolysis of z-LLL-AMC, but not suc-LLVY-AMC, was decreased in cytosolic extracts in the presence of recombinant HSP90 (Fig. 4, A and B). Furthermore, the inhibitory effect of HSP90 on z-LLL-AMC hydrolysis was inhibited by preincubation with 2 μM geldanamycin, an inhibitor of the biological functions of HSP90. These observations confirmed that HSP90 could act as a functional endogenous inhibitor in H69 cells. We next asked whether TGF-β alters expression of HSP90. However, no significant alteration in HSP90 protein levels was observed during incubation with TGF-β (Fig. 4, C and D). Furthermore, incubation with geldanamycin did not alter growth inhibition or substrate hydrolysis by TGF-β (data not shown). In combination, these findings suggest that although HSP90 may selectively modulate proteasomal LLL-AMC hydrolysis in vitro, the inhibitory effect of TGF-β on this activity is mediated by an HSP90-independent mechanism in vivo.
Inhibit INHIBITION OF PROTEASOMAL ACTIVITY

The effects of TGF-β on a wide variety of epithelial cell types, incubation with TGF-β decreased H69 cell proliferation in a concentration-dependent manner (Fig. 5A). In contrast, incubation with IL-6, a known biliary epithelial cell mitogen, resulted in an increase in proliferation under the same conditions. Although TGF-β can induce apoptosis in hepatic epithelia, TGF-β did not significantly increase nuclear fragmentation or activation of caspases-3 or -8 in H69 cells over the same range of concentrations (data not shown). Thus growth inhibition by TGF-β involves altered cell cycle progression rather than an increase in apoptosis. Proteasomal degradation has been implicated in the modulation of expression of the cdk inhibitors p21WAF1/CIP1 and p27KIP1, both of which have been implicated in cell cycle arrest by TGF-β. Quantitative immunoblot analysis indicated that incubation with TGF-β did not significantly alter the expression of either p16 or p21WAF1/CIP1 but increased expression of p27KIP1 (Fig. 5B). Preincubation with the antioxidant U-83836E decreased the effect of TGF-β on p27 expression (Fig. 6, A and B) and on proliferation (Fig. 6C). TGF-β was shown previously to increase p27KIP1 by altered degradation rather than via transcriptional mechanisms (15). Thus manipulation of proteasomal degradation can modulate growth inhibition in response to TGF-β. Furthermore, the direct regulation of proteasomal activity may have functional implications for TGF-β-mediated cellular processes other than growth inhibition.

Modulation of proteasomal z-LLL-AMC activity by exogenous inhibitors. The cell-permeant peptide aldehyde z-LLL-CHO, or MG132, has been used widely in studies evaluating proteasomal degradation of a variety of proteins including transcription factors, cell cycle regulatory proteins, and enzymes. This inhibitor has been shown to reversibly inhibit chymotrypsin-like proteasomal activity. As predicted on the basis of their similar structures, z-LLL-CHO also inhibits z-LLL-AMC hydrolysis. In vitro studies performed on cytosolic extracts revealed that z-LLL-CHO potently inhibited z-LLL-AMC hydrolysis with an IC50 of 24 nM. In contrast, z-LLL-CHO was a weaker inhibitor of succinylleucylvaline-AMC or z-LLE-AMC hydrolysis (IC50 of 45 and 78 μM, respectively). Incubation of cells with z-LLL-CHO resulted in increased p27KIP1 protein expression (Fig. 7, A and B). Moreover, an effect on cellular growth inhibition was also noted (Fig. 7C). These observations provide additional support for an important role of proteasomal activity in mediating growth arrest in response to TGF-β.

DISCUSSION

Our studies demonstrate a selective effect of TGF-β on a specific proteasome-associated hydrolytic activity characterized by z-LLL-AMC hydrolysis. The relationship of this observation to cell cycle regulation is emphasized by alterations in p27KIP1 protein expression and growth inhibition resulting from the modulation of proteasomal function. Thus the direct regulation of TGF-β increases expression of p27KIP1 and inhibits cell growth. To assess the functional consequence of proteasomal inhibition by TGF-β, we next determined the effects of TGF-β on cell growth and apoptosis.

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specific proteasome-associated hydrolytic activity represents a potential mechanism of cell cycle control. The cell cycle is a precisely controlled process regulated by a complex program involving the temporal expression and degradation of regulatory proteins such as the cyclins, cdk, and cdk inhibitors. Proteolytic degradation of many of these regulatory proteins occurs by the proteasomal complex and is crucial to normal cell cycle progression. Thus the proper control of proteasomal activity is extremely critical. Indeed, in yeast, several
proteasome mutants exhibit cell cycle arrest (9, 10). Recent studies identified and defined a sophisticated, evolutionary conserved system involving ubiquitination that targets proteins for degradation by the proteasome. These targeting systems are believed to represent the primary mechanism for the regulation of degradation of key cell cycle regulatory proteins by the proteasome. However, the presence of alternate mechanisms controlling proteasomal degradation is suggested by the ability of the proteasome to degrade proteins such as c-jun and ornithine decarboxylase without their prior marking by ubiquitin (12, 20). Furthermore, proteasomal activity can be modulated in vitro by multiple diverse stimuli such as fatty acids, SDS, or polylysine. The critical role of degradative mechanisms is further highlighted by the large numbers of genes that are transcriptionally expressed during the cell cycle (36). Thus the presence of a mechanism for the direct regulation of proteasomal function in cell cycle control is not surprising. Although reported in vitro studies with Xenopus extracts have shown that proteasomal degradation of a peptide sub-

strate remains constant throughout the cell cycle, we are not aware of any previous reports that describe the regulation of specific proteasomal hydrolytic activities (16).

The signaling pathways and mechanisms responsible for direct regulation of proteasomal function remain unknown. Several different hydrolytic activities have been identified in human proteasomes. The overall structure of the proteasome consists of four stacked rings, with each of the two end rings containing seven α-subunits and the two central rings containing seven β-subunits. The active sites are formed by the amino-terminal threonine residues of the β-subunits, which face a central cavity in this cylindrical particle (22). Analysis of yeast mutants defective in various hydro-

![Graph A](https://via.placeholder.com/150)

**Fig. 6.** Modulation of TGF-β-induced growth inhibition and altered p27 expression. A: representative immunoblot of the protein levels of p27 and β-actin in the presence or absence of 10 ng/ml TGF-β in cells pretreated for 30 min with U-83836E (100 μM) or cycloheximide (CHX; 100 μg/ml). B: cells were treated with 10 ng/ml TGF-β (filled bars) or diluent (open bars) for 24 h in the presence or absence of the antioxidant U-83836E (100 μM) or the protein synthesis inhibitor cycloheximide (100 μg/ml). Quantitative data (means ± SE) of p27 expression relative to β-actin from 3 studies are shown. C: cells were treated as before, and the proliferation index was assessed after 24 h. Data represent means ± SE from 4 separate experiments.

![Graph B](https://via.placeholder.com/150)

![Graph C](https://via.placeholder.com/150)

**Fig. 7.** Inhibition of proteasomal hydrolysis increases p27 expression and inhibits growth. A: cells were treated with the proteasomal inhibitor Chz-Leu-Leu-leucinal (z-LLL-CHO or MG132) for 24 h. Immunoblot analysis was then performed on equivalent amounts of protein with antibodies to p27. A representative immunoblot is shown. B: quantitative immunoblot analysis of p16, p21, and p27 expression in cells treated with 50 nM z-LLL-CHO for 24 h. C: cells were incubated with varying concentrations of z-LLL-CHO, and the proliferation index was assessed after 24 h. The results represent means ± SE from 4 separate experiments.
lyzing activities has revealed that hydrolytic activity is associated with the β-subunits. The α-subunits act as a template for correct β-subunit assembly as well as binding regulatory complexes such as PA28 or REG and PA700, which alter proteasomal proteolytic activities. PA28 is a potent activator that differentially increases peptide hydrolysis by the proteasome. The peptidase activity and substrate specificity of the 20S proteasome can be influenced by association with these regulatory complexes. Thus perturbed proteasomal hydrolysis may result from structural or functional alterations in either α- or β-subunits. Altered expression of the proteasome and associated regulatory proteins has been described in some physiological conditions such as muscle atrophy due to increased rates of global protein degradation (3). Alterations in the intracellular localization of the proteasome have also been observed in a cell cycle-specific manner (2). Thus potential mechanisms of selective regulation of z-LLL-AMC hydrolysis by TGF-β may involve altered expression of proteasomal subunits, regulatory proteins, or altered intracellular distribution of functionally distinct proteasomal complexes.

Multiple studies have demonstrated a relationship between protein oxidation and proteolysis, and the 26S proteasome has been shown to be susceptible to oxidative inactivation (28, 37). The cellular generation of ROI by TGF-β has been shown to involve induction of NADH oxidase in fibroblasts. A plausible mechanism for the involvement of ROI is the inactivation of critical subunits involved in z-LLL-AMC hydrolysis. Alternatively, TGF-β may increase the expression of a specific protein inhibitor that selectively affects specific proteasomal components. TGF-β can directly alter the expression of the β-catalytic proteasome Z subunit (38). However, decreased expression of proteasomal components has not been observed during oxidative impairment of proteasomal function (13, 28). Thus additional studies will be required to ascertain whether the Z subunit participates in the selective hydrolysis of z-LLL-AMC or can be oxidatively modified.

There is considerable emerging evidence that implicates the proteasome in antigen processing. Proteasomal inhibitors can block the generation of class I antigenic peptides and their presentation to cytotoxic lymphocytes. Interferon (IFN)-γ stimulates antigen presentation and has been shown to stimulate the expression of proteasomal regulators PA28 or 11S (REG). IFN-γ and TNF-α also alter the proteasome subunit composition by replacing the X, Y, and Z catalytic β-subunits with other subunits, LMP7, LMP2, and LMP10, respectively (1, 11). These alterations in the β-catalytic subunit composition of the proteasome are expected to alter the hydrolytic activities in a manner that would favor generation of class I antigenic peptides. TGF-β has profound effects as a regulator of the immune system and as a potent suppressor of immune cell activity. Indeed, TGF-β has been implicated in a broad range of pathogenic mechanisms involving primary effects on immune cells (33). However, the specific gene targets mediating TGF-β regulation of immune cell behavior remain poorly understood. We speculate that modulation of proteasomal function may mediate the immunoregulatory effects of TGF-β. Studies to ascertain the role of TGF-β on proteasomal structure and function are warranted to ascertain the potential involvement of the proteasome in immune function regulation by TGF-β. The proteasome plays a role in numerous intracellular processes other than cell growth regulation, and dysregulated proteasomal function has been implicated in some disease states. Thus identification of the precise mechanisms by which specific proteasomal activity is regulated in physiological or pathophysiological settings should provide new insights into diverse cellular processes.

DISCLOSURES

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