Role of the proteasome in the downregulation of transcription factors NFκB and C/EBP in macrophages from tumor hosts

GISELLE PERRY[1], VIYAYA IRAGAVARAPU-CHARYULU[3], EDWARD W. HARHAJ[1,2] and MARTA TORROELLA-KOURI[1,2]

[1]Department of Microbiology and Immunology, University of Miami School of Medicine; [2]Sylvester Cancer Center, Miami, FL 33236; [3]Department of Biomedical Sciences, Florida Atlantic University, Boca Raton, FL 33431, USA

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Abstract. Macrophages from mice bearing advanced mammary tumors are critically impaired in their immune functions, exhibiting reduced expression at the mRNA and protein levels of the crucial transcription factors, nuclear factor κB (NFκB) and CCAAT enhancer binding protein (C/EBP). We have previously shown that tumor-derived factors such as transforming growth factor ß (TGFß) and prostaglandin E_2 (PGE_2) modulate NFκB and C/EBP expression in macrophages. Transcriptional, post-transcriptional, translational and/or post-translational mechanisms may also play a role in altered levels of NFκB and C/EBP in macrophages from tumor hosts, contributing to impaired inflammatory response. One of the post-translational mechanisms that may tune down or recycle proteins in cells is the proteasomal pathway. Since upregulation of ubiquitin/proteasomal pathways has been described under cancer-induced cachexia, we examined the possible role of this proteolytic machinery in the decrease of NFκB and C/EBP proteins in macrophages from tumor hosts. Using MG-132 proteasome inhibitor to block the proteasome machinery in macrophages from normal and tumor-bearing animals we found that macrophages from tumor hosts display higher ubiquitination and proteolysis compared to those from normal mice and also that NFκB and C/EBP downregulation is reversed in these treated cells. Thus, proteasome degradation may contribute, at least in part, to NFκB and C/EBP impairment in macrophages from tumor-bearers.

Introduction

Inflammation and immune suppression are two opposing responses of the immune system linked in different ways to cancer: earlier stages of tumor development are associated with chronic inflammation, while established cancers induce immune suppression (1,2). Macrophages are key players of the inflammatory response and exhibit significant roles in the different stages of tumorigenesis. The release of mutation-inducing free radicals, such as nitrogen and oxygen intermediates as part of their cytotoxic/inflammatory response contributes to tumor initiation (3). Macrophages also play important roles in tumor progression by releasing factors that promote angiogenesis, invasion, extracellular matrix remodeling and metastasis (4). However, while tumor development is modulated by macrophages, the tumor likewise can shape macrophage behavior, with development of macrophages that exhibit immune suppressive traits, enabling tumor progression (5,6).

We have previously shown that peritoneal macrophages from mice with well-established mammary tumors are critically impaired in their immune functions (7-10). These cells exhibit decreased levels of inflammatory cytokines, important defects in the corresponding signaling pathways, increased apoptosis and less differentiation than their counterparts in normal mice (11). One of the most essential deficiencies found in these macrophages is a reduced function of nuclear factor κB (NFκB) and CCAAT enhancer binding protein (C/EBP) transcription factors, likely due to their decreased expression (12). These two molecules are crucial in transcriptional regulation of inflammatory cytokine expression. Furthermore, NFκB, a transcription factor that is constitutively upregulated in tumor cells, is considered to be one of the fundamental molecular links between inflammation and cancer (13). Nevertheless, we demonstrated that in macrophages from tumor-bearing mice NFκB is down-regulated, providing additional evidence for the existence of tumor-induced immune suppression. Furthermore, we showed that this phenomenon is not only valid in macrophages from our D1-DMBA3 mouse mammary tumor-bearers, but we confirmed that it is a more general event present in macrophages from mice with other solid malignancies as well (11).

Among the different factors secreted by the DA-3 cells and other tumors that might modulate NFκB and C/EBP expression, we provided evidence that transforming growth factor ß ($TGFß_1$) and prostaglandin E_2 (PGE_2) are associated with a reduced expression of these transcription factors in

Correspondence to: Dr Marta Torroella-Kouri, University of Miami School of Medicine, P.O. Box 016960 (R-138), Miami, FL 33101, USA
E-mail: mtorroella@med.miami.edu

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macrophages. We have also demonstrated that NFκB and C/EBP are diminished at the mRNA and protein levels in macrophages from tumor hosts (11). Thus, transcriptional, post-transcriptional, translational and/or post-translational mechanisms might explain the decreased expression of NFκB and C/EBP in macrophages of tumor hosts, hence contributing to their impaired inflammatory response.

There are two main protein degradation systems in cells, autophagy, which is a mechanism for bulk protein degradation in lysosomes (14,15) and the proteasomal pathway, which is a mechanism for degradation of individual proteins tagged with polyubiquitin in proteasomes (16). Proteasomes are large protein complexes, located in the nucleus or the cytoplasm inside all eukaryote cells. The main function of the proteasome is to degrade unnecessary or damaged (misfolded) proteins by proteolysis and also to modify or activate proteins. An example of the latter is the proteasomal activation of NFκB by processing p105 into p50 and p100 into p52 via internal proteolysis.

Proteins are generally tagged either for proteasomal degradation or modification by another small protein called ubiquitin. The tagging reaction is catalyzed by enzymes called ubiquitin ligases (17). Once a protein is tagged with a single ubiquitin molecule, this may be a signal to other ligases to attach additional ubiquitin molecules, resulting in a poly-ubiquitin chain. Depending on the type of ligation between ubiquitin molecules, the substrate will be targeted to destruction or modification (18). The overall system of ubiquitination and proteasomal degradation is known as the ubiquitin-proteasome system (UPS). UPS is essential for many cellular processes, including the cell cycle, regulation of gene expression and responses to oxidative stress.

Increased proteasome activity has been implicated in autoimmune syndromes and also in cancer (19,20). Enhanced proteasome activity results in the activation of NFκB. Due to its role in generating the activated form of NFκB, which contributes to anti-apoptotic and pro-inflammatory cytokine expression, proteasomal activity has been associated with inflammatory and autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis. Since upregulation of ubiquitin/proteasomal pathway has also been described under cancer-induced cachexia (21), we decided to examine the possible role of this proteolytic machinery in the decreased expression of NFκB and C/EBP proteins in macrophages from tumor hosts. Our results using MG-132 proteasome inhibitor to block the proteasome machinery in macrophages from normal and tumor-bearing animals suggest that macrophages from tumor hosts display higher ubiquitination and proteolysis than the corresponding cells from normal mice. Also, that proteasome degradation may contribute, at least in part, to NFκB and C/EBP impairment in macrophages from tumor bearers.

Materials and methods

Animals and tumors. BALB/c mice of 10-14 weeks of age, maintained by brother-sister mating in our facilities, were used. The D1-DMBA-3 mammary adenocarcinoma and the DA-3 cell line isolated from the *in vivo* tumor were maintained and used as previously described (22). The Institutional Animal Care and Use Committee approved the animal experiments.

Macrophage collection and culture. Age and gender matched normal mice and 4-week post implantation tumor-bearing mice were injected i.p. with 1.5 ml of 3% thioglycollate (Difco Laboratories, MD). On day 4, peritoneal elicited macrophages from normal animals (N-PEMs) and from tumor-bearers (T-PEMs) were obtained and cultured as previously described (12).

Reagents. RPMI-1640 containing 10% FCS, 2 mM L-gluthamine with 100 U of penicillin and 100 μg/ml of streptomycin (all from Hyclone, UT) was used as complete culture medium in all experiments. Dimethylsulfoxide (DMSO) and TGF-ß signaling inhibitor SB-431542 were purchased from Sigma-Aldrich (St. Louis, MO), whereas recombinant human TGF-$ß_1$ was from Peprotech (Rocky Hill, NJ) and proteasome inhibitor MG-132 was from A.G. Scientific, Inc. (San Diego, CA).

Western blot analysis. N-PEM and T-PEM (10^7) were adhered to plastic tissue culture dishes in plain RPMI for 2 h at 37˚C/5% CO_2, washed and cultured in complete RPMI for the indicated amounts of time, whole cell extracts were obtained and Western blot analysis was performed as previously described (12). Rabbit α-mouse polyclonal antibodies from Santa Cruz Biotechnologies were used as the primary antibodies for NFκB (p50, p65 and c-rel), C/EBP (α and ß) and ubiquitin, whereas goat α-rabbit IgG-HRP was used as the secondary antibody. Filters were stripped using Restore™ Plus Western Blot Stripping Buffer (Thermo Scientific, Waltham, MA) and rabbit α-mouse actin polyclonal antibody (Sigma-Aldrich) was used to standardize the signals.

Treatment of macrophages with TGFß$_1$ and TGF-ß signaling inhibitor SB-431542. Solutions of recombinant TGFß$_1$ and of SB-431542 were freshly prepared before use. Adhered N-PEMs were cultured for 20 h in complete medium containing recombinant TGFß$_1$ (100 ng/ml), TGFß signaling inhibitor SB-431542 (10 μM) and both together. Cells were then lysed, whole cell extracts were obtained and Western blot analysis was performed to detect expression of NFκB.

Treatment of macrophages with proteasome inhibitor MG-132. Adhered N-PEMs and T-PEMs were cultured in complete medium containing freshly prepared MG-132 (25 μM for 20 h) or similar volumes of DMSO. Cells were then lysed, whole cell extracts were obtained and Western blot analysis was performed to detect expression of NFκB, C/EBP and ubiquitin.

Statistical analysis. Paired t-test was used to analyze statistical significance and error bars represent SEM. Differences were considered statistically significant when $P<0.05$.

Results

Pre-treatment of macrophages with TGFß signaling inhibitor partially restores TGFß-induced downregulation of NFκBp65.

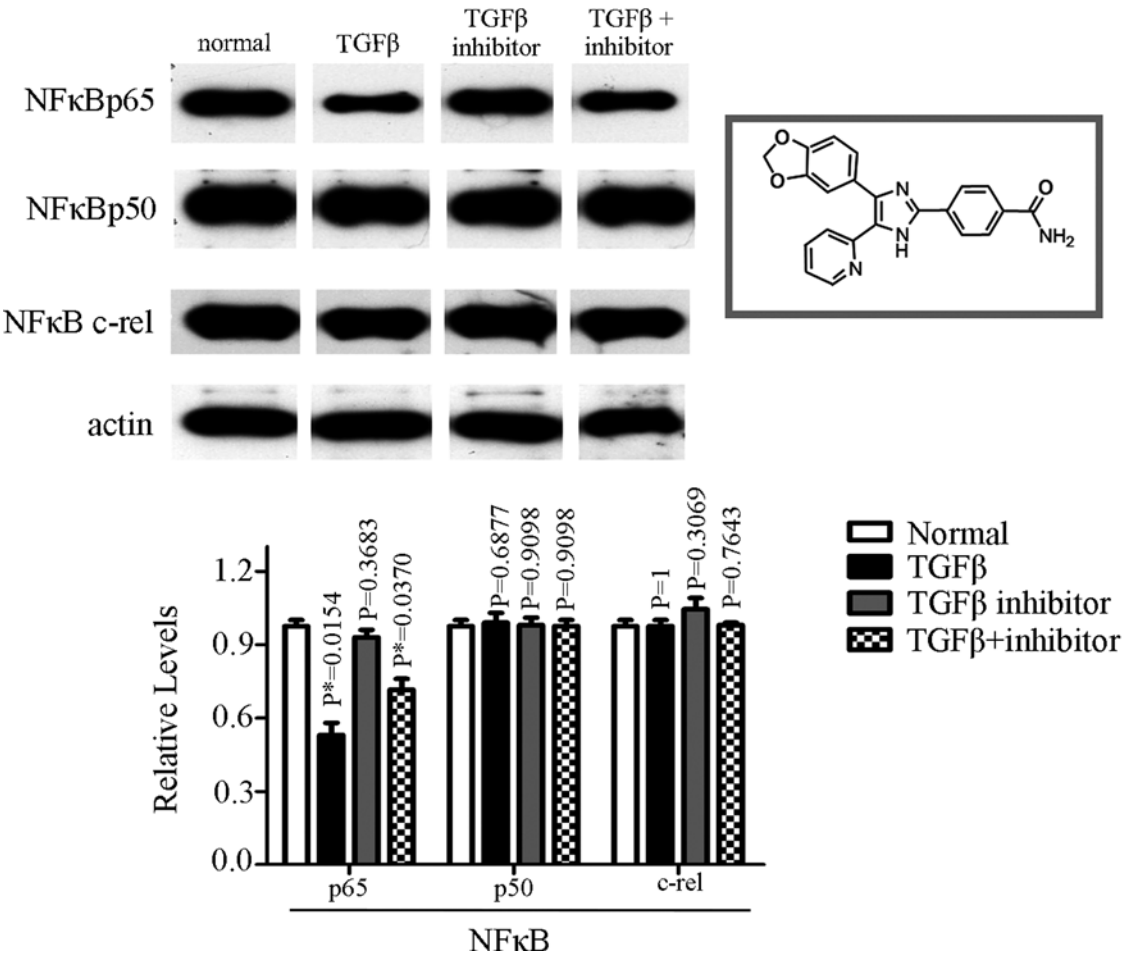

Figure 1. Inhibition of TGF-ß signaling partially reverses TGF-ß-induced NFκBp65 downregulation. Macrophages from normal mice ($10x10^6$) were incubated for 20 h in complete medium containing recombinant $TGFß_1$ (100 ng/ml), $TGFß_1$ signaling inhibitor SB-431542 (10 μM; chemical structure shown) and both together. Cells were then lysed, whole cell extracts were obtained and Western blot analysis was performed to detect expression of NFκB proteins. Values in the densitometry were normalized with ß-actin and represent mean ± SE from three separate experiments (untreated macrophages or controls were arbitrarily assigned a value of 1.0 in all the cases for comparison with treated samples).

Our previous studies demonstrated that *ex vivo* culture of N-PEMs with increasing concentrations of recombinant $TGFß_1$ resulted in NFκBp65 protein downregulation (11). We now examined whether this decreased expression of NFκBp65 could be reversed upon inhibition of TGFß signaling. To do this, we used the small molecule SB-431542 which blocks signaling downstream from TGFß type I receptor (23). As seen in Fig. 1, treatment of N-PEMs with recombinant $TGFß_1$ specifically downregulates NFκBp65 and simultaneous culture of N-PEMs with TGFß and the SB-431542 TGFß signaling inhibitor partially blocks TGFß signaling, resulting in a moderate reversion of NFßBp65 downregulation. As expected, SB-431542 by itself did not modulate NFκB expression. Importantly, viability was not affected by treatment with SB-431542, as assessed by trypan blue exclusion (data not shown).

Increased ubiquitination and proteolysis takes place in macrophages from tumor bearers. As mentioned, upregulation of the ubiquitin-proteasomal pathway has been reported under conditions of cancer-induced cachexia. NFκBp105 is normally processed to its active form NFκBp50 by means of a physiological proteasomal proteolysis mechanism. However, increased and sustained activity of the proteasomal machinery in macrophages from tumor bearers might also result in abnormal degradation of NFκB and other proteins. To verify if indeed there is enhanced ubiquitination and proteolysis in T-PEMs, we treated N-PEMs and T-PEMs with and without the proteasome inhibitor MG-132 and studied the resulting expression of ubiquitin in these cells. When comparing untreated N-PEMs with untreated T-PEMs, our results indicate (Fig. 2) that higher proteolysis is occurring in T-PEMs, as revealed by lesser amounts of polyubiquitin in T-PEMs, suggesting that it is being more rapidly degraded together with the ubiquitin-tagged proteins by the actively functioning proteasome complex in these cells. On the other hand, when comparing the two cell types upon treatment with the MG-132 proteasome inhibitor, increased amounts of accumulated polyubiquitin in T-PEMs suggest enhanced ubiquitination (ubiquitin accumulation) in T-PEMs, due to a block in proteolysis.

Augmented proteasome activity in macrophages from tumor-bearers may account for NFκB and C/EBP downregulation in these cells. We hypothesized that increased proteasomal activity in T-PEMs may contribute to the diminished levels

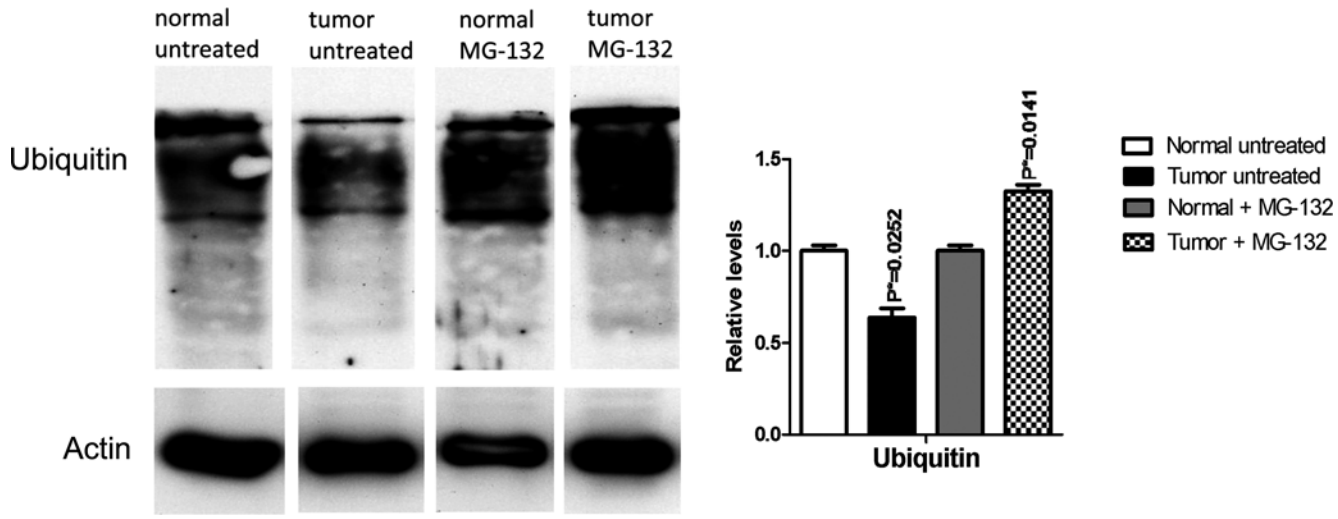

Figure 2. Increased ubiquitination and proteolysis occur in macrophages from tumor bearing mice. Macrophages from normal and 4-week tumor bearing mice ($10x10^6$) were incubated for 20 h in complete medium containing 25 μM of the proteasome inhibitor MG-132 or similar volumes of DMSO. Cells were lysed, whole cell extracts were obtained and Western blot analysis was performed to detect expression of ubiquitin. Densitometry values were normalized with ß-actin and represent mean ± SE from three separate experiments (normal untreated and treated macrophages were arbitrarily assigned a value of 1.0 for comparison with tumor samples).

of NFκB and C/EBP proteins in these cells. To test this idea we treated N-PEMs and T-PEMs with the proteasome inhibitor MG-132 and examined the expression of the different NFκB and C/EBP proteins in these two macrophage populations. As Fig. 3 shows, the reduced expression of NFκB (p50, p65 and c-rel) and C/EBP (α and ß) observed in resting T-PEMs is significantly reversed in all cases upon their culture with MG-132, although the effect is especially observed for NFκB p65, c-rel and C/EBPα. This may suggest that enhanced proteasome-induced proteolysis plays an important role in NFκB and C/EBP deficiencies in T-PEMs, but that additional mechanisms may be also important in NFκBp50 and C/EBPß impairments in these cells. Interestingly, with the exception of NFκBp50, there is increased NFκB and C/EBP protein expression in MG-132-treated N-PEMs as well, suggesting that a certain amount of proteolysis also occurs in macrophages from normal mice, although the proteasome effect is much more amplified in T-PEMs, likely accounting for a great deal of the transcription factor protein defect in these cells. There were no differences in NFκB and C/EBP expression in macrophages grown in complete media as compared to cells grown in DMSO. Cell viability was not affected by treatment with MG-132 (results not shown).

Discussion

Proteolytic degradation of eukaryotic cell proteins by the UPS is the result of a highly complex and tightly controlled series of reactions that are central to the regulation of basic cellular features, such as development, differentiation, proliferation, cell cycling, gene expression, signal transduction, apoptosis and senescence, as well as antigen presentation, inflammation and stress response. This process is carried out by a complex cascade of enzymes and displays a high degree of specificity towards its numerous substrates. Non-proteolytic post-translational modifications of proteins represent another layer of regulation of cellular homeostasis of central importance. In addition, the UPS functions as a quality control mechanism that selectively removes abnormal and damaged proteins, which would otherwise form toxic intracellular inclusions as seen in various neurodegenerative diseases. The UPS has been identified as the cell's major tool for extralysosomal cytosolic and nuclear protein degradation. The 26S proteasome, a large multicatalytic multisubunit protease complex, constitutes the central proteolytic machinery of the UPS and is responsible for the degradation and proteolytic processing of many different cell proteins. Cell proteins destined to undergo processing by the UPS must be targeted for recognition and subsequent degradation by the 26S proteasome (24) by covalent attachments of multiple monomers of the 76 amino acid, 8.5 kDa polypeptide ubiquitin, which is highly conserved and present in the cytoplasm of all eukaryotic cells. Proteins are rapidly degraded into small peptides in the proteasome and ubiquitin molecules are cleaved off the protein immediately prior to destruction and are recycled for further use.

The most prominent role of ubiquitin is labeling proteins for proteasomal degradation, although modification to modulate stability, function and intracellular localization of a wide variety of proteins synthesized as inactive precursors are also regulated by this molecule. A prerequisite for the selective degradation of intracellular proteins by the UPS is the attachment of a chain of ubiquitin monomers to the targeted protein. Polyubiquitinated proteins with the poly-merization at ubiqutin lysine 48 are targeted for degradation by the proteolytic 26s proteasome, where proteins are cut progressively into small peptides of 6 to 12 amino acids that are subsequently released and rapidly hydrolysed to amino acids by cytosolic exopeptidases. The release of ubiquitin from the substrate protein makes ubiquitin available for recycling in the proteolytic pathway.

One would expect that aberrations in such a complex system may be implicated in the pathogenesis of many diseases. In view of this complexity, it is not surprising that the UPS can undergo substantial deregulation that contributes

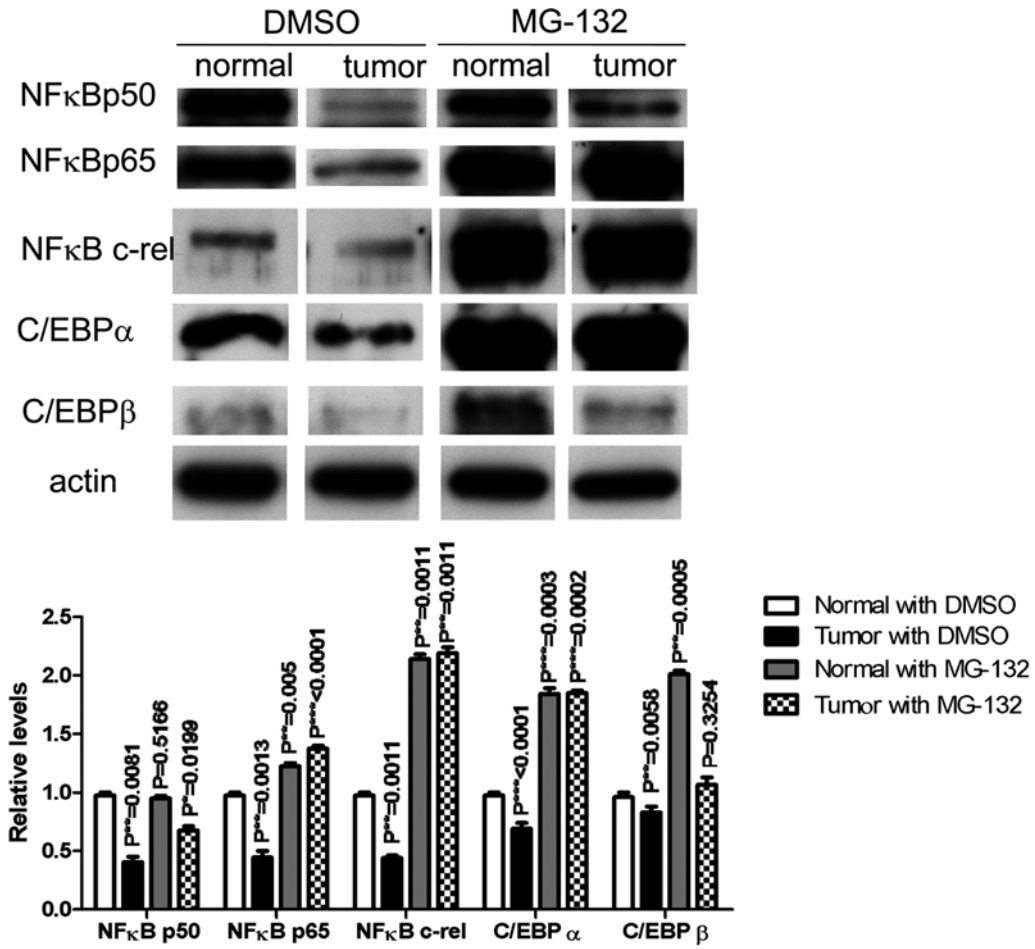

Figure 3. Enhanced proteasomal-induced proteolysis may account for NFκB and C/EBP deficit in macrophages from tumor hosts. Macrophages from normal and 4-week tumor-bearing mice ($10x10^6$) were incubated for 20 h in complete medium containing 25 μM of the proteasome inhibitor MG-132 or similar volumes of DMSO. Cells were lysed, whole cell extracts were obtained and Western blot analysis was performed to detect expression of NFκB and C/EBP. Densitometry values were normalized with ß-actin and represent mean ± SE from three separate experiments (normal DMSO-treated macrophages were arbitrarily assigned a value of 1.0 for comparison with other samples).

to the pathogenesis of various human diseases, such as cancer, neurodegenerative, autoimmune, genetic and metabolic disorders. Importantly, the findings that inhibition of proteasome activity induces apoptosis selectively in cancer cells and interferes with essential functions of immune cells (24) have led to the exploitation of the UPS as a molecular target for cancer therapy and immune modulation. On the other hand, proteasome inhibitors represent a powerful tool for dissecting the role of the UPS in cellular physiology and also have practical applications in cancer therapy (25). They have already played an essential role in advancing our understanding of importance of the proteasome in biology.

We have previously shown that macrophages from tumor-bearing mice have impaired gene and protein expression of NFκB and C/EBP and that tumor-secreted TGF-ß is associated with these deficiencies (11,12). Among other mechanisms that might explain these defects, we hypothesized that enhanced proteasomal degradation in macrophages from tumor hosts may contribute to this effect. To provide additional evidence of the role of TGF-ß in abating NFκB protein expression, we treated macrophages from normal mice with recombinant TGF-$ß_1$ and the TGF-ß signaling inhibitor SB-431542, and measured NFκB proteins in normal macrophages. Small molecules, such as SB-431542, are unlikely to completely block signaling (26), thus it was not surprising to realize that the simultaneous culture with TGF-$ß_1$ together with SB-431542 could only partially reverse NFκBp65 down-regulation. Recent studies of the Smad family proteins, which are the key signal transducers of the TGF-ß family ligands, have revealed the ability of Smads to interact with various components of the 26S proteasome system (27). In breast cancer cell lines, TGF-ß increases the activity of the proteasome (28). We speculate that TGF-ß-induced modulation of proteasomal function may in part mediate the immunosuppressive effects of TGF-ß in macrophages by accelerating degradation of inflammatory mediators . Further studies to elucidate the role of TGF-ß on proteasome function in macrophages are required to assess the potential involvement of the proteasome in immune regulation by TGF-ß, particularly as it regards to increased NFκB and C/EBP proteolysis.

To examine the consequences of inhibiting proteasome activity in macrophages from tumor hosts, we used MG-132, a peptide aldehyde proteasome inhibitor. Our results show that upon blocking proteasome activation in macrophages from tumor-bearing animals, excess ubiquitination and proteolysis were inhibited and the impaired NFκB and C/EBP protein

expression was restored in the dysfunctional cells. Importantly, however, even after proteasome inhibition, NFκBp50 and C/EBPß remain downregulated in macrophages from tumor-bearers, suggesting the existence of additional mechanisms of transcriptional, post-transcriptional or even translational nature, accounting for the deficit of these two proteins in macrophages from tumor-bearing mice. Interestingly, a less pronounced effect is also observed in macrophages from normal mice, which exhibit increased protein expression upon proteasome inhibition in all the cases except with NFκBp50. A certain level of NFκB and C/EBP proteolysis may normally exist as a homeostatic mechanism for breakdown and recycling of intracellular proteins in macrophages. However, our results suggest that this process is remarkably accelerated in macrophages from tumor hosts, probably due to the presence of tumor-derived factors such as TGFß.

The proteasome is also critical to the regulation of LPS-induced signaling in macrophages, controlling functions such as TLR signaling and thus NFκB activation. Hence, proteasome inhibition results in a conversion to an anti-inflammatory phenotype in LPS-activated macrophages (29). If this is the case, our data implicate that proteasome inhibition in resting macrophages, particularly the ones from tumor-bearers, results in an opposite phenotype to that reported with proteasome inhibition in LPS-activated macrophages, i.e., resting macrophages, especially from tumor-bearers, exhibit enhanced UPS activity and increased proteolysis of several proteins such as the pro-inflammatory NFκB and C/EBP, thus, proteasome inhibition in resting macrophages results in a pro-inflammatory phenotype.

Macrophages infected by Leishmania have STAT1 degraded by means of the UPS (30). Similar to that observed with parasites, tumors may subvert the microbicidal or inflammatory functions of macrophages to promote their own survival and propagation. We have shown that not only NFκB and C/EBP but also several other inflammatory cytokines and signaling intermediates such as STAT1, MAK kinases and IRAK1 are impaired in macrophages from tumor hosts (11). It will be important to examine whether the abnormal increase in ubiquitination and proteolysis that we have shown here in macrophages from tumor hosts might explain the deficit in some of these other proteins as well. The use of proteasome inhibitors will then be extremely useful to reverse immune suppression in dysfunctional macrophages from tumor hosts.

Acknowledgements

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References

1. Balkwill F and Mantovani A: Inflammation and cancer: back to Virchow? Lancet 357: 539-545, 2001.
2. North RJ: Down-regulation of the antitumor immune response. Adv Cancer Res 45: 1-43, 1985.
3. Balkwill F, Charles KA and Mantovani A: Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell 7: 211-217, 2005.
4. Pollard JW: Tumour-educated macrophages promote tumour progression and metastasis. Nat Rev Cancer 4: 71-78, 2004.
5. Biswas SK, Sica A and Lewis CE: Plasticity of macrophage function during tumor progression: regulation by distinct molecular mechanisms. J Immunol 180: 2011-2017, 2008.
6. Mantovani A, Sozzani S, Locati M, Allavena P and Sica A: Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol 23: 549-555, 2002.
7. Torroella-Kouri M, Keith JC, Ivanova M and Lopez DM: IL-11-induced reduction of C/EBP transcription factor binding may contribute to the IL-12 downregulation in tumor-bearing mice. Int J Oncol 22: 439-448, 2003.
8. Dinapoli MR, Torroella-Kouri M, Perry G and Lopez DM: Diminished PKC activity and decreased binding of transcription factors are involved in the impaired production of nitric oxide by macrophages from tumor-bearing mice. Int J Mol Med 15: 503-512, 2005.
9. Handel-Fernandez ME, Cheng X, Herbert LM and Lopez DM: Down-regulation of IL-12, not a shift from a T helper-1 to a T helper-2 phenotype, is responsible for impaired IFN-gamma production in mammary tumor-bearing mice. J Immunol 158: 280-286, 1997.
10. DiNapoli MR, Calderon CL and Lopez DM: The altered tumoricidal capacity of macrophages isolated from tumor-bearing mice is related to reduce expression of the inducible nitric oxide synthase gene. J Exp Med 183: 1323-1329, 1996.
11. Torroella-Kouri M, Silvera R, Rodriguez D, Caso R, Shatry A, Opiela S, Ilkovitch D, Schwendener RA, Iragavarapu-Charyulu V, Cardentey Y, Strbo N and Lopez DM: Identification of a subpopulation of macrophages in mammary tumor-Bearing mice that are neither M1 nor M2 and are less differentiated. Cancer Res 69: 4800-4809, 2009.
12. Torroella-Kouri M, Ma X, Perry G, Ivanova M, Cejas PJ, Owen JL, Iragavarapu-Charyulu V and Lopez DM: Diminished expression of transcription factors nuclear factor kappaB and CCAAT/enhancer binding protein underlies a novel tumor evasion mechanism affecting macrophages of mammary tumor-bearing mice. Cancer Res 65: 10578-10584, 2005.
13. Karin M: Nuclear factor-kappaB in cancer development and progression. Nature 441: 431-436, 2006.
14. Nakatogawa H, Suzuki K, Kamada Y and Ohsumi Y: Dynamics and diversity in autophagy mechanisms: lessons from yeast. Nat Rev Mol Cell Biol 10: 458-467, 2009.
15. White E and DiPaola RS: The double-edged sword of autophagy modulation in cancer. Clin Cancer Res 15: 5308-5316, 2009.
16. Jung T, Catalgol B and Grune T: The proteasomal system. Mol Aspects Med 30: 191-296, 2009.
17. Tsukamoto S and Yokosawa H: Targeting the proteasome pathway. Expert Opin Ther Targets 13: 605-621, 2009.
18. Sun F, Kanthasamy A, Anantharam V and Kanthasamy AG: Mitochondrial accumulation of polyubiquitinated proteins and differential regulation of apoptosis by polyubiquitination sites Lys-48 and -63. J Cell Mol Med (In press).
19. Arlt A, Bauer I, Schafmayer C, Tepel J, Muerkoster SS, Brosch M, Roder C, Kalthoff H, Hampe J, Moyer MP, Folsch UR and Schafer H: Increased proteasome subunit protein expression and proteasome activity in colon cancer relate to an enhanced activation of nuclear factor E2-related factor 2 (Nrf2). Oncogene (In press).
20. Fissolo N, Kraus M, Reich M, Ayturan M, Overkleeft H, Driessen C and Weissert R: Dual inhibition of proteasomal and lysosomal proteolysis ameliorates autoimmune central nervous system inflammation. Eur J Immunol 38: 2401-2411, 2008.
21. Muscaritoli M, Bossola M, Battista Doglietto G and Rossi Fanelli F (eds): The ubiquitin/proteasome system in cancer cachexia. In: Cachexia and Wasting, A Modern Approach. Springer-Verlag, 2006.
22. Medina D and DeOme KB: Response of hyperplastic alveolar nodule outgrowth-line D1 to mammary tumor virus, nodule-inducing virus, and prolonged hormonal stimulation acting singly and in combination. J Natl Cancer Inst 42: 303-310, 1969.
23. Waghabi MC, de Souza EM, de Oliveira GM, Keramidas M, Feige JJ, Araujo-Jorge TC and Bailly S: Pharmacological inhibition of transforming growth factor beta signalling decreases infection and prevents heart damage in acute chagas disease. Antimicrob Agents Chemother (In press).

24. Berges C, Haberstock H, Fuchs D, Sadeghi M, Opelz G, Daniel V and Naujokat C: Proteasome inhibition activates the mitochondrial pathway of apoptosis in human CD4(+) T cells. J Cell Biochem (In press).
25. Vaziri SA, Grabowski DR, Hill J, Rybicki LR, Burk R, Bukowski RM, Ganapathi MK and Ganapathi R: Inhibition of proteasome activity by bortezomib in renal cancer cells is p53 dependent and VHL independent. Anticancer Res 29: 2961-2969, 2009.
26. Hjelmeland MD, Hjelmeland AB, Sathornsumetee S, Reese ED, Herbstreith MH, Laping NJ, Friedman HS, Bigner DD, Wang XF and Rich JN: SB-431542, a small molecule transforming growth factor-beta-receptor antagonist, inhibits human glioma cell line proliferation and motility. Mol Cancer Ther 3: 737-745, 2004.
27. Wang T: The 26S proteasome system in the signaling pathways of TGF-beta superfamily. Front Biosci 8: d1109-d1127, 2003.
28. Petrel TA and Brueggemeier RW: Increased proteasome-dependent degradation of estrogen receptor-alpha by TGF-beta1 in breast cancer cell lines. J Cell Biochem 88: 181-190, 2003.
29. Cuschieri J, Gourlay D, Garcia I, Jelacic S and Maier RV: Implications of proteasome inhibition: an enhanced macrophage phenotype. Cell Immunol 227: 140-147, 2004.
30. Forget G, Gregory DJ and Olivier M: Proteasome-mediated degradation of STAT1alpha following infection of macrophages with Leishmania donovani. J Biol Chem 280: 30542-30549, 2005.