likely to explain side effects of specific treatment elements and is important as well. It was beyond the scope of this study and will be extremely challenging, but one wonders if similar findings will be made if minimal residual leukemic cells are studied.

Meanwhile, this study encourages similar efforts focusing on other drugs. Given the large interindividual variation in drug sensitivity, the complicated biological processes that determine their antileukemic activity and toxicity, and their importance in the treatment of childhood acute leukemias, obvious candidates are cytarabine, l-asparaginase, and glucocorticoids.5-7 The understanding of the efficacy of other drugs and in a wide range of cancers will also benefit from this type of integrated research. In the longer term, this approach is not feasible for routine use in multicenter clinical studies. Therefore, customized chips should be developed, which likely need to be disease- and treatment-specific. Ultimately, this type of research will enable more individualized and tailored chemotherapy of cancer, aiming at more effective and less toxic therapy.

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Comment on Zapata et al, page 4595

TRAF3 in B cells: too much, too little, too bad

P. Leif Bergsagel  MAYO CLINIC

In this issue of Blood, Zapata and colleagues investigate a novel transgenic mouse model overexpressing TRAF3 in B cells and surprisingly find plasmacytosis and hypergammaglobulinemia, even though TRAF3 is reported to be a tumor suppressor gene in MM.

Homozygous inactivation of tumor necrosis factor (TNF)–receptor-associated factor 3 (TRAF3) was recently reported in 13% of multiple myeloma (MM) patients.1,2 It has been found to be a negative regulator of NIK, which, as expected, is elevated in MM with TRAF3 inactivation. NIK is a key enzyme regulating processing of NFKB2 p100 to p52 that dimerizes with relB and results in transcriptional activation of NFKB targets. Highlighting the importance of NIK stabilization, additional mutations with similar functional consequences occur in MM, including biallelic deletions encompassing both cIAP1 and cIAP2, as well as biallelic deletions of TRAF2, all contributing together with TRAF3 to form a NIK negative regulatory complex.3 Based on constitutive p100 processing, and the presence of nuclear p52-relB complexes in TRAF2 or TRAF3 deficient B cells, they have been described as “inhibitors” of the alternative NF-kB pathway.4 Whereas tumors with dysregulated overexpression of TRAF3 have not been reported, amplification and overexpression of cIAP1/2 occurs in various tumors and has been shown to be critical in liver cancer.5

Consistently, mice with conditional inactivation of TRAF3 in B cells develop splenomegaly and lymphadenopathy due to B-cell expansion, and hypergammaglobulinemia with increased plasma cell numbers.6 On the other hand, the TRAF3 transgenic mice described in the current report have lower levels of NIK with splenic hypotrophy and reduced numbers of B cells.7 The unexpected finding, however, is that elevated TRAF3 in these mice also results in hypergammaglobulinemia and plasmacytosis. Zapata et al have shown that this phenotype is not due to increased B-cell proliferation, but suggest it may be the result of heightened Toll-like receptor (TLR)–mediated B-cell differentiation. Interestingly, the mice develop a high incidence of solid tumors (67%) with age, primarily squamous cell carcinoma of the tongue, which the authors suggest may result from the chronic inflammation present in these mice. As noted, this represents the first model of spontaneous carcinogenesis initiated by B-cell mediated chronic inflammation. A potential limitation of these studies is that only a single transgenic founder line was examined in detail, and the phenotype may potentially relate to the site of transgene insertion in this founder.

Additional studies of these fascinating mice will be required to better understand the mechanisms underlying the many surprising observations these mice afford.

Conflict-of-interest disclosure: The author declares no competing financial interests.
RNA into the language of protein.1 RDPs generate a formidable 2 million proteins to collectively refer to as the 20S proteasome.2 These 4 rings are flanked on top and on the bottom by the 14 distinct -subunit rings. In fact, of the 14 distinct -subunits that comprise the 20S proteasome (a-1, a-2, ..., a-7 and b-1, b-2, ..., b-7), only 3 of these subunits contain true protease function, namely the b-1, b-2, and b-5 subunits. These functional threonyne proteases are unique among the vast numbers of different proteases found in the cytosol as the 20S proteases are processive in nature, catalyzing more than one cleavage site per substrate. With their active sites oriented inward toward the lumen of the 20S core, they cleave all recognized sites within a single substrate, generating enormous quantities of oligopeptides. These proteases are characterized by their distinctly different proteolytic functions (ie, different cleavage sites) and are referred to as the chymotrypsin-like (b-1), trypsin-like (b-2), and peptidylglutamyl-peptide hydrolyzing (PGPH; b-5) activities, respectively. The latter of these enzymatic functions is also referred to as the postacidic or caspase-like activity.

Upon stimulation with interferon- (IFN-) or tumor necrosis factor- (TNF-), a change occurs in the protease subunit composition, leading to replacement of the b-1, b-2, and b-5 subunits with new proteolytically distinct subunits called the LMP or bi-subunits. While each of these bi-subunits is genetically homologous to a specific constitutively expressed b-subunit, and can be readily inserted in place of the standard b-1, b-2, and b-5 subunits, it possesses a distinctly different proteolytic function, cleaving proteins at different cleavage sites. Specifically, the b1i/LMP2 replaces the constitutively expressed b1-homolog, the b2i/LMP10 replaces the b2 homolog, and the b5i/LMP7 replaces the b5 homolog. In addition to the replacement of the b-subunits, there is a different regulatory subunit referred to as 11S or PA28. The complete replacement of the b-subunits with the bi-subunits, and replacement of the 19S cap with the PA28 regulator now creates a unique structure often referred to as the immunoproteasome.

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**LYMPHOID NEOPLASIA**

Comment on Kuhn et al, page 4667

**Nimble micromanagement of macromolecules**

Owen A. O’Connor COLUMBIA UNIVERSITY

Intracellular protein homeostasis is managed by a complex series of enzymatic reactions, the terminal steps of which are catalyzed by dynamic protein aggregates called proteasomes, and more recently, immunoproteasomes.

The sheer enormity of the numbers defies comprehension, dwarfing even the most aggressive congressional proposal to bail out the economy. Eighty to 90% of all intracellular protein is degraded through the ubiquitin–proteasome pathway. Of these proteins, the proteasome degrades about 500,000 different “slowly degraded protein” (or SDPs) substrates per minute, per cell. That would be 3 x 10^7 protein substrates per hour in a single cell. Interestingly, about 20% to 30% of all cellular protein synthesized in mammalian cells is degraded with a half-life of less than 10 minutes. These proteins are generally referred to as rapidly degraded polypeptides (or RDPS). A major fraction of RDPS includes defective ribosomal products (often referred to as DRPs). DRPs are defective proteins arising from the sometimes less than perfect process of translating genetic information from RNA into the language of protein.3 RDPS generate another 1.3 x 10^6 substrates degraded by the proteasome per minute, per cell. Collectively, the management of both SDPs and the RDPS generates over 2 million proteins to be degraded by the proteasome, per minute, per cell. This remarkable level of proteolytic activity produces more than 10^9 oligopeptides per minute per cell. These oligopeptides are then metabolized by endopeptidases and aminopeptidases to regenerate the pool of amino acids required for protein biogenesis, a process that transpires in only seconds.

The proteasome is a distinctive aggregate of globular proteins arranged in 4 symmetrical rings, each consisting of 7 a- and 7 b-subunits. The 4 rings form a barrel-like structure with a central lumen. The barrel is flanked on top and on the bottom by the a-subunit ring while the 2 inner rings compose the b-subunit rings. These 4 rings are collectively referred to as the 20S proteasome. They house the proteases responsible for the catalytic functions of the proteasome and may, by itself, contribute to the degradation of nonubiquitylated substrates. The degradation of ubiquitylated substrates requires the addition of the 19S regulators to the 2 ends of the 20S proteasome, generating the 26S proteasome. The 19S regulator is also an aggregate of proteins that performs a number of vital functions. This regulator mediates the deubiquitylation of protein substrates, unfolds proteins from their complex tertiary structure into their primary structure, and then threads that unfolded, linearized protein into the central lumen of the 20S core. The core is where the protein meets its final fate, being degraded into smaller oligopeptides typically ranging in size from 3 to 23 amino acids. The process, as one might imagine, is fairly energy intensive, requiring the hydrolysis of ATP via ATPases integral to the 19S regulator.

The critical function of the proteasome, of course, is to degrade proteins back into the simpler building blocks from which they were assembled. This process is catalyzed through the function of discrete proteases embedded in the b-subunit rings. In fact, of the 14 distinct subunits that comprise the 20S proteasome (a-1, a-2, ..., a-7 and b-1, b-2, ..., b-7), only 3 of these subunits contain true protease function, namely the b-1, b-2, and b-5 subunits. These functional threonyne proteases are unique among the vast numbers of different proteases found in the cytosol as the 20S proteases are processive in nature, catalyzing more than one cleavage site per substrate. With their active sites oriented inward toward the lumen of the 20S core, they cleave all recognized sites within a single substrate, generating enormous quantities of oligopeptides. These proteases are characterized by their distinctly different proteolytic functions (ie, different cleavage sites) and are referred to as the chymotrypsin-like (b-1), trypsin-like (b-2), and peptidylglutamyl-peptide hydrolyzing (PGPH; b-5) activities, respectively. The latter of these enzymatic functions is also referred to as the postacidic or caspase-like activity.

Upon stimulation with interferon- (IFN-γ) or tumor necrosis factor- (TNF-α), a change occurs in the protease subunit composition, leading to replacement of the b-1, b-2, and b-5 subunits with new proteolytically distinct subunits called the LMP or bi-subunits. While each of these bi-subunits is genetically homologous to a specific constitutively expressed b-subunit, and can be readily inserted in place of the standard b-1, b-2, and b-5 subunits, it possesses a distinctly different proteolytic function, cleaving proteins at different cleavage sites. Specifically, the b1i/LMP2 replaces the constitutively expressed b1-homolog, the b2i/LMP10 replaces the b2 homolog, and the b5i/LMP7 replaces the b5 homolog. In addition to the replacement of the b-subunits, there is a different regulatory subunit referred to as 11S or PA28. The complete replacement of the b-subunits with the bi-subunits, and replacement of the 19S cap with the PA28 regulator now creates a unique structure often referred to as the immunoproteasome.
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