

INTERVIEW about

Immunomodulatory effects
of proteasome inhibitors

with

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What is known about proteasome inhibitors?

Alessio Nencioni & Peter Brossart: Proteasome inhibitors are small molecule compounds that inhibit the proteolytic activity of the proteasome, an intracellular multiprotein complex deputed to the degradation of ubiquitinated proteins (1,2). Protein degradation via the proteasome plays a key role in numerous cellular activities including antigen processing, cell cycle regulation, signal transduction, and cell death control. Therefore proteasome inhibition has important repercussions on all of these functions. Numerous proteasome inhibitors are presently available (1). Most of these compounds specifically inhibit the chymotrypsin-like activity of the proteasome while minimally affecting the trypsin- and the caspase-like activities. Some of them act as irreversible inhibitors whereas others such as bortezomib (Velcade, formerly PS-341) act in a reversible fashion. Few proteasome inhibitors

have already entered clinical studies mostly for the treatment of cancerous disorders and the Food and Drug Administration has recently approved bortezomib for multiple myeloma patients who have received at least one previous line of therapy (2,3). How exactly proteasome inhibitors exert their antitumor activity remains unclear. Inhibition of NF- κ B through impeded I κ B α degradation has long been invoked as a key effect (1,2). However, other mechanisms are also likely to come into play (1,2,4,5). In particular, it has recently been observed that mature plasma cells exhibit reduced proteasome activity due to proteasome downregulation, which makes these cells more susceptible to proteasome inhibitor-induced apoptosis (6). If this were also true for malignant plasma cells, such a phenomenon would proffer a nice explanation for the anti-myeloma activity of bortezomib.

Do proteasome inhibitors have effects on the immune system?

Alessio Nencioni & Peter Brossart: Proteasome inhibitors have the potential to interfere with several aspects of an immune response. Antigen processing for presentation on MHC class I molecules requires proteasome activity, although other proteases participate in this process and may be able to partially substitute for impaired proteasome activity (7). In addition, ubiquitination and proteasomal degradation play a key role in NF- κ B signaling, which mediates numerous aspects of immune cell activation. Finally, cell cycle, cell migration, and apoptosis in immune cells also use the proteasome for the regulated turnover of their components.

Indeed, numerous studies in the mouse model suggest that proteasome inhibitors may have

anti-inflammatory and immunosuppressive effects *in vivo*. Proteasome inhibitors alleviate inflammation and immune-mediated tissue damage in different models of immune disorders including allograft rejection, colitis, psoriasis, diabetes, experimental autoimmune encephalomyelitis and arthritis (8-10). The one exception in this scenario is represented by graft-versus-host disease (GVHD), where inflammation in the intestine is exacerbated by bortezomib when the inhibitor is given starting from a few days (3 to 5) after the transplant or over a prolonged period (11,12). Conversely, this effect is not observed when the drug is given immediately after transplant as in this case inflammation actually improves. The reasons for this unexpected outcome are

unclear but appear to be unrelated to NF- κ B inhibition (12).

At the cellular level, proteasome inhibitors were shown to inhibit proliferation and cytokine secretion by T lymphocytes and to induce T cell apoptosis (13). These effects may become more pronounced in activated vs. resting T cells due to the increased dependence on NF- κ B activation of the formers. Proteasome inhibitors' effects on B cells are less well defined and may include induction of cell death upon exposure to high inhibitor concentrations.

Our studies have focused on the effects of proteasome inhibition in dendritic cells (DCs), specialized antigen-presenting cells that present antigens to T lymphocytes and initiate antigen-specific immune response (14,15). We found that proteasome inhibition strongly affects both DC function and survival. Bortezomib prevents the upregulation of co-stimulatory molecules and the increase in migratory and immunostimulatory capacity that is normally

triggered by lipopolysaccharide (LPS) or by endogenous cytokines such as TNF- α and CD40-ligand. Cytokine secretion (including IL-12 and TNF- α) in response to LPS is also reduced by bortezomib. At the molecular level, the proteasome inhibitor blocks LPS-induced nuclear translocation of the NF- κ B protein RelB, which is crucial for DC differentiation and function. In addition, bortezomib also affects activation of Erk and of the interferon response factors IRF-3 and IRF-8 through mechanisms that remain unclear. Finally, when given at higher concentrations (>1 ng/ml), Velcade also induces apoptosis in DCs, possibly by activating the mitochondrial apoptosis pathway and the caspase cascade (15).

All that being said, whether proteasome inhibitors actually affect antigen presentation and overall immune function in humans *in vivo* remains unclear. Here, hints for an immunomodulatory activity of proteasome inhibitors include the induction of lymphopenia in a fraction of the patients treated with bortezomib, and cases of Herpes Zoster re-activation following bortezomib administration, particularly in patients who were given the proteasome inhibitor after bone marrow transplantation (16-19). Future clinical studies will hopefully shed more light on the effects of proteasome inhibitors on the human immune system.

Do you believe that proteasome inhibitors can be used for therapeutic purposes in immune disorders?

Alessio Nencioni & Peter Brossart: Given the promising results obtained in the preclinical studies it is probable that proteasome inhibitors will enter clinical trials for autoimmune and inflammatory diseases. In particular, the effects of these compounds on plasma cells may make them good

candidates for the treatment of disorders where tissue damage is mediated through antibody production (auto-antibodies, immune complexes). On the other hand, the usefulness of proteasome inhibitors in the treatment of GVHD is questionable given the risk of GVHD exacerbation.

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