Editorial

The proteasome system: a neglected tool for improvement of novel therapeutic strategies?

In this decade the proteasome system has clearly established itself as being the central cellular enzymatic system which, at many different levels, regulates the cellular metabolism and cell fate by proteolysis. This is achieved by highly sophisticated signalling mechanisms such as ubiquitinylation of substrates, phosphorylation, dephosphorylation, controlled substrate binding, activation etc, which allow the tight control of this multi-component (consisting of more than 50 different proteins) multi-catalytic enzyme system and as such the turnover or activation of proteins.¹⁻⁴

Among the many processes for which proteasome function has been firmly established, and I name only the most prominent ones, are cell proliferation or cell cycle control, the activation of the transcription factor NF κ B which is involved in the inflammatory response and MHC class I antigen processing as part of the cellular immune surveillance system. Just mentioning these three examples, it becomes evident that the proteasome system plays a key role in inflammatory events, tumorigenesis, chronic infectious diseases as well as disorders which are caused by either enhanced or partially impaired cellular protein breakdown such as autoimmune diabetes and neurodegenerative diseases.

One can therefore imagine that by supporting or interfering with functions of the proteasome system one ought to be able to challenge various disorders at a central point of their control. If so, one may ask whether one has to take the proteasome system into consideration for therapeutic strategies and whether the proteasome system is suitable for acting as drug target? As one may expect for such a complex biological system the answers to these questions are not straightforward and are, based on our present still limited but rapidly increasing knowledge, in fact, yes and no. During the past few years various types of more or less proteasome-specific peptide aldehyde inhibitors have been developed which penetrate the plasma membrane and act by binding to the six active sites within the lumen of the proteolytically active 20S proteasome core.¹ Of all the inhibitors available so far the fungus-derived β -lacton lactacystin is the most specific inhibitor. With the help of such inhibitors one can stop cell proliferation at the G2 stage, down-regulate NF κ B activation or even impair antigen presentation. Thus, some of the inhibitors are already being used in preclinical trials for tumor therapy. While all this appears to be encouraging the major drawback of this first generation

of proteasome inhibitors is that they are highly toxic and that they do not discriminate between the different active sites of the enzyme complex.

The central role of the proteasome system in cell metabolism certainly makes it a sensitive target for inhibitors. Owing to the shutdown of cellular proteolysis most of these inhibitors not only slow down or stop cell proliferation, and as such may represent putative tools for cancer therapy when used at nontoxic concentrations, but can also induce apoptosis as shown in several cases. Thus, while the proteolytic core of the proteasome system is well suited for serving as a drug target, the future will demand drugs which are either targeted for specific active sites and therefore interfere only with certain proteolytic functions of the proteasome system or drugs which act at the level of regulatory components of the system such as the 18 protein component proteasome activator PA700 which is responsible for the regulation of substrate binding or the proteasome modulator PA28 which enhances the MHC class I antigen processing/presentation function of the system.^{2,3,5} Indeed, there already exist commonly used drugs such as cyclosporine or F506 which influence proteasome activity and are able to suppress the function of the important proteasome modulator complex PA28. This complex, for example, activates the cellular immune response by enhancing the presentation of antigens bound by MHC class I molecules and its down-regulation may be involved in suppressing graft rejection.

As part of the immunological surveillance of viral infections and tumor growth, MHC class I molecules present endogenously derived viral or self peptides of 8-10 amino acids in length at the surface of the plasma membrane to CD8⁺ T lymphocytes. Often the inability of a tumor cell (Burkitt's lymphoma, melanoma) or virally infected cell to mount a cytotoxic T cell response is due to limited peptide supply. This is either the result of epitope mutations and in consequence impaired peptide generation or due to the down-regulation of the antigen generation and antigen presentation machinery. Peptide vaccination strategies using peptides tailored to bind efficiently to MHC class I molecules have been one approach to overcome some of these problems. However, the success rates, particularly in animal models have, apart from a few exceptions, largely been beyond expectation. One major reason for this is that the tailored peptides are rapidly inactivated by extracellular or intracellular exopeptidases before they can reach their target. With the aim of developing improved therapeutic tools or vaccination strategies a growing number of laboratories have started to study, in great detail, the MHC class I ligand

generation properties of the proteasome system. The question may therefore be asked whether the proteasome system has the potential to offer a feasible alternative to improve cytotoxic T cell response.

The ability of the proteasome system to supply MHC class I ligands is based on its inherent property to generate, in most cases, the correct C-terminal anchor residue of the MHC class I ligands with high efficiency and to produce peptides of the correct size for MHC class I binding. In addition, IFN- γ induces synthesis of the PA28 complex and three catalytic subunits of the proteasome triggering the system to higher efficiency.⁵

In vitro experiments, in which larger synthetic polypeptides containing defined 9mer MHC class I epitopes were used as proteasome substrates, demonstrated that purified proteasomes are able to generate immunodominant peptides and that the peptide-specific CTL response of *in vitro* digests was indistinguishable from the CTL response obtained when the same peptides were expressed in mouse cells. Based on these observations first experiments using larger synthetic polypeptides as vaccine in mouse models resulted in a greatly improved CTL response compared with the application of the short 9mer ligand. Also, diminished MHC class I epitope generation due to unfavorable flanking sequences can be rescued *in vitro* and *in vivo* by flanking the epitope of choice with Ala residues making epitope generation more efficient.

Similarly, *in vitro* and *in vivo* studies have demonstrated that antigen processing and presentation is greatly enhanced when the proteasome activator PA28 is either added to the *in vitro* assay or overexpressed *in vivo*. These experiments provide hope since they also show that PA28 greatly improves the CTL response without the up-regulation of any other component of the MHC class

I antigen presentation pathway, probably by simply enhancing the peptide supply. This raises the possibility that by combination of the PA28 modulator with a substrate of choice such as native protein or Ala-flanked epitope repeats, a DNA string epitope vaccine can be designed which will allow efficient generation of viral and tumor antigens by the cellular proteasome system. Finally, owing to its intrinsic enzymatic properties the proteasome system will, in future, also allow identification of class I ligands which do not possess the canonical MHC class I binding motifs.

Thus, one can conclude that a number of laboratories have started challenging research programs with the aim of exploiting the proteasome system for therapeutic strategies. However, one also has to appreciate that considering the complexity of the system it will require some time before appropriate proteasome-based vaccines and substrate-specific inhibitors or activators will be available.

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