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The immunoproteasome in antigen processing and other immunological functions

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Treatment of cells with interferon-γ leads to the replacement of the constitutive catalytic proteasome subunits β1, β2, and β5 by the inducible subunits LMP2 (β1i), MECL-1 (β2i), and LMP7 (β5i), respectively, building the so-called immunoproteasome. The incorporation of these subunits is required for the production of numerous MHC class-I restricted T cell epitopes. Recently, new evidence for an involvement of the immunoproteasome in other facets of the immune response emerged. Investigations of autoimmune diseases in animal models and a genetic predisposition of β5i in human autoimmune disorders suggest a crucial function of the immunoproteasome in proinflammatory diseases. The recent elucidation of the high-resolution structure of the immunoproteasome will facilitate the design of immunoproteasome selective inhibitors for pharmacological intervention.

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Introduction

The 20S proteasome is a large intracellular multicatalytic protease consisting of α and β subunits that build a barrel-shaped complex of four rings with seven subunits each [1,2••]. In cells of hematopoietic origin, or during an immune response in the context of interferon-γ (IFN-γ) or tumor necrosis factor-α (TNF-α) stimulation, the three catalytically active β subunits (β1, β2, and β5) are replaced by the inducible catalytic subunits LMP2 (β1i), MECL-1 (β2i), and LMP7 (β5i) during proteasome neosynthesis. The immunological benefit of the resulting 'immunoproteasome' is attributed to structural changes in substrate binding pockets [2••] and an altered cleavage pattern of the multicatalytic complex, thus optimizing quality and quantity of the generated peptides for presentation on MHC class I molecules [3–6]. Because of a pivotal role in class-I ligand generation, the immunoproteasome shapes the naïve CD8-T-cell repertoire in the thymus and cytotoxic T-cell responses in the periphery [7,8,9•,10–12]. Recently, novel functions of immunoproteasomes in autoimmune diseases, virus induced neuroinflammation, T cell expansion, T helper cell differentiation, and cytokine production have been proposed [13••,14–16]. In this review we discuss the latest findings on the immunoproteasome in antigen processing as well as these novel functions.

The immunoproteasome in antigen processing

The major histocompatibility complex (MHC) class-I restricted pathway of antigen processing allows the presentation of intracellular antigens to cytotoxic T lymphocytes. The main protease involved in this process is the proteasome [17–19]. It is generally assumed that the immunoproteasome improves quality and quantity of generated class-I ligands. Indeed, a proteomic analysis of MHC-I associated peptides derived from wild type (WT) and $\beta2i^{-/-}/\beta5i^{-/-}$-double deficient mouse dendritic cells (DC) demonstrated that immunoproteasomes dramatically increase the abundance and diversity of class-I ligands [20]. The recently solved crystal structures of the constitutive proteasome and immunoproteasome of the mouse at 2.9 Å provides an explanation for enhanced antigen processing by immunoproteasomes [2••] (Table 1). The β1i substrate-binding channel is lined with hydrophobic amino acids, which enhances the production of MHC-I epitopes ending with small, nonpolar residues. The β5i-mediated peptide bond hydrolysis might be kinetically favored by an increased hydrophilicity of the active site and additional hydrogen bonds shaping the oxyanion hole. From a structural point of view, the exchange of β2/β2i is not obvious, and it is quite an enigma why, nevertheless, β2i deficient mice are protected from experimental colitis [14] and why β2i influences homeostatic proliferation [21].

Analysis of the T cell response in murine cytomegalovirus (MCMV) infected β5i-deficient mice revealed a critical role for immunoproteasomes [22]. Interestingly, all MCMV-derived $CD8^+$ T cell epitopes tested were affected by the loss of β5i, suggesting that the virus has evolved a primary sequence poorly processed by constitutive proteasomes. The authors hypothesized that DCs containing both immunoproteasomes and constitutive proteasomes elicit the acute MCMV-specific T cell response, whereas the chronic MCMV infection is maintained in cells expressing constitutive proteasomes.

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Table 1

Immunoproteasome subunit-induced alterations in the 20S proteasome (information derived from Ref. [2••])

Immunoproteasome	Alteration – immuno vs. constitutive subunit	Alteration in substrate binding channel	Consequences
MECL-1 (β2i)	D53E	Identical substrate binding channel.	The rationale for the incorporation of subunit β2i into the immunoproteasome remains elusive.
LMP2 (β1i)	T20V, T31F, R45L, and T52A	Increase in hydrophobicity of S1 pocket. Diminishes S1 pocket in size.	$CD8^+$ T cell epitopes with non-polar C-termini such as Ile, Leu, or Val are produced. These epitopes are better suited for presentation on MHC-I molecules. Peptide bond hydrolysis preferentially occurs after small, hydrophobic, and branched residues.
	T22A, and A27V in LMP2; Y114H in β2i	Decrease size and increase polarity of S3 pocket.	Altered amino acid preference at P3.
LMP7 (β5i)	Ala20, Met45, Ala49, and Cys52 in S1 pocket are unchanged.	Hydrophobic character of S1 pocket is maintained.	Both β5 and β5i are responsible for the chymotrypsin-like activity of the proteasome.
	Gly48 Ser or Cys	Shallow S2 pocket.	Limits size of P2 amino acids.
	Ala27Ser	Restricts size of S3 pocket and endows it with a more hydrophilic character.	Limits P3 amino acids to small hydrophilic amino acids.
	Distinct conformation of Met45	Results in spacious S1 pocket in β5i.	β5i can accommodate larger amino acids in S1 compared to β5.
	A46S, V127T	Increase the hydrophilicity surrounding the active site nucleophilic Thr1Oγ of β5i.	Elevated polarity might favor peptide bond hydrolysis.
	SerOγ, Thr127Oγ, and Gly47NH	Build unique hydrogen network.	Stabilization of the tetrahedral transition state during catalysis.

Hence, with the help of expression of immunoproteasome-dependent epitopes, MCMV may evade immune recognition leading to viral persistence.

The structural properties rather than the proteolytic activity of immunoproteasome subunits are needed for the generation of some epitopes [23,24], but the underlying mechanisms have remained elusive. In a recent study, the presentation of the male HY Ag-derived epitope $UTY_{246–254}$ and the influenza virus matrix M1 58–66 epitope were analyzed, which both were dependent on the structure of β1i or β5i, respectively, but not on their catalytic activity [25•]. With different proteasome inhibitors it was shown that β5i protects matrix M1 58–66 from cleavage by β5 and β1i protects $UTY_{246–254}$ from cleavage by β1, proposing a novel mechanistic basis for the function of immunoproteasome subunits (Figure 1).

Using newly developed immunoproteasome subunit-specific antibodies, Guillaume *et al.* isolated and characterized human 20S proteasomes that are intermediate between the standard and the immunoproteasome [26••]. Rather than jointly incorporating β1i, β2i, and β5i into immunoproteasomes, intermediate proteasomes incorporate only one (β5i) or two (β2i and β5i) immunoproteasome subunits. The existence of intermediate proteasomes is consistent with the rules of cooperative assembly of immunoproteasome subunits [27–29]. Depending on the investigated organ, the intermediate proteasomes represent between 30% and 50% of the total proteasome content. Not unexpectedly, the intermediate proteasomes have different cleavage properties in the generation of class I peptides [26••,30]. The existence of 4 different types of proteasomes within cells broadens the MHC-I-presented peptidome. It is conceivable that an asymmetric hybrid proteasome, consisting of immunoproteasome and constitutive proteasome, exists. Nevertheless, Guilllaume *et al.* did not find asymmetrical β5/β5i proteasomes in melanoma cells and kidney samples [26••].

Mice deficient for one or two immunoproteasome catalytic subunits have relatively modest changes in antigen presentation (summarized in [10]). To investigate the antiviral immune response in mice devoid of immunoproteasome activity, we analyzed the lymphocytic choriomeningitis virus specific T cell response in $\beta1i^{-/-}/\beta2i^{-/-}$ double-deficient mice treated with the β5i-selective inhibitor ONX 0914 to generate mice devoid of immunoproteasome activity [11]. Mice devoid of immunoproteasome activity could mount a strong CTL-response, although the T cell response to some epitopes was slightly altered compared to WT mice. Interestingly, β1i and β2i are needed for the generation of the lymphocytic choriomeningitis virus (LCMV)-derived epitope $NP_{205–212}$, whereas β5i destroys $NP_{205–212}$ in β1i/β2i deficient cells. A more pronounced phenotype with respect to antigen presentation was observed in genetically engineered mice completely lacking immunoproteasome subunits [31••]. Similar to

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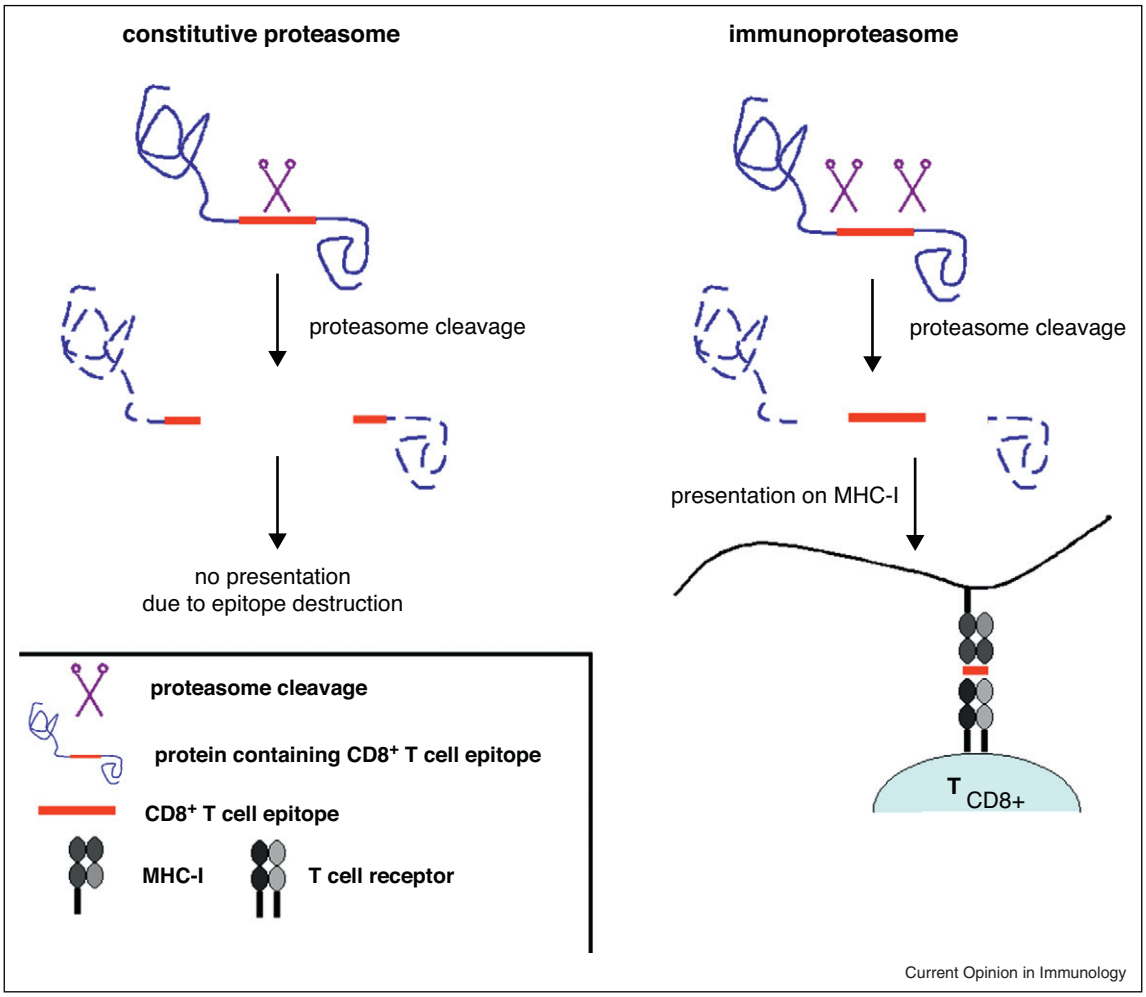
Figure 1



The immunoproteasome protects a $CD8^+$ T cell epitope. A protein containing a $CD8^+$ T cell epitope (in red) is destroyed by the constitutive proteasome. The induction of the immunoproteasome subunits and the replacement of their corresponding constitutive subunits protects this T cell epitope from the destruction by the constitutive proteasome and the peptide can be presented to cytotoxic T cells (T_{CD8+}) [25•].

β5i-deficient mice [32], MHC-I surface expression in triply deficient mice was reduced by approx. 50%. Presentation of numerous $CD8^+$ T cell epitopes derived from different antigens was markedly changed in triple-deficient mice. Interestingly, most investigated epitopes were poorly presented in cells completely lacking immunoproteasome subunits, except for the LCMV-derived epitope $GP_{276–286}$, which elicited a significantly increased CTL-response in LCMV-infected triple-deficient mice. An increased presentation of this T cell epitope was already previously observed in β1i and β5i single-deficient mice [7,33], whereas β2i-deficient mice demonstrated an increased $GP_{276–286}$-CTL-response owing to alterations in the T cell repertoire [8]. Mass spectrometric analysis of MHC-I bound peptides on splenocytes derived from β1i/β2i/β5i triple-deficient or WT mice revealed marked changes in the MHC-I peptide repertoire [31••]. Approx. 1/3 of the detected peptides were uniquely presented on WT cells, 1/3 uniquely on triple-deficient cells, and 1/3 was presented on both cell types. Interestingly, triple-deficient mice rejected adoptively transferred WT splenocytes, whereas adoptively transferred immunoproteasome-deficient cells were tolerated in WT mice. A similar observation was made with WT skin grafted onto $\beta5i^{-/-}$ mice, but not vice versa [4]. Why the immunoproteasome-deficient transplants are not rejected from WT mice, although they present approx. 1/3 unique MHC-I peptides, has remained elusive and needs further investigation.

Other immunological functions of the immunoproteasome

In recent years it became apparent that immunoproteasomes do not only function to change the processing of MHC-I ligands, but also possess additional immunological functions. An involvement of the immunoproteasome in NF-κB activation has remained controversial [34–37].

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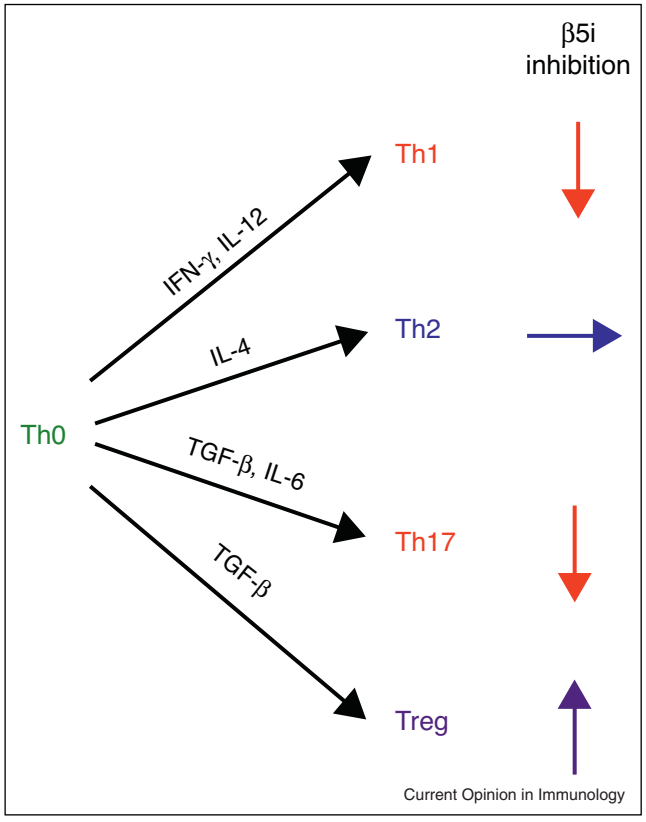
Using β1i and β5i specific inhibitors, Jang *et al.* recently demonstrated that immunoproteasomes are not essential for canonical NF-κB activation [38]. In 2001, Chen *et al.* reported that immunoproteasomes are major determinants of the hierarchy of T cell epitopes during antiviral CTL responses. Already in this study it was noted that adoptively transferred β1i-deficient T cells were not able to expand in influenza virus infected WT hosts [9•], but this phenomenon was suspected to rely on the rejection of the adoptively transferred cells [39]. The inability of immunoproteasome subunit-deficient T cells to expand in a virus infected WT host was also observed by Moebius *et al.*, who provided strong evidence that the loss of β5i-deficient T cells after transfer was not a consequence of graft rejection by the host [15]. Hence, the immunoproteasome possesses a so far uncharacterized function in controlling T cell expansion in an infected host and therefore might qualify as a potential new target for the suppression of undesired pro-inflammatory T cell responses. Indeed, with the help of a β5i-selective inhibitor (named PR-957 and later renamed to ONX 0914), the autoreactive immune responses in two mouse models of arthritis and a model of diabetes could be suppressed [13••]. Additionally, a new function of immunoproteasomes in cytokine production and T helper cell differentiation was proposed [13••]. Furthermore, β5i inhibition prevented experimental colitis [14], murine lupus like disease [40], and Hashimoto's thyroiditis [41]. Not merely inhibition, but also genetic deficiency of immunoproteasome subunits attenuates inflammatory bowel disease in mouse models, suggesting a special contribution of the immunoproteasome in the etiology of inflammatory bowel diseases [14,42,43]. Disparate results have been obtained in mouse models of murine autoimmune encephalomyelitis (EAE). Frausto *et al.* demonstrated that the immunoproteasome is not required for the establishment of myelin oligodendrocyte glycoprotein-induced EAE in β1i-deficient mice [44], whereas Seifert *et al.* reported an exacerbation of EAE symptoms in $\beta5i^{-/-}$ mice [45•]. Additionally, it was demonstrated that immunoproteasomes are required for the efficient degradation of poly-ubiquitylated proteins and the preservation of cell viability under cytokine-induced oxidative stress [45•,46]. However, how an immunoproteasome subunit should control substrate access to the 26S proteasomes has remained elusive especially because the high-resolution crystal structures of the 20S constitutive – and immunoproteasome of the mouse did not reveal any difference in the α-rings where proteasome regulators bind [2••].

Several recent human genetics studies support the involvement of immunoproteasomes in inflammatory disorders [47,48•,49•]. Genetic mapping of patients with an autosomal-recessive auto-inflammatory syndrome characterized by joint contractures, muscle atrophy, microcytic anemia, and panniculitis-induced lipodystrophy syndrome (JMP syndrome) revealed a point mutation (T75M) in PSMB8, the gene encoding for β5i, leading to a disruption of the tertiary structure of β5i [47]. Patients bearing a G176V mutation in the PSMB8 gene, suffered from a newly recognized type of Japanese autoinflammatory syndrome with lipodystrophy (JASL). The mutation manifested in low β5i expression causing increased p38 phosphorylation, which resulted in increased IL-6 production [48•]. Similarly, Arima *et al.* found that a G201V mutation in the PSMB8 gene causes the autoinflammatory disorder Nakajo-Nishimura syndrome [49•]. The mutation disrupts the β-sheet structure of β5i, resulting in accumulation of poly-ubiquitylated and oxidized proteins within cells expressing immunoproteasomes. Furthermore, a strong association between human type 1 diabetes and two single nucleotide polymorphisms in the PSMB8 gene demonstrated a correlation of autoimmune diseases with genetic alteration of β5i [50•]. The authors also showed that β2i/β5i double-deficient mice develop $CD8^+$ T cell-mediated early-stage multiorgan autoimmunity following irradiation and bone marrow reconstitution, suggesting that immunosubunits also play an important function in the prevention of $CD8^+$ T cell-mediated autoimmune reactions [50•] as has been hypothesized previously [51].

Figure 2



Influence of LMP7 on T helper cell differentiation. Depending on the cytokine environment naïve T helper cells (Th0) differentiate into Th1, Th2, Th17, or regulatory T cells (Treg). ↑: enhanced differentiation; →: no influence; ↓: reduced differentiation [52•].

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How does the immunoproteasome exert its effect in autoimmune diseases? A likely explanation could be through the regulation of inflammatory cytokines or T helper cell differentiation. Indeed, selective inhibition or genetic ablation of β5i resulted in diminished Th1 and Th17 differentiation, enhanced development of regulatory T cells, but no effect on Th2 differentiation [13••,43,52•] (Figure 2). Muchamuel *et al.* first demonstrated that selective inhibition of β5i blocked the production of IL-23 by activated monocytes and the production of IFN-γ and IL-2 by T cells, whereas the inhibition of β5 did not substantially affect cytokine release [13••]. Mixed proteasomes expressed in $\beta1i^{-/-}$ mice decrease cytokine production by DCs, which supports the notion of immunoproteasomes playing a role in cytokine production [53]. PMA/ionomycin stimulation of $\beta2i^{-/-}/\beta5i^{-/-}$ derived splenocytes demonstrated that immunoproteasomes regulate the expression of IFN-γ, IL-4, IL-10, IL-2Rβ, GATA3, and T-bet [54]. Furthermore, LPS-stimulated thioglycollate-elicited macrophages from immunoproteasome-deficient mice were found to produce markedly reduced NO levels owing to defects in the TRIF/TRAM and IRF-3 pathway [55].

Conclusions

The severe phenotype in MHC-I ligand generation of triply immunoproteasome-deficient mice [31••] and the existence of intermediate immunoproteasomes diversifying the MHC-I repertoire [26••] emphasize the important role of the immunoproteasome in antigen processing. The development of a specific inhibitor of β5i has revealed a new function of immunoproteasomes in inflammatory autoimmune disorders. However, how the immunoproteasome is mechanistically involved in the newly described processes has remained unclear so far. We propose that the immunoproteasome might selectively processes a factor that is required for regulating cytokine production and T helper cell differentiation, but such a factor remains to be identified. Though selective inhibitors have been described, the recently solved immunoproteasome crystal structures will promote the structure-guided design of new inhibitory lead structures [2••]. Finally, clinical investigations, with either ONX 0914 or other immunoproteasome inhibitors will show whether the promising pre-clinical findings can be translated to human medicine.

Conflict of interest

C.J.K. is an employee of and shareholder in Onyx Pharmaceuticals. M.B. and M.G. have no financial conflicts of interest.

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