OPINION

Proteasomes in immune cells: more than peptide producers?

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Abstract | When cells are stimulated with pro-inflammatory cytokines, most of their constitutively expressed proteasomes are replaced with immunoproteasomes, which increase the production of peptides for presentation on MHC class I molecules. In addition, cortical thymic epithelial cells selectively express a type of proteasome known as the thymoproteasome that is required for the positive selection of thymocytes. Here, we discuss how these specialized types of proteasome shape the T cell receptor repertoire of cytotoxic T lymphocytes and propose that immunoproteasomes have functions, in addition to antigen processing, that influence cytokine production and T cell differentiation, survival and function. We also discuss how inhibitors of immunoproteasomes can suppress undesired T cell responses in autoimmune diseases.

Antigen recognition by cytotoxic T lymphocytes (CTLs) occurs through the interaction of their T cell receptors (TCRs) with peptide–MHC class I complexes. The peptides presented by MHC class I molecules are derived either from endogenous proteins in the direct presentation pathway or from proteins taken up from the extracellular environment during cross-presentation[1]. In both direct and cross-presentation pathways, the proteasome is the protease that determines the carboxy-terminal anchor residues of MHC class I-binding peptides. The proteasome produces peptides of 8–9 amino acids that can bind directly to the peptide-binding cleft of MHC class I molecules and it also produces amino-terminally extended precursor peptides that are processed further by aminopeptidases in the cytoplasm or endoplasmic reticulum[2] (FIG. 1).

The proteasome is an evolutionarily ancient enzyme and is present in a simplified form in archaebacteria[3]. Because of the evolutionary conservation of the proteasome, it has been proposed that the entire MHC class I-restricted antigen presentation pathway has evolved to accommodate the peptides that the proteasome generates[4]. The proteasome consists of a central proteolytic unit, known as the 20S proteasome, and the 19S regulator, which together make up a 26S structure[5]. Moreover, the interferon-γ (IFNγ)-inducible heteroheptameric regulator proteasome activator 28 (PA28) which is composed of PA28α (also known as PSME1) and PA28β (also known as PSME2) subunits[6,7], can associate with the 26S proteasome to form the 'hybrid' proteasome[8]. Evidence from mutant cell lines[9,10] and mice[11] shows that PA28 influences antigen processing by either affecting peptide cleavage[12,13] or facilitating the release of peptide products from the proteasome complex[14].

The cylindrical 20S proteasome consists of four heteroheptameric rings: two outer rings composed of seven α-type structural subunits and two inner rings composed of seven β-type structural and proteolytic subunits. Most mammalian tissues express 'constitutive' proteasomes, in which the proteolytic activity is mediated by proteasome subunit β1 (also known as PSMB6, Y and δ), which cleaves after acidic residues (caspase-like activity), proteasome subunit β2 (also known as PSMB7, Z and MC14), which cleaves after basic residues (trypsin-like activity), and proteasome subunit β5 (also known as PSMB5, X, MB1 and ε), which cleaves after hydrophobic residues (chymotrypsin-like activity) (FIG. 2).

With the exception of β5, the proteolytic activities of the constitutive proteasome subunits do not fully match the requirements for the generation of MHC class I ligands. Human MHC class I molecules accommodate peptides with hydrophobic residues (products of β5-mediated cleavage) and occasionally basic residues (products of β2-mediated cleavage) at their C-termini, whereas mouse MHC class I molecules only accommodate peptides with hydrophobic C-terminal residues. Peptides with acidic C-terminal residues (products of β1-mediated cleavage) have an inappropriate C-terminus and cannot function as MHC class I ligands in mice or humans[15].

In the early 1990s, two additional β-type proteasome subunits, designated proteasome subunit β1i (also known as PSMB9 and LMP2) and proteasome subunit β5i (also known as PSMB8 and LMP7) were identified[16–19]. These subunits, which are highly homologous to β1 and β5, respectively, are encoded by genes in the MHC class II region adjacent to the genes encoding transporter associated with antigen processing 1 (TAP1) and TAP2, and the expression of β1i and of β5i are strongly and synergistically induced by the pro-inflammatory cytokines IFNγ and tumour necrosis factor (TNF)[20]. Subsequently, another cytokine-inducible proteasome subunit with homology to β2, proteasome subunit β2i (also known as PSMB10, LMP10 and MECL1), was found outside the MHC region[21–23]. After stimulation with IFNγ and/or TNF, expression of these three inducible 'immunosubunits' is strongly upregulated and the neosynthesis of 20S proteasomes is switched almost exclusively to the generation of a type of proteasome known as the immunoproteasome[24,25]. Indeed, by eight days after infection of mice with a virus, bacterium or fungus, constitutive proteasomes in the liver and other tissues are almost completely replaced by immunoproteasomes[26,27]. However, despite nearly two decades of research, the specific reasons for this exchange of proteasome subunits are not completely understood.

The pool of MHC class I ligands that is generated by the immunoproteasome is both distinct from and more efficient at

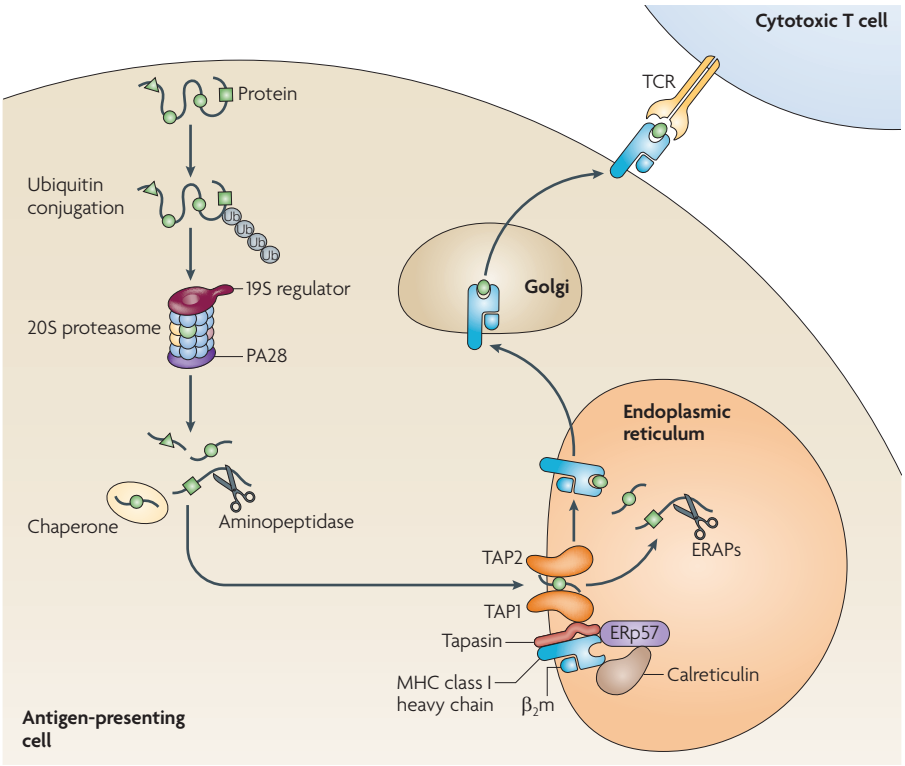


Figure 1 | **Antigen processing in the MHC class I-restricted pathway.** Proteins that are synthesized in the cell (direct presentation) or are released from endosomes (cross-presentation) are polyubiquitylated in the cytoplasm and degraded by hybrid proteasomes consisting of the 20S proteasome core, the 19S regulator and PA28. The peptides that are produced are either of the ideal length for binding to MHC class I molecules (8–9 amino acids) or are amino-terminally extended precursors that can be further cleaved by aminopeptidases in the cytoplasm (such as leucine aminopeptidase, puromycin-sensitive aminopeptidase, bleomycin hydrolase and tripeptidyl peptidase II). Chaperones (such as heat shock protein 70 (HSP70), HSP90α and TRiC) can stabilize the peptides in the cytoplasm to prevent their rapid degradation (for example by tripeptidyl peptidase II or thimet oligopeptidase). Transporter associated with antigen processing 1 (TAP1) and TAP2, which are attached to nascent MHC class I chains through tapasin, transport the peptides into the endoplasmic reticulum (ER), where they can be further trimmed at the N-terminus by ER aminopeptidase 1 (ERAP1) and ERAP2. The oxidoreductase ERp57 ensures the maintenance of disulphide bridges in the MHC class I loading complex. Note that the carboxyl terminus of a peptide ligand for MHC class I molecules is mainly determined by proteasomal cleavage. The binding of peptides with high affinity to the MHC class I heavy chain–β_2-microglobulin (β_2m) complex induces a final folding and release of the MHC class I molecule from the ER lumenal chaperone calreticulin to allow exit from the ER and migration through the Golgi to the plasma membrane. TCR, T cell receptor.

CTL activation than the ligand pool generated by the constitutive proteasome[28,29]. This is a result, in part, of the replacement of β1 with β1i, which leads to the elimination of the caspase-like activity of β1 and enhancement of the chymotrypsin-like activity of β1i and therefore to the generation of peptides with hydrophobic C-terminal residues[25,30–34]. Mice lacking one or more of the inducible immunosubunits have been generated and infected with commonly used laboratory strains of viruses, bacteria and fungi[35,36]. These studies have indicated roles for the immunoproteasome in shaping the CTL repertoire and in pathogen clearance that have, until recently (see later), been ascribed to alterations in the MHC class I ligands that are generated.

In cortical thymic epithelial cells (cTECs), which are involved in the positive selection of T cells in the thymus, a third type of specialized proteasome (the thymoproteasome) has been discovered that, in addition to the immunosubunits β1i and β2i, also contains the cTEC-specific proteasome subunit β5t (also known as PSMB11), which seems to be essential for the positive selection of $CD8^+$ T cells[37].

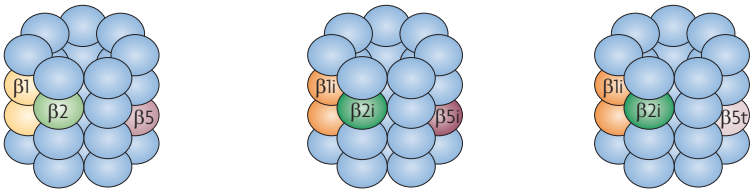
Immunoproteasomes and thymoproteasomes are thought to function in shaping CTL responses at the level of antigen presentation. In this article, we review these insights into the biology of immune-associated proteasomes and propose, based on recent data, that immunoproteasomes also have a role in the control of cytokine production and T cell differentiation. The therapeutic implications of these immunoproteasome functions that are independent of antigen processing in immune responses are also discussed.

Immunoproteasome-deficient mice

The discovery of the genes encoding β1i and β5i in the MHC region led researchers to assume that they would have important functions in the immune response, but initial functional and phenotypic analyses of knockout mice were disappointing. β1i-deficient mice generated normal CTL responses to Sendai virus and to ovalbumin and cleared lymphocytic choriomeningitis virus (LCMV) infection; CTL responses to the LCMV epitopes GP33, GP276 and NP396 were unaltered in these mice[38]. The CTL response to influenza virus infection in β1i-deficient mice was skewed towards the sub-dominant epitopes of the virus (PB1F2.61 and NS2.114) and away from two immunodominant epitopes (NP366 and PA224)[39]. Despite the initial report that splenocytes from β5i-deficient mice generate a decreased number of CTLs specific for the male minor antigen HY[36], we observed normal responses to all dominant LCMV epitopes and normal kinetics of viral clearance in β5i-deficient mice[38]. However, after challenge with recombinant vaccinia virus or a DNA vaccine encoding the LCMV glycoprotein, an increased response to the LCMV epitope GP276 was detected in β5i-deficient mice, which indicates that the immunoproteasome down-regulates the presentation of this epitope in wild-type mice[38,40].

A role for β5i in the clearance of pathogens was first shown in knockout mice after infection with *Listeria monocytogenes*[41]. Similar to LCMV infection, infection with *L. monocytogenes* results in the upregulation of immunoproteasome expression and the replacement of constitutive proteasomes in the liver[26]. Although *L. monocytogenes*-specific CTLs were generated at a normal frequency in β5i-deficient mice, the clearance of bacteria from the liver was not apparent by day 10, by which time bacterial burden in the spleen of wild-type mice had decreased[41]. This result underscores the necessity to induce immunoproteasomes at sites of infection for pathogen clearance, most probably because the effective CTL response is focused on immunoproteasome-dependent pathogen epitopes.

An even more prominent phenotype was seen when β5i-deficient mice were infected with the protozoan parasite *Toxoplasma gondii*[42]. In contrast to wild-type mice, β5i-deficient mice succumbed to infection and



Constitutive proteasome	Immunoproteasome	Thymoproteasome
β1 (PSMB6, Y, δ)	β1i (PSMB9, LMP2)	β1i (PSMB9, LMP2)
β2 (PSMB7, Z, MC14)	β2i (PSMB10, LMP10, MECL1)	β2i (PSMB10, LMP10, MECL1)
β5 (PSMB5, X, MB1, ε)	β5i (PSMB8, LMP7)	β5t (PSMB11)

Figure 2 | **Subunit composition of the active sites of the constitutive proteasome, immunoproteasome and thymoproteasome.** The proteolytic subunits of the constitutive proteasome are β1 (also known as PSMB6, Y and δ), β2 (also known as PSMB7, Z and MC14) and β5 (also known as PSMB5, X, MB1 and ε). The proteolytic immunoproteasome subunits are β1i (also known as PSMB9 and LMP2), β2i (also known as PSMB10, LMP10 and MECL1) and β5i (also known as PSMB8 and LMP7). The proteolytic thymoproteasome subunits are β1i, β2i and β5t (also known as PSMB11). Compared with the constitutive proteasome, the immunoproteasome has a strongly decreased caspase-like activity and an increased chymotrypsin-like activity, whereas the thymoproteasome has a decreased chymotrypsin-like activity.

this correlated with decreased production of IFNγ by parasite-specific $CD8^+$ T cells. Immunodominant epitopes of the *T. gondii*-specific CTL response have not been identified and it remains to be shown whether the decreased generation of activated CTLs in β5i-deficient mice is a result of the lack of presentation of β5i-dependent *T. gondii* epitopes or whether additional functions of β5i might explain this phenotype. Taken together, these results show that the requirement for immunoproteasomes for pathogen elimination varies markedly between infection models and further testing of immunoproteasome-deficient mice is required to fully appreciate the contribution of individual proteasome subunits to the immune response to infectious agents.

Stimulation of cells with IFNγ or TNF typically leads to a tenfold upregulation of the cell surface expression of MHC class I molecules[43]. Through increased ligand production, switching to the generation of immunoproteasomes provides the larger pool of peptides that is required to allow the increased number of MHC class I molecules to finalize their folding in the endoplasmic reticulum and to migrate to the cell surface. Evidence of a role for β5i in contributing to the increased cell surface expression of MHC class I molecules has been obtained in β5i-deficient mice, which have a 50% decrease in cell surface expression of MHC class I molecules by lymphocytes and monocytes compared with wild-type mice[36]. Remarkably, no decrease in the cell surface expression of MHC class I molecules has been observed in β1i- or β2i-deficient mice[35,44]. The normal level of MHC class I expression on the cell surface of β1i- but not β5i-deficient splenocytes (as determined by flow cytometry) is surprising as β1i contributes to the generation of MHC class I-binding peptides by replacing the caspase-like activity of β1 with the chymotrypsin-like activity of β1i. These data indicate that β5i produces higher affinity MHC class I ligands than does β5, which was not predicted from structural models of the two subunits[33].

Decreased cell surface MHC class I expression might be predicted to affect the number of $CD8^+$ T cells in β5i-deficient mice. However, a surprising finding was a 20–30% decrease in the number of $CD8^+$ T cells compared with $CD4^+$ T cells in the thymus, blood and spleen of β1i- or β2i-deficient mice, but not β5i-deficient mice[35,45]. As these findings did not correlate with MHC class I expression levels, it is possible that they were a result of intrinsic T cell effects. Indeed, this has been elegantly shown using bone marrow chimaeras in which wild-type recipient mice received equal numbers of cells from wild-type and from β5i and β2i double-deficient donors. The decreased ratio of $CD8^+$ to $CD4^+$ T cells was maintained in the thymus and periphery for β5i and β2i double-deficient donor cells in wild-type recipient mice, whereas wild-type donor cells had normal numbers of both cell subsets[46]. As the two donor populations were selected by the same wild-type thymus and expanded in the same wild-type periphery, this phenomenon cannot be attributed to a difference in antigen presentation. Rather, it seems that $CD8^+$ T cells deficient in β5i and β2i expand less readily than wild-type $CD8^+$ T cells. This finding clearly hints at a previously overlooked function of immunoproteasome subunits in the proliferative expansion of $CD8^+$ T cells.

TCR repertoire formation

The cells that are responsible for negative selection and TCR repertoire formation in the thymus — thymic dendritic cells and medullary thymic epithelial cells (mTECs) — constitutively express high levels of immunoproteasomes (FIG. 3). By contrast, cTECs, which support the positive selection of T cells, also express immunoproteasome subunits but only after systemic infection or the administration of IFNγ[47]. Therefore, it is not surprising that immunoproteasomes shape the repertoire of $CD8^+$ T cells in the thymus. It has been shown that the lack of NP366-specific CTLs in β1i-deficient mice infected with influenza virus is not owing to the inability of β1i-deficient splenocytes to present the epitope for the activation of CTLs, as was previously reported[35], but instead is owing to the lack of NP366-specific precursor T cells in the periphery of knockout mice[39]. In addition, β2i-deficient mice infected with LCMV mounted a normal CTL response to most LCMV epitopes but the response to GP276 was markedly decreased[44]. Again, this defect was not caused by the inability of β2i-deficient antigen-presenting cells (APCs) to process and present the GP276 epitope to CTLs but by a decrease in the number of GP276-specific precursor T cells in the knockout mice.

Differences in T cell selection in the thymus can be best observed in TCR-transgenic mice. $CD8^+$ T cells from OT-1 mice, which express a TCR that is specific for the ovalbumin epitope SIINFEKL presented by the MHC class I molecule $H–2K^b$, did not undergo positive selection in the absence of β5i (REF. 48). *In vitro* processing of the $Cpα1_{92–99}$ self peptide derived from the F-actin capping protein Cpα1, which contributes to the positive selection of OT-1 cells, can be mediated by immunoproteasomes but not by constitutive proteasomes. Furthermore, repeated injection of β5i-deficient OT-1 mice with synthetic $Cpα1_{92–99}$ peptide rescued the positive selection of OT-1 cells, which emphasizes the role of β5i in this process.

Although this finding strongly supports the involvement of immunoproteasomes (and β5i in particular) in positive selection, it is difficult to reconcile the result with the failure to detect β5i mRNA and protein in cTECs from non-infected mice[47] and with the recent finding that β5i is replaced by the cTEC-specific subunit β5t in these cells (see below).

Thymoproteasomes in T cell selection

The interest in the role of immune-type proteasomes in TCR repertoire selection has recently been boosted by the discovery of a seventh active subunit of the

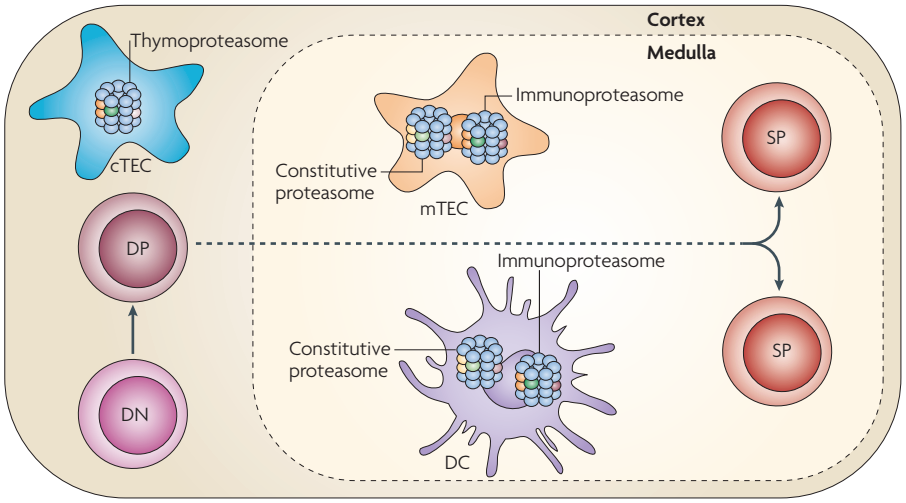


Figure 3 | **Proteasomes in positive and negative selection in the thymus.** Positive selection occurs at the double-positive (DP; $CD4^+CD8^+$) thymocyte stage and is mediated by cortical thymic epithelial cells (cTECs). These highly specialized antigen-presenting cells express a unique type of proteasome, known as the thymoproteasome, which contains the active site subunits β1i, β2i and the cTEC-specific subunit β5t. Low-affinity interactions with the T cell receptor for the positive selection of CD8 single-positive (SP) thymocytes probably rely on a spectrum of weak peptide–MHC class I ligands with hydrophilic carboxy-termini that are generated by the thymoproteasome. Medullary thymic epithelial cells (mTECs) and dendritic cells (DCs) mediate the negative selection of self-reactive thymocytes at the boundary of the cortex and medulla. Both cell types express high levels of immunoproteasomes containing the active site subunits β1i, β2i and β5i, as well as constitutive proteasomes containing β1, β2 and β5. The negative selection of CD8 SP thymocytes relies on high-affinity peptide–MHC class I ligands and should involve peptides generated from self antigens by both types of proteasome that are encountered in the periphery (the constitutive proteasome and the immunoproteasome).

mammalian proteasome known as β5t, which is expressed exclusively by cTECs in mice[37] and humans[49]. β5t from mouse thymus lysates co-immunoprecipitates with β1i and β2i, but not with β1 or β2 proteasome subunits[37]. This β1i–β2i–β5t-containing proteasome has been designated the thymoproteasome to distinguish it from the β1i–β2i–β5i-containing immunoproteasome (FIG. 2). In contrast to the classical immunosubunits (β1i and β2i), expression of β5t is not induced by IFNγ. $Ly51^+$ cTECs were shown to express low levels of the proteasome subunits β1, β2, β5 and β5i, whereas β1i, β2i and β5t were highly expressed[37]. This result is in conflict with an earlier report that showed that mouse cTECs express the constitutive proteasome subunits β1, β2 and β5 and only express the immunosubunits β1i, β2i and β5i after infection or IFNγ stimulation *in vivo*[47]. These disparate results could be explained by differences in infection status and cytokine levels in the mice or by the presence of other cell types in the cTEC preparations. Further investigation of the proteasome subunits that are expressed by cTECs from naive and infected mice is warranted to determine if inducing the expression of β5i can replace β5t in proteasome assembly in cTECs as it does for β5 in other tissues.

Why does a highly specialized cell type that has evolved to mediate positive selection in the thymus require a unique β5-type proteasome subunit? Examination of the S1 pocket of β5t, which accommodates the amino acid directly before the polypeptide cleavage site, shows that it is lined with hydrophilic residues rather than the hydrophobic residues that are present in β5 and β5i (REF. 37). This change in the S1 pocket decreases the chymotrypsin-like activity (cleavage after hydrophobic residues) of β5t by 60–70%, and probably affects the pool of available ligands for MHC class I molecules that are generated. Peptides with hydrophilic C-termini (such as those produced by β5t) are predicted to be poor ligands for MHC class I molecules, and although there are normal numbers of double-negative, double-positive and CD4 single-positive (SP) thymocytes in β5t-deficient mice, the number of CD8 SP thymocytes and peripheral $CD8^+$ T cells is decreased by 75% in these mice[37] (FIG. 3).

The expression profile, biochemical properties and effect on thymic selection of β5t are all consistent with a role for this proteasome subunit in the positive selection of $CD8^+$ T cells, but one finding has remained puzzling: low-affinity MHC class I ligands should negatively affect the cell surface expression of MHC class I molecules but this correlation was not found for β5t-deficient cTECs[50]. Why do β5t-deficient cTECs have such a marked deficit in positive selection when the level of MHC class I cell-surface expression is not changed? It was speculated that the β5t-dependent peptide repertoire of cTECs is better suited for positive selection and that the peptide repertoire on which positive selection occurs should be different from the peptide repertoire of negatively selecting APCs[50]. The requirement of a special peptide population for positive selection is not fully consistent with the demonstration that a single peptide can positively select a large and diverse TCR repertoire[51]. One possible explanation could be that cTECs have mechanisms to better stabilize MHC class I molecules complexed with low-affinity peptide ligands. Alternatively, the β5t-generated peptide ligands could achieve a higher affinity for MHC class I molecules by the use of MHC anchor positions other than the C-terminal position; an experimental determination of the dissociation rates of MHC class I ligands in β5t-expressing cells should clarify this issue. Together, these results show that it is probable that the production of low-affinity peptide ligands for MHC class I molecules underlies the phenotype of β5t-deficient mice, but there is some room for alternative mechanisms.

Immunoproteasomes in T cell survival

In addition to its role in shaping the antigenic peptide repertoire presented by MHC class I molecules, the immunoproteasome might have other roles in regulating immune responses. As mentioned earlier, after adoptive transfer into wild-type mice, T cells from β1i-deficient mice fail to proliferate in response to influenza virus infection, despite the robust proliferation of host T cells[39]. It has been argued that this could be attributed to the rejection of β1i-deficient donor T cells by the wild-type host because the donor cells present a different peptide repertoire on their MHC class I molecules[52]. An argument against such a rejection phenomenon is provided by the finding that skin from β5i-deficient mice transplanted onto wild-type mice was not rejected by the host[53]. Strikingly, when β2i-deficient T cells were transferred into LCMV-infected wild-type mice, they also did not survive[44]. As the cell surface expression level of MHC class I molecules is not altered in β1i- or β2i-deficient mice and as no major change in the specificity of proteasomal cleavage could be observed in β2i-deficient mice[44], we think that the disappearance of β1i- or

β2i-deficient T cells after transfer into virus-infected wild-type mice is not the result of rejection but instead reflects the requirement of these immunoproteasome subunits for the survival of T cells in a pro-inflammatory environment. Based on this notion, we postulate that the immunoproteasome could be a suitable drug target for the suppression of overactive T cell responses such as are found in many autoimmune diseases.

β5i inhibition blocks autoimmunity

The data generated from knockout mice highlight two important aspects of the biology of the immunoproteasome: first, the inducible subunits of the immunoproteasome have non-redundant immunoregulatory functions; and second, compensatory mechanisms and altered proteasome structure in subunit-deficient cells might complicate the search for specific pathways that are regulated by the immunoproteasome. A need for specific inhibitors of immunoproteasome subunits is evident. Small molecule inhibitors of the proteasome have been used in research since 1994 (REF. 54) and the dipeptide boronate proteasome inhibitor bortezomib (Velcade; Millennium Pharmaceuticals) is used for the treatment of malignant diseases, such as multiple myeloma[55]. However, these inhibitors do not selectively target immunoproteasome subunits and, so far, their clinical use has been restricted to the treatment of cancer owing to drug side effects.

Recently, a cell-permeable ketoepoxide-based immunoproteasome inhibitor, designated PR-957, which selectively inhibits β5i in both human and mouse cells at concentrations that do not target other proteasome subunits, has been developed[56]. The selectivity of PR-957 was verified by its ability to down-regulate MHC class I cell surface expression by 50% in wild-type but not β5i-deficient mice and to suppress the presentation of β5i-dependent peptide epitopes, such as $Uty_{246–254}$ (derived from the male minor antigen HY) and LCMV GP33, without affecting the presentation of β5i-independent epitopes.

As PR-957 suppressed the presentation of the LCMV GP33 epitope *in vivo*, we investigated whether PR-957 could prevent diabetes in RIP–GP mice, which express a fragment of the LCMV glycoprotein epitope in β-islet cells under the control of the rat insulin promoter (RIP) and develop diabetes after infection with LCMV[57]. Treatment with PR-957 completely prevented the onset of disease after virus challenge in RIP–GP mice, highlighting the role of β5i in the production of the immunodominant LCMV GP33 epitope[56], which could not be fully appreciated in experiments with β5i-deficient mice most probably owing to the concomitant lack of β1i and β2i[38].

The role of β5i in immune responses is not restricted to T cells. We found that selectively targeting β5i in human peripheral blood mononuclear cells (PBMCs) blocked the production of several pro-inflammatory cytokines, including interleukin-6 (IL-6), IL-23 and TNF[56]. The level of inhibition varied for each cytokine but was equivalent in PBMCs derived from healthy volunteers and from patients with active rheumatoid arthritis, which indicates that immunomodulation through targeting β5i might be feasible in patients with rheumatoid arthritis. The suppression of IL-6 and IL-23 production is intriguing given that these cytokines have a crucial role in the development and/or maintenance of T helper 17 (T_H17) cells, which are involved in the pathogenesis of several autoimmune diseases, including rheumatoid arthritis, inflammatory bowel disease and psoriasis[58]. Interestingly, β2i-deficient mice have been shown to be protected from dextran sulphate sodium-induced colitis, which is a T cell-independent mouse model of inflammatory bowel disease[59]. PR-957 suppressed the development of T_H1 and T_H17 cells *in vitro* from both mouse and human naive T cells but did not affect the differentiation of regulatory T cells and T_H2 cells[56] (E. Suzuki, C.J.K., M.B. and K. Kalim, unpublished observations).

We extended these *in vitro* data to show that PR-957 could block disease progression in mouse models of rheumatoid arthritis. Inhibition of β5i suppressed disease symptoms in both collagen-induced arthritis (CIA) and collagen antibody-induced arthritis (CAIA) and was more effective than the soluble TNF antagonist etanercept[56]. PR-957 suppressed disease symptoms at doses of less than one-tenth the maximum tolerated dose, a therapeutic window that is not achievable with non-selective inhibitors. The efficacy of PR-957 in the T cell-independent CAIA model highlights the immunoregulatory role of β5i outside of antigen presentation that, consistent with *in vitro* data, involves multiple immune effector cell types. Therapeutic activity of PR-957 in mouse models of colitis, dermatitis and lupus has also been noted (T. Muchamuel, C.J.K., M.B. and M.G., unpublished observations).

Conclusions and perspectives

Despite the initial studies with immunoproteasome-deficient mice that indicated a mild phenotype, more recent studies involving pathogen infection models have shown that immunoproteasome subunits have distinct functions in pathogen elimination[41,42]. Further studies with both knockout mice and selective inhibitors will enable a more complete understanding of how the immunoproteasome and thymoproteasome shape the antigenic repertoire and regulate the CTL response to infectious agents. However, questions remain about the role of specific subunits in immune responses. It is unclear why β5i but not β1i or β2i deficiency decreases the cell surface expression of MHC class I molecules but the number of $CD8^+$ T cells is decreased in β1i- or β2i-deficient mice, but not β5i-deficient mice. What are the pathways that are regulated by the immunoproteasome that underlie its role in the proliferative expansion of T cells in a pro-inflammatory environment? Are these pathways similar to the pathways that are regulated by β5i during cytokine production by T cells and monocytes?

The development of the specific β5i inhibitor PR-957 has opened new and interesting avenues of research into immunoproteasome biology. Further work is required to fully understand the unique role of the immunoproteasome in the induction and maintenance of inflammation. The suppression of T_H1 and T_H17 cell differentiation by PR-957 indicates that the immunoproteasome is a possible target for therapeutic intervention in several autoimmune diseases. However, it is unclear how the immunoproteasome is mechanistically involved in these processes. We propose that the immunoproteasome selectively processes a factor that is required for regulating cytokine production. Clearly, more research, including the development of selective inhibitors of β1i and β2i, is required. Clinical investigation with PR-957 in autoimmune disease will bring the immunoproteasome forward as a drug target and extend these interesting preclinical findings. From subtle phenotypes in gene-deficient mice to a drug target in inflammatory diseases, the immunoproteasome is back in the limelight.

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Competing interests statement

The authors declare no competing financial interests.

DATABASES

UniProtKB: http://www.uniprot.org
IFNγ | PA28α | PA28β | proteasome subunit β1 | proteasome subunit β1i | proteasome subunit β2 | proteasome subunit β2i | proteasome subunit β5 | proteasome subunit β5i | proteasome subunit β5t | TAP1 | TAP2 | TNF

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