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The immunoproteasome and thymoproteasome: functions, evolution and human disease

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The basic principle of adaptive immunity is to strictly discriminate between self and non-self, and a central challenge to overcome is the enormous variety of pathogens that might be encountered. In cell-mediated immunity, immunological discernment takes place at a molecular or cellular level. Central to both mechanisms of discernment is the generation of antigenic peptides associated with MHC class I molecules, which is achieved by a proteolytic complex called the proteasome. To adequately accomplish the discrimination between self and non-self that is essential for adaptive immunity and self-tolerance, two proteasome subtypes have evolved via gene duplication: the immunoproteasome and the thymoproteasome. In this Review, we describe various aspects of these immunity-dedicated proteasomes, from their discovery to recent findings.

Selective proteolysis in cells is achieved by the proteasome, an ATP-dependent protease complex that degrades proteins that have been covalently modified with ubiquitin molecules. The proteasome is a highly sophisticated supramolecular complex of 2.5 MDa composed of a catalytic core particle (CP) and regulatory particles (RPs) (Fig. 1a)[1,2]. Because the proteasome did not fit neatly into the category of known proteolytic enzymes, its precise molecular nature was unclear for many years. In the latter half of the 1980s, the CP (20S proteasome) was first isolated and named the 'proteasome'[3], and analysis of the primary structure of the eukaryotic CP and RP by cDNA cloning took approximately 15 years to complete. The crystal structure of the CP was determined for yeast[4] and mammalian[5] proteasomes, but it was very difficult to obtain an atomic-level structure of the RP. Recently, the higher-order structure of the 26S proteasome, consisting of the CP and two RPs, was solved by single-particle analysis with cryo-electron microscopy, and consequently, the mechanism of the active proteasome was fully established[6–12].

Discovery of immunity-related isoforms of the proteasome

Concurrently with these structural studies, research concerning the roles of the proteasome in physiology and pathology has benefited from increased interest in the ubiquitin system[13,14]. In the decades since the first description of the proteasome, the ubiquitin–proteasome system (UPS) has been shown to be involved in the control of nearly all biological processes in cells. Among the many functions of the UPS is a prominent role in immunity. The dimeric TAP transporter, a pump that transports antigenic peptides from the cytoplasm to the endoplasmic reticulum, was discovered in 1990 in a study of antigen presentation (MHC class I pathway) in cell-mediated immunity[15,16], and shortly thereafter it was found that the proteasome genes *PSMB9* (*LMP2*) and *PSMB8* (*LMP7*) were located in the vicinity of the *TAP* genes in the MHC class II genomic region[17]. In 1994, the proteasome was identified as essential for the MHC class I–restricted antigen-processing pathway[18]. At the same time, the function of the CP was found to fluctuate with interferon (IFN)-γ treatment[19–22], and it was observed that substitution of CP subunits occurred in response to treatment with this cytokine. The resulting IFN-γ-generated proteasome isoform was termed the 'immunoproteasome' to emphasize its specialized role in processing intracellular antigens for presentation to the immune system[23,24]. In 2007, a search of expressed sequence tags led to the discovery of a unique proteasomal subunit expressed specifically in the thymus. The proteasome isoform containing this subunit became known as the 'thymoproteasome', and its role was demonstrated in thymic positive selection[25]. It is now widely accepted that these two immune-type proteasomes are responsible for establishing and triggering cell-mediated immunity by promoting the development and response of $CD8^+$ T cells.

The immunoproteasome in antigen presentation

The constitutive CP of the proteasome consists of a cylindrical stack of four rings, two outer α-rings and two inner β-rings. Each ring is composed of seven structurally similar α or β subunits, of which β1, β2 and β5 have proteolytic activities (Fig. 1b)[1]. The immunoproteasome has a specialized CP with altered peptide-cleavage properties, enabling more efficient antigen processing for presentation on MHC class I molecules. The three immunoproteasome subunits, β1i (LMP2), β2i (MECL-1) and β5i (LMP7), are cooperatively and preferentially incorporated in place of the constitutive counterparts, β1, β2 and β5, during de novo formation of the CP, partly owing to direct binding of β5i to the assembly chaperone POMP (also known as UMP1) (Fig. 1b)[26–28]. This is reflected in the rapid assembly rate of the immunoproteasome, which is approximately four times faster than assembly of the constitutive CP, enabling a rapid response to immune and inflammatory stimuli[27]. The immunoproteasome is constitutively expressed in hematopoietic cells and induced in non-immune cells following exposure to proinflammatory cytokines such as IFN-γ, IFN-α or IFN-β, and tumor necrosis factor (TNF), of which IFN-γ is the most potent in its effects on immunoproteasome induction[19,29,30]. IFN-γ also induces expression of PA28αβ, a ring-shaped heteroheptameric complex that binds to the end of both

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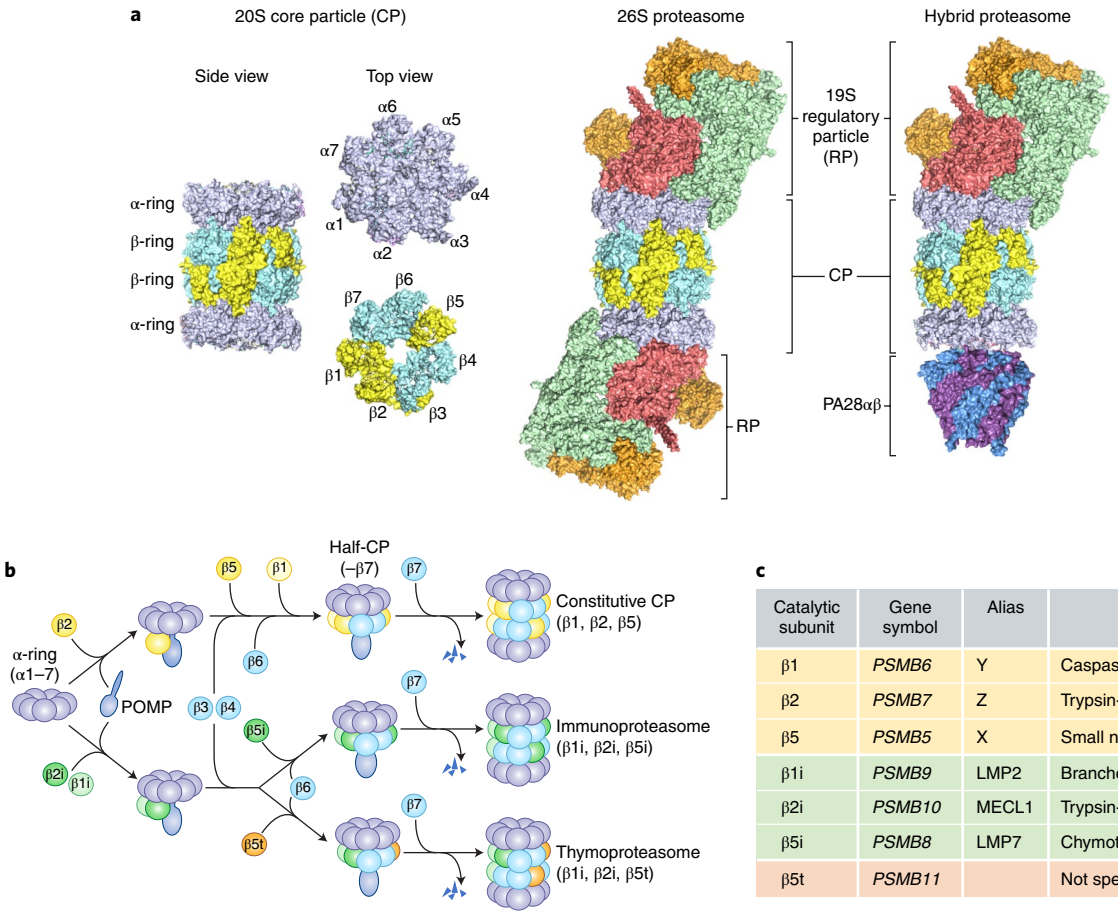

Catalytic subunit	Gene symbol	Alias	Peptidase activity
β1	*PSMB6*	Y	Caspase-like
β2	*PSMB7*	Z	Trypsin-like
β5	*PSMB5*	X	Small neutral amino acid preferring
β1i	*PSMB9*	LMP2	Branched-chain amino acid preferring
β2i	*PSMB10*	MECL1	Trypsin-like
β5i	*PSMB8*	LMP7	Chymotrypsin-like
β5t	*PSMB11*		Not specified

Fig. 1 | Structures and diversity of the proteasome. a, Model structure of the proteasome. The 20S core particle (CP) is activated through binding of regulators. The CP is capped on one or both sides with a 19S regulatory particle (RP), which is required for degradation of ubiquitinated proteins. The CP with RPs is called the '26S proteasome'. The IFN-γ-inducible complex PA28αβ can bind to the CP together with one RP to form the 'hybrid proteasome'[120]. There also exist 'football-like' proteasomes in which PA28αβ associates with both ends of the CP. The structures shown were adopted and modified from the Protein Data Bank (human 26S proteasome, 5GJR; mouse PA28αβ, 5MX5)[12,32]. **b**, Diversity of the CP. In most organisms equipped with adaptive immunity, specialized CPs dedicated to immunity are formed according to cell type and cellular environment. IFN-γ exposure induces the synthesis of three immunosubunits, β1i, β2i and β5i, which are incorporated into a newly formed CP in place of their constitutive counterparts, β1, β2 and β5, to form the immunoproteasome. In the thymus, the cTEC-specific subunit β5t is incorporated in place of β5 or β5i along with β1i and β2i to form the thymoproteasome. Stepwise assembly of the CP is assisted by several proteasome assembly chaperones; of these, POMP guides the initial step of β-ring formation and is involved in preferential formation of the immune-type CPs[121]. Dimerization of half-CPs (–β7) occurs upon the incorporation of β7 to form mature CPs, accompanied by degradation of POMP. **c**, Catalytic subunits of the CP in proteasomes, immunoproteasomes and thymoproteasomes and their properties.

the constitutive CP and immunoproteasome with the same affinity (Fig. 1a)[31,32]. PA28αβ has a limited role in presentation of specific epitopes but promotes overall supply of MHC class I–binding peptides, independently of the immunoproteasome[33,34].

Crystal structures of the mouse constitutive CP and immunoproteasome revealed differences between the constitutive subunits (β1, β2, β5) and the immunosubunits (β1i, β2i, β5i) that provide an explanation for efficient antigen processing by the immunoproteasome for binding MHC class I. The cleavage preferences of the catalytic subunits are determined by the nature of their substrate specificity pockets. Whereas the pocket of β1 accommodates an acidic P1 residue (caspase-like activity), that of β1i binds with a hydrophobic P1 residue, exerting a branched-chain amino acid–preferring (BrAAP) activity. The active sites of both β5 and β5i are surrounded by nonpolar environments, but the specificity pocket of β5i (chymotrypsin-like activity) is significantly larger than that of β5 (small neutral amino acid–preferring activity), enabling accommodation of a bulky hydrophobic P1 residue (Fig. 1c)[35]. Accordingly, the immunoproteasome produces more antigenic peptides with C-terminal hydrophobic residues, which fit better in the cleft of the MHC class I molecule. By contrast, the substrate-binding pockets of β2 and β2i are essentially identical, showing the same trypsin-like activity. There are also mosaic CPs in which only one (β5i) or two (β5i and β1i) of the immunosubunits are incorporated, comprising 30–50% of the CP[36]. These mosaic CPs broaden the repertoire of the antigens presented to $CD8^+$ T cells.

Mice deficient in each single immunoproteasome subunit exhibited defects in MHC class I antigen presentation that were rather more modest than one might expect[37–39]. Recently, mice deficient in all three immunoproteasome subunits were generated[40], and these mice showed strongly impaired and altered MHC class I epitope presentation. Most of the MHC class I epitopes tested were poorly presented in the triply deficient mice, and only half of the MHC class I–binding peptides were shared between wild-type and triply deficient splenocytes, demonstrating the prominent role of the immunoproteasome in antigen processing[40].

Roles of the immunoproteasome beyond antigen processing

It is reasonable to expect antigen-presenting cells, both professional and non-professional, to express the immunoproteasome for

efficient antigen processing. Unexpectedly, T cells also constitutively express the immunoproteasome. Indeed, studies have uncovered functions of the immunoproteasome beyond antigen presentation. T cells from immunoproteasome-subunit-deficient mice show impaired proliferation and survival when transferred into virus-infected wild-type mice. These observations cannot be attributed to graft rejection through antigen presentation, suggesting a T cell–intrinsic role for the immunoproteasome in the expansion and maintenance of T cell populations during an immune response[41,42]. Additionally, immunoproteasome activity promotes differentiation of proinflammatory T helper type 1 (T_H1) and type 17 (T_H17) cells while suppressing induction of regulatory T cells, and also promotes synthesis of the cytokines IL-2, IFN-γ and TNF in activated T cells[43,44]. How the immunoproteasome exerts these functions is unknown, but the CP might somehow select substrates such as the NF-κB precursor p105 for degradation or limited processing, or there may be a thus far unrecognized role of short peptides produced by the immunoproteasome. These possibilities are intriguing, but at present there is no concrete evidence to support them.

These emerging roles of the immunoproteasome in activated immune cells in an inflammatory environment have made an immunoproteasome-specific inhibitor a rational candidate for treatment of various immune diseases. Indeed, the covalent β5i-selective inhibitor ONX0914 (formerly called PR-957) prevented experimental colitis and colitis-associated cancer, lupus- and rheumatoid arthritis–like disease, Hashimoto's thyroiditis, acute myocarditis, microglial activation following central nervous system injury and allograft rejection in mouse models, without apparent toxicity[44–51]. More recently, DPLG3, a non-covalent β5i-specific inhibitor, and LU-005i, a pan-immunoproteasome inhibitor that targets all three active subunits, have shown therapeutic efficacy in immune diseases in mice[52,53]. Because the immunoproteasome is highly expressed in immune cells, immunoproteasome-specific inhibitors selectively affect the function of activated immune cells while sparing other cell types that would be damaged by treatment with bortezomib, an anticancer drug approved by the US Food and Drug Administration (FDA) that inhibits the β5 subunit of both the constitutive CP and the immunoproteasome. Although these studies are at the preclinical stage, they hold great promise for the treatment of immune diseases.

The thymoproteasome in killer T cell development

The thymoproteasome was first described in 2007, and this originated from the finding that a non-intronic sequence proximal to the *Psmb5* locus in the mouse genome, encoding the β5 subunit, encodes a protein termed β5t (PSMB11)[25]. β5t is structurally homologous to β5 and β5i (PSMB8), and the genomic proximity of the genes for β5 and β5t is conserved in many mammalian species[25,54]. β5t is abundant specifically in cortical thymic epithelial cells (cTECs) in mice and humans[25,55–57]. In cTECs, β5t is incorporated into the CP along with β1i and β2i, forming a cTEC-specific subtype of proteasome termed the thymoproteasome (Fig. 1b)[25,58].

Lineage-tracing experiments in the mouse confirmed that transcription of β5t is highly specific to TECs and that nearly all medullary TECs (mTECs), which do not express β5t, are derived from progenitors that transiently transcribe β5t[57,59,60]. Direct promotion of β5t transcription by Foxn1, a transcription factor specifically expressed in TECs and skin hair cells, contributes to specific expression of β5t in TECs[54,61].

In contrast to β5 and β5i, whose substrate-binding pockets are mostly composed of hydrophobic amino acids, β5t contains many hydrophilic amino acids in its substrate pocket[25]. Consequently, thymoproteasomes exhibit a unique substrate specificity in endopeptidase proteolysis and thus produce a distinct spectrum of peptide fragments[25,58,62]. Indeed, thymoproteasome-expressing cells display a unique set of peptides associated with cell-surface MHC class I molecules[62].

Mice deficient in β5t appear to develop normally and have typically sized thymuses[25]. The corticomedullary structure of the thymus is undisturbed, and the cellularity of cTECs is normal[25,63]. cTECs in β5t-deficient mice lack thymoproteasomes and instead express immunoproteasomes[63]. Strikingly, the abundance of $CD8^+$ T cells in β5t-deficient mice is reduced to approximately 20% of that in normal mice, indicating that β5t-containing thymoproteasomes are essential for optimal production of $CD8^+$ T cells[25,57,63,64]. $CD8^+$ T cells in β5t-deficient mice have an altered T cell receptor (TCR) repertoire[63,64] as well as altered TCR responsiveness[65], indicating that β5t-containing thymoproteasomes are essential for optimizing repertoire formation and fine-tuning the responsiveness of $CD8^+$ T cells. No defects in $CD4^+$ T cells, including regulatory T cells, or in self-tolerance of T cells have been observed in β5t-deficient mice. Thus, thymoproteasomes specifically expressed in cTECs are essential for inducing optimal positive selection of $CD8^+$ T cells.

Mechanisms of thymoproteasome-mediated T cell selection

How thymoproteasomes affect $CD8^+$ T cells remains unknown. Because proteasome-mediated protein degradation primarily provides peptides associated with MHC class I molecules[66] and thymoproteasomes specifically affect MHC class I–restricted $CD8^+$ T cells but not MHC class II–restricted $CD4^+$ T cells, it is likely that thymoproteasome-generated self-peptides associated with MHC class I molecules expressed by cTECs contribute to the positive selection of $CD8^+$ T cells (Fig. 2a). Two hypotheses have been proposed to explain how these self-peptides optimize the positive selection of $CD8^+$ T cells, although it is also possible that thymoproteasomes may regulate $CD8^+$ T cell selection through alternate mechanisms[67–69].

The peptide-switch hypothesis posits that, because thymoproteasomes containing β5t are exclusively expressed in cTECs, cTECs are capable of displaying a unique set of MHC class I–associated self-peptides, and this difference in self-peptides is required for optimal positive selection of $CD8^+$ T cells. It is possible that the difference in the self-peptides displayed by cTECs from the self-peptides displayed by other cell types permits the survival of T cells that are positively selected by cTECs by escaping negative selection by other cells that express identical self-peptides (Fig. 2b). Supporting this hypothesis, severe loss of $CD8^+$ T cells and elevated negative selection of $CD8^+$-lineage thymocytes in mice deficient in all four cell-type-specific proteasome components (β1i, β2i, β5i and β5t) has been reported[70]. However, another study showed that the generation of $CD8^+$ T cells in the absence of β5t was comparably defective even when the difference in proteasomal β5 and β5i subunit composition was genetically created between cTECs and other cell types, indicating that the peptide switch cannot entirely explain why positive selection of $CD8^+$ T cells depends on thymoproteasomes[64].

Positive selection of $CD8^+$ T cells is selectively induced in immature thymocytes that are engaged in low-affinity TCR–peptide–MHC interactions[71,72]. In the low-affinity motif hypothesis, thymoproteasomes contribute to positive selection by providing structural features in MHC class I–associated self-peptides for low-affinity TCR interactions (Fig. 2c). Indeed, MHC class I–associated peptides in thymoproteasome-expressing embryonic fibroblasts are enriched in acidic amino acids at the second position from the C terminus and proline at the third amino acid from the C terminus, suggesting that thymoproteasome-expressing cTECs may also display these motifs in MHC class I–associated peptides, resulting in more advantageous low-affinity interactions with TCRs and thus positive selection of $CD8^+$ T cells[62].

Evolution of the proteasome and its specialized isoforms

Accumulated evidence indicates that adaptive immunity emerged in a common ancestor of vertebrates (Fig. 3). All classes of jawed ver-

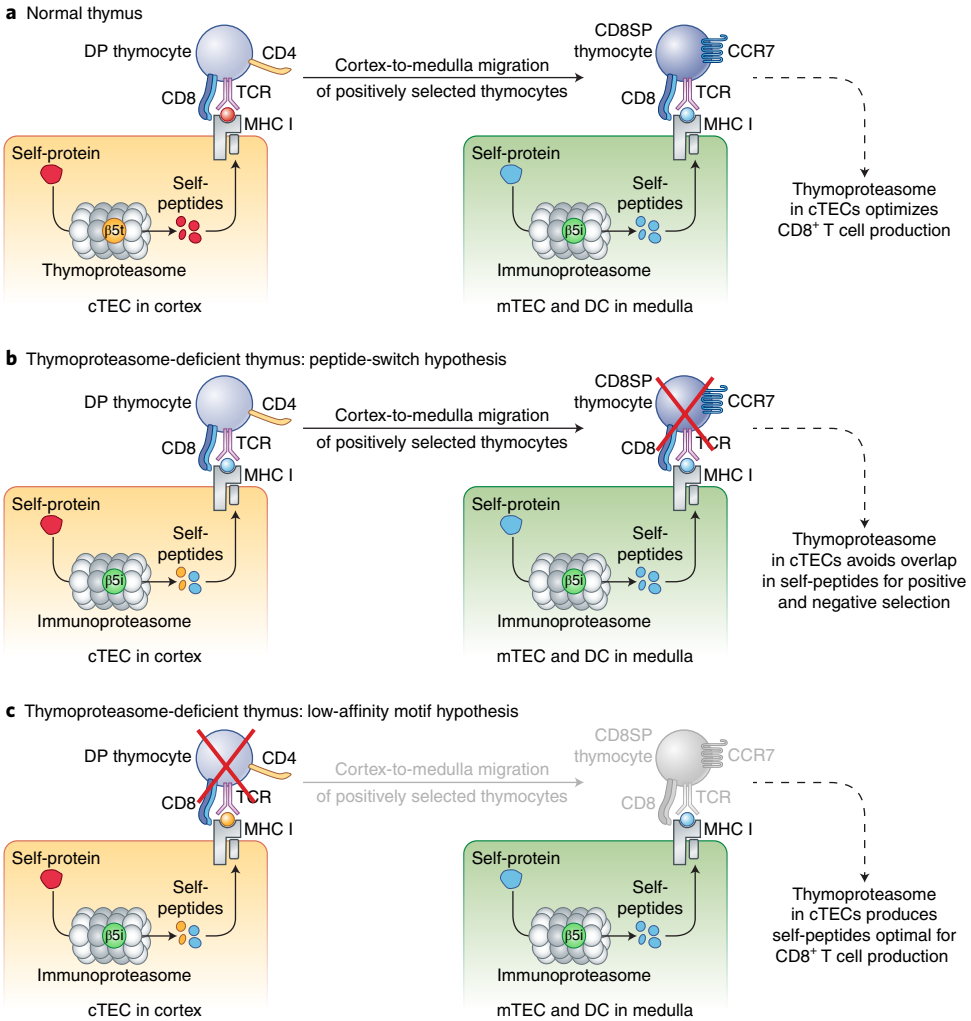

Fig. 2 | Mechanisms of thymoproteasome-mediated positive selection. a, In a normal thymus, cortical thymic epithelial cells (cTECs) express β5t-containing thymoproteasomes, which produce a unique set of self-peptides associated with MHC class I molecules displayed by cTECs. $CD4^+CD8^+$ thymocytes that are generated in the thymic cortex and that recognize the thymoproteasome-dependent self-peptide–MHC class I complexes at a low affinity are positively selected to differentiate into $CD4^-CD8^+$ thymocytes and to begin expressing the chemokine receptor CCR7. With help from CCR7-mediated attraction, positively selected thymocytes migrate to the thymic medulla, where they interact with additional self-antigen-presenting cells, including medullary thymic epithelial cells (mTECs) and dendritic cells (DCs), which primarily express β5i-containing immunoproteasomes and which negatively select self-antigen-reactive T cells for the establishment of self-tolerance. Only $CD4^-CD8^+$ thymocytes that do not receive negative-selection-inducing high-affinity TCR signals provided by mTECs and DCs are capable of export out of the thymus to form a functionally competent and self-tolerant repertoire of $CD8^+$ T cells. **b**, Peptide-switch hypothesis. In β5t-deficient mice, β5i-containing immunoproteasomes are expressed in cTECs as well as in mTECs and DCs in the thymus. Overlap between positive-selection-inducing peptides in the cortex and negative-selection-inducing peptides in the medulla causes medullary negative selection of positively selected thymocytes and thus loss of $CD8^+$ T cells. In this hypothesis, the difference in MHC class I–associated peptides between cTECs and other antigen-presenting cells in the thymus is the key to explaining how positive selection of $CD8^+$ T cells depends on the thymoproteasome. **c**, Low-affinity motif hypothesis. Thymoproteasomes contribute to the positive selection of $CD8^+$ T cells by providing structural features in MHC class I–associated self-peptides for low-affinity TCR interactions. In β5t-deficient mice, cTECs are unable to display a set of self-peptides that carry these low-affinity motifs; therefore, positive selection in the cortex is defective before thymocyte migration to the medulla.

tebrates (gnathostomes) ranging from cartilaginous fishes to mammals possess adaptive immunity in which TCRs, B cell receptors (BCRs) and MHC molecules function as antigen recognition molecules[73]. By contrast, jawless vertebrates, represented by lampreys and hagfish, use variable lymphocyte receptors (VLRs) as antigen receptors instead of TCRs and BCRs[74–76]. VLRs are members of the leucine-rich repeat (LRR) family and generate diversity comparable to that of gnathostome antigen receptors by assembling highly variable LRR modules through a gene-conversion-like mechanism. Consistent with the fact that VLRs are structurally unrelated to TCRs, jawless vertebrates have neither MHC class I nor MHC class II molecules. Because immunoproteasomes and thymoproteasomes generate peptides tailored for binding to MHC class I molecules, it is reasonable to assume that these specialized forms of the proteasome occur only in jawed vertebrates. Phylogenetic studies have shown that this is indeed the case.

Evolution of immunoproteasomes

The genes encoding the three catalytic β subunits of immunoproteasomes, β1i, β2i and β5i, are derived from more ancient genes encoding the β1, β2 and β5 subunits of the constitutive CP, respectively. They seem to have emerged not from independent gene duplication events, but from two rounds of whole-genome duplication (2R-WGD) known to have taken place before the emergence

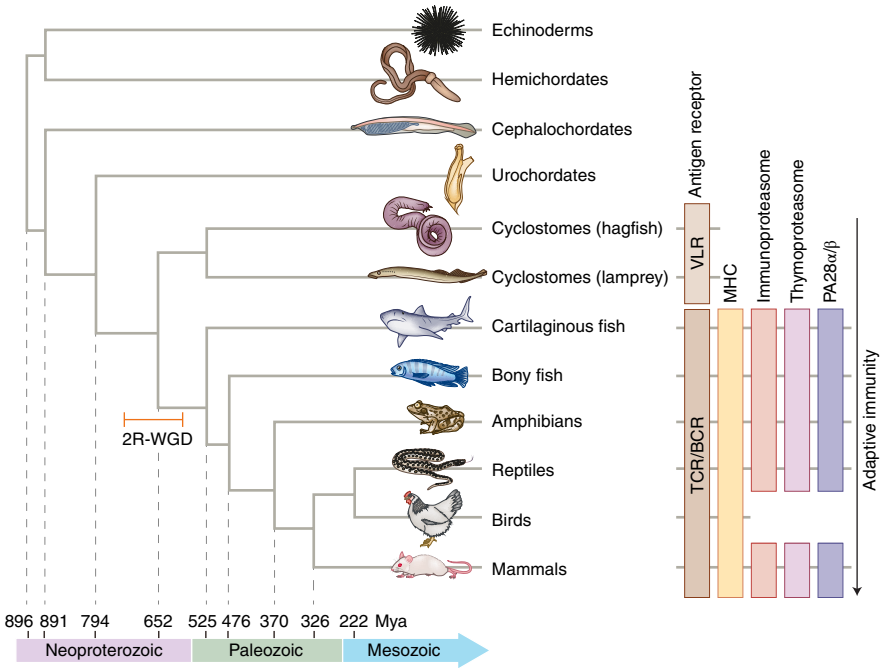

Fig. 3 | Evolution of adaptive immunity and proteasomes. Adaptive immunity based on lymphocytes exists only in vertebrates. All classes of jawed vertebrates have MHC molecules and use TCRs and BCRs as antigen receptors, whereas jawless vertebrates such as lampreys and hagfish lack MHC molecules and use VLRs as antigen receptors. Immunoproteasomes, thymoproteasomes and PA28αβ occur only in jawed vertebrates. Birds appear to have lost these components altogether for unknown reasons. Divergence times are based on molecular data compiled by Blair and Hedges[122]. BCR, B cell receptor; Mya, million years ago; PA28, proteasome activator 28; 2R-WGD, two rounds of whole-genome duplication; TCR, T cell receptor; VLR, variable lymphocyte receptor.

of a common ancestor of jawed vertebrates but after the emergence of protochordates such as amphioxus and tunicates[77,78]. This was initially proposed on the basis of the observation that the human MHC region, in which the genes for β1i and β5i are located, has paralogous regions (thought to be remnants from 2R-WGD) on three different chromosomes and that one of the MHC-paralogous regions contains the gene encoding β2[79,80]. Because the β1, β2 and β5 subunits of the CP are more closely related to one another than they are to other non-catalytic subunits, it was assumed that the genes encoding β1, β2 and β5 arose by tandem duplication from their common ancestor. Subsequent studies showed that the gene encoding β2i is also encoded in the MHC region in *Xenopus* and that the *Xenopus* β1 subunit is encoded in one of the MHC-paralogous regions[81], thus reinforcing the idea that the genes for β1i, β2i and β5i emerged by 2R-WGD.

Evolution of thymoproteasomes

The *PSMB11* gene, which encodes the thymoproteasome subunit β5t, is located adjacent to the *PSMB5* gene, which encodes the constitutive β 5 subunit, in all classes of jawed vertebrates for which genome sequence information is available[82]. From this, we can infer that *PSMB11* emerged in a common ancestor of jawed vertebrates by tandem duplication from *PSMB5*. A notable feature of *PSMB11* is that it lacks introns, unlike all other β-subunit genes. The exon–intron organization of gnathostome *PSMB5* differs completely from that of other β-subunit genes. This abnormality in gene structure is unique to gnathostome *PSMB5* genes, as the *PSMB5* genes of jawless vertebrates and invertebrates have the structure typical of β-subunit genes. It has been proposed that *PSMB5* lost all of its introns in a jawed vertebrate ancestor by homologous recombination with a reverse-transcriptase product of a spliced mRNA and that, following the tandem duplication of intronless *PSMB5* in a jawed vertebrate ancestor, *PSMB11* has remained intronless but *PSMB5* acquired introns at completely different positions[82].

An enigma in proteasome evolution

$CD8^+$ T cell production is severely impaired in thymoproteasome-deficient mice[25], and presentation of MHC class I epitopes is markedly reduced in immunoproteasome-deficient mice[40]. Mice that lack both thymoproteasomes and immunoproteasomes display a profound defect in the generation of $CD8^+$ T cells[70]. Taken together, these observations indicate that these two specialized forms of proteasomes have an essential role in antigen presentation and/or thymic selection. In this regard, it is striking that birds, including chickens, turkeys and zebra finches, apparently have neither thymoproteasomes nor immunoproteasomes[82–85]. Because reptiles have both, loss of thymoproteasomes and immunoproteasomes must have taken place in an avian lineage. Additionally, birds apparently lack PA28αβ[86], indicating that they lost major proteasome subunits involved in antigen presentation by MHC class I molecules (Fig. 3). These findings reinforce the previous observation that birds generally have a smaller repertoire of immune-related genes than mammals[87]. Some alleles of dominantly expressed chicken MHC class I BF2 molecules bind peptides with an acidic residue at the C terminus[88]. This is consistent with the absence of immunoproteasomes. Although the T cell repertoire in chickens is much less complex than that in mammals, the essential features of T cell development are conserved between chickens and mammals[89]. Any compensatory or adaptive mechanisms that birds might have evolved for the loss of thymoproteasomes and immunoproteasomes merit detailed study.

The immunoproteasome and thymoproteasome in human diseases

Because proteasome function is essential for every single cell, human diseases caused by mutations in proteasome genes were not identified until recently. However, identification of a mutation in an immunoproteasome subunit gene linked to human disorders led to the rapid discovery of many mutations in proteasome genes.

The proteasome in autoinflammatory syndromes

Studies have identified autosomal recessive mutations within the *PSMB8* gene, which encodes β5i, as the cause of a spectrum of autoinflammatory disorders that manifest as recurrent spiking fever, skin rash or erythema, and lipodystrophy starting in early childhood. These include Nakajo–Nishimura syndrome (NNS); joint contractures, muscle atrophy, microcytic anemia and panniculitis-induced lipodystrophy (JMP) syndrome; Japanese autoinflammatory syndrome with lipodystrophy (JASL); and chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE)[90–93]. This spectrum of disorders is referred to as proteasome-associated autoinflammatory syndrome (PRAAS)[94,95], and the diseases are characterized by impaired proteasome activity in immunoproteasome-expressing cells mainly due to defects in CP assembly, leading to higher p38 phosphorylation and elevated IL-6 and type I IFN signaling through an unidentified pathway. Symptoms do not seem to be the result of specific impairment of the immunoproteasome or β5i, but rather a decrease in proteasome activity itself, as mutations in the constitutive CP subunit genes *PSMA3* (encoding α7) and *PSMB4* (β7), as well as the immunoproteasome gene *PSMB9* (β1i) and the general CP assembly chaperone gene *POMP* (*UMP1*), have all been identified as causes of PRAAS[96].

Human genomic variations in *PSMB11*

In humans, many genomic variations in the *PSMB11* locus, which encodes β5t, have been noted. Among them, the most frequent minor allele (G49S of the single-nucleotide polymorphism rs34457782) affects the post-translational processing of β5t protein to its catalytically active state[97]. Introduction of this variation to the mouse genome revealed that heterozygotes showed reduced β5t expression in cTECs, and homozygous mutants further exhibited a reduction in the cellularity of $CD8^+$ T cells. A cohort study has revealed no severe health problems in many heterozygous and several homozygous human individuals[97], although one study reported the association of this polymorphism with Sjögren's syndrome[98]. Long-term analysis of health status, particularly in homozygotes, is expected to improve understanding of thymoproteasome-dependent positive selection of $CD8^+$ T cells in humans.

Expression of β5t in thymoma

Thymomas are relatively rare neoplasms arising from TECs or cells in the process of differentiating into TECs, with a variable number of non-neoplastic lymphocytes. Histologically, they are classified into type A, type B (B1 to B3) and type AB (a variable mixture of type A and type B components)[99]. The β5t expression pattern differs markedly depending on histologic type: β5t is expressed in most cases of type B thymoma, but not in type A thymomas[100]. In type AB thymomas, β5t is expressed in type B but not in type A components[101]. Morphologically, type A and type B thymomas exhibit differentiation into mTECs and cTECs, respectively. Therefore, β5t retains its normal physiologic expression pattern in the context of a thymoma and is a reliable marker for detecting neoplastic epithelial cells differentiating into cTECs.

Perspectives

In this Review, we have thoroughly described the immunoproteasome and the thymoproteasome, including recent findings about their molecular evolution and mechanisms of action. These specialized isoforms of the proteasome are derived from the CP of the constitutive proteasome and serve prominent functions in the processing of endogenous antigens in the killer T cell response.

The killer T cell response is initiated by the generation of an antigenic peptide that is a ligand of MHC class I, which is responsible for designating self-identity at the molecular level. The proteasome has a crucial role in the processing of intracellular self-antigens[66]. When a pathogen invades and causes secretion of cytokines, the immunoproteasome is rapidly induced along with a series of antigen-presentation molecules (for example, MHC class I and TAP), and consequently, antigen processing is accelerated in a highly sophisticated manner[77,102]. Additionally, the proteasome also catalyzes the process of MHC class I presentation of extracellular antigens via cross-presentation involving autophagy[103]. Intriguingly, a study suggests that the immunoproteasome enhances the cross-presentation pathway[104].

The production of non-self-antigen peptides is essential for initiating an efficient killer T cell response. There is a long history of research on tumor antigens, and researchers sought to identify cancer-specific antigens such as mutant gene products so that T cells could attack cancer cells[105]. Neoepitopes and cryptic peptides that serve as non-self tumor antigens should accelerate the immune response by facilitating the killer T cell response[106,107]. How the immunoproteasome participates in the processing of these tumor-associated non-self antigens is an important topic for study in the future.

The DRiP (defective ribosomal product) hypothesis and the concept of 'immunoribosomes', a subset of ribosomes specialized for generating immunologically relevant DRiPs[108,109], are relatively new ideas concerning antigen presentation by the proteasome. Although it was long believed that newly synthesized proteins adopt a 3D structure immediately after translation, a considerable number of nascent proteins leaving the ribosome fail to fold and are quickly ubiquitinated and subsequently degraded by the proteasome. The hypothesis that these DRiP-derived peptides are used as the major source of antigenic peptides provides a reasonable explanation for rapid induction of the killer T cell response after viral infection despite the fact that viral proteins tend to be very stable. This controversial hypothesis will surely form the basis of many future studies[110], and it will be interesting to see how the immunoproteasome is involved in utilization of DRiPs in the antiviral immune response.

Proteasome-catalyzed peptide splicing (PCPS) is a recently discovered mechanism for proteasome-mediated generation of $CD8^+$ T cell epitopes that expands the diversity of the epitopes presented by MHC class I molecules[111,112]. This splicing process was shown to occur in the proteasome through a transpeptidation reaction involving an acyl-enzyme intermediate. Surprisingly, recent analysis has shown that one-third of the self-peptides presented on the cell surface are generated by PCPS[113], which, if true, implies a large expansion of killer T cell responses, including antitumor immunity, and could transform the field. There have been reports that the immunoproteasome is responsible for PCPS[114], but thus far there is no knowledge of a relationship between the thymoproteasome and PCPS. The immunological significance of PCPS in humans, particularly in relation to the immune-type proteasomes, is unknown, and there are many open questions in the field.

Neoantigens, cryptic peptides, DRiPs and PCPS are all expected to contribute greatly to expansion of the diversity and pool size of antigenic peptides, which has promising implications for immunotherapy. In particular, when the number of tumor-specific T cells increases, immune checkpoint inhibitors may be more effective. Conversely, the ineffectiveness of checkpoint inhibitors in some patients may be attributed to defects in processing and presentation of tumor antigens. Therefore, research on the immune-type proteasomes may contribute to improving the efficacy of immune checkpoint inhibitors as well as therapies for other immune diseases.

It has become apparent that the thymoproteasome is essential for the optimal production of $CD8^+$ killer T cells, but isolation of peptides that can actually induce positive selection will be required for definitive determination of the mechanism. Although such study is currently underway, it is extremely difficult owing to the small number of cTECs in the mouse thymus and the sensitivity limit on MHC-eluted peptide analysis by mass spectrometry. Identification of these peptides will greatly improve understanding of the molecular mechanism of positive selection and address fundamental questions about adaptive immunity.

Positive selection of MHC class II–restricted T cells is likely quite different from the case for CD8+ killer T cells because it involves CD4+ T cells and their interaction with antigen-presenting cells such as dendritic cells, macrophages and B cells to promote and regulate immune responses, including cytokine and antibody production. Considering the diverse repertoire of CD4+ T cells, it is not surprising that there is a similar mechanism in the principle of antigen processing for CD4+ T cell repertoire formation in the thymus. Interestingly, a group of thymus-specific endosomal–lysosomal proteases, including cathepsin L and TSSP, are expressed in cTECs. Mice lacking these molecules are defective in CD4+ T cells in the thymus and secondary immune organs, suggesting that these molecules are involved in the positive selection of CD4+ T cells[115]. Cathepsin L and TSSP may contribute to the production of unique MHC class II–binding self-peptides that optimally induce positive selection of CD4+ T cells, analogous to the role of the thymoproteasome in positive selection of CD8+ T cells. In this regard, it is interesting to note that the members of the cathepsin family involved in the production of MHC class II–binding peptides, such as cathepsin D, and cathepsins involved in CD4+ T cell development, cathepsins L1 and L2, also appear to have emerged by 2R-WGD thought to have taken place in an ancestor of the jawed vertebrate lineage[116].

Tertiary and quaternary structures of the immunoproteasome have been determined, and numerous attempts have been made to produce inhibitors on the basis of the atomic structure[117–119]. The development of an inhibitor specific for the thymoproteasome has also been attempted but has not yet succeeded. Development of inhibitors specific to the immune-type proteasomes would provide a promising tool for treating cancer and immunological diseases in the future.

Gene duplication has a key role in the evolution of biological systems. This is also the case with the immune system. As described here, genes encoding immunoproteasome and thymoproteasome subunits arose by duplication of existing genes encoding constitutive subunits nearly concomitantly with the emergence of MHC genes. This suggests that the two immune-type proteasomes are inseparable from the emergence of MHC-based adaptive immunity and strongly suggests their biological importance in antigen presentation by MHC class I molecules.

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Competing interests

The authors declare no competing interests.

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