Tissue-specific proteasomes in generation of MHC class I peptides and CD8+ T cells

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Abstract

Thymoproteasomes and immunoproteasomes are two types of tissue-specific proteasomes, which contribute to the production of MHC class I (MHC-I)-associated peptides that are important for the development and function of CD8+ cytotoxic T cells. Thymoproteasomes are specifically expressed by cortical thymic epithelial cells and are important for MHC-I-dependent positive selection of developing thymocytes, whereas immunoproteasomes are abundant in many other cells, including hematopoietic cells and medullary thymic epithelial cells. Here we summarize the role of these two tissue-specific proteasomes, focusing on their functions in the development of CD8+ T cells in the thymus.

Introduction

The immune system protects an organism from pathogenic invasion and thus requires machinery to distinguish self-components from foreign substances. In the case of cytotoxic T cell-mediated response, which is important for immune response to cancer, virus, and other pathogens, T-cell antigen-receptors (TCRs) expressed by CD8+ T cells function by recognizing short antigenic peptides presented by MHC class I (MHC-I) molecules. The MHC-I-associated peptides are mostly derived from the proteasome-dependent degradation of ubiquitinated proteins in the cytoplasm and the nucleus, transported by the TAP transporter to the lumen of the endoplasmic reticulum (ER), trimmed by amino terminal proteases in the cytoplasm and ER, and associated with MHC-I molecules. Thus, proteasomes are a key player in the generation of MHC-I-associated peptides (1).

The immunoproteasome, which is a type of tissue-specific proteasomes, contains β1i (encoded by *Psmb9*), β2i (encoded by *Psmb10*), and β5i (encoded by *Psmb8*) catalytic

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subunits and is expressed constitutively in hematopoietic cells and induced in most somatic cells upon exposure to proinflammatory cytokines. The immunoproteasome expressed in various antigen-presenting cells, including dendritic cells (DCs), plays a major role in antigen processing for CD8^{+} T cell-mediated immune response (2). Importantly, the development of CD8^{+} T cells, which occurs in the thymus, requires TCR signals triggered by the interaction between TCRs and peptide-MHC-I complexes displayed in the thymic cortex. Another type of tissue-specific proteasomes, the thymoproteasome, which contains β1i, β2i, and β5t (encoded by *Psmb11*) catalytic subunits and is expressed specifically in cortical thymic epithelial cells (cTECs), is essential for the optimal development of CD8^{+} T cells in the thymus (2, 3).

In this article, we summarize our understanding of the role of these two tissue-specific proteasomes, i.e., immunoproteasomes and thymoproteasomes, in the development of CD8^{+} T cells in the thymus. We also discuss possible link among these tissue-specific proteasomes, the thymus, and the adaptive immune system from a perspective of comparative biology.

Thymoproteasome for thymic development of CD8^{+} T cells

The thymoproteasome is expressed exclusively in cTECs. β5t is one of the three catalytically active subunits of the thymoproteasome and the only subunit that is specifically expressed in cTECs. In this regard, the expression specificity of the β5t subunit in cTECs is responsible for the cTEC-specific expression of the thymoproteasome. The cTEC-specific expression of β5t is partially ascribed to transcription factor Foxn1 (4), which is expressed specifically in epithelial cells of the thymus and the skin. β5t has a unique enrichment of hydrophilic amino acids in its substrate-binding pocket, unlike other proteolytic proteasome components including β5 and β5i, which have many hydrophobic amino acids in their substrate-binding pockets (3). Consequently, the thymoproteasome exhibits a unique substrate specificity in endopeptidase activity and thus produces a unique spectrum of peptides distinct from those generated by other types of proteasomes including the immunoproteasome (5, 6). The thymoproteasome differs from the immunoproteasome in terms of proteolytic activity, not only in substrate specificity but also in quantitative kinetics to produce peptides (7). Importantly, MHC-I-associated peptides detected by mass spectrometric analysis are partially overlapped but clearly different between embryonic fibroblasts that are engineered to express thymoproteasomes and immunoproteasomes (5), suggesting that thymoproteasome-expressing cTECs display a unique spectrum of MHC-I-associated peptides that are not available in any other cells in the body.

Studies of β5t-deficient mice have demonstrated that β5t-containing thymoproteasomes are essential for the development of an optimal number and a functionally competent TCR repertoire of CD8^{+} T cells. Indeed, the number of CD8^{+} T cells in β5t-deficient mice is reduced to 20 to 30% of the number in control mice, and the TCR repertoire and the antigen responsiveness of the remaining CD8^{+} T cells are altered and impaired by β5t-deficiency (3, 8–10). It is reasonable to speculate that the thymoproteasome-dependent unique spectrum of MHC-I-associated peptides are responsible for the optimal development of a functionally competent TCR repertoire of CD8^{+} T cells. However, technical limitations in the isolation

of cTECs from mice (11–13) have hindered the mass spectrometric identification of thymoproteasome-dependent MHC-I-associated peptides displayed by cTECs. Thus, it remains unknown how the thymoproteasome contributes to the optimal development of CD8$^+$ T cells. The possible contribution of thymoproteasome-dependent mechanisms other than the production of a unique spectrum of MHC-I-associated peptides has been suggested (14, 15) but has remained controversial (16–18).

Recently it was revealed that the defective development of CD8$^+$ T cells in β5t-deficient mice was detectable as early as the CD4$^+$CD8$^+$ CD69$^+$CCR7$^-$ cortical thymocyte stage, without the contribution of the thymic medulla and without apoptosis-dependent negative selection processes (19, 20). These results demonstrated that the thymoproteasome optimized CD8$^+$ T cell development by directing the positive selection of CD8$^+$ T cells in the thymic cortex, independent of the negative selection that occurs either in the thymic cortex and thymic medulla.

Immunoproteasome for thymic development of CD8$^+$ T cells

Unlike the thymoproteasome that is expressed exclusively in cTECs, the immunoproteasome is expressed in the body by many cell types, including hematopoietic antigen-presenting cells. In the thymus, the immunoproteasome-expressing cells include developing thymocytes, DCs, and medullary TECs (mTECs). In mice deficient in immunoproteasome-specific subunit β5i, thymoproteasome expression in cTECs is not affected and the development of CD8$^+$ T cells is not reduced (21). However, alterations in the TCR repertoire generated in the thymus are detected in CD8$^+$ T cells of β5i-deficient mice (22, 23). How β5i affects the TCR repertoire of CD8$^+$ T cells remains unclear.

Mice deficient in all three immunoproteasome catalytic subunits β1i, β2i, and β5i show reduced surface expression of MHC-I molecules in various hematopoietic cells, an altered spectrum of MHC-I-associated peptides in spleen cells, and a reduced number of CD8$^+$ T cells (24, 25), indicating that these immunoproteasome components are important for the generation of MHC-I-associated peptides and the development of CD8$^+$ T cells. Because β1i and β2i are also the components of the thymoproteasome, the impaired development of CD8$^+$ T cells in the triple-knockout mice may be, at least in part, due to defects in the expression and function of the thymoproteasome in cTECs.

It was further shown that mice lacking all four tissue-specific catalytic subunits β1i, β2i, β5i, and β5t were severely defective in the generation of CD8$^+$ T cells, indicating that the immunoproteasome and the thymoproteasome are essential for the development of the vast majority of CD8$^+$ T cells (25). The implication of these findings in the context of the possible contribution of the "proteasome switch" to the development of CD8$^+$ T cells and the classical "peptide switch" hypothesis for the thymic selection of T cells are discussed elsewhere (20, 26).

Tissue-specific proteasomes and vertebrate immune system

Ubiquitously expressed standard proteasomes are shared in all eukaryotes and archaea. On the other hand, tissue-specific immunoproteasomes and thymoproteasomes are restricted in

vertebrate species that carry the adaptive immune system (2, 27). The adaptive immune system is equipped with antigen-recognition receptors, i.e., immunoglobulins and TCRs, which generate diverse antigen-recognition specificities through the V(D)J recombination and the gene conversion (28). Antigen-recognition by TCRs requires MHC-dependent antigen presentation, which requires antigenic peptide processing. Accordingly, genes that encode immunoglobulins, TCRs, MHC molecules, TAP transporter, immunoproteasomes, and thymoproteasomes are present only in the vertebrate species that carry the adaptive immune system. Those vertebrate species include cartilaginous fish, bony fish, amphibians, reptiles, and mammals, including mouse and human (27).

The specific presence of the adaptive immune system-specific genes in these vertebrate species coincides with the presence of the thymus, an organ responsible for producing T cells. Thus, it is interesting to point out that thymoproteasome β5t-encoding gene *Psmb11*, which is specifically expressed in cTECs, emerges concomitantly with the thymus in vertebrate species. β5t and the thymus are synchronized evolutionary. *Psmb11* lacks introns and is located next to *Psmb5*, which encodes standard proteasome subunit β5. It was speculated that *Psmb11* arose by tandem duplication from *Psmb5* through a reverse transcriptional mechanism (27).

It is also interesting to note that the genome sequences from bird species currently available in public NCBI database do not include the genes that encode β1i, β2i, β5i, and β5t. Thus, both the thymoproteasome and the immunoproteasome are not evident in birds, even though birds are equipped with the thymus and the adaptive immune system (27). Indeed, mass spectrometric analysis of chicken 20S proteasomes purified from lymphoid tissues, including the thymus and the spleen, failed to provide any evidence for the presence of thymoproteasomes or immunoproteasomes (29). The lack of genes involved in the immune system in birds is not limited to these tissue-specific proteasomes but includes lymphotoxins and another MHC-I-processing molecule tapasin (30). The lack of lymphotoxin genes coincides with the absence of lymph nodes in birds (30), as lymphotoxin signals are essential for the development of lymph nodes in mice (31). Consequently, the immune responses in birds may be somewhat limited or impaired in comparison with those in other vertebrate species including mammals. It is possible, however, that the immune system in birds is equipped with the machinery to compensate for the lack of thymoproteasomes and immunoproteasomes, for example, by gaining their unique strategies like the development of the Bursa of Fabricius.

Thymoproteasome subunit β5t as a thymus-specific and vertebrate-specific molecule

The appearance of thymoproteasome subunit β5t expressed specifically in cTECs coincides with the emergence of the thymus in vertebrate species. To gain an insight into the evolutionary relationship between β5t and the thymus, and to seek additional molecular pathways associated with vertebrate-specific development and function of the thymus, we performed global taxonomy analysis of molecules highly expressed in mouse TECs using NCBI taxonomy database. Among 199 molecules that are significantly more abundant in

cTECs than mTECs in both mRNA expression and protein expression (18), we noticed that 10 molecules were specifically present in vertebrate species (Table 1). In parallel, we also analyzed 202 molecules that are significantly more abundant in mTECs than cTECs (18) and extracted 23 vertebrate-specific molecules (Table 1). It should be noted that β5t (*Psmb11*) stood out as a cTEC-specific and vertebrate-specific molecule, which functionally contributes to the processing of MHC-associated antigens in the thymus. It is also interesting to note that in addition to β5t, calpain 1 is extracted as a vertebrate-specific molecule that is abundant in cTECs. Similar to proteasomes, calpain 1 is a cytoplasmic and non-lysosomal protease. However, calpain 1 is a Ca^{2+}-dependent protease that is expressed broadly but not specifically in cTECs. A study has shown that the inhibition of calpain 1 reduced MHC class II (MHC-II)-associated presentation of a peptide derived from cytoplasmic, but not endocytosed, glutamate decarboxylase (GAD) to MHC-II-restricted GAD-specific T cells, suggesting that calpain 1 contributes to the processing of MHC-II-associated endogenous peptides (32). In addition to calpain 1, calpain 2 is also abundant in cTECs (26). Uncovering the role of calpains in the thymus is awaited in future studies. Nevertheless, the taxonomy analysis has highlighted the uniqueness of β5t as a thymus-specific and vertebrate-specific molecule.

Perspectives

Accumulating evidence has highlighted the important role of two tissue-specific proteasomes, the immunoproteasome and the thymoproteasome, in the generation and function of CD8^{+} T cell-mediated immunity. However, many key questions remain unanswered. Among them, it appears most important and interesting to identify MHC-I-associated peptides expressed by thymic antigen-presenting cells, including cTECs and mTECs. The identification of MHC-I-associated peptides expressed by cTECs, which critically promote β5t-dependent positive selection in the thymic cortex, will likely improve our understanding of the nature of T-cell positive selection occurring in the thymus. The limited availability of isolated cTECs (11–13) has been a hindrance to the biochemical analysis of MHC-I-associated peptides, although genetically engineered mice carrying an enlarged, yet functionally capable, thymus (18) may be a useful tool for biochemical identification of β5t-dependent MHC-I-associated peptides that induce positive selection in the thymus.

Acknowledgments

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Highlights

- Thymoproteasomes and immunoproteasomes contribute to the development of CD8$^+$ T cells.

- Thymoproteasomes optimize the positive selection of CD8$^+$ T cells in the thymic cortex.

- Thymoproteasome component β5t is a thymus-specific and vertebrate-specific molecule.

Table 1.

Vertebrate-specific Molecules Abundant In cTECs Or mTECs

Abundance	Gene Symbol	Protein Name
cTECs	Arhgap45	Rho GTPase Activating Protein 45
	Capn1	Calpain 1
	Clic5	Chloride Intracellular Channel 5
	Coro1a	Coronin 1A
	Ehhadh	Enoyl-CoA Hydratase And 3-Hydroxyacyl CoA Dehydrogenase
	Mbnl3	Muscleblind Like Splicing Regulator 3
	Palm3	Paralemmin 3
	Psmb11	Proteasome Subunit Beta 11 (Beta5t)
	Pygb	Glycogen Phosphorylase B
	Scn4b	Sodium Voltage-Gated Channel Beta Subunit 4
mTECs	Alox5ap	Arachidonate 5-Lipoxygenase Activating Protein
	Bspry	B Box And SPRY Domain-Containing Protein
	Ckmt1	Creatine Kinase, Mitochondrial 1A
	Cttnbp2nl	CTTNBP2 N-Terminal Like
	Fabp6	Fatty Acid Binding Protein 6
	Gsta2	Glutathione S-Transferase Alpha 2
	Gsdma	Gasdermin A
	Gsta1	Glutathione S-Transferase A1
	Il4i1	Interleukin 4 Induced 1
	Krt13	Keratin 13
	Lxn	Latexin
	Ly6d	Lymphocyte Antigen 6 Family Member D
	Mydgf	Myeloid Derived Growth Factor
	Nectin4	Nectin Cell Adhesion Molecule 4
	Prph	Peripherin
	Samd4b	Sterile Alpha Motif Domain Containing 4B
	Sephs2	Selenophosphate Synthetase 2
	Sncg	Synuclein Gamma
	Soat2	Sterol O-Acyltransferase 2
	Stap2	Signal Transducing Adaptor Family Member 2
	Tppp3	Tubulin Polymerization Promoting Protein Family Member 3
	Trpm5	Transient Receptor Potential Cation Channel Subfamily M Member 5
	Zfp41	Zinc Finger Protein 41