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Thymoproteasome Shapes Immunocompetent Repertoire of CD8$^+$ T Cells

Takeshi Nitta,[1] Shigeo Murata,[3] Katsuhiro Sasaki,[4] Hideki Fujii,[5] Adiratna Mat Ripen,[1] Naozumi Ishimaru,[2] Shigeo Koyasu,[5,6] Keiji Tanaka,[4] and Yousuke Takahama[1,*]
[1]Division of Experimental Immunology, Institute for Genome Research
[2]Department of Pathology, Institute for Health Bioscience
University of Tokushima, Tokushima 770-8503, Japan
[3]Laboratory of Protein Metabolism, Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo 113-0033, Japan
[4]Laboratory of Frontier Science, Tokyo Metropolitan Institute of Medical Science, Tokyo 156-8506, Japan
[5]Department of Microbiology and Immunology, Keio University School of Medicine, Tokyo 160-8582, Japan
[6]Research Center for Science Systems, Japan Society for the Promotion of Science, Tokyo 102-8472, Japan
*Correspondence: takahama@genome.tokushima-u.ac.jp
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SUMMARY

How self-peptides displayed in the thymus contribute to the development of immunocompetent and self-protective T cells is largely unknown. In contrast, the role of thymic self-peptides in eliminating self-reactive T cells and thereby preventing autoimmunity is well established. A type of proteasome, termed thymoproteasome, is specifically expressed by thymic cortical epithelial cells (cTECs) and is required for the generation of optimal cellularity of CD8$^+$ T cells. Here, we show that cTECs displayed thymoproteasome-specific peptide-MHC class I complexes essential for the positive selection of major and diverse repertoire of MHC class I-restricted T cells. CD8$^+$ T cells generated in the absence of thymoproteasomes displayed a markedly altered T cell receptor repertoire that was defective in both allogeneic and antiviral responses. These results demonstrate that thymoproteasome-dependent self-peptide production is required for the development of an immunocompetent repertoire of CD8$^+$ T cells.

INTRODUCTION

Most T lymphocytes are generated in the thymus. By entering the thymus and interacting with the microenvironment of the thymic cortex, lymphoid progenitor cells are induced to develop into thymocytes that express T cell receptor (TCR), as well as coreceptors CD4 and CD8 (double-positive, DP) (Scollay et al., 1988). Newly generated DP thymocytes that express a virgin set, or the germline repertoire, of TCRs are motile, seeking TCR engagement by interacting with peptide-major histocompatibility complex (MHC) expressed in the cortical microenvironment (Bousso et al., 2002; Li et al., 2007). DP thymocytes that receive weak signals of low-avidity (i.e., affinity × number per cell) TCR engagement are induced to survive and further develop into mature T cells that express large amounts of TCRs and either one of CD4 or CD8 (single-positive, SP) (Ashton-Rickardt et al., 1993, 1994; Hogquist et al., 1994; Sebzda et al., 1994; Takahama et al., 1994; Alam et al., 1996). This process is referred to as positive selection and is assumed to contribute to the enrichment of an immunocompetent, i.e., useful and self-protective, repertoire of self-MHC-restricted foreign-antigen-reactive T cells (Kisielow et al., 1988; von Boehmer, 1994; Allen, 1994; Starr et al., 2003). In contrast, DP thymocytes that receive strong signals of high-avidity TCR engagement are deleted, a process referred to as negative selection (Kappler et al., 1987; Palmer, 2003). It is well appreciated that negative selection is essential for eliminating self-reactive T cells and thereby preventing autoimmunity (Strasser, 2005; Siggs et al., 2006).

Unlike negative selection, the physiological and pathological importance of positive selection is still controversial. Positive selection was originally identified as the thymic process that determines the MHC-restriction specificity of T cells (Bevan, 1977; Zinkernagel et al., 1978) and is assumed to contribute to enriching an inherently rare T cell repertoire that is useful in the body harboring a given combination of MHC polymorphisms. However, it was shown that the germline TCR repertoire before positive and negative selection is inherently conserved to be MHC reactive (Zerrahn et al., 1997; Merkenschlager et al., 1997). It was also shown that a single MHC-peptide ligand identified in B lymphoma cells could induce positive selection of a diverse repertoire of T cells (Ignatowicz et al., 1996; Fukui et al., 1997). Based on these results, along with the structural analysis of TCR-MHC-peptide interactions, it is proposed that the specificity of TCR for peptides is not demanding during positive selection and that rather than positive selection, it is the subsequent negative selection that establishes the MHC-restriction specificity and the peptide specificity of peripheral T cells (Marrack and Kappler, 1997; Huseby et al., 2005; Dai et al., 2008; Huseby et al., 2008). However, those T cells generated in mice expressing single MHC-peptide ligands show markedly reduced cellularity and an unusual TCR repertoire that occasionally causes autoimmunity (Ignatowicz et al., 1996; Huseby et al., 2005; Oono et al., 2001). Thus, it is unclear whether the positive selection detectable in those single MHC-peptide-expressing mice represents positive selection occurring in the normal body. More importantly, it remains unanswered whether

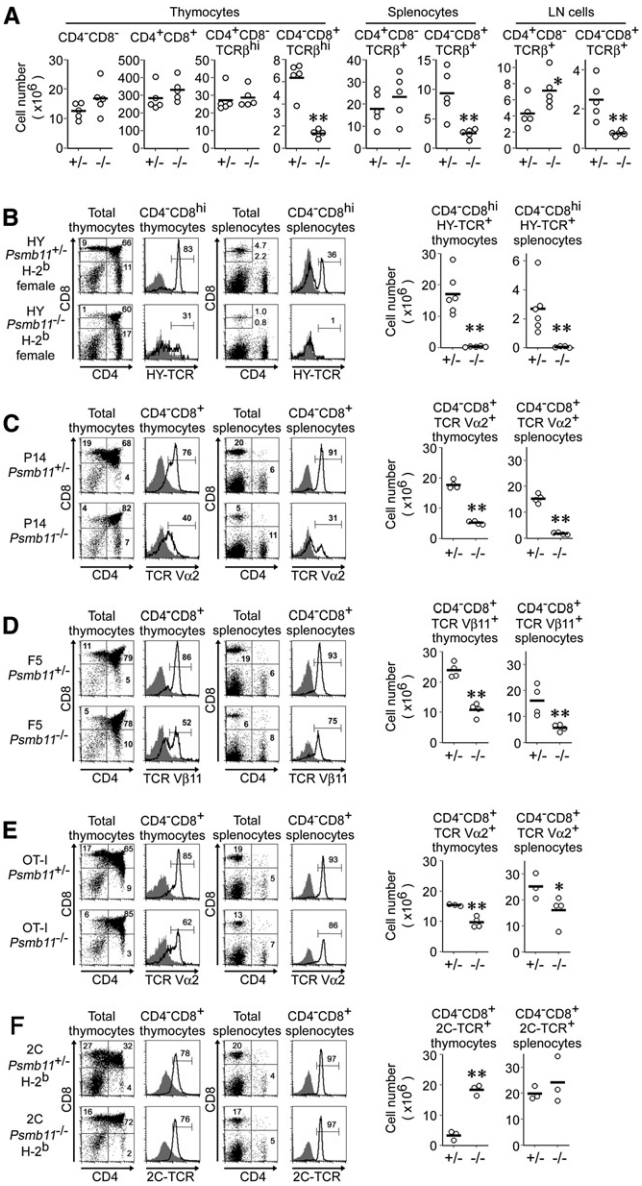

Figure 1. β5t Regulates Positive Selection of Diverse, but Not All, TCR Specificities of CD8⁺ T Cells

(A) Numbers (per mouse) of thymocytes, splenic T cells, and lymph node T cells in indicated populations were determined by flow cytometry in 3- to 6-week-old *Psmb11*$^{+/-}$ or *Psmb11*$^{-/-}$ mice. Data of individual mice (circles) and means (bars) are shown (n = 5).

(B–F) Thymocytes and splenocytes from HY-TCR-transgenic H-2^b female mice (B), as well as P14-TCR- (C), F5-TCR- (D), OT-I-TCR- (E), and 2C-TCR- (F) transgenic H-2^b mice, were analyzed by flow cytometry for CD4 and CD8. Histograms show TCR expression profiles (solid lines) obtained by staining with an antibody specific for HY-TCR (T3.70) (B), TCR Vα2 (C), TCR Vβ11 (D), TCR Vα2 (E), or 2C-TCR (1B2) (F), overlaid with control staining profiles (shaded lines), of the indicated cell populations. Numbers indicate percentage of cells within indicated areas. Graphs indicate cell numbers (per mouse) of indicated populations in individual mice (circles) and their means (bars) (n = 3 to 6). *p < 0.05; **p < 0.01. See also Figure S1.

self-peptides displayed in the thymus play a role in positive selection of an immunocompetent repertoire of T cells.

We previously identified β5t, a proteasome subunit that is specifically expressed in thymic cortical epithelial cells (cTECs) (Murata et al., 2007). Proteasomes are multicatalytic protease complexes that are responsible for the degradation of cytoplasmic proteins and the production of antigen peptides presented by MHC class I molecules (Brown et al., 1991; Rock et al., 1994). The β5 catalytic subunits of the proteasome are responsible for its chymotrypsin-like activity, producing peptides that possess at their carboxyl termini hydrophobic residues that can bind efficiently to MHC class I molecules (Heinemeyer et al., 1993; Fehling et al., 1994; Rock and Goldberg, 1999). β5t-containing proteasomes, termed thymoproteasomes, exhibit low chymotrypsin-like activity compared with the other types of proteasomes, i.e., β5-containing standard proteasomes or β5i-containing immunoproteasomes (Murata et al., 2007). Interestingly, β5t-containing cTEC-specific thymoproteasomes are essential for the generation of the optimal cellularity of CD8$^+$ T cells (Murata et al., 2007). However, the mechanism by which thymoproteasomes regulate T cell development has yet to be disclosed.

In this study, we examined how thymoproteasomes regulate T cell development. Our results showed that thymoproteasomes were essential for the positive selection of major and diverse, but not all, repertoire of CD8$^+$ T cells. We also found that thymoproteasomes conferred on cTECs the ability to express MHC class I -peptide complexes that were capable of generating major repertoire of CD8$^+$ T cells. cTECs in thymoproteasome-deficient mice compensatively assembled immunoproteasomes and expressed an altered set of MHC class I -peptide complexes that fail to positively select most repertoires of CD8$^+$ T cells. In addition, CD8$^+$ T cells generated in the absence of thymoproteasomes displayed an altered TCR repertoire that was defective in allogeneic and antiviral responses. Thus, this study reveals a unique role of cTEC-specific protein degradation that is essential for the cTEC-specific production of self-peptide-MHC class I complexes, and these complexes are required for the development of an immunocompetent and self-protective repertoire of CD8$^+$ T cells.

RESULTS

β5t Regulates Positive Selection of Major Repertoire of CD8⁺ T Cells

In β5t-deficient (*Psmb11*$^{-/-}$) mice, the numbers of CD8$^+$ T cells in the spleen and lymph nodes were markedly reduced to 27% and 31% (ratio between averages, n = 5), respectively, of those in normal mice (Figure 1A). The number of CD4$^-$CD8$^+$ single-positive (CD8SP) thymocytes was also reduced to 21% (n = 5) of the control, whereas the numbers of CD4$^-$CD8$^-$ double-negative (DN), CD4$^+$CD8$^+$ DP, and CD4$^+$CD8$^-$ (CD4SP) thymocytes were unchanged (Figure 1A), indicating that β5t specifically regulates the CD8 lineage, but not the CD4 lineage of T cell development beyond the DP stage. Other lineages of immune cells, including TCRγδ$^+$ cells, NK cells, NKT cells, macrophages, dendritic cells, B cells, and CD8αα$^+$ intraepithelial lymphocytes, showed no decreases in their numbers in *Psmb11*$^{-/-}$ mice (Figure S1A available online). In order to examine how β5t specifically affects CD8$^+$ T cell development, *Psmb11*$^{-/-}$ mice were crossed with TCR-transgenic mice. In HY-TCR-transgenic