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REVIEW

Thymus-specific serine protease, a protease that shapes the CD4 T cell repertoire

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Abstract

The lifespan of T cells is determined by continuous interactions of their T cell receptors (TCR) with self-peptide-MHC (self-pMHC) complexes presented by different subsets of antigen-presenting cells (APC). In the thymus, developing thymocytes are positively selected through recognition of self-pMHC presented by cortical thymic epithelial cells (cTEC). They are subsequently negatively selected by medullary thymic epithelial cells (mTEC) or thymic dendritic cells (DC) presenting self-pMHC complexes. In the periphery, the homeostasis of mature T cells is likewise controlled by the interaction of their TCR with self-pMHC complexes presented by lymph node stromal cells while they may be tolerized by DC presenting tissue-derived self-antigens. To perform these tasks, the different subsets of APC are equipped with distinct combination of antigen processing enzymes and consequently present specific repertoire of self-peptides. Here, we discuss one such antigen processing enzyme, the thymus-specific serine protease (TSSP), which is predominantly expressed by thymic stromal cells. In thymic DC and TEC, TSSP edits the repertoire of peptide presented by class II molecules and thus shapes the CD4 T cell repertoire.

Keywords Thymus-specific serine protease (TSSP) · CD4 T cell tolerance · CD4 T cell-positive selection · Autoimmunity · Dendritic cells · Thymic epithelial cells

Introduction

The functional T cell repertoire is shaped in the thymus by positive and negative selection through interactions with self-peptide-major histocompatibility complexes (self-pMHC) expressed by thymic epithelial cells and bone marrow (BM)-derived antigen-presenting cells (APC). For class II-restricted T cells, the size and diversity of the CD4 T cell compartment are determined by the complexity of the self-peptide repertoire presented by MHC class II molecules in the thymus (Ebert et al. 2009; Marrack and Kappler 1997; Perrigoue et al. 2009).

MHC class II αβ heterodimers assemble in the endoplasmic reticulum and there engage with the chaperon invariant chain (Ii) that allows proper routing of MHC class II dimers within the endocytic pathway and protects the peptide-binding site from loading with endogenous peptides. Once in MHC class II loading compartments, the Ii is progressively cleaved by endosomal proteases leading to the generation of complexes of MHC class II with the class II invariant chain peptide (CLIP) in the peptide-binding groove (Hsing and Rudensky 2005). The ordered proteolysis of Ii can be mediated by different cysteine proteases such as the Cathepsins (Cat)-S and -L and the asparaginyl endopeptidase (AEP). These proteases show some level of tissue-specific expression with cortical thymic epithelial cells (cTEC) predominantly expressing Cat-L while other APC mainly expressing Cat-S and AEP.

The generation of the peptides for loading on MHC class II molecules similarly results from sequential proteolysis of endosomal proteins. Different cysteine proteases among which Cat-S, -L, -F, and -H and AEP and aspartyl proteases

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Cat-D and -E have been linked to Ag processing (Hsing and Rudensky 2005). Some indirect evidence suggests, however, that serine proteases and metalloproteases may also contribute to this latter process (Musson et al. 2003, 2006). Thymus-specific serine protease (TSSP) is one such serine protease that plays a unique role in editing the peptide repertoire in thymic stromal cells.

Identification of TSSP

TSSP was identified in the late 1990s independently by two different laboratories, as highly expressed in the thymus from RAG- and CD3ε-deficient mice blocked at an early stage of thymocyte maturation (Carrier et al. 1999) and as a gene located within the major histocompatibility complex (MHC) locus on human chromosome 6 (Bowlus et al. 1999). Both laboratories showed high expression of *PRSS16*, the gene encoding TSSP, in cTEC, and a high similarity of TSSP protein sequence with the human lysosomal prolylcarboxypeptidase (PRCP). Remarkably, considering these two main features, the same name was given to this new putative protease in both species by the two research groups, which also came to the same hypothesis of its possible function in antigen presentation by MHC class II molecules in the thymus thus in CD4 T cells selection.

TSSP protein (509 and 514 amino acids in mouse and human, respectively) was thereafter also named protease serine #16 (PRSS16), with respect to its suspected enzymatic activity due to the presence of three amino acids (serine, aspartate, histidine) in regions highly similar to the ones forming the catalytic site of PRCP. TSSP/PRSS16, thereafter called TSSP, is the third member of the S28 group of serine proteases (S28.003), the first member being the lysosomal PRCP (S28.001) which cleaves C-terminal amino acids linked to proline, and the second the dipeptidyl peptidase-2/7 (DPP2/7; S28.002) that cleaves X-proline dipeptides from the N-terminus of proteins. Thus, it is possible that TSSP could be an exopeptidase as PRCP and DPP2/7. As TSSP was shown to localize in the endosomal compartment of cTECs (Bowlus et al. 1999; Cheunsuk et al. 2002a), this putative protease could be involved in the generation and/or the trimming of MHC class II-bound peptides from self-proteins. As yet, the specific enzymatic function of TSSP is still unknown.

The two research groups which discovered TSSP independently generated *Prss16*-genetically inactivated mouse models (Cheunsuk et al. 2005; Gommeaux et al. 2009). TSSP-deficient mice are viable, fertile, without any obvious immune defect, including a normal thymic structure as well as normal numbers of thymocytes and T cells in the periphery, showing no quantitative impact of TSSP deficiency. Notwithstanding, crossing with T cell receptors (TCR) transgenic mice unveiled the impact of TSSP absence in the maturation of a subset of CD4 thymocytes, demonstrating for the first time that TSSP is qualitatively involved in CD4 T cells selection (Gommeaux et al. 2009).

Prss16 expression pattern

The *Prss16* gene is highly conserved between mammals and other vertebrates and emerged as a paralogue of evolutionary older members of the S28 family of serine protease (Boehm 2009; Cheunsuk et al. 2002a). *Prss16* is localized on chromosome 13 in mouse and *PRSS16* is localized in the syntenic region in the extended HLA region on chromosome 6 in human. As discussed above, initial studies showed that in mice and humans, *Prss16* is predominantly expressed by cTEC (Bowlus et al. 1999; Carrier et al. 1999; Cheunsuk et al. 2002b; Viret et al. 2011b). cTEC expression was detected by day 14 of embryonic life, the earliest time point analyzed (Carrier et al. 1999). Northern blot analysis showed some low level of *PRSS16* expression in the human pancreas and thyroid that was not detected in mouse tissues (Bowlus et al. 1999; Carrier et al. 1999). Expression of human *PRSS16* in the pancreas, breast mammary tissue, and thyroid was confirmed by the genotype-tissue expression project (Achenbach et al. 2002) and by human *PRSS16* EST (UniGene database, NCBI). Concerning other non-immune cell types, *PRSS16* seems to be weakly expressed in the human neuronal tissue (UniGene database, NCBI). Indeed, some SNP in the extended major histocompatibility complex region on chromosome 6 were associated with schizophrenia in human (Shi et al. 2009; Stefansson et al. 2009). In agreement with neuronal expression, *Prss16* expression was found in rat embryonic forebrain neuron progenitors (Girgenti et al. 2012). Lastly, a weak expression of *Prss16* was reported in murine colon epithelial cells (Brisson et al. 2015).

Further analysis of *Prss16* expression pattern with more sensitive RT-PCR and RNA-seq analysis revealed low level of expression in different immunologically relevant tissues. Hence, we showed that *Prss16* is expressed at low levels by mature single-positive $CD4^+CD8^-$ thymocytes and by peripheral naïve or LPS-activated B cells (Viret et al. 2011b). We also detected low levels of *Prss16* expression in all thymic dendritic cells (DC) subsets but not in peripheral DC even upon activation by Toll-like receptor (TLR) agonists (Viret et al. 2011b). Expression of *Prss16* by thymic DC was recently shown to decrease with age in non-obese diabetic (NOD) mice (Kroger et al. 2016). Interestingly, human conventional $XCR1^+$ thymic cDC1 also express *PRSS16* mRNA (Serre et al. 2017). *Prss16* mRNAs were also detected in activated microglial cells, but whether it is expressed by other monocyte/macrophage subsets has not been examined (Serre et al. 2017). For further insight into *Prss16* expression pattern, we explored different human and mouse transcriptomic

databases (ImmGen Project; NCBI; Gene Expression Commons) and RNA-seq data of some key thymic cell populations. Beside the expression in cTEC, both microarray and RNA-seq analysis revealed some expression of *Prss16* in MHC-II^{high} EPCAM^{high} mature medullary thymic epithelial cells (mTEC) (Meredith et al. 2015; Sansom et al. 2014). Its expression level seems to be correlated with surface MHC-II expression and so with mTEC maturation (Sansom et al. 2014). Furthermore, mTEC transcriptomic data revealed that *Prss16* expression does not correlate with AIRE expression. Transcriptomic analysis also revealed *Prss16* expression in BM neutrophils and their direct precursors (Seita et al. 2012; Shay and Kang 2013). TSSP is therefore expressed by different immune cells but predominantly by thymic stromal cells.

TSSP shapes the CD4 T cell repertoire

TSSP in positive selection of CD4 T cells

As discussed above, in cTEC, TSSP localizes in LAMP2-expressing endosomes suggesting a role in the class II presentation pathway (Bowlus et al. 1999; Guiraud et al. unpublished observation). In contrast to Cat-L, TSSP is not required for Ii processing in cTEC (Cheunsuk et al. 2005; Gommeaux et al. 2009). In addition, lack of TSSP expression does not affect the level of class II expression by cTEC. In agreement with the lack of a major role of TSSP in the generation of self-pMHC complexes in cTEC, the generation of the polyclonal CD4 and CD8 T cell compartment was overall normal in TSSP-deficient C57BL/6 (B6) mice (Cheunsuk et al. 2005; Gommeaux et al. 2009). Indeed, the number of TCRαβ double-positive CD4$^+$CD8$^+$, single-positive CD4$^+$CD8$^-$, and CD4$^-$CD8$^+$ thymocytes and the number of CD4$^+$ and CD8$^+$ T cells in the periphery of TSSP-deficient B6 mice was comparable to that of wild type (WT) B6 mice. However, the positive selection of transgenic Marilyn CD4$^+$ T cells specific for the male DBY antigen, of OTII CD4$^+$ T cells specific for the ovalbumin (OVA) 323–339 peptide or of 1H3.1 CD4$^+$ T cells specific for the Eα_{52-68} peptide was impaired in TSSP-deficient B6 mice (Gommeaux et al. 2009; Viret and Guerder, unpublished observation). In contrast, the positive selection of transgenic OTI CD8$^+$ T cells specific for the OVA$_{257-264}$ peptide and transgenic Matahari CD8$^+$ T cells specific for the UBY male antigen were not affected by TSSP deficiency suggesting that TSSP has no role in the class I presentation pathway in cTEC (Gommeaux et al. 2009; Carrier et al. unpublished observation). Hence, TSSP expression by cTEC is required for the positive selection of some but not all class II-restricted CD4 T cells, suggesting that TSSP may, in cTEC, contribute to the generation of some self-peptide presented by class II molecules.

We also analyzed the impact of TSSP deficiency in positive selection of TCR αβ CD4 T cells in NOD mice that express the I-A^{g7} class II molecule. I-A^{g7} shares the I-A^d α chain but has a unique β chain with substitutions of the highly conserved Proβ56 and Aspβ57 with Hisβ56 and Serβ57. These substitutions greatly modify the P1 and P9 pockets of the peptide-binding groove thus altering the stability of pMHC complexes and conferring unique peptide binding features to I-A^{g7} (Latek et al. 2000). As observed in B6 mice, TSSP deficiency has no quantitative impact on the positive selection of CD4$^+$ and CD8$^+$ T cells expressing polyclonal TCRαβ chains (Viret et al. 2011b). Furthermore, the Vβ-segment usage by mature peripheral CD4$^+$ and CD8$^+$ T cells is unaffected by TSSP deficiency. To gain further insight into the role of TSSP in CD4$^+$ T cell development, we analyzed the intrathymic development of CD4$^+$ T cells expressing nine distinct TCRαβ chains specific for islet self-antigens and one non-self-reactive TCRαβ-specific for hen egg lysozyme (HEL). We found that the positive selection of 2 of those 10 distinct TCR was altered in TSSP-deficient NOD mice (Table 1, (Viret et al. 2011a, b, 2015)). The positive selection of CD4$^+$ T cells expressing the BDC-6.9 TCR specific for the islet amyloid polypeptide (IAPP) was impaired in NOD mice with TSSP deficiency confined to the radio-resistant TEC compartment, suggesting that TSSP permits the generation of a peptide required for positive selection of the BDC-6.9 TCR. In contrast, the results suggest that the positive selection of the BDC-10.1 TCR specific for chromogranine A (ChgA) was increased by TSSP deficiency. Indeed, we found that the number of CD4$^+$CD8$^+$ DP and CD4$^+$CD8$^-$ SP BDC-10.1 thymocytes was increased in mice with TSSP deficiency confined to the TEC compartment. Furthermore, in mice with TSSP-deficient TEC, immature CD24^{high}TCR^{low} and mature CD24^{low}TCR^{high} BDC-10.1 thymocytes expressed increased levels of CD5 as compared to immature and mature BDC-10.1 thymocytes from WT mice, suggesting that TSSP-deficient cTEC may expressed increased ligand density or affinity that may favor the development or survival of the BDC-10.1 thymocytes. Hence, TSSP may also limit the generation of the peptide required for positive selection of the BDC-10.1 TCR. Interestingly, the positive selection of the BDC-5.2.9 and BDC-2.5 TCR, two additional TCR specific for IAPP and ChgA, respectively, was not impacted by TSSP deficiency. The distinct effect of TSSP on the positive selection of two TCR with the same antigen specificity but distinct TCR VαVβ segments usage strongly suggest that TSSP edits the peptide repertoire presented by class II molecules in cTEC.

TSSP in negative selection of CD4 T cells

Among the different thymic stromal cells, both mTEC and thymic DC induce the negative selection of developing thymocytes. mTEC express a large array of parenchymal self-

Table 1 Effect of TSSP deficiency on the intra-thymic development of distinct CD4$^+$ T cells

Mouse strain	TCR	Specificity	Positive selection	Negative selection		References
			TEC TSSP KO	TEC TSSP KO	DC TSSP KO	
B6	Marilyn	DBY	Impaired	NO	NO	1
	OTII	$OVA_{323–339}$	Impaired	NT	NT	1
	1H3.1	$E\alpha_{52–68}$	Impaired	NT	NT	2
NOD	PA21.14.HA	$HEL_{11–25}$	Normal	Deletion	Deletion	3
	12.4.1	$Insulin_{9–23}$	Normal	NO	NO	2
	PA18.9H7	$IA2\beta_{755–777}$	Normal	Deletion	Deletion	4
	PA19.5E11	$GAD65_{206–220}$	Normal	NO	NO	4
	1A4	$GAD65_{217–236}$	Normal	NO	NO	2
	BDC-2.5	$ChgA_{358–371}$	Normal	NO	NO	5
	BDC-10.1	$ChgA_{358–371}$	Increased	Deletion	Deletion	5
	BDC-6.9	IAPP	Impaired	NO	NO	5
	BDC-5.2.9	IAPP	Normal	NO	NO	5
	NY4.1	Unknown	Normal	Deletion	Deletion	4

Impaired positive selection reduced development of the corresponding CD4$^+$ T cells in TSSP-deficient as compared to TSSP-sufficient mice; *Increased positive selection* increased generation of the corresponding CD4$^+$ T cells in TSSP-deficient as compared to TSSP-sufficient mice; *Normal positive selection* comparable development of the corresponding CD4$^+$ T cells in TSSP-deficient and TSSP-sufficient mice; *Deletion* of the corresponding CD4$^+$ T cells in TSSP-deficient mice; *NO* increased deletion of the corresponding CD4$^+$ T cells in TSSP-deficient mice as compared to TSSP-sufficient NOD mice; *NT* Not tested; *1* Gommeaux et al. 2009; *2* Viret and Guerder, unpublished observation; *3* Viret et al. 2011a; *4* Viret et al. 2011b; *5* Viret et al. 2015

antigens due to the expression of two transcription factors, AIRE and Fezf2 (Anderson et al. 2002; Derbinski et al. 2001; Takaba et al. 2015). mTEC can directly display these self-pMHC complexes and induce the negative selection of developing CD4$^+$ T cells (Klein et al. 2014). Alternatively, mTEC may handle these self-antigens to neighboring DC that will process them in the class II pathway and induce the negative selection of developing CD4$^+$ T cells (Klein et al. 2014). Given the expression of TSSP in thymic DC and mTEC and its role in editing the peptide repertoire presented by class II molecule in cTEC, we explored its role in negative selection of autoreactive CD4 T cells.

NOD mice develop spontaneous diabetes that faithfully reproduce human type 1 diabetes (T1D). The autoreactive repertoire of NOD mice has therefore been extensively studied to identify islet antigens contributing to diabetes development. Many of those are found in the secretory granules of β cells such as insulin (Ins), glutamic acid decarboxylase (GAD) 65, insulinoma-associated protein 2 (IA-2), phogrin (IA-2β), ChgA, S100β, and islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP). Most of these antigens are recognized by CD4$^+$ T cells and the corresponding immunodominant class II epitope have been characterized (Lieberman and DiLorenzo 2003). In addition, diabetogenic CD4$^+$ T cell clones specific for these islet antigens have been generated thus offering a number of tools to examine how TSSP may impact central tolerance.

We therefore examined the development of a large panel of CD4$^+$ T cells expressing a self-reactive TCR specific of these β cell antigens (Table 1). Out of nine such TCR, three were deleted in TSSP-deficient NOD mice (Viret et al. 2011b, 2015). Using mixed BM chimeras, we showed that thymic DC but also radio-resistant TEC induced the deletion of these β cell-reactive CD4$^+$ T cells. We initially thought that, in this latter case, cTEC were inducing the negative selection of the corresponding TCR. However, the recent observation that mTEC also express *Prss16* suggests that deletion of these self-reactive CD4$^+$ T cells may be mediated by mTEC, an issue that remains to be addressed.

Unexpectedly, among the two $ChgA_{358–371}$-specific CD4 T cell clones (BDC-2.5 and BDC-10.1), only the BDC-10.1 clone was deleted in TSSP-deficient NOD mice (Viret et al. 2015). While both clones react with the same $ChgA_{358–371}$ peptide, they show some distinctive antigen binding features and the diabetogenic potential of BDC-10.1 CD4 T cells is higher than that of BDC-2.5 CD4 T cells suggesting that the BDC-10.1 TCR may have a higher avidity for its natural ligand (Burton et al. 2008). Higher avidity of the BDC-10.1 TCR as compared to the BDC-2.5 TCR for ChgA or a mimotope may explain the observed difference in negative selection of the two TCR in TSSP-deficient NOD mice. The antigen recognized by these two TCR has however remained enigmatic. In a seminal study, Stadinski et al. showed that both TCR react with a naturally occurring proteolytic cleavage product of ChgA, the WE14 bioactive hormone generated in the secretory granules of endocrine cells (Stadinski et al. 2010). WE14 is, however, a weak agonist of both T cell clones at least in part because the peptide fills only half of the I-A^{g7}

peptide binding groove leaving the MHC-II positions 1 to 4 unoccupied (Stadinski et al. 2010). It was proposed that the WE14 may be converted to a high affinity ligand upon post-transcriptional modifications with the transglutaminase enzyme (Delong et al. 2012). More recently, it was shown that, in β cells secretory vesicles, WE14 covalently binds to a fragment of the insulin C-peptide to generate a hybrid insulin peptide (HIP2.5) that is a very potent agonist of the BDC-2.5 TCR (Delong et al. 2016). Whether any of these modifications occurs in mTEC has not been investigated, yet mTEC express several transglutaminase mRNA (ImmGen Project database), and whether they contribute to the differential effect of TSSP deficiency on the negative selection of the two ChgA reactive clones remains to be examined.

We also found that TSSP-deficient NOD mice are less responsive to the class II immunodominant S100β_{1-15} epitope, another antigen found in β cell secretory granules (Serre et al. 2015). As observed for the other self-antigens, hyporesponsiveness of TSSP-deficient NOD mice to S100β_{1-15} was imposed during CD4$^+$ T cell development in the thymus. In-depth analysis of the polyclonal TCRαβ repertoire of S100β_{1-15}-reactive CD4$^+$ T cells showed that TSSP-deficiency led to the deletion of high-avidity T cells expressing a private TCRαβ repertoire.

Altogether, these different studies suggest that in the NOD mouse, the protease TSSP limits the generation of some self-ligands involved in negative selection of some self-reactive CD4$^+$ T cells. Hence, TSSP would have a quantitative impact on the amount of some self-pMHC complexes displayed by thymic DC and mTEC. We did not find self-reactive CD4$^+$ T cells that were more efficiently deleted in WT NOD mice as compared to TSSP-deficient NOD mice. Likewise, when analyzing the polyclonal CD4$^+$ T cell response to self-peptide immunization, we did not find any that induced higher response in TSSP-deficient mice as compared to WT mice, suggesting that TSSP does not positively contribute to the generation of some self-peptide for class II presentation in thymic DC or mTEC. A more global approach is however required to conclude on that point.

TSSP contributes to organ-specific autoimmune diseases

TSSP expression in thymic stromal cells predisposes to T1D

PRSS16 was initially described as a gene of the extended HLA region linked to a diabetes susceptibility locus in humans (Lie et al. 1999; Viken et al. 2009). Several polymorphisms within the promoter region, some exons, and the 3′UTR have been detected in healthy individuals as well as a 15-bp deletion observed in 17% of healthy individuals (Lie et al. 2002). Whether these polymorphisms affect the level or pattern of *PRSS16* expression in mTEC, cTEC, or thymic DC is unknown. Yet, the level of *PRSS16* mRNA in thymic DC varies greatly among healthy individuals (Serre et al. 2017). Indeed, different individuals segregate in two groups with either low or high expression of TSSP mRNA in thymic DC, regardless of the sex or age of the donors.

To assess the role of TSSP in diabetes development, we introgressed the TSSP mutation into the NOD background for up to 20 generations (Viret et al. 2011b). TSSP-deficient NOD mice did not show quantitative defect in all immune cell compartments and mount normal CD4$^+$ and CD8$^+$ T cell responses against most non-self-antigens tested (Viret et al. 2011a). Quite remarkably, TSSP-deficient NOD mice were completely protected from spontaneous diabetes and severe insulitis (Viret et al. 2011b). Hence, TSSP-deficiency affects an essential and early event in disease initiation. Diabetes protection was a property of the CD4$^+$ T cell compartment and was induced during CD4$^+$ T development by either TEC or DC lacking TSSP expression. Given the major impact of TSSP deficiency on the negative selection of the islet-reactive CD4$^+$ T cell compartment (see above), the reduced diabetes incidence of TSSP-deficient NOD mice likely results from increased negative selection of autoreactive CD4$^+$ T cells.

T1D development is thought to proceed in two steps. First, CD4$^+$ T cells response to an essential autoantigen is induced by yet unknown mechanism. One such essential initiating antigen is thought to be insulin (Nakayama et al. 2005). This conclusion was based on the observation that NOD mice with an alanine to tyrosine substitution at position 16 of the Ins1$_{9-23}$ epitope (Tyr16Ala), that abolishes recognition by CD4$^+$ T cells, were protected from T1D. However, both CD4$^+$ and CD8$^+$ T cell responses to insulin are initiated before insulitis and both CD4$^+$ and CD8$^+$ T cells recognize the same Ins1$_{9-23}$ epitope (Nakayama et al. 2005; Wong et al. 1999). Furthermore, the Tyr16Ala mutation in the Ins1$_{9-23}$ epitope abolishes recognition by both CD4$^+$ and CD8$^+$ T cells (Nakayama et al. 2005; Wong et al. 1999). It is therefore unclear whether protection from diabetes in these mice is due to impaired CD4$^+$ or CD8$^+$ T cell response to insulin or both. The recent observation that ChgA-deficient NOD mice remains disease-free and develop only mild insulitis suggest, however, that ChgA is likewise an essential antigen for diabetes development (Baker et al. 2016). Following this initial insult, T cells specific for several additional islet antigen are activated and contribute to the almost complete destruction of the islets of Langerhans. The remarkable effect of TSSP-deficiency on diabetes development did not correlate with CD4$^+$ T cell tolerance to insulin. We found however that up to 30% of the islet-reactive CD4$^+$ T cells were deleted in TSSP-deficient mice NOD mice (Table 1), including some TCR specific for ChgA. In addition, the high-avidity self-reactive CD4$^+$ T cell repertoire may be more efficiently deleted

in TSSP-deficient mice. Diabetes protection of TSSP-deficient NOD mice therefore more likely results from a global reduction of the frequency of multiple islet-reactive $CD4^+$ T cells.

TSSP increases experimental autoimmune encephalomyelitis severity

TSSP deficiency also affected the development of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS) (Serre et al. 2017). EAE is another $CD4^+$ T cell-mediated autoimmune disease, induced by myelin oligodendrocyte glycoprotein (MOG) 35–55 immunization (Serre et al. 2017). Although disease incidence and time of onset were comparable, disease severity was markedly reduced in TSSP-deficient NOD mice as compared to their WT counterpart. In this case too, reduced disease severity was imposed during thymic selection and correlated with a reduced frequency of $MOG_{35–55}$-reactive $CD4^+$ T cells, further suggesting that lack of TSSP expression by thymic stromal cells increases deletion of the corresponding $CD4^+$ T cells (Serre et al. 2017). Although *Prss16* mRNA was detected in activated microglial cells, such expression had no significant impact on disease development or severity.

EAE can also be induced in B6 mice by $MOG_{35–55}$ immunization. It was therefore possible to assess whether TSSP had a similar effect on negative selection in B6 mice as compared to NOD mice. Quite remarkably, TSSP deficiency did not reduce the frequency of $MOG_{35–55}$-reactive CD4 T cells nor did it impact the disease course following $MOG_{35–55}$ immunization in B6 mice (Serre et al. 2017). Importantly, the level of *Prss16* expression in both TEC and DC is comparable in the two strains of mice (Serre et al. 2017). At this stage, the reason for the distinct effect of TSSP-deficiency in B6 and NOD mice on the $MOG_{35–55}$–reactive $CD4^+$ T cell repertoire can only be surmise. The specific features of the $I\text{-}A^{g7}$ class II molecules contribute to pMHC complexes instability and thus, may increase peptide dwell time in the endosomal compartment of NOD DC and consequently its exposure to the protease TSSP. In addition, while the same $MOG_{35–55}$ peptide induces EAE in B6 and NOD mice, the $I\text{-}A^{b}$ core peptide corresponds to $MOG_{38–50}$ while the $I\text{-}A^{g7}$ core peptide corresponds to $MOG_{42–55}$ thus allowing for possible subtle effects of TSSP (Mayo and Quinn 2007; Mendel Kerlero de Rosbo and Ben-Nun 1996). Although the function and enzymatic characteristics of TSSP are still unclear, TSSP belongs to the family of proline-specific dipeptidyl peptidases (DPP), a family of Xaa-Pro aminopeptidase (Bezerra et al. 2012; Leiting et al. 2003). The $I\text{-}A^{g7}$ core peptide $MOG_{42–55}$ but not the B6 core peptide contains in its amino-terminal end a Ser42-Pro43 dipeptide that may be trimmed by TSSP thereby destabilizing the pMHC complex or reducing TCR avidity (Carson et al. 1997). Further characterization of the enzymatic property and precise function of TSSP are clearly required to determine whether this may be the mechanism by which TSSP impacts central tolerance to $MOG_{35–55}$ or other self-Ags.

Taken together, the results obtained in the NOD and B6 mice suggest that the level of TSSP expression modifies the risk factors conferred by some MHC class II haplotype. Given that the MHC class II allele confers the highest susceptibility to MS and T1D, it was therefore interesting to find that humans segregate in two groups with either high or low levels of expression of *PRSS16* in thymic DC. Regarding T1D, the human susceptibility molecules HLA-DQ shares the Aspβ57 substitution with $I\text{-}A^{g7}$ and most known MHC class II-restricted auto-Ags are similar between mice and humans suggesting that TSSP may confer T1D susceptible by similar mechanisms in mice and humans (Lieberman and DiLorenzo 2003). The HLA DRB1*15:01, which confers the highest risk factor to MS, also present several polymorphisms within the β1 domain which reorganizes the different pockets of the peptide-binding groove and which leads to distinctive peptide-binding characteristics as reported for myelin-derived peptides for instance (Smith et al. 1998). Interestingly, the HLA DRB1*15:01 binding epitope of myelin basic protein (MBP), $MBP_{85–95}$ (ENPVVHFFKNIVTPR), contains a Pro at position 3. Whether this confers higher susceptibility to proteolytic cleavage by TSSP and thus alters the loading of these MBP peptides or other myelin-derived peptides on HLA DRB1*15:01 remains to be determined. While polymorphisms within *PRSS16* have been linked to T1D susceptibility in humans, there are yet no polymorphism within *PRSS16* that have been linked to MS susceptibility, though our study suggests a possible role of TSSP in disease severity which was not assessed in published genome-wide association studies (GWAS). It remains, however, possible that defects lie within the regulators of TSSP expression as reported for the transcription factor ZNF804a that regulates expression of *PRSS16* in the brain and is, together with *PSS16*, associated with susceptibility to schizophrenia and bipolar disorders (Girgenti et al. 2012).

TSSP in other pathologies than T1D and MS

Besides the studies discussed above, few publications dealing with TSSP are found in the literature. Several GWAS suggest a putative impact of the gene encoding TSSP in some physiological features of body motion: exercise (De Moor et al. 2009) and gait speed (Ben-Avraham et al. 2017), as well as the neurological disorder schizophrenia (Girgenti et al. 2012; Shi et al. 2009).

The participation of TSSP in the cancerous pathology was mentioned in some publications. Some studies suggest a role of TSSP in cancer, as belonging to a molecular signature of metastasis formation in cervical cancer patients (Fernandez-

Retana et al. 2017) or potentially involved in cancer-associated post-translational modifications due to its homology with DPP-IV protease (Busek et al. 2004). Regarding thymic tumors, TSSP was reported to be either underexpressed or highly expressed, depending on the lymphoid or stromal origin of tumoral cells, respectively (Lin and Aplan 2007; Strobel et al. 2014).

Contrary to autoimmune diseases in which the absence of TSSP is protective, our group revealed a deleterious impact of TSSP absence in cancer (Brisson et al. 2015). We first observed a high susceptibility of aged TSSP-deficient B6 mice to develop spontaneous hepatocellular carcinoma and lymphoma. We then showed that TSSP is protective against the development of inflammation-induced colorectal tumors, through its role in the maturation of $CD4^+$ T cells. Considering that cancer is mostly a pathology of elderly, and that aging is characterized by a systemic inflammation (the so-called inflam-aging), we can postulate that TSSP could play a role in the prevention of immunosenescence characterized by a thymic involution and the decrease of immune functions including anti-tumoral immunity. Restoration of TSSP function in a non-autoimmune prone genetic context could thus be a new avenue in the prevention of aging-associated immunodeficiency.

Concluding remarks

Since its discovery 20 years ago, TSSP has revealed at least part of its functions (Fig. 1). The different studies suggest that TSSP is a protease of the class II presentation pathway that shapes, by yet unknown mechanisms, the peptide repertoire presented by TEC and DC in the thymus. Among the different proteases of the class II presentation pathway, TSSP presents quite unique features as it is the first example of a protease with a restricted impact on T cell repertoire selection and a major role in the development of autoreactive T cells. It was therefore surprising that a protease with such deleterious effect may be maintained through evolution. Some studies showed that the deletion of self-reactive T cells introduces holes in the functional T cell repertoire and may impair responsiveness to some foreign antigens (Vidovic and Matzinger 1988). This is due to the degenerated interaction of the TCR with multiple pMHC complexes. The same was found in TSSP-deficient mice that show unresponsiveness to one foreign antigen out of seven tested (Viret et al. 2011a). Thus, by limiting central tolerance, TSSP diversify the functional CD4 T cell repertoire and may increase the chance to fight infection. In addition, beyond its deleterious impact in the context of autoimmune disorders, we demonstrated a protective role of TSSP in the context of aging- and inflammation-related

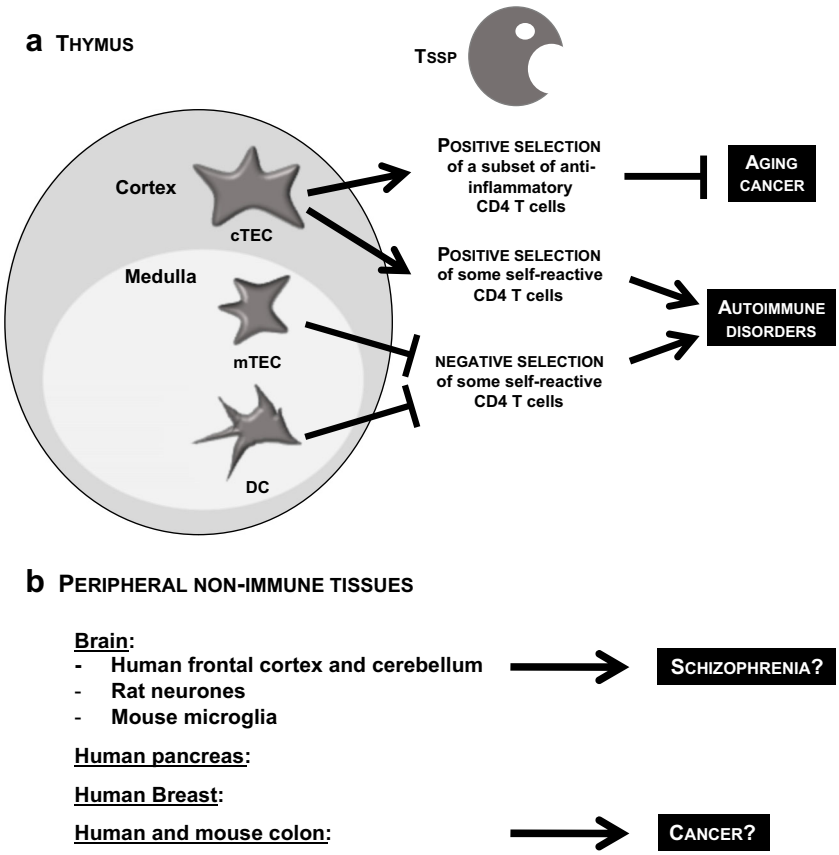

Fig. 1 **a** TSSP expression pattern in thymic stromal cells: The different effects of TSSP on intra-thymic $CD4^+$ T cell selection and consequences on immune-mediated pathologies are presented. **b** TSSP expression pattern in non-immune tissues extracted from public databases and possible link to pathologies

carcinogenesis, suggesting a selective advantage for its function in the thymus and potentially in other organs.

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Compliance with ethical standard

Conflict of interest The authors declare that they have no competing interests.

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