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REVIEW

Dysregulation of Thymic Clonal Deletion and the Escape of Autoreactive T Cells

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Abstract Events ongoing in the thymus are critical for deleting developing thymocytes specific for tissue antigens, and establishing self-tolerance within the T cell compartment. Aberrant thymic negative selection, however, is believed to generate a repertoire with increased self-reactivity, which in turn can contribute to the development of T cell-mediated autoimmunity. In this review, mechanisms that regulate the efficacy of negative selection and influence the deletion of autoreactive thymocytes will be discussed.

Keywords Antigen-presenting cells · Autoimmunity · Central tolerance · Thymocytes

Introduction

Self-tolerance within the T cell compartment is established and maintained by events ongoing in the thymus and periphery. Central tolerance mediated via clonal deletion (i.e. negative selection) ensures that much of the T cell repertoire is purged of autoreactive specificities in the thymus (Kappler et al. 1987; Kisielow et al. 1988; Klein et al. 2009; Kyewski and Klein 2006; Siggs et al. 2006; Sospedra et al. 1998). Peripheral events intrinsic and extrinsic to T cells regulate autoreactive T precursors that failed to be deleted during thymic negative selection. Defects in either central or peripheral tolerance may result in T cell-mediated autoimmunity such as type 1 diabetes (T1D) (Anderson and Bluestone 2005; Aoki et al. 2005; Chatenoud and Bach 2005; D'Alise et al. 2008; Kishimoto and Sprent 2001; You et al. 2008), multiple sclerosis (MS) (Costantino et al. 2008), and rheumatoid arthritis (RA) (Anderson and Isaacs 2008). Inefficient negative selection is thought to lead to the escape of an increased frequency of T cells with enhanced affinity for self-antigens. Once established, this repertoire may be further influenced by aberrant peripheral tolerance (Anderson and Isaacs 2008; Decallonne et al. 2003; Oh et al. 2010; Venken et al. 2010; Zhang et al. 2001), which when coupled with environmental triggers promotes expansion and differentiation of pathogenic autoreactive T effectors. The complexity of events associated with central and peripheral tolerance is in part reflected by the polygenic nature of T cell-mediated autoimmune diseases. Several chromosomal loci have been identified that are associated with susceptibility and resistance to T1D, MS, and RA (Bowes and Barton 2008; Kim and Polychronakos 2005; Tajouri et al. 2007; Todd 2010; Ziegler and Nepom 2010). The causative genes in the majority of these chromosomal loci have yet to be identified. However, genes that have been defined typically play a key role in regulating T cell recognition of and responses to antigen (Ebers et al. 1996; Hahn et al. 2005; Haines et al. 1996; Holmdahl 2000; Suri et al. 2008; Taneja and David 2010; von Boehmer 2009; Wucherpfennig et al. 1997; Zanelli et al. 2000).

The relative contribution of aberrant central versus peripheral tolerance in promoting autoimmunity is ill-defined, and is likely to vary depending on the respective autoimmune disease. Nevertheless, dysregulated central tolerance would be predicted to play a role in most T

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cell-mediated autoimmune diseases by establishing a T cell repertoire "prone" to self-antigen recognition. In the current review, we will discuss how defects in cellular and molecular events associated with thymic negative selection may contribute to the escape of autoreactive thymocytes.

Thymic Clonal Deletion: the Process and the Players

T cells develop in the thymus following a series of programmed events, some of which involve cognate interactions with thymic antigen-presenting cells (APC) (Germain 2002; Klein et al. 2009; Takahama 2006). T cells differentiate from progenitors residing in the liver early in life, and then from the bone marrow shortly after birth. Upon entering the thymus T cell progenitors undergo differentiation which is both antigen-independent and -dependent. Early antigen-independent events involve double-negative thymocytes, which lack expression of the T cell receptor (TCR) and the CD4 and CD8 co-receptors. Developmental cues provided by the thymic cortical region promote TCR gene rearrangement and upregulation of CD4 and CD8 surface expression by the thymocytes. These immature double-positive (DP) thymocytes subsequently undergo antigen-dependent positive and negative selection events mediated by specific types of thymic APC. DP thymocytes that express TCR which bind with a relativity weak affinity/avidity to peptide-MHC class I or class II complexes (pMHC) presented by cortical thymic epithelial cells (TEC) undergo positive selection upon receiving signals needed for survival and differentiation into single-positive (SP) $CD4^+$ and $CD8^+$ thymocytes. Conversely, DP thymocytes that express a rearranged TCR that fails to bind self-pMHC complexes undergo apoptosis due to "neglect". Positively selected thymocytes, which upregulate expression of chemokine receptor 7 (CCR7), traffic to the medulla where the cognate CCL19 and CCL21 chemokines are produced and negative selection is mediated (Davalos-Misslitz et al. 2007; Misslitz et al. 2004; Nitta et al. 2009; Ueno et al. 2004). Here, thymocytes interact with medullary TEC (mTEC) and/or medullary dendritic cells (mDC) which present a diverse spectrum of self-pMHC complexes (Anderson et al. 2002; Klein et al. 2009). Apoptosis is induced in thymocytes expressing TCR that bind self-pMHC with a relatively high affinity/avidity (Siggs et al. 2006). In this way, the TCR repertoire is purged of thymocytes exhibiting increased affinity for self-peptides. In a minor pool of $CD4^+$ thymocytes, differentiation into natural immunoregulatory T cells characterized by expression of the transcription factor FoxP3 may also be promoted by interactions with increasing avidity/affinity (Coutinho et al. 2005). Mature SP $CD4^+$ and $CD8^+$ thymocytes that survive negative selection and representing $\sim$3–5% of all thymocytes finally exit the thymus and populate the periphery.

As noted above mTEC and mDC are the key thymic APC mediating negative selection. Notably, mTEC are specialized to express a broad range of tissue specific antigens (TSA) normally found in the periphery (Anderson et al. 2002; Coutinho et al. 2005; Derbinski et al. 2001; Derbinski et al. 2008; Gabler et al. 2007; Gillard and Farr 2006). On the other hand, mDC mostly acquire TSA via uptake of apoptotic mTECs or migrating from the periphery into the thymus (Klein et al. 2009; Koble and Kyewski 2009; Millet et al. 2008). mTEC and mDC are characterized as "mature" APC based on their high expression of MHC class I and II, and co-stimulatory molecules such as CD80, CD86 and CD40, thereby enabling these cells to be potent effectors of clonal deletion of autoreactive thymocytes (Derbinski et al. 2001; Gray et al. 2007; Koble and Kyewski 2009; Millet et al. 2008). The relative contribution of mTEC and mDC in mediating negative selection is currently unclear. However, altered composition and/or function of mTEC and mDC either in various autoimmune models or in patients can have marked effects on the efficacy of negative selection, and the development of autoreactive thymocytes.

mTEC and the Deletion of Autoreactive Thymocytes

mTEC are a dynamic pool of thymic stromal cells that have a high proliferative capacity, and undergo distinct stages of maturation marked by changes in their overall capacity to stimulate thymocytes (Gabler et al. 2007; Gillard and Farr 2006). Immature mTEC express low levels of CD40, CD80, CD86, MHC class II, and TSA (Derbinski et al. 2001; Derbinski et al. 2005; Gray et al. 2006; Gray et al. 2007). In contrast, mature mTEC display a phenotype conducive to efficient thymocyte deletion, characterized by increased surface expression of CD40, CD80, CD86, and MHC class II, as well as an increase in the level and repertoire of TSA expressed (Derbinski et al. 2001; Derbinski et al. 2005; Gray et al. 2006; Gray et al. 2007). The latter is in part attributed to upregulation of the autoimmune regulator protein (AIRE), a transcription factor that drives the expression of genes encoding a variety of TSA by mTEC. These TSA include proteins restricted to the pancreas, stomach, eye, salivary gland, muscle, and the thyroid, among other tissues (Anderson et al. 2002; Derbinski et al. 2001; Kyewski and Peterson 2010; Mathis and Benoist 2007; Mathis and Benoist 2009). However, not all TSA genes expressed by mTEC are regulated by AIRE. Somewhat surprising is that only 1–2% of mTEC express a given TSA, raising the question of how a relatively small number of mTEC can efficiently delete particular clonotypes

(Derbinski et al. 2008; Gillard and Farr 2006; Villasenor et al. 2008). Here it is believed that negative selection is aided by the high motility of thymocytes, which make numerous contacts with mTEC over a period of 4–5 days while in the medulla (Rooke et al. 1997; Scollay and Godfrey 1995).

Altered mTEC function resulting in reduced negative selection has been typically described in the context of dysregulated expression of TSA. This is best illustrated by studies examining the role of AIRE in central tolerance. Genetically manipulated mice deficient in AIRE expression and exhibiting diminished TSA expression by mTEC develop multiorgan autoimmunity characterized by T cell infiltrates in numerous organs (Anderson et al. 2002; Jiang et al. 2005). In addition to reduced expression of TSA, AIRE deficiency may affect antigen processing by mTEC, thereby further limiting presentation of self-peptides. Noteworthy is that the organs targeted and the severity of autoimmunity vary depending on the genetic background of AIRE-deficient mice, indicating that additional genetic "modifiers" shape the repertoire of developing autoreactive thymocytes (Jiang et al. 2005). In this instance, MHC is very likely to be a key "modifier" in view of the strong association of specific alleles of MHC/HLA with susceptibility to various tissue-specific autoimmune diseases. Further underscoring the importance of AIRE in thymic selection is the observation that mutations in the AIRE gene lead to the development of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), a genetic disorder in which patients present with various autoimmune pathologies including thyroiditis, T1D, oophoritis, and hepatitis (Mathis and Benoist 2009; Villasenor et al. 2005).

Noteworthy is that a temporal window exists during which AIRE-mediated events play a critical role in establishing T cell tolerance. Inducible expression in a transgenic mouse model showed that AIRE is required to block autoimmunity up until shortly after birth but is dispensable in adult mice (Guerau-de-Arellano et al. 2009). This finding suggests that in general, central tolerance plays a more predominant role early in life when peripheral mechanisms of T cell tolerance are still being established.

Whereas AIRE deficiency results in global dysregulation of TSA expression, reduced expression of select TSA by mTEC has also been associated with autoimmunity. For example, susceptibility to T1D is strongly linked to polymorphisms in variable number of tandem repeats (VNTRs) found within the insulin promoter, which correlate with the level of insulin mRNA expression in the thymus. T1D is characterized by the destruction of the insulin producing β cells in the pancreas, and insulin is a key autoantigen driving the diabetogenic T cell response. Individuals at risk for T1D have a VNTR allele composed of 26–63 repeats and exhibit decreased insulin mRNA expression relative to subjects with a VNTR allele composed of 140–210 repeats (Pugliese et al. 1997; Vafiadis et al. 1997). Limited thymic insulin expression is expected to reduce the efficacy of clonal deletion, and promote increased escape of insulin-specific T cells with enhanced affinity. This scenario is consistent with a study demonstrating that the lack of insulin expression by mTEC leads to development of T1D in mice (Fan et al. 2009). Myasthenia gravis, in which the neuromuscular system is targeted, is associated in a subset of patients with a point mutation in the *CHRNA1* gene encoding the α-subunit of the muscle acetylcholine receptor. The mutation prevents the binding of the interferon regulatory factor 8 to the *CHRNA1* gene, consequently reducing transcription in mTEC (Giraud et al. 2007). TSA-specific mRNA processing has also been shown to influence the efficacy of negative selection of autoreactive thymocytes. The gene encoding proteolipid protein, an autoantigen used to induce experimental autoimmune encephalomyelitis, undergoes mRNA splicing in SJL/L mice that removes a key immunodominant epitope that in C57BL/6 mice clonally deletes the corresponding proteolipid protein peptide-specific thymocytes (Anderson et al. 2000; Klein et al. 2000).

Finally, aberrant development of mTEC may also impact the efficacy of negative selection. Molecules such as lymphotoxin β receptor, RANK and CD40 expressed by mTEC play key roles in regulating maturation and/or maintenance of this thymic APC population (Boehm et al. 2003; Chin et al. 2003; Derbinski and Kyewski 2005; Tykocinski et al. 2008). Therefore, dysregulation of these molecules and associated signaling pathways could deleteriously impact the size of the pool and the stimulatory capacity of mTEC, leading to an increased frequency of autoreactive T cells in the periphery.

mDC and the Deletion of Autoreactive Thymocytes

Several studies have shown that mDC play an important role in thymic negative selection (Brocker et al. 1997; Gallegos and Bevan 2004; Ohnmacht et al. 2009). For example, selective depletion of thymic DC in a transgenic mouse model results in an increased frequency of SP thymocytes and ensuing autoimmunity (Ohnmacht et al. 2009). The general consensus is that mDC complement the function of mTEC and effectively "extend the range" of clonal deletion of TSA-specific thymocytes. Indeed, evidence suggests that mDC are more efficient at negatively selecting $CD4^+$ SP thymocytes than mTEC due to an increased stimulatory capacity (Gallegos and Bevan 2004; Humblet et al. 1994; Ohnmacht et al. 2009; Proietto et al. 2008).

Simplistically, mDC can be divided into two major subsets, namely conventional DC (cDC) which have a high capacity to process and present antigens, and plasmacytoid DC (pDC) which are the major producers of type 1 interferons. Murine cDC in the thymus are further subdivided based on the expression of CD8α and Sirpα (e.g. $CD8\alpha^+Sirp\alpha^-$, $CD8\alpha^-Sirp\alpha^+$) (Wu and Shortman 2005; Klein et al. 2009). $CD8\alpha^+Sirp\alpha^-$ cDC, which comprise up to 50% of mDC, are derived directly from thymic precursors, whereas immature $CD8\alpha^-Sirp\alpha^+$ cDC and pDC and/or corresponding precursors enter the thymus from the blood under steady state conditions (Donskoy and Goldschneider 2003; Li et al. 2009).

mDC acquire TSA in a variety of mechanisms, which in part are determined by the thymic- versus blood-derived source of mDC. For example, parabiosis experiments in which partner mice express distinct allotypic markers demonstrated that blood-derived $CD8\alpha^-Sirp\alpha^+$ cDC and pDC residing in the medulla can present TSA obtained in the periphery (Donskoy and Goldschneider 2003; Li et al. 2009). Blood-derived DC residing in the medulla have also been reported to transcribe genes encoding TSA such as proinsulin; consequently these DC may serve a tolerogenic role in both the thymus and the periphery (Garcia et al. 2005). Importantly, "migrant" mDC effectively expand the repertoire of TSA presented in the medulla.

mDC also gain TSA from intra-thymic sources. Recently investigators have shown that mDC can efficiently present TSA expressed by mTEC by either 'nibbling' portions of mTEC membrane that contain pMHC complexes or by direct transfer of cytosolic content (Gallegos and Bevan 2004; Humblet et al. 1994; Koble and Kyewski 2009; Millet et al. 2008; Zhang et al. 2003). In addition, mDC engulf apoptotic mTEC and cross-present TSA-derived peptides. Mature mTEC have a high turnover rate and AIRE exhibits pro-apoptotic properties which may ensure a continuous source of TSA that can be cross-presented by mDC (Gray et al. 2007). It is noteworthy that thymic-derived $CD8\alpha^+Sirp\alpha^-$ cDC which predominate in the medulla exhibit an enhanced capacity to cross-present antigens derived from apoptotic cells relative to other DC subsets. In addition to mDC, blood-derived $CD8\alpha^-Sirp\alpha^+$ cDC residing in the cortex of the thymus have been reported to mediate negative selection of DP thymocytes. Cortical $CD8\alpha^-Sirp\alpha^+$ cDC are found near small vessels or inside perivascular regions and filter antigens entering the thymus through the blood (Baba et al. 2009). In this way cortical blood-derived $CD8\alpha^-Sirp\alpha^+$ cDC are thought to primarily mediate negative selection to ubiquitous self-antigens whereas mDC promote clonal deletion to TSA (McCaughtry et al. 2008).

The role for DC in clonal deletion has largely been established by studies manipulating thymic DC in bone marrow chimera and/or transgenic models. Only limited data are available defining defects within the pool of thymic DC that directly impact thymic negative selection in spontaneous or induced models of T cell-mediated autoimmunity. This is attributed to the relatively low numbers of thymic DC and the complexity in DC subsets making analyses problematic. It is also unclear whether $CD8\alpha^+Sirp\alpha^-$ cDC, $CD8\alpha^-Sirp\alpha^+$ cDC and pDC have distinct roles in mediating thymic negative selection under steady state conditions. Support for this scenario is provided by recent findings indicating that murine $CD8\alpha^-Sirp\alpha^+$ cDC exhibit an increased efficacy to induce FoxP3-expressing Treg in vitro compared to thymic-derived $CD8\alpha^+Sirp\alpha^-$ cDC, due to a more mature phenotype (Proietto et al. 2008). If indeed a "division of labor" exists among the respective mDC subsets, then dysregulation in the number and composition of the different mDC would be expected to influence the efficacy of thymic negative selection.

Dysregulation of the APC function of mDC would also be predicted to influence the efficacy of clonal deletion. A diminished capacity to endocytose, process and/or cross-present TSA derived from mTEC, for instance, should limit the efficacy of thymic negative selection. Similarly, diminished clonal deletion would occur if mDC failed to provide sufficient stimulatory signals to drive thymocyte apoptosis. In general, the capacity of DC to process and present antigen and stimulate T cells is dependent on the maturation status of the DC. Under steady-state conditions DC typically exhibit an immature phenotype marked by the ability to readily endocytose antigen but a limited capacity to stimulate T cells ($CD40^{lo}$, $CD80^{lo}$, $CD86^{lo}$, MHC^{lo}). Upon activation DC maturation is induced, resulting in: (1) upregulation of co-stimulatory and MHC molecules, thereby establishing a phenotype suited to efficiently stimulate T cells, and (2) a concomitant reduction in the capacity to endocytose antigen. Notably, DC may exhibit varying degrees of maturation depending on the nature and magnitude of the activating stimuli. Reports have shown that the maturation status of mDC correlates with increased development of autoreactive thymocytes. For instance, mDC in nonobese diabetic (NOD) mice, a spontaneous model of T1D, express reduced levels of CD86 and CD40 compared to mDC from a congenic mouse line that fails to develop diabetes (Decallonne et al. 2005). Furthermore, treatment of NOD mice with an analog of vitamin D results in upregulation of CD40 and CD86, an increase in the frequency of $CD8\alpha^+$FasL mDC, and an enhanced capacity of mDC to induce thymocyte apoptosis in vitro. In a transgenic model of systemic lupus erythematosus in which T cells express a TCR specific for a nucleosomal epitope, the development of nephritis varies depending on the genotype of the mice. In animals developing nephritis, mDC exhibit reduced levels of co-stimulatory molecule and MHC expression, and an increased frequency of

autoreactive thymocytes relative to mice protected from autoimmunity (Michaels et al. 2005).

Interestingly, a recent study indicates that the stimulatory capacity of mDC is actively regulated under steady state conditions. NOD mice lacking expression of the receptor tyrosine kinase MerTK fail to develop diabetes (Wallet et al. 2008; Wallet et al. 2009). MerTK is an important receptor for binding to and mediating the inhibitory effect apoptotic cells have on DC (Sen et al. 2007; Wallet et al. 2009). The latter is a mechanism for establishing and maintaining self-tolerance within the DC compartment (Sen et al. 2007; Wallet et al. 2009). Importantly, the lack of diabetes in MerTK-deficient NOD mice correlates with an enhanced capacity of mDC to mediate thymic negative selection. Although the subset composition and surface phenotype of mDC in MerTK-deficient NOD mice are unaltered, microarray analyses indicate that these mDC nevertheless display a "more" mature phenotype that correlates with enhanced thymocyte clonal deletion (Wallet et al. 2009).

Properties Intrinsic to Thymocytes that Promote the Escape of Autoreactive T cells

In addition to altered thymic APC, properties intrinsic to thymocytes have been reported to reduce the efficacy of clonal deletion, thereby promoting increased escape of autoreactive thymocytes into the periphery. For instance, dysregulation of early TCR signaling pathways and induction of apoptosis have been implicated in aberrant thymocyte negative selection.

It is well documented that clonal deletion is governed by the strength of signaling transduced by TCR upon binding of self-pMHC. Accordingly, attenuated TCR signaling in thymocytes has been linked to the development of autoimmunity. An example of this is seen in the SKG mouse, a spontaneous mutant line which develops an RA-like disease (Sakaguchi et al. 2003). SKG mice have a point mutation in the C-terminus of the SH2-domain of ZAP-70. ZAP-70 is a Src family tyrosine kinase that phosphorylates the TCR CD3 and ζ subunits and plays a critical role in early signaling in both thymocytes and mature T cells. The mutated ZAP-70 results in reduced TCR signaling, thereby limiting thymocyte activation and subsequent deletion of the arthritogenic thymocytes. Polymorphisms in the *PTPN22* gene have been associated with a broad range of autoimmune disorders, including T1D, RA, MS and myasthenia gravis (Begovich et al. 2004; Criswell et al. 2005; Smyth et al. 2006; Vandiedonck et al. 2006). The lymphoid tyrosine phosphatase encoded by *PTPN22* normally downregulates TCR signaling and T cell activation via dephosphorylation of ZAP-70 and the tyrosine kinase LCK (Cohen et al. 1999). Elevated levels of the active form of the phosphatase are detected for *PTPN22* variants associated with autoimmune susceptibility, thereby promoting a hyporesponsive phenotype in mature T cells (Rieck et al. 2007; Vang et al. 2005). Variant *PTPN22* would be expected to similarly induce hyporesponsive thymocytes and limit thymic negative selection; this hypothesis, however, still needs to be tested. Abnormal activity of other negative regulators of early TCR signaling, such as the Cbl proteins, a family of E3 ubiquitin ligases, may also contribute to aberrant thymocyte responsiveness upon binding to self-pMHC complexes.

Dysregulation of pathways inducing apoptosis in thymocytes have as well been implicated in reduced thymic negative selection. Defective negative selection in NOD mice correlates with DP thymocytes expressing reduced levels of Bim, a pro-apoptotic member of the Bcl-2 family (Liston et al. 2004). Indeed, thymocytes are protected from apoptosis in mice deficient of Bim expression (Bouillet et al. 1999; Bouillet et al. 2002). Nur77, an orphan steroid receptor, is another pro-apoptotic molecule shown to regulate thymocyte negative selection. For instance, autoreactive thymocytes are resistant to apoptosis in Nur77 deficient mice, whereas overexpression of Nur77 enhances thymocyte apoptosis (Calnan et al. 1995). Nur77 may act in part by binding to and blocking the anti-apoptotic function of Bcl-2 (Thompson and Winoto 2008). Notably, reduced expression of Nur77 in thymocytes has been reported in a number of autoimmune diseases.

SP $CD4^+$ thymocytes play an important role in regulating the development of mature mTEC. As noted earlier, mTEC maturation is regulated by RANK- and CD40-dependent signaling events (Derbinski and Kyewski 2005; Rossi et al. 2007; Akiyama et al. 2008; Tykocinski et al. 2008). On the other hand, SP $CD4^+$ thymocytes express the corresponding ligands for these receptors, namely RANKL and CD40L. Blocking the interaction between SP $CD4^+$ thymocytes and mTEC and the binding of RANK to RANKL and CD40 to CD40L leads to a marked decrease in the number of mature mTEC. The highly motile nature of SP $CD4^+$ thymocytes is believed to ensure that sufficient contacts are made to maintain the pool of mature mTEC. Accordingly, dysregulation of the crosstalk between SP $CD4^+$ thymocytes and mTEC, due for instance to reduced expression of CD40L and/or RANKL and/or limited thymocyte migration in the medulla, may also contribute to aberrant clonal deletion.

Concluding Remarks

To date, evidence directly implicating dysregulation of thymic negative selection in specific T cell-mediated

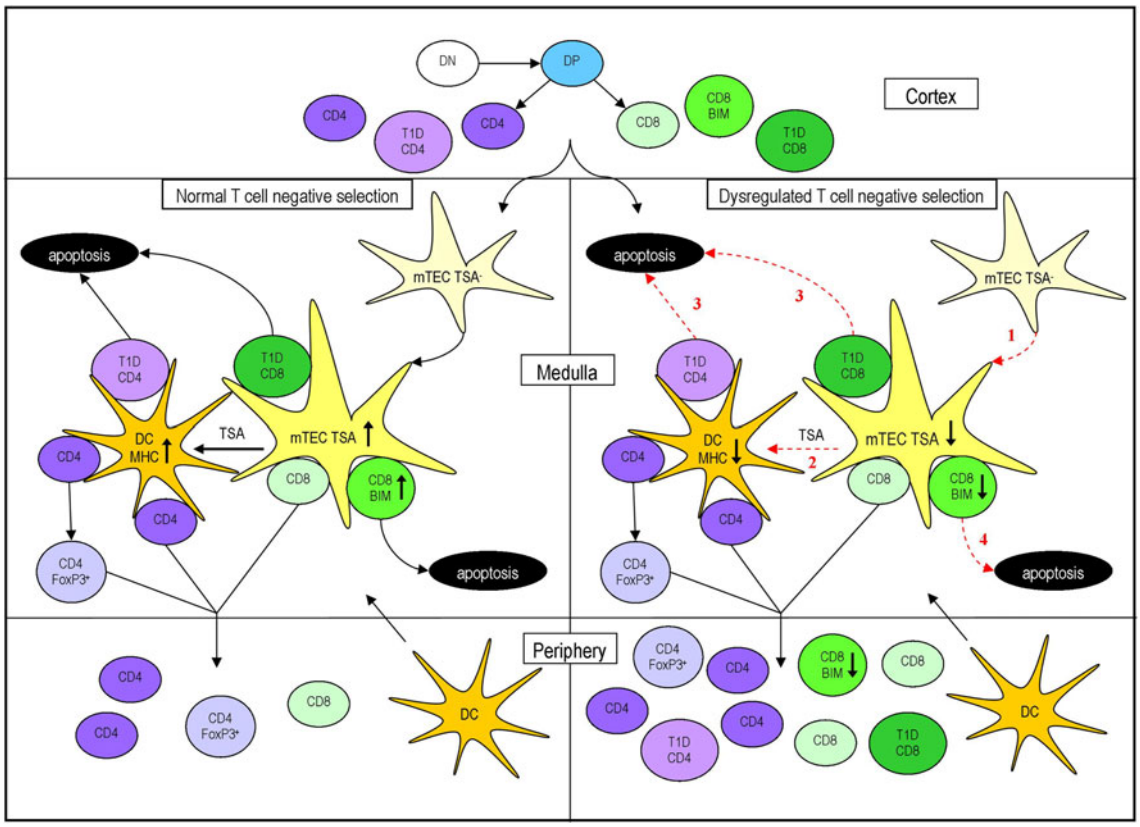

Fig. 1 Dysregulated negative selection events promoting the escape of autoreactive thymocytes into the periphery. Events involved in negative selection ongoing in the medulla of the thymus are depicted in a "normal" (*left panel*) and an "autoimmune prone" (*right panel*) thymus. Following positive selection, the polyclonal $CD4^+$ SP and $CD8^+$ SP thymocytes migrate from the cortex to the medulla. In the normal thymus, immature mTEC differentiate into mature $AIRE^+$ mTEC now capable of expressing a broad spectrum of TSA. Thymic resident DC and DC that have entered the thymus following circulation in the periphery also directly and cross-present a high level of TSA to the developing thymocytes after the uptake of dying mTEC. The effective presentation of autoantigens by mTEC and DC in the normal thymus results in the deletion of autoreactive thymocytes with a high affinity/avidity for self-antigens via apoptosis. Additionally, thymocytes that received apoptotic signals should upregulate pro-apoptotic molecules such as BIM inducing their cell death. A small subset of autoreactive $CD4^+$ SP thymocytes may escape apoptosis by differentiating into natural Treg that express the transcription factor FoxP3. The SP thymocytes that survive the process of negative selection leave the thymus and comprise the peripheral T cell compartment. In contrast, in the dysregulated thymus several processes (indicated by *broken lines*) could be altered leading to increased escape of autoreactive thymocytes. *1* One potential scenario is that mTEC differentiation is compromised decreasing the frequency of $AIRE^+$ mTEC, thereby diminishing the absolute level of TSA being presented to developing thymocytes. *2* Furthermore, this mutation would diminish the level of TSA available to DC to cross-present to SP thymocytes. *3* The low presentation of TSA may reduce TCR signaling in high affinity SP thymocytes, thereby resulting in decreased induction of apoptosis. *4* Alternatively, there may be T cell intrinsic deficiencies, such as the inability to upregulate expression of the pro-apoptotic protein BIM. Minimal BIM upregulation would prevent SP thymocytes entering the apoptotic pathway. These deficiencies alone or in concert allow an increased frequency of autoreactive T cells with enhanced affinity to enter into the periphery

autoimmune diseases is limited at best. Insight into possible mechanisms impacting the efficacy of negative selection has been obtained largely through the use of various model systems to manipulate the cell types and events associated with thymic negative selection. Nevertheless, it is very likely that aberrant clonal deletion contributes to the escape of autoreactive thymocytes (Kishimoto and Sprent 2001). Defects affecting the efficacy of clonal deletion can be categorized in two general ways. The first reflects "global" dysregulation of key molecular and cellular events associated with T cell recognition of and responses to antigen. An example of this is the association between specific alleles of MHC/HLA and several T cell mediated autoimmune diseases (Ebers et al. 1996; Hahn et al. 2005; Haines et al. 1996; Holmdahl 2000; Suri et al. 2008; Taneja and David 2010; von Boehmer 2009; Wucherpfennig et al. 1997; Zanelli et al. 2000). The intrinsic binding properties of specific alleles of MHC/HLA affect the repertoire of peptides presented by APC regardless of whether the APC reside in the thymus or periphery. Similarly, aberrant regulation of TCR signaling, seen with mutant ZAP70 for instance, affects responses of both thymocytes and mature T cells. The second "class" of defects influencing the efficacy of negative selection is

those that affect specific thymic cell types and consequently events involved in thymic clonal deletion. A possible example of this is the development of autoreactive T cells in APECED due to mutations in the *AIRE* gene, and altered mTEC function and maturation (Fig. 1). The current challenge is to define the "global" and "thymus-specific" defects that affect thymocyte selection, in addition to determining the nature of the dysregulation, and ultimately generate therapeutic treatments to increase the efficiency of negative selection in those autoimmune prone individuals to shift the balance back to self-tolerance.

References

Akiyama T, Shimo Y, Yanai H et al (2008) The tumor necrosis factor family receptors RANK and CD40 cooperatively establish the thymic medullary microenvironment and self-tolerance. Immunity 29:423–437

Anderson MS, Bluestone JA (2005) The NOD mouse: a model of immune dysregulation. Annu Rev Immunol 23:447–485

Anderson AE, Isaacs JD (2008) Tregs and rheumatoid arthritis. Acta Reumatol Port 33:17–33

Anderson AC, Nicholson LB, Legge KL et al (2000) High frequency of autoreactive myelin proteolipid protein-specific T cells in the periphery of naive mice: mechanisms of selection of the self-reactive repertoire. J Exp Med 191:761–770

Anderson MS, Venanzi ES, Klein L et al (2002) Projection of an immunological self shadow within the thymus by the aire protein. Science 298:1395–1401

Aoki CA, Borchers AT, Ridgway WM et al (2005) NOD mice and autoimmunity. Autoimmun Rev 4:373–379

Baba T, Nakamoto Y, Mukaida N (2009) Crucial contribution of thymic Sirp alpha + conventional dendritic cells to central tolerance against blood-borne antigens in a CCR2-dependent manner. J Immunol 183:3053–3063

Begovich AB, Carlton VE, Honigberg LA et al (2004) A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. Am J Hum Genet 75:330–337

Boehm T, Scheu S, Pfeffer K et al (2003) Thymic medullary epithelial cell differentiation, thymocyte emigration, and the control of autoimmunity require lympho-epithelial cross talk via LTbetaR. J Exp Med 198:757–769

Bouillet P, Metcalf D, Huang DC et al (1999) Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity. Science 286:1735–1738

Bouillet P, Purton JF, Godfrey DI et al (2002) BH3-only Bcl-2 family member Bim is required for apoptosis of autoreactive thymocytes. Nature 415:922–926

Bowes J, Barton A (2008) Recent advances in the genetics of RA susceptibility. Rheumatology 47:399–402

Brocker T, Riedinger M, Karjalainen K (1997) Targeted expression of major histocompatibility complex (MHC) class II molecules demonstrates that dendritic cells can induce negative but not positive selection of thymocytes in vivo. J Exp Med 185:541–550

Calnan BJ, Szychowski S, Chan FK (1995) A role for the orphan steroid receptor Nur77 in apoptosis accompanying antigen-induced negative selection. Immunity 3:273–282

Chatenoud L, Bach JF (2005) Regulatory T cells in the control of autoimmune diabetes: the case of the NOD mouse. Int Rev Immunol 24:247–267

Chin RK, Lo JC, Kim O et al (2003) Lymphotoxin pathway directs thymic Aire expression. Nat Immunol 4:1121–1127

Cohen S, Dadi H, Shaoul E et al (1999) Cloning and characterization of a lymphoid-specific, inducible human protein tyrosine phosphatase, Lyp. Blood 93:2013–2024

Costantino CM, Baecher-Allan C, Hafler DA (2008) Multiple sclerosis and regulatory T cells. J Clin Immunol 28:697–706

Coutinho A, Caramalho I, Seixas E et al (2005) Thymic commitment of regulatory T cells is a pathway of TCR-dependent selection that isolates repertoires undergoing positive or negative selection. Curr Top Microbiol Immunol 293:43–71

Criswell LA, Pfeiffer KA, Lum RF et al (2005) Analysis of families in the multiple autoimmune disease genetics consortium (MADGC) collection: the PTPN22 620W allele associates with multiple autoimmune phenotypes. Am J Hum Genet 76:561–571

D'Alise AM, Auyeung V, Feuerer M et al (2008) The defect in T-cell regulation in NOD mice is an effect on the T-cell effectors. Proc Natl Acad Sci USA 105:19857–19862

Davalos-Misslitz AC, Rieckenberg J, Willenzon S et al (2007) Generalized multi-organ autoimmunity in CCR7-deficient mice. Eur J Immunol 37:613–622

Decallonne B, van Etten E, Giulietti A et al (2003) Defect in activation-induced cell death in non-obese diabetic (NOD) T lymphocytes. J Autoimmun 20:219–226

Decallonne B, van Etten E, Overbergh L et al (2005) 1Alpha, 25-dihydroxyvitamin D3 restores thymocyte apoptosis sensitivity in non-obese diabetic (NOD) mice through dendritic cells. J Autoimmun 24:281–289

Derbinski J, Kyewski B (2005) Linking signalling pathways, thymic stroma integrity and autoimmunity. Trends Immunol 26:503–506

Derbinski J, Schulte A, Kyewski B et al (2001) Promiscuous gene expression in medullary thymic epithelial cells mirrors the peripheral self. Nat Immunol 2:1032–1039

Derbinski J, Gabler J, Brors B et al (2005) Promiscuous gene expression in thymic epithelial cells is regulated at multiple levels. J Exp Med 202:33–45

Derbinski J, Pinto S, Rösch S et al (2008) Promiscuous gene expression patterns in single medullary thymic epithelial cells argue for a stochastic mechanism. Proc Natl Acad Sci USA 105:657–662

Donskoy E, Goldschneider I (2003) Two developmentally distinct populations of dendritic cells inhabit the adult mouse thymus: demonstration by differential importation of hematogenous precursors under steady state conditions. J Immunol 170:3514–3521

Ebers GC, Kukay K, Bulman DE et al (1996) A full genome search in multiple sclerosis. Nat Genet 13:472–476

Fan Y, Rudert WA, Grupillo M et al (2009) Thymus-specific deletion of insulin induces autoimmune diabetes. EMBO J 28:2812–2824

Gabler J, Arnold J, Kyewski B (2007) Promiscuous gene expression and the developmental dynamics of medullary thymic epithelial cells. Eur J Immunol 37:3363–3372

Gallegos AM, Bevan MJ (2004) Central tolerance to tissue-specific antigens mediated by direct and indirect antigen presentation. J Exp Med 200:1039–1049

Garcia CA, Prabakar KR, Diez J et al (2005) Dendritic cells in human thymus and periphery display a proinsulin epitope in a transcription-dependent, capture-independent fashion. J Immunol 175:2111–2122

Germain RN (2002) T-cell development and the CD4-CD8 lineage decision. Nat Rev Immunol 2:309–322

Gillard GO, Farr AG (2006) Features of medullary thymic epithelium implicate postnatal development in maintaining epithelial

heterogeneity and tissue-restricted antigen expression. J Immunol 176:5815–5824
Giraud M, Taubert R, Vandiedonck C et al (2007) An IRF8-binding promoter variant and AIRE control CHRNA1 promiscuous expression in thymus. Nature 448:934–937
Gray DH, Seach N, Ueno T et al (2006) Developmental kinetics, turnover, and stimulatory capacity of thymic epithelial cells. Blood 108:3777–3785
Gray D, Abramson J, Benoist C et al (2007) Proliferative arrest and rapid turnover of thymic epithelial cells expressing Aire. J Exp Med 204:2521–2528
Guerau-de-Arellano M, Martinic M, Benoist C et al (2009) Neonatal tolerance revisited: a perinatal window for Aire control of autoimmunity. J Exp Med 206:1245–1252
Hahn M, Nicholson MJ, Pyrdol J et al (2005) Unconventional topology of self peptide-major histocompatibility complex binding by a human autoimmune T cell receptor. Nat Immunol 6:490–496
Haines JL, Ter-Minassian M, Bazyk A et al (1996) A complete genomic screen for multiple sclerosis underscores a role for the major histocompatability complex. The Multiple Sclerosis Genetics Group. Nat Genet 13:469–471
Holmdahl R (2000) Association of MHC and rheumatoid arthritis. Why is rheumatoid arthritis associated with the MHC genetic region? An introduction. Arthritis Res 2:203–204
Humblet C, AYu Rudensky, Kyewski B (1994) Presentation and intercellular transfer of self antigen within the thymic microenvironment: expression of the E alpha peptide-I-Ab complex by isolated thymic stromal cells. Int Immunol 6:1949–1958
Jiang W, Anderson MS, Bronson R et al (2005) Modifier loci condition autoimmunity provoked by Aire deficiency. J Exp Med 202:805–815
Kappler JW, Roehm N, Marrack P (1987) T cell tolerance by clonal elimination in the thymus. Cell 49:273–280
Kim MS, Polychronakos C (2005) Immunogenetics of type 1 diabetes. Horm Res 64:180–188
Kishimoto H, Sprent J (2001) A defect in central tolerance in NOD mice. Nat Immunol 2:1025–1031
Kisielow P, Bluthmann H, Staerz UD et al (1988) Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature CD4+ 8+ thymocytes. Nature 333:742–746
Klein L, Klugmann M, Nave KA et al (2000) Shaping of the autoreactive T-cell repertoire by a splice variant of self protein expressed in thymic epithelial cells. Nat Med 6:56–61
Klein L, Hinterberger M, Wirnsberger G et al (2009) Antigen presentation in the thymus for positive selection and central tolerance induction. Nat Rev Immunol 9:833–844
Koble C, Kyewski B (2009) The thymic medulla: a unique microenvironment for intercellular self-antigen transfer. J Exp Med 206:1505–1513
Kyewski B, Klein L (2006) A central role for central tolerance. Annu Rev Immunol 24:571–606
Kyewski B, Peterson P (2010) Aire, master of many trades. Cell 140:24–26
Li J, Park J, Foss D et al (2009) Thymus-homing peripheral dendritic cells constitute two of the three major subsets of dendritic cells in the steady-state thymus. J Exp Med 206:607–622
Liston A, Lesage S, Gray DH et al (2004) Generalized resistance to thymic deletion in the NOD mouse; a polygenic trait characterized by defective induction of Bim. Immunity 21:817–830
Mathis D, Benoist C (2007) A decade of AIRE. Nat Rev Immunol 7:645–650
Mathis D, Benoist C (2009) Aire. Annu Rev Immunol 27:287–312
McCaughtry TM, Baldwin TA, Wilken MS et al (2008) Clonal deletion of thymocytes can occur in the cortex with no involvement of the medulla. J Exp Med 205:2575–2584
Michaels MA, Kang HK, Kaliyaperumal A et al (2005) A defect in deletion of nucleosome-specific autoimmune T cells in lupus-prone thymus: role of thymic dendritic cells. J Immunol 175:5857–5865
Millet V, Naquet P, Guinamard RR (2008) Intercellular MHC transfer between thymic epithelial and dendritic cells. Eur J Immunol 38:1257–1263
Misslitz A, Pabst O, Hintzen G et al (2004) Thymic T cell development and progenitor localization depend on CCR7. J Exp Med 200:481–491
Nitta T, Nitta S, Lei Y et al (2009) CCR7-mediated migration of developing thymocytes to the medulla is essential for negative selection to tissue-restricted antigens. Proc Natl Acad Sci USA 106:17129–17133
Oh S, Rankin AL, Caton AJ (2010) CD4+ CD25+ regulatory T cells in autoimmune arthritis. Immunol Rev 233:97–111
Ohnmacht C, Pullner A, King SB et al (2009) Constitutive ablation of dendritic cells breaks self-tolerance of CD4 T cells and results in spontaneous fatal autoimmunity. J Exp Med 206:549–559
Proietto AI, van Dommelen S, Zhou P et al (2008) Dendritic cells in the thymus contribute to T-regulatory cell induction. Proc Natl Acad Sci USA 105:19869–19874
Pugliese A, Zeller M, Fernandez A Jr et al (1997) The insulin gene is transcribed in the human thymus and transcription levels correlated with allelic variation at the INS VNTR-IDDM2 susceptibility locus for type 1 diabetes. Nat Genet 15:293–297
Rieck M, Arechiga A, Onengut-Gumuscu S et al (2007) Genetic variation in PTPN22 corresponds to altered function of T and B lymphocytes. J Immunol 179:4704–4710
Rooke R, Waltzinger C, Benoist C et al (1997) Targeted complementation of MHC class II deficiency by intrathymic delivery of recombinant adenoviruses. Immunity 7:123–134
Rossi SW, Kim MY, Leibbrandt A et al (2007) RANK signals from CD4(+)3(−) inducer cells regulate development of Aire-expressing epithelial cells in the thymic medulla. J Exp Med 204:1267–1272
Sakaguchi N, Takahashi T, Hata H et al (2003) Altered thymic T-cell selection due to a mutation of the ZAP-70 gene causes autoimmune arthritis in mice. Nature 426:454–560
Scollay R, Godfrey DI (1995) Thymic emigration: conveyor belts or lucky dips? Immunol Today 16:268–273 (discussion 273–274)
Sen P, Wallet MA, Yi Z et al (2007) Apoptotic cells induce Mer tyrosine kinase-dependent blockade of NF-kappaB activation in dendritic cells. Blood 109:653–660
Siggs OM, Makaroff LE, Liston A (2006) The why and how of thymocyte negative selection. Curr Opin Immunol 18:175–183
Smyth DJ, Howson JM, Payne F et al (2006) Analysis of polymorphisms in 16 genes in type 1 diabetes that have been associated with other immune-mediated diseases. BMC Med Genet 7:20
Sospedra M, Ferrer-Francesch X, Dominguez O et al (1998) Transcription of a broad range of self-antigens in human thymus suggests a role for central mechanisms in tolerance toward peripheral antigens. J Immunol 161:5918–5929
Suri A, Levisetti MG, Unanue ER (2008) Do the peptide-binding properties of diabetogenic class II molecules explain autoreactivity? Curr Opin Immunol 20:105–110
Tajouri L, Fernandez F, Griffiths LR (2007) Gene expression studies in multiple sclerosis. Curr Genomics 8:181–189
Takahama Y (2006) Journey through the thymus: stromal guides for T-cell development and selection. Nat Rev Immunol 6:127–135
Taneja V, David CS (2010) Role of HLA class II genes in susceptibility/resistance to inflammatory arthritis: studies with humanized mice. Immunol Rev 233:62–78
Thompson J, Winoto A (2008) During negative selection, Nur77 family proteins translocate to mitochondria where they associate with Bcl-2 and expose its proapoptotic BH3 domain. J Exp Med 205:1029–1036

Todd JA (2010) Etiology of type 1 diabetes. Immunity 32:457–467
Tykocinski LO, Sinemus A, Kyewski B (2008) The thymus medulla slowly yields its secrets. Ann NY Acad Sci 1143:105–122
Ueno T, Saito F, Gray DH et al (2004) CCR7 signals are essential for cortex-medulla migration of developing thymocytes. J Exp Med 200:493–505
Vafiadis P, Bennett ST, Todd JA et al (1997) Insulin expression in human thymus is modulated by INS VNTR alleles at the IDDM2 locus. Nat Genet 15:289–292
Vandiedonck C, Capdevielle C, Giraud M et al (2006) Association of the PTPN22*R620W polymorphism with autoimmune myasthenia gravis. Ann Neurol 59:404–407
Vang T, Congia M, Macis MD et al (2005) Autoimmune-associated lymphoid tyrosine phosphatase is a gain-of-function variant. Nat Genet 37:1317–1319
Venken K, Hellings N, Liblau R et al (2010) Disturbed regulatory T cell homeostasis in multiple sclerosis. Trends Mol Med 16:58–68
Villasenor J, Benoist C, Mathis D (2005) AIRE and APECED: molecular insights into an autoimmune disease. Immunol Rev 204:156–164
Villasenor J, Besse W, Benoist C et al (2008) Ectopic expression of peripheral-tissue antigens in the thymic epithelium: probabilistic, monoallelic, misinitiated. Proc Natl Acad Sci USA 105:15854–15859
von Boehmer H (2009) Central tolerance: essential for preventing autoimmune disease? Eur J Immunol 39:2313–2316
Wallet MA, Sen P, Flores RR et al (2008) MerTK is required for apoptotic cell-induced T cell tolerance. J Exp Med 205:219–232
Wallet MA, Flores RR, Wang Y et al (2009) MerTK regulates thymic selection of autoreactive T cells. Proc Natl Acad Sci USA 106:4810–4815
Wu L, Shortman K (2005) Heterogeneity of thymic dendritic cells. Semin Immunol 17:304–312
Wucherpfennig KW, Catz I, Hausmann S et al (1997) Recognition of the immunodominant myelin basic protein peptide by autoantibodies and HLA-DR2-restricted T cell clones from multiple sclerosis patients. Identity of key contact residues in the B-cell and T-cell epitopes. J Clin Invest 100:1114–1122
You S, Alyanakian MA, Segovia B et al (2008) Immunoregulatory pathways controlling progression of autoimmunity in NOD mice. Ann NY Acad Sci 1150:300–310
Zanelli E, Breedveld FC, de Vries RR (2000) HLA class II association with rheumatoid arthritis: facts and interpretations. Hum Immunol 61:1254–1261
Zhang J, Bardos T, Mikecz K et al (2001) Impaired Fas signaling pathway is involved in defective T cell apoptosis in autoimmune murine arthritis. J Immunol 166:4981–4986
Zhang M, Vacchio MS, Vistica BP et al (2003) T cell tolerance to a neo-self antigen expressed by thymic epithelial cells: the soluble form is more effective than the membrane-bound form. J Immunol 170:3954–3962
Ziegler AG, Nepom GT (2010) Prediction and pathogenesis in type 1 diabetes. Immunity 32:468–478