The why and how of thymocyte negative selection

Owen Marc Siggs, Lydia Elizabeth Makaroff and Adrian Liston

The generation of T cell receptor (TCR) sequence diversity is the strength of adaptive immunity, yet is also the Achilles' heel. To purge highly self-reactive T cells from the immune system, generation of diversity has coevolved with a mechanism of negative selection. Recent studies have revealed new insights addressing the why and how of negative selection by examining situations in which negative selection has failed in human and animals models of autoimmunity. Both thymocyte extrinsic and intrinsic mechanisms are required to restrict the TCR repertoire to a non-autoreactive set. Negative selection also ensures that T cells emerge with receptors that are focussed on the peptide moiety of MHC-peptide complexes.

Addresses

John Curtin School of Medical Research and School of Biochemistry and Molecular Biology, The Australian National University, Canberra 2601, Australia

Corresponding author: Liston, Adrian (adrian.liston@anu.edu.au)

Current Opinion in Immunology 2006, **18**:175–183

This review comes from a themed issue on
Lymphocyte development
Edited by Kristin Hogquist and Harinder Singh

Available online 3rd February 2006

0952-7915/\$ – see front matter

© 2005 Elsevier Ltd. All rights reserved.

DOI 10.1016/j.coi.2006.01.001

Introduction

The generation of T cell receptor (TCR) sequence diversity by subunit selection and junctional addition of nucleotides provides a wide repertoire of T cells, which are capable of anticipating all manner of pathogenic entities. Burnet [1] was the first to propose that this same process would generate 'forbidden' clones able to recognize self-antigens, and that deletion of these clones would be essential to prevent autoimmunity. Experimental evidence demonstrates that self-reactive T cells are indeed purged from the repertoire during a developmental window in the thymus [2]. Further studies have demonstrated that negative selection occurs primarily in the thymic medulla, via presentation of self-antigen-MHC complexes on dendritic cells, forcing any thymocytes with strong reactivity to undergo apoptosis. Recent genetic dissection of autoimmune diseases has emphasized the importance this process has in the phenomenon of *horror autotoxicus*.

This review focuses on the recent advances in understanding the importance of negative selection in shaping the T cell repertoire, and how genetic defects in this process increase susceptibility to autoimmune disease.

The why of negative selection

The removal of strongly self-reactive T cells has long been considered the sole purpose of negative selection, yet a recent study suggests that negative selection may play a more fundamental role in moulding the T cell repertoire [3^{••}]. It has been suggested that unmodified germline TCR sequences have an inherent high affinity for MHC, with reductions in affinity generated during the TCR rearrangement processes. Reduction from an original high affinity is a more efficient process for producing TCRs that only recognise MHC presenting specific peptides, compared with chance increase in affinity from a low germline state. Huseby and colleagues [3^{••}] demonstrated that by limiting negative selection but leaving positive selection intact, a repertoire of T cells develops that is highly cross-reactive for many different peptide-MHC complexes. This study demonstrates that, after TCR rearrangement, most T cells display a very high affinity for MHC, validating the high basal affinity hypothesis.

It is therefore hypothesized that negative selection is essential to remove those TCRs with insufficient deviation from the original high affinity for conserved MHC. Under this new model, the onus is on negative selection to 'push down' affinity, rather than on positive selection to 'pull up' affinity. The role of negative selection consequently becomes one of culling the T cells bearing the default TCR, with unmodified high affinity to MHC. This leaves only those T cells with TCRs that have a low enough affinity for MHC that they are only able to bind with the added help of a specific interaction with a unique MHC-peptide conformation, thus creating the specificity of the adaptive immune branch [3^{••}].

Negative selection therefore appears to have two roles — the first to bias the repertoire towards TCRs that are specific for a single MHC-peptide complex, and the second to eliminate undesirable clones with the potential to drive autoimmunity.

The how of negative selection: thymocyte-extrinsic factors

A most basic requirement of negative selection is the thymic expression and presentation of self-antigen to developing thymocytes. For ubiquitous antigens this prerequisite presents no difficulty; for 'organ-specific'

antigens, however, it has long been debated whether trace thymic expression is biologically relevant, with few antigens validated by deletion studies [4].

The importance of thymic peripheral antigen expression has been emphasized within the past few years through the study of the rare human disorder autoimmune polyendocrinopathy syndrome type 1 (APS-1), which is characterised by a spectrum of autoimmune diseases, caused by mutations in the *AIRE* gene [5,6]. A seminal study by Anderson and colleagues [7] demonstrated that *Aire*-deficient mice had reduced expression of peripheral antigens in medullary thymic epithelial cells (mTECs). An estimated ~2000–3000 tissue-specific antigens are expressed in human and murine mTECs, breaking established anatomical, developmental and sex constraints [8,9]. Of these, ~500 may be Aire-dependent, including the archetypical autoantigen insulin [7,9]. For the insulin promo-

ter-driven hen egg lysozyme (HEL) antigen, a reduction in thymic expression caused by *Aire*-deficiency is sufficient to allow a dramatic reduction in the efficiency of negative selection, causing an increase in susceptibility to autoimmune disease [10^{••},11] (Figure 1). A recent report does, however, indicate that the role of Aire may not be as simple as currently proposed [12[•]].

Using HEL as a reporter of the insulin promoter, the expression of antigen in the thymic stroma is both necessary and sufficient for deletion [13], but presentation has not been investigated. Gallegos and Bevan [14^{••}] have recently explored this aspect of the process, using the neo-self antigen ovalbumin under the control of the insulin promoter. They found that, although mTECs provide the antigen, thymic dendritic cells are capable of capturing this antigen and presenting it to both MHC class I- and II-restricted thymocytes to induce negative

Figure 1

Thymocyte-extrinsic pathways required for negative selection. Organ-specific antigens, such as insulin, are transcribed by specialized epithelial cells in the thymic medulla (mTECs) in an Aire-dependent manner. Protein antigens are presented at the cell surface to thymocytes as a peptide-MHC complex, induce negative selection of MHC class-I restricted thymocytes, and may also negatively select MHC class II-restricted thymocytes. These antigens are also 'shared' with local resident dendritic cells, and following processing can induce deletion of MHC class II-restricted thymocytes, and of class I-restricted thymocytes by cross-presentation of antigen upon MHC class I.

selection. This indirect route of antigen presentation was in fact required for deletion of MHC class II-restricted thymocytes [14**] (Figure 1).

A recent study has cautioned against using thymic expression as the sole indicator of effective negative selection. Allen and colleagues [15**] used transgenic mouse systems to investigate tolerance towards the gastric self-antigen H/K α . Unlike its partner subunit, H/K β , H/K α is expressed in the thymus, although this expression is insufficient to cause deletion of H/K α -reactive CD4⁺ T cells. H/K α -reactive T cells fail to be deleted even when H/K α is overexpressed as a transgene, indicating that in this case the limiting factor of negative selection is not expression. Conversely, transgenic expression of H/K β in the thymus induced deletion of H/K α -reactive T cells [15**], demonstrating that the limiting factor is instead the presence of the partner subunit, known to be essential for stable membrane insertion of H/K α [16]. This could indicate the necessity of the chaperone unit in antigen processing, or, in light of the Gallegos and Bevan study, could imply that the absence of a surface complex prevents thymic dendritic cells from capturing the antigen and mediating deletion of MHC class II-restricted thymocytes. In either scenario, for self-antigens with complex biosynthesis pathways, expression of single subunits alone may not provide sufficient antigen processing for effective negative selection.

The how of negative selection: thymocyte-intrinsic factors

According to the clonal selection theory, an innocuous T cell repertoire requires elimination of T cells with a high affinity for self-antigens. It now appears to be likely that discrimination between high and low affinity TCRs is achieved by interpretation of TCR signal strength upon contact with peptide-MHC. Enhanced TCR/CD3 signalling creates a concomitant increase in negative selection, as characterised by mice deficient in the negative signalling regulators c-Cbl [17] or calcium-modulating ligand (CAML) [18]. Equally, reduced activity of positive signalling mediators can impair negative selection. The most pertinent recent examples include the proximal kinase zeta-chain-associated protein 70 (ZAP-70) and downstream substrates linker for activation of T cells (LAT) and SH2-domain-containing leukocyte protein of 76 kDa (SLP-76). Male HY-TCR transgenic mice show reduced levels of negative selection when combined with variants of ZAP-70 or LAT [19*,20], and the same appears to be true of a SLP-76 variant in the context of superantigen-mediated deletion (OM Siggs *et al.*, unpublished).

As all of these variants affect both positive and negative selection, one would expect signal bifurcation to occur downstream of the proximal TCR signalling complex. Yet, when confronted with a peptide-MHC complex, how

can a single receptor differentiate between high and low affinity ligands? One explanation is that the TCR itself is the signal 'discriminator'. Engagement of the TCR/CD3 complex has been shown to reveal a proline-rich motif in CD3 ϵ . However, both positive selection and negative selection ligands induce the same conformational alteration [21], thus signal bifurcation does not appear to occur at the level of conformation change. The most likely model is that TCR-induced signalling kinetics are differentially interpreted by molecules downstream of the TCR, resulting in positive or negative selection.

A well-established point at which positive and negative selection signals can segregate is at the level of extracellular signal-related kinase (ERK) and Jun amino-terminal kinase (JNK) or p38 activation (Figure 2). Selective disruption of calcineurin and ERK activity specifically affects positive selection [22*], whereas inhibition of JNK and p38 phosphorylation can uniquely impair negative selection [23]. The observation by Gong *et al.* [23] that ERK has a lower activation threshold than either JNK or p38 could therefore account for the distinction of positive and negative selection signals on the basis of signal intensity.

But what is causing these differential activation kinetics? It may simply be that high affinity ligands engage more TCRs simultaneously, or engage the same number for a longer period of time. Prolonged engagement might allow the completion of a unique sequence of biochemical events [24], or recruitment of unique signalling molecules. Strong evidence in favour of the latter has come recently from the Cantor group [25**], implicating the kinase misshapen NIKs-related kinase (MINK) in such a unique complex. Deficiency of the kinase MINK compromises negative selection, but not positive selection, with a reduction in phosphorylation of JNK and not ERK.

How does death occur?

Regardless of the mechanism, the strong negative selection signal will at some point activate a biochemical pathway distinct from positive selection, and culminate in thymocyte apoptosis. Key effector molecules in this process are the Bcl-2 family members Bim, Bax and Bak. Deficiency of Bim [26], or combined deficiency of Bax and Bak [27], can cripple negative selection. As apoptosis induced by Bim overexpression requires Bax and Bak, it implies that Bax and Bak function downstream of, or in collaboration with, Bim [28,29]. Another critical effector is the nuclear receptor Nur77 [30]; although the primary pro-apoptotic function of Nur77 seems to be at the transcriptional level [31], the recently described interaction of Nur77 with Bcl-2 may also be a contributing factor [32].

Precisely how these proteins are activated to initiate thymocyte apoptosis is beginning to be understood,

Figure 2

Thymocyte-intrinsic pathways required for negative selection. Upon binding of high-affinity peptide-MHC ligand, self-reactive TCRs propagate a signal through the ZAP-70 kinase, which in turn activates several downstream adaptor proteins including LAT and SLP-76. The outcome of this proximal signal is a transient burst of ERK activation, along with activation of JNK and p38. Through a process which may or may not require ERK, JNK or p38, activated PKD phosphorylates HDAC7, causing its nuclear exclusion and triggering transcription of *nur77* by the MEF2D transcription factor. HDAC exclusion and transcriptional derepression may also occur at the *Bim* locus via the E2F1 transcription factor. Nur77 may exert its proapoptotic effects through transcriptional modifications, or by interaction with the Bcl-2 protein. Bim acts in concert with Bax and Bak to disrupt mitochondrial stability. Both Nur77 and Bim may be further regulated by post-translational modifications. Dotted lines represent speculative interactions. The TCR-CD3 complex is shown in purple, kinases in light blue, and proximal signal transducers in grey.

and might involve the remodelling of chromatin at key genetic loci, enabling transcription of apoptotic mediators. TCR-mediated signals promote nuclear exclusion of

the histone deacetylase HDAC7, which would otherwise inhibit the activity of the transcription factor myocyte enhancer factor 2D (MEF2D) on the *nur77* promoter

[33,34]. This nuclear exclusion of HDAC7 is mediated by protein kinase D (PKD) and is independent of calcium signalling [35]. The redundancy of calcium signalling is consistent with recent findings that the calcineurin pathway is not required for negative selection [22].

Another study implicated HDAC activity in the regulation of Bim expression [36]. The transcription factor E2F1, which is required for negative selection [37], is recruited to the *Bim* promoter by HDAC inhibition, leading to enhanced *Bim* expression and Bim-dependent cell death [36]. One could imagine a scenario in which strong TCR signals inhibit HDAC function by activating kinases such as PKD, leading to the alteration of chromatin structure, enabling the activation of MEF2D and E2F1 transcription factors and culminating in the expression of proapoptotic molecules including Bim and Nur77 (Figure 2). Simultaneously, TCR signalling might contribute to the post-translational activation of apoptotic effectors, antagonising inhibitory modifications such as phosphorylation of Bim [38] or Nur77 [39,40], to initiate the apoptotic response (Figure 2).

Defective negative selection in the NOD mouse

Over the past year several new insights into negative selection have come from the dissection of negative selection in the non-obese diabetic (NOD) mouse. NOD mice have a general susceptibility to autoimmunity, with a thymocyte-intrinsic defect in MHC class II- [41,42] and class I-restricted negative selection [43]. Research in our laboratory indicates a general quantitative (~10-fold) decrease in negative selection efficiency, which is not limited to membrane-bound or soluble proteins, organ-specific or systemic promoters, or to specific thymocyte developmental stages [44].

One possibility for the NOD defect in negative selection is a reduced efficiency of TCR signalling in NOD thymocytes [45], similar to the *ZAP-70*, *LAT* and *SLP-76* mutants described earlier. Alternatively, the defect in negative selection could be due to a specific lesion in the apoptotic pathway, explaining the reduced response of NOD T cells to various apoptotic stimuli, for example dexamethasone [46]. Transcriptional analysis favours the negative selection-specific model over the broad TCR signalling model. TCR signalling appears to be largely intact, with normally responsive calcineurin and ERK pathways in NOD thymocytes [44], whereas negative selection-specific changes, such as expression of apoptotic mediators *nur77* and *bim*, are reduced [44,47]. The NOD defect is thus likely to occur downstream of the positive selection/negative selection partition.

Further understanding of the molecular mechanism may ensue through analysis of the genetics of the NOD defect. Currently, two studies have taken different approaches to

this problem and show contrasting results. An *in vivo* defect in thymocyte negative selection mapped in a backcross to NOD as a recessive trait weakly linked to six loci that have all previously been associated with diabetes susceptibility (including *Idd5* and *Idd13*, but not *Idd3*) [44]. The polarity for defective deletion and diabetes susceptibility were perfectly concordant, such that the diabetes susceptible allele was also the defective thymic deletion allele at each locus, regardless of whether the susceptible allele originated from the NOD or B10 strain. The backcross strategy used *in vivo* would nevertheless be unable to detect any dominant NOD-derived alleles associated with defective negative selection. By contrast, dominant NOD-derived defects in thymocyte death *in vitro* were identified in an intercross, and these were strongly linked to *Idd5* and *Idd3* [47]. In the *in vivo* thymocyte negative selection model used in our studies [44], *Idd3/5* congenic NOD mice have no protection from the defect in negative selection (A Liston *et al.*, unpublished), and the trait is recessive even when self-antigen is expressed at very high levels in the thymus (A Liston *et al.*, unpublished). Genetic analysis in a third system and generation of congenics able to correct the trait are likely to be required to resolve this discrepancy.

Linkage of negative selection defects to autoimmunity

Genetic defects in thymocyte-intrinsic negative selection can occur in two disparate forms: first, defects in negative selection-specific pathway components; and second, defects in common TCR signalling pathway components. It is intuitive that defects in negative selection-specific pathway components could result in enhanced susceptibility to autoimmune disease, with an increased representation of autoreactive clones in the peripheral repertoire. However, it is less intuitive that a defect in common TCR-signalling pathway components could be linked to disease, as the successful generation of tolerance indicates that the signalling threshold for negative selection in the thymus is at least equivalent to the signalling threshold for activation in the periphery. Thus, defects in common signalling machinery could be expected to remain 'susceptibility neutral', with reduced negative selection balanced by reduced peripheral activation (Figure 3a). This assumption, however, does not take into consideration the differential evolutionary pressure placed upon thymic and peripheral antigen expression, whereby evolutionary pressure requires sufficient tolerogenic levels in the thymus and functional levels in the periphery.

In the case of organ-specific antigens, thymic expression is generally extremely low. The thymic level for insulin is low enough to be a limiting factor for negative selection [10,48,49], and a ~twofold decrease in expression is sufficient to increase susceptibility to autoimmunity [10,50,51]. By contrast, the requirement of physiological

Figure 3

Model for the association of TCR signalling mutants with autoimmunity. A self-reactive TCR of a given affinity is capable of relaying 100 arbitrary units of signalling when saturated with cognate ligand, where it is assumed that 50 units of signalling is sufficient for thymic negative selection or peripheral activation. Thymic and peripheral signalling capacities are given for a range of signalling mutants, from 100 (wild-type) to 0 (null mutant), although mutants exist with signalling capacities beyond 100 (e.g. c-Cbl or CAML-deficient T cells). **(a)** In conditions where ligand is not rate-limiting, wild-type self-reactive T cells will be deleted with high efficiency, avoiding the strong activation these cells are capable of in the periphery. Slight decreases in signalling capacity will still allow both negative selection and peripheral activation, while large decreases in signalling capacity will block negative selection, but also prevent peripheral activation. **(b)** In conditions in which ligand is expressed in the thymus at levels sufficient for negative selection (rate-limiting), but peripheral ligand is present at high functional levels (non rate-limiting), the potential for autoimmunity appears. In this situation, wild-type thymocytes would still undergo negative selection, precluding their peripheral activation. Moderate reductions in signalling, however, enable thymocytes to escape negative selection, in spite of their capability to be activated by high peripheral antigen levels (shaded area). More severe reductions in signalling prevent both negative selection and peripheral activation.

function means that peripheral expression may be many orders of magnitude higher. For this subset of antigens it is conceivable that a minor reduction in TCR signalling capacity could prevent negative selection, yet still allow peripheral activation (Figure 3b). We therefore predict that defects in both negative selection-specific pathways

and common TCR signalling pathways are associated with autoimmune disease.

Conclusions

Recent results linking defects in negative selection to autoimmunity have emphasized the importance of this

pathway in the suppression of autoimmunity. The dissection of the underlying genetic and cellular defects of APS-1 have demonstrated that defects in the thymocyte-extrinsic pathways of negative selection are capable of causing severe autoimmune disease. New evidence indicates that the Aire-dependent pathway is even more fragile than previously appreciated, and that even subtle modification of thymic antigen expression may dramatically increase susceptibility to autoimmune disease. In a similar manner, research on the autoimmune-prone NOD mouse has demonstrated a link between thymocyte-intrinsic defects in negative selection and susceptibility to autoimmunity. It is currently unknown if similar defects exist in the human population, due to the difficulty of measuring the efficiency of negative selection in a polygenic repertoire. A recent report, however, suggests a method to screen for negative selection defects in humans, using a similar negative selection model to that originally used to prove negative selection in mice [52[•]]. A comprehensive screen of the human population, particularly autoimmune patients, would enable a determination of the role of thymocyte-intrinsic genetic lesions of negative selection in susceptibility to autoimmune disorders.

Acknowledgements

We thank Chris Goodnow and Aude Fahrer for stimulating discussion of the topic and reviewing the manuscript. The authors' work has been supported by grants from the Juvenile Diabetes Research Foundation, National Health and Medical Research Council, Wellcome Trust, and the National Institutes of Health.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Burnet M: **Auto-immune disease. II. Pathology of the immune response.** *Br Med J* 1959, **5154**:720-725.
2. Kappler JW, Roehm N, Marrack P: **T cell tolerance by clonal elimination in the thymus.** *Cell* 1987, **49**:273-280.
3. Huseby ES, White J, Crawford F, Vass T, Becker D, Pinilla C, Marrack P, Kappler JW: **How the T cell repertoire becomes peptide and MHC specific.** *Cell* 2005, **122**:247-260.
- This comprehensive study examined the effect of a reduction of negative selection on TCR specificity, by examining the proliferative response of mature immortalised T cells resulting from various thymic developmental conditions. When negative selection (via haematopoietic-derived antigen-presenting cells), but not positive selection (mediated by cortical thymic epithelial cells), was mediated by a single MHC-peptide complex, the resultant TCRs were much more degenerate for both the peptide and MHC. This indicates that pressure exerted by negative selection is responsible for the exquisite TCR specificity of peripheral T cells.
4. Kyewski B, Derbinski J, Gotter J, Klein L: **Promiscuous gene expression and central T-cell tolerance: more than meets the eye.** *Trends Immunol* 2002, **23**:364-371.
5. Nagamine K, Peterson P, Scott HS, Kudoh J, Minoshima S, Heino M, Krohn KJ, Lalot MD, Mullis PE, Antonarakis SE *et al.*: **Positional cloning of the APECED gene.** *Nat Genet* 1997, **17**:393-398.
6. Aaltonen J, Horelli-Kuitunen N, Fan JB, Bjorsen P, Perheentupa J, Myers R, Palotie A, Peltonen L: **High-resolution physical and transcriptional mapping of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy locus on chromosome 21q22.3 by FISH.** *Genome Res* 1997, **7**:820-829.
7. Anderson MS, Venanzi ES, Klein L, Chen Z, Berzins S, Turley SJ, Von Boehmer H, Bronson R, Dierich A, Benoist C *et al.*: **Projection of an Immunological Self-Shadow Within the Thymus by the Aire Protein.** *Science* 2002, **298**:1395-1401.
8. Gotter J, Brors B, Hergenbahn M, Kyewski B: **Medullary epithelial cells of the human thymus express a highly diverse selection of tissue-specific genes colocalized in chromosomal clusters.** *J Exp Med* 2004, **199**:155-166.
9. Derbinski J, Gabler J, Brors B, Tierling S, Jonnakuty S, Hergenbahn M, Peltonen L, Walter J, Kyewski B: **Promiscuous gene expression in thymic epithelial cells is regulated at multiple levels.** *J Exp Med* 2005, **202**:33-45.
10. Liston A, Gray DHD, Lesage S, Fletcher AL, Wilson J, Webster KE, Scott HS, Boyd RL, Peltonen L, Goodnow CC: **Gene dosage limiting role of Aire in thymic expression, clonal deletion and organ-specific autoimmunity.** *J Exp Med* 2004, **200**:1015-1026.
- The 3A9 hen egg lysozyme (HEL)-reactive MHC class II-restricted TCR transgenic system is used here to track the effect of Aire deficiency upon negative selection. Using HEL under the insulin promoter, loss of a single copy of Aire is sufficient to reduce negative selection and increase susceptibility to spontaneous autoimmunity. These data demonstrate that the insulin promoter is activated in limiting quantities in the thymus, that the Aire pathway is sensitive even to subtle changes, and that defective expression and negative selection is directly linked to susceptibility to autoimmunity.
11. Liston A, Lesage S, Wilson J, Peltonen L, Goodnow CC: **Aire regulates negative selection of organ-specific T cells.** *Nat Immunol* 2003, **4**:350-354.
12. Anderson MS, Venanzi ES, Chen Z, Berzins SP, Benoist C, Mathis D: **The cellular mechanism of Aire control of T cell tolerance.** *Immunity* 2005, **23**:227-239.
- This study used a similar system to [10^{••}] to analyse the tolerogenic role of Aire, using OT-I and OT-II TCR transgenic mice crossed to the insulin promoter-driven transgenic ovalbumin. It was demonstrated that thymic deletion and resistance to autoimmunity were Aire-dependent, however surprisingly ovalbumin transcription was Aire-independent. This indicates an additional role for Aire in the tolerance pathway, albeit a perplexing one in light of [14^{••}], data indicating that the Aire-expressing epithelial cells are not active presenters of ovalbumin to OT-II thymocytes.
13. Liston A, Lesage S, Gray DH, Boyd RL, Goodnow CC: **Genetic lesions in T-cell tolerance and thresholds for autoimmunity.** *Immunol Rev* 2005, **204**:87-101.
14. Gallegos AM, Bevan MJ: **Central tolerance to tissue-specific antigens mediated by direct and indirect antigen presentation.** *J Exp Med* 2004, **200**:1039-1049.
- In this paper the route of thymic peripheral antigen presentation is explored. Membrane-bound ovalbumin under the insulin promoter was expressed by medullary thymic epithelial cells, which were capable of deleting OT-I transgenic CD8⁺ T cells and Vβ5 transgenic polyclonal CD8⁺ T cells, but not OT-II transgenic CD4⁺ T cells. Conversely, thymic dendritic cells, which are unable to express ovalbumin, were capable of capturing the antigen and deleting both CD4⁺ and CD8⁺ T cells.
15. Allen S, Read S, Dipaolo R, McHugh RS, Shevach EM, Gleeson PA, van Driel IR: **Promiscuous thymic expression of an autoantigen gene does not result in negative selection of pathogenic T cells.** *J Immunol* 2005, **175**:5759-5764.
- This study crossed the A23 H/Kα-reactive TCR transgenic strain to H/Kα- and H/Kβ-overexpressing strains. It elegantly demonstrated that the paucity of negative selection towards H/Kα is not due to limiting H/Kα expression, but rather due to a lack of the essential partner subunit H/Kβ.
16. Beggah AT, Beguin P, Bamberg K, Sachs G, Geering K: **beta-subunit assembly is essential for the correct packing and the stable membrane insertion of the H,K-ATPase alpha-subunit.** *J Biol Chem* 1999, **274**:8217-8223.
17. Thien CB, Blystad FD, Zhan Y, Lew AM, Voigt V, Andoniou CE, Langdon WY: **Loss of c-Cbl RING finger function results in high-intensity TCR signaling and thymic deletion.** *EMBO J* 2005, **24**:3807-3819.
18. Tran DD, Edgar CE, Heckman KL, Sutor SL, Huntoon CJ, van Deursen J, McKean DL, Bram RJ: **CAML is a p56Lck-**

- interacting protein that is required for thymocyte development.** *Immunity* 2005, **23**:139-152.
19. Sommers CL, Lee J, Steiner KL, Gurson JM, Depersis CL, El-Khoury D, Fuller CL, Shores EW, Love PE, Samelson LE: **Mutation of the phospholipase C-gamma1-binding site of LAT affects both positive and negative thymocyte selection.** *J Exp Med* 2005, **201**:1125-1134.
 - References [19*,20] apply the HY-TCR transgenic model of negative selection to assay the respective effects of missense variants of *LAT* and *ZAP-70* in negative selection. Although female HY-TCR transgenic mice with either mutation show a partial block in positive selection, male HY-TCR transgenics exhibit a profound defect in negative selection.
 20. Sakaguchi N, Takahashi T, Hata H, Nomura T, Tagami T, Yamazaki S, Sakihama T, Matsutani T, Negishi I, Nakatsuru S *et al.*: **Altered thymic T-cell selection due to a mutation of the ZAP-70 gene causes autoimmune arthritis in mice.** *Nature* 2003, **426**:454-460.
 21. Gil D, Schrum AG, Alarcon B, Palmer E: **T cell receptor engagement by peptide-MHC ligands induces a conformational change in the CD3 complex of thymocytes.** *J Exp Med* 2005, **201**:517-522.
 22. Neilson JR, Winslow MM, Hur EM, Crabtree GR: **Calcineurin B1 is essential for positive but not negative selection during thymocyte development.** *Immunity* 2004, **20**:255-266.
 - In MHC class I-restricted (HY), MHC class II-restricted (5C.C7) and *in vitro* (α CD3 + α CD28) systems, this study demonstrates that calcineurin B1 is dispensable for thymocyte negative selection, but essential for positive selection.
 23. Gong Q, Cheng AM, Akk AM, Alberola-Ila J, Gong G, Pawson T, Chan AC: **Disruption of T cell signaling networks and development by Grb2 haploid insufficiency.** *Nat Immunol* 2001, **2**:29-36.
 24. McKeithan TW: **Kinetic proofreading in T-cell receptor signal transduction.** *Proc Natl Acad Sci USA* 1995, **92**:5042-5046.
 25. McCarty N, Paust S, Ikizawa K, Dan I, Li X, Cantor H: **Signaling by the kinase MINK is essential in the negative selection of autoreactive thymocytes.** *Nat Immunol* 2005, **6**:65-72.
 - Through lentiviral RNAi-mediated knockdown of MINK expression, the authors demonstrate a unique role for MINK in negative selection, but not positive selection, by superantigen, OT-II + peptide and HY-TCR transgenic assays.
 26. Bouillet P, Purton JF, Godfrey DI, Zhang LC, Coultas L, Puthalakath H, Pellegrini M, Cory S, Adams JM, Strasser A: **BH3-only Bcl-2 family member Bim is required for apoptosis of autoreactive thymocytes.** *Nature* 2002, **415**:922-926.
 27. Rathmell JC, Lindsten T, Zong WX, Cinalli RM, Thompson CB: **Deficiency in Bak and Bax perturbs thymic selection and lymphoid homeostasis.** *Nat Immunol* 2002, **3**:932-939.
 28. Zong WX, Lindsten T, Ross AJ, MacGregor GR, Thompson CB: **BH3-only proteins that bind pro-survival Bcl-2 family members fail to induce apoptosis in the absence of Bax and Bak.** *Genes Dev* 2001, **15**:1481-1486.
 29. Cheng EH, Wei MC, Weiler S, Flavell RA, Mak TW, Lindsten T, Korsmeyer SJ: **BCL-2, BCL-X(L) sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis.** *Mol Cell* 2001, **8**:705-711.
 30. Woronicz JD, Calnan B, Ngo V, Winoto A: **Requirement for the orphan steroid receptor Nur77 in apoptosis of T-cell hybridomas.** *Nature* 1994, **367**:277-281.
 31. Winoto A, Littman DR: **Nuclear hormone receptors in T lymphocytes.** *Cell* 2002, **109**(Suppl):S57-S66.
 32. Lin B, Kolluri SK, Lin F, Liu W, Han YH, Cao X, Dawson MI, Reed JC, Zhang XK: **Conversion of Bcl-2 from protector to killer by interaction with nuclear orphan receptor Nur77/TR3.** *Cell* 2004, **116**:527-540.
 33. Dequiedt F, Kasler H, Fischle W, Kiermer V, Weinstein M, Herdier BG, Verdin E: **HDAC7, a thymus-specific class II histone deacetylase, regulates Nur77 transcription and TCR-mediated apoptosis.** *Immunity* 2003, **18**:687-698.
 34. Woronicz JD, Lina A, Calnan BJ, Szychowski S, Cheng L, Winoto A: **Regulation of the Nur77 orphan steroid receptor in activation-induced apoptosis.** *Mol Cell Biol* 1995, **15**:6364-6376.
 35. Dequiedt F, Van Lint J, Lecomte E, Van Duppen V, Seufferlein T, Vandenheede JR, Wattiez R, Kettmann R: **Phosphorylation of histone deacetylase 7 by protein kinase D mediates T cell receptor-induced Nur77 expression and apoptosis.** *J Exp Med* 2005, **201**:793-804.
 - This study identifies PKD as the necessary link between TCR signalling and nuclear exclusion of HDAC7, leading to expression of the pro-apoptotic nuclear receptor Nur77.
 36. Zhao Y, Tan J, Zhuang L, Jiang X, Liu ET, Yu Q: **Inhibitors of histone deacetylases target the Rb-E2F1 pathway for apoptosis induction through activation of proapoptotic protein Bim.** *Proc Natl Acad Sci USA* 2005, **102**:16090-16095.
 - Tumour cells with enhanced E2F1 activity are known to be sensitive to apoptosis induced by HDAC inhibitors (HDACIs). The authors of this study reveal that this sensitivity is due to enhanced recruitment of E2F1 to the *Bim* promoter, and that RNAi knockdown of *Bim* could prevent HDACI-mediated apoptosis.
 37. Garcia I, Murga M, Vicario A, Field SJ, Zubiaga AM: **A role for E2F1 in the induction of apoptosis during thymic negative selection.** *Cell Growth Differ* 2000, **11**:91-98.
 38. Harada H, Quearry B, Ruiz-Vela A, Korsmeyer SJ: **Survival factor-induced extracellular signal-regulated kinase phosphorylates BIM, inhibiting its association with BAX and proapoptotic activity.** *Proc Natl Acad Sci USA* 2004, **101**:15313-15317.
 39. Masuyama N, Oishi K, Mori Y, Ueno T, Takahama Y, Gotoh Y: **Akt inhibits the orphan nuclear receptor Nur77 and T-cell apoptosis.** *J Biol Chem* 2001, **276**:32799-32805.
 40. Pekarsky Y, Hallas C, Palamarchuk A, Koval A, Bullrich F, Hirata Y, Bichi R, Letofsky J, Croce CM: **Akt phosphorylates and regulates the orphan nuclear receptor Nur77.** *Proc Natl Acad Sci USA* 2001, **98**:3690-3694.
 41. Kishimoto H, Sprent J: **A defect in central tolerance in NOD mice.** *Nat Immunol* 2001, **2**:1025-1031.
 42. Lesage S, Hartley SB, Akkaraju S, Wilson J, Townsend M, Goodnow CC: **Failure to censor forbidden clone of CD4 T cells in autoimmune diabetes.** *J Exp Med* 2002, **196**:1175-1188.
 43. Choisy-Rossi CM, Holl TM, Pierce MA, Chapman HD, Serreze DV: **Enhanced pathogenicity of diabetogenic T cells escaping a non-MHC gene-controlled near death experience.** *J Immunol* 2004, **173**:3791-3800.
 - The MHC class I-restricted diabetogenic clone A14 shows reduced levels of negative selection on the NOD background.
 44. Liston A, Lesage S, Gray DHD, O'Reilly LA, Strasser A, Fahrner AM, Boyd RL, Wilson J, Baxter A, Gallo EM *et al.*: **Generalised resistance to thymic deletion in the NOD mouse: a polygenic trait characterized by defective induction of Bim.** *Immunity* 2004, **21**:817-830.
 - This study looks at the negative selection defect in the NOD mouse, using the HEL-reactive MHC class II-restricted 3A9 TCR transgenic system. Defective negative selection is observed when HEL is expressed under a range of promoters. The trait is linked to six diabetes-associated loci, including *Idd5* and *Idd13/Bim*, and is characterised by a defect in the ability to upregulate Bim at the mRNA and protein level.
 45. Salojin K, Zhang J, Cameron M, Gill B, Arreaza G, Ochi A, Delovitch TL: **Impaired plasma membrane targeting of Grb2-murine son of sevenless (mSOS) complex and differential activation of the Fyn-T cell receptor (TCR)-zeta-Cbl pathway mediate T cell hyporesponsiveness in autoimmune nonobese diabetic mice.** *J Exp Med* 1997, **186**:887-897.
 46. Bergman ML, Duarte N, Campino S, Lundholm M, Motta V, Lejon K, Penha-Goncalves C, Holmberg D: **Diabetes protection and restoration of thymocyte apoptosis in NOD Idd6 congenic strains.** *Diabetes* 2003, **52**:1677-1682.
 47. Zucchelli S, Holler P, Yamagata T, Roy M, Benoist C, Mathis D: **Defective central tolerance induction in NOD mice: genomics and genetics.** *Immunity* 2005, **22**:385-396.
 - This independent study used an alternative approach to [44**] to analyse negative selection in the NOD mouse. Addition of a mimotope peptide in foetal thymic organ culture of MHC class II-restricted BDC2.5 TCR trans-

genic thymocytes results in apoptosis and conversion to the CD8 $\alpha\alpha$ lineage. In this system NOD mice demonstrated defective negative selection, with reduced upregulation of *Nur77* and *Bim*. The trait was dominant, and linked to either *Idd3* or *Idd5*, depending on whether mimotope peptide was added.

48. Jaeckel E, Lipes MA, Von Boehmer H: **Recessive tolerance to preproinsulin 2 reduces but does not abolish type 1 diabetes.** *Nat Immunol* 2004, **5**:1028-1035.
49. Steptoe RJ, Ritchie JM, Harrison LC: **Transfer of hematopoietic stem cells encoding autoantigen prevents autoimmune diabetes.** *J Clin Invest* 2003, **111**:1357-1363.
50. Vafiadis P, Bennett ST, Todd JA, Nadeau J, Grabs R, Goodyer CG, Wickramasinghe S, Colle E, Polychronakos C: **Insulin expression in human thymus is modulated by INS VNTR alleles at the IDDM2 locus.** *Nat Genet* 1997, **15**:289-292.
51. Pugliese A, Zeller M, Fernandez A Jr, Zalcberg LJ, Bartlett RJ, Ricordi C, Pietropaolo M, Eisenbarth GS, Bennett ST, Patel DD: **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* 1997, **15**:293-297.
52. Meylan F, De Smedt M, Leclercq G, Plum J, Leupin O, Marguerat S, Conrad B: **Negative thymocyte selection to HERV-K18 superantigens in humans.** *Blood* 2005, **105**:4377-4382.

This study demonstrates that the human superantigen HERV-K18 is expressed in the thymus on DP thymocytes and corresponds with low peripheral numbers of V β 7⁺ CD4⁺ T cells, suggesting a method to screen for negative selection defects in humans.

Elsevier joins major health information initiative

Elsevier has joined with scientific publishers and leading voluntary health organizations to create patientINFORM, a groundbreaking initiative to help patients and caregivers close a crucial information gap. patientINFORM is a free online service dedicated to disseminating medical research and is scheduled to launch in 2005.

Elsevier will provide the voluntary health organizations with increased online access to our peer-reviewed biomedical journals immediately upon publication, together with content from back issues. The voluntary health organizations will integrate the information into materials for patients and link to the full text of selected research articles on their websites.

patientINFORM has been created to allow patients seeking the latest information about treatment options online access to the most up-to-date, reliable research available for specific diseases.

'Not only will patientINFORM connect patients and their caregivers with the latest research, it will help them to put it into context. By making it easier to understand research findings, patientINFORM will empower patients to have a more productive dialogue with their physicians and make well-informed decisions about care', said Harmon Eyre, M.D., national chief medical officer of the American Cancer Society.

For more information, visit www.patientinform.org