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Apoptosis during negative selection of autoreactive thymocytes

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Recent investigations have solidified the importance of negative selection in controlling autoimmunity. Loss of autoimmune regulator (AIRE), required for thymic stromal-cell differentiation and thymic expression of peripheral antigens, results in multi-organ autoimmunity. Mice with *AIRE/Foxp3* double mutations suffer from exacerbated autoimmunity when compared with mice with only one mutation, supporting the important contributions of both central and peripheral tolerance. In thymocytes, Cbl is a negative regulator of thymocyte apoptosis while MINK, a MEKK kinase, is required for negative selection. This is consistent with the requirement of JNK, p38 and possibly ERK5 MAP kinases in thymocyte apoptosis. ERK5 induces the Nur77 orphan steroid receptor family members. In cell lines, Nur77 interaction with Bcl-2 turns Bcl-2 into a pro-apoptotic molecule. This and other possibilities will be discussed to explain the unresolved finding that negative selection is defective in *Bim*^{-/-} but is not efficiently blocked in Bcl-2 transgenic mice.

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Introduction

During development, autoreactive thymocytes are eliminated through apoptosis in a process termed negative selection (central tolerance). This process has always been thought to be important to prevent autoimmunity, but concrete proof did not come until recently. Indeed, given that mice possessing mutations in the *Fas*, *IL-2*, or *Foxp3* gene develop severe autoimmunity despite normal negative selection, some have argued that peripheral tolerance is more important than central tolerance and that T cell negative selection might be dispensable for development of a normal immune system (for example, see [1]). This argument was bolstered by examples of apparently little or no autoimmunity and only a partial inhibition of thymocyte apoptosis in mice harboring a

mutation in a single gene involved in negative selection. Nonetheless, the identification of autoimmune regulator (AIRE) as a protein important for expression of non-thymic antigens on stromal cells has started to bring credence to the importance of negative selection [2]. AIRE-deficient mice suffer from multiple organ autoimmunity. In this review, we will discuss AIRE and its importance in thymic stromal cells. In addition, we will discuss advances in our understanding of how T cells might distinguish positive from negative selection signals. Recent elucidation of signaling proteins important for negative selection in T cells will be reviewed.

AIRE, thymic epithelial cells, and the importance of central tolerance

AIRE was first identified as a gene defective in human patients suffering from multiple organ autoimmunity autoimmune polyendocrinopathy (APECED) [3,4]. Similar to human APECED patients, AIRE-deficient mice exhibit autoimmunity in multiple organs [2]. AIRE is primarily expressed in medullary thymic epithelial cells (mTECs). Interestingly, mTECs express many 'tissue-specific antigens' usually not found in thymocytes or T cells. Expression of these antigens is lost in *AIRE*^{-/-} mTEC. Subsequent experiments showed that this results in generation of autoreactive T cells, which attack various organs [5,6]. For example, Anderson et al. crossed ovalbumin-specific T-cell receptor (TCR) transgenic mice (OT-I or OT-II) to transgenic mice expressing OVA under the control of a 'pancreas-specific' promoter (the insulin promoter-driven expression of OVA [RIP-OVA] was also seen in mTECs). They showed that deletion of OVA-specific thymocytes occurred only in wild-type but not in *AIRE*^{-/-} mice [6]. Indeed, all of these *AIRE*^{-/-} mice (OVA transgenic/OVA-TCR/*AIRE*^{-/-}) develop diabetes because of defective negative selection and not because of defective function in regulatory T cells [6].

To further show the important role of negative selection, mice with double *AIRE/Foxp3* mutations were generated in C57Bl/6 background. While *Foxp3*-mutant mice that have lost their regulatory T cell function can survive up to 50 days, *AIRE*^{-/-}/*Foxp3*-mutant mice die within 28 days because of accelerated autoimmunity [7**]. Thus, both peripheral and central tolerance play indispensable roles in preventing the immune system from attacking self-organs. Equally interesting is the extensive autoimmune damages detected specifically in the liver and lung of these mice but not in other organs examined. It is possible that another, yet-to-be identified AIRE-independent mechanism operates to remove thymocytes that are autoreactive to organs other than the lung and liver. The

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significance of thymic expression of peripheral antigens for controlling autoimmunity was further illustrated in a study of an eye antigen interphotoreceptor retinoid-binding protein (IRBP) [8^{••}]. IRBP was identified as a dominant eye-specific protein lost in AIRE-deficient animals. Amazingly, the transfer of IRBP-deficient thymic stromal cells into nude mice resulted in eye-specific autoimmunity [8^{••}]. Thus, the absence of a single peripheral antigen in the thymic stroma, even in the presence of functional regulatory T cells (Tregs) and peripheral tolerance (nude mice are defective in thymic stromal cells and hence transfer of the IRBP-deficient stromal cells should restore generation of mature T cells, including Tregs), could still lead to defects in negative selection and consequently to autoimmunity.

How AIRE works is still not clear. AIRE is regulated transcriptionally by NF- κ B2 (p52) and thus $p52^{-/-}$ mice also exhibit defective negative selection [9[•]]. AIRE contains several zinc finger domains and thus one possible scenario is that AIRE acts as a 'general' transcriptional regulator for promiscuous gene expression in mTECs. However, one study reported that although AIRE deficiency did not affect expression of the RIP-OVA transgene in thymic stromal cells, the negative selection of OVA-specific T cells was still impaired [6]. In another study, negative selection of fodrin-specific T cells was shown to be defective in $AIRE^{-/-}$ mice despite its continued expression in thymic stromal cells [10]. To explain these results, an alternative hypothesis was put forward where, instead of acting as a general transcriptional activator, AIRE regulates differentiation of thymic stromal cells [11,12]. Several lines of experimental data support this alternative hypothesis. First, distinct expression patterns of 'tissue-restricted' genes were observed among different types of thymic epithelial cells [11], suggesting that not all thymic epithelial cells are equal. More recently, it was found that genes associated with stem cells and progenitor cells, for example, *Nanog*, *Oct4*, and *Sox2*, are expressed in a subset of thymic epithelial cells in an AIRE-dependent manner [13[•]]. Thus, AIRE might regulate thymic epithelial differentiation and indirectly affect expression of 'tissue-specific' genes in different thymic stromal cell types.

Signaling molecules regulating negative selection

Cbl proteins are ubiquitin ligases thought to negatively regulate TCR signaling. Inactivation of both *c-Cbl* and *Cbl-b*, two of the Cbl family members, leads to enhanced negative selection [14[•]]. In female H-Y TCR transgenic mice, where the majority of thymocytes normally undergo positive selection to become CD8⁺ cells, depletion of CD4⁺CD8⁺ double-positive (DP) thymocytes was instead noted in *c-Cbl/Cbl-b* double-deficient background. Interestingly, although most membrane-proximal events are unaffected in *c-Cbl⁻¹/Cbl-b⁻¹*

thymocytes, NF- κ B was found to be constitutively phosphorylated. However, the effect of Cbl deficiency on two important downstream molecules involved in negative selection, Nur77 and Bim (see below), was not analyzed in this paper. In a somewhat similar finding, thymocytes initially develop normally in a strain of mice expressing a mutant form of *c-Cbl* with an impaired RING finger domain (*c-Cbl C379A* knockin mice). Notably, as the mice age, thymic cellularities drop precipitously [15[•]]. In six-week-old mice, for example, their thymi are 10% the size of the wild-type littermate controls. Membrane-proximal TCR signaling events are also normal in these *c-Cbl C379A* mice, and the levels of Bim and its related pro-apoptotic and anti-apoptotic Bcl-2 family members remain unaffected. By contrast, phosphorylation of AKT is highly elevated. However, this latter event is not likely to directly enhance thymocyte apoptosis because phosphorylated (activated) AKT is normally associated with inhibition of apoptosis [16,17], and expression of a constitutively activated AKT protein in T cells does not induce apoptosis in transgenic mice [18,19]. Thus, other changes in pro-apoptotic or anti-apoptotic pathways must account for the age-dependent depletion of thymocytes in these *c-Cbl C379A* knockin mice. These data point to an intricate balance of positive and negative forces that shape apoptotic responses during T cell development.

In the context of negative selection, Cbl proteins may influence signaling pathways involving not only Bim, a pro-apoptotic member of the Bcl-2 family, but also the Nur77 family of orphan steroid nuclear receptors as well as the regulators of the mitogen-activated protein (MAP) kinase cascades. Misshapen-Nck-interacting kinase-related kinase (MINK) (MAPKKK6 or ASK2) is a member of the Ste20 kinase family acting as an upstream activator of the MAP kinase cascades. MINK was shown to activate both the c-Jun N-terminal kinase (JNK) and p38 MAP kinase pathways [20]. In a recent paper, MINK expression was found to be the highest in DP thymocytes, and MINK-deficient mice displayed defective negative selection in several models of negative selection [21[•]]. This might be related to the ability of MINK to regulate JNK activation and the subsequent Bim expression because both are severely compromised in MINK-deficient thymocytes. However, others have reported that inhibition of JNK or p38 activities with pharmacological inhibitors did not lead to changes in Bim expression levels [22[•]]. Thus, the effects of MINK on JNK and Bim are most likely the results of MINK regulating two independent pathways.

How these molecular events translate into differential effects of positive versus negative selection during T cell development remains a mystery. A small difference in ligand affinity for the TCR is responsible for shifting the threshold from positive to negative selection signals [23[•]]. For example, conformational changes at the TCR might

reflect subtle differences of ligand–receptor avidity [24^{*}]. Consistent with this notion, a CD3-specific antibody detects TCR/CD3 complexes on the surfaces of T cells undergoing negative selection but not, if at all, those on thymocytes undergoing positive selection [24^{*}]. This conformational change may subsequently translate to activation of MINK and other MAP kinase pathways. In addition to JNK and p38, there are two additional classes of MAP kinases: extracellular signal-related kinase 1/2 (ERK1/2) and ERK5. ERK1/2 is required for positive but not negative selection [25] while JNK and p38 are required for negative selection but not for positive selection [26]. The role of ERK5 in T cell development has not been fully characterized, but constitutively activated ERK5 can activate Nur77 transcription [27], suggesting that the ERK5 pathway may participate in negative selection. Recently, ERK1/2 were found to localize differentially between positively and negatively selected thymocytes [23^{*}]. ERK1/2 are found in the membrane for cells receiving negative selection signals but are evenly distributed in the cytoplasm of positively selected thymocytes. The physiological significance of this differential localization remains to be investigated. Nonetheless, this observation is consistent with the model wherein high avidity interactions with a negatively selecting ligand induce a ‘stronger’ signal and recruit ERK1/2 to the plasma membrane better than low avidity stimulation by a positively selecting ligand.

If Bim is the only BH3 molecule involved in negative selection, why is Bcl-2 overexpression unable to efficiently inhibit negative selection?

The NOD strain of mice suffers from diabetes. This is partly because of defective negative selection. Using DNA microarray analysis, two groups have examined the gene expression profiles of wild-type and NOD mice. Both groups have identified Bim and Nur77 among genes that are upregulated in stimulated wild-type but not NOD CD4⁺CD8⁺ thymocytes [28–30]. These results are consistent with previous functional studies for the roles of Bim and Nur77 family in negative selection [31–34]. The relationship between Bim and Nur77 family members, if any, however, is not clear. Interestingly, Nur77 was reported to translocate from the nucleus to mitochondria in LNCaP cells and in peripheral T cells [35,36]. The phenomenon was independently confirmed in other cancer cell lines [37–39]. There, Nur77 associates with Bcl-2 through a linker region between the BH3 and BH4 domains of Bcl-2, exposing the BH3 domain and converting Bcl-2 into a pro-apoptotic molecule [36]. Nur77 is also capable of interacting with two other Bcl-2 family members, Bcl-B and Bcl-2A1 [40]. However, neither of them possesses a BH4–BH3 linker region. How Nur77 can convert them to pro-apoptotic molecules is not clear. If Nur77 interacts with Bcl-2 or Bcl-2A1 during negative selection, and in fact Bcl-2A1 is expressed

abundantly in DP thymocytes [41], Bim and Nur77 may constitute two distinct pathways in negative selection that converge at the mitochondria. This hypothesis could explain the disparity between the effects of Bim deficiency and Bcl-2 overexpression on negative selection. Although negative selection is defective in *Bim*^{-/-} mice, it is only inefficiently blocked by overexpression of Bcl-2, a normally anti-apoptotic downstream negative regulator of Bim [31,42–44]. Overexpression of Bcl-2 was sufficient to block most Bim-mediated apoptosis (irradiation, glucocorticoid, and death-by-neglect) but was unable to efficiently inhibit negative selection. One formal possibility is that expression of Bcl-2 is not high enough in these transgenic mice to prevent negative selection (but is high enough to block all other forms of apoptosis). To make such a stoichiometric argument, we speculate that a Bcl-2-interacting protein is specifically induced during negative selection to prevent Bcl-2 from antagonizing Bim completely or that another BH3-only protein that binds to Bcl-2 poorly [45] is induced during negative selection and whose expression is affected in *Bim*^{-/-} mice. Another possibility, given the potential for Bcl-2 to switch from an anti-apoptotic to pro-apoptotic molecule, is that induction of Nur77 in negatively selected thymocytes promotes Bcl-2 to mediate apoptosis in developing thymocytes. Although expressed at low levels in DP thymocytes, Bcl-2 has been shown to be induced during negative selection [46]. Thus, in the presence of Nur77, Bcl-2 is unable to block the Bim action. However, one recent report suggested that Nur77 does not translocate to mitochondria in anti-CD3/CD28-stimulated DP thymocytes [47]. Nur77 can also transcriptionally upregulate several apoptotic genes, including *FasL* and *TRAIL*, two of the apoptotic TNF family members and a novel gene *NDG1* [48]. Furthermore, a dominant negative Nur77 protein, which resides mostly in the nucleus, can inhibit negative selection. Although this dominant negative protein might be able to inhibit the endogenous Nur77 translocation to mitochondria via homodimerization [49], it is also likely to inhibit transcription of apoptotic genes like *FasL* [50]. Thus, Nur77 (and its family member Nor-1) might act through two distinct mechanisms, one via the nucleus and the other via the mitochondria. Additional experiments are necessary to elucidate the signal transduction pathways of negative selection.

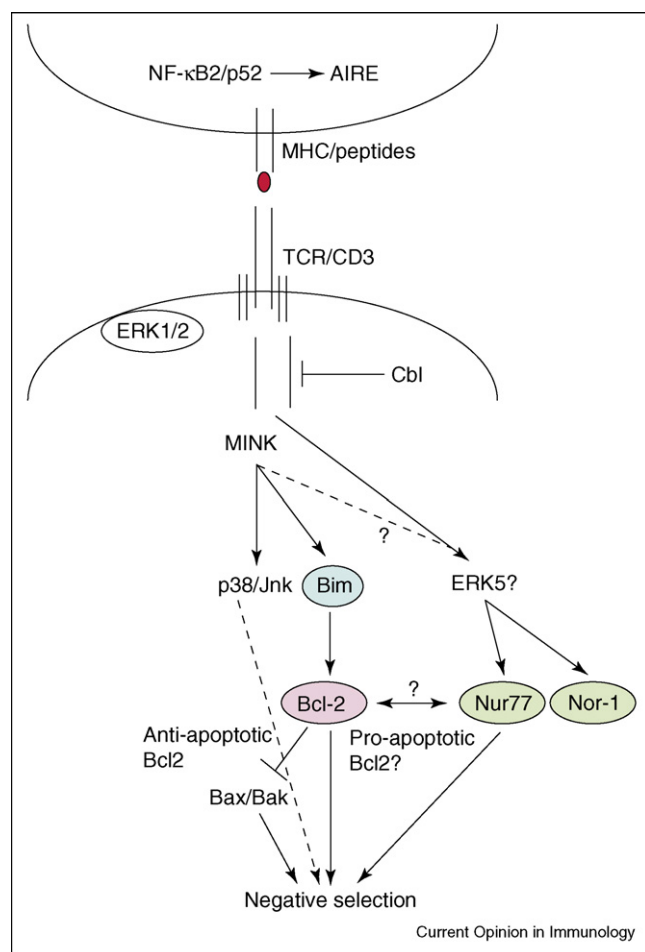
Conclusions

As evidenced by the genetic data, central and peripheral tolerance work together to prevent autoimmune diseases. The observation that Bim and Nur77 levels are suppressed in thymocytes in multiple autoimmune disease models, although mostly correlative, render further support for their roles in negative selection. With the potential for Bcl-2 to act as a pro-apoptotic molecule, we can now consider a modified model wherein Bim/Bcl-2 pathway functionally, and perhaps synergistically, interacts

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with the MAPK/Nur77 pathway (Figure 1). These intracellular molecular events are triggered by the interaction between TCR and its ligand, and whether these interactions result in strictly quantitatively or qualitatively distinct signals remains unresolved. Nonetheless, the crucial role of AIRE in the formulation of antigenic repertoire as well as functional differentiation of thymic stromal cells and their contribution to thymocyte negative selection, suggest that further studies are required to understand the cellular context in which thymocytes differentiate.

Figure 1



A schematic diagram of proteins and the pathways involved in negative selection. NF-κB2 regulates AIRE expression in the thymic stromal cells. AIRE in turn controls expression of 'tissue-specific' antigens, either directly or indirectly through regulation of thymic stromal cell differentiation. Thymocytes receiving negative selection signals initiate a cascade of signaling pathways, including sequestration of ERK1/2, activation of the MINK kinase and the downstream kinases like JNK, p38 and ERK5, which are negatively regulated by Cbl. Expression of apoptotic effector molecules like Bim, Nur77, and the Nur77 family member, Nor-1 is affected by activities of these kinases. Nur77 may interact with Bcl-2 at the mitochondria and turns the normally anti-apoptotic protein into a pro-apoptotic molecule. However, further experimentation is necessary to see whether this actually occurs in thymocytes.

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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. Walker LS, Abbas AK: **The enemy within: keeping self-reactive T cells at bay in the periphery.** *Nat Rev Immunol* 2002, **2**:11-19.
 2. Anderson MS, Venanzi ES, Klein L, Chen Z, Berzins SP, 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.
 3. The-Finnish-German-APECED-Consortium: **An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. The Finnish-German APECED Consortium. Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy.** *Nat Genet* 1997, **17**:399-403.
 4. Nagamine K, Peterson P, Scott HS, Kudoh J, Minoshima S, Heino M, Krohn KJ, Lalioti MD, Mullis PE, Antonarakis SE *et al.*: **Positional cloning of the APECED gene.** *Nat Genet* 1997, **17**:393-398.
 5. 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.
 6. 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.
 7. Chen Z, Benoist C, Mathis D: **How defects in central tolerance •• impinge on a deficiency in regulatory T cells.** *Proc Natl Acad Sci U S A* 2005, **102**:14735-14740.
- Foxp3-deficient mice exhibit impressive autoimmunity while autoimmune diseases in AIRE-deficient mice are less dramatic. These results had led to a notion that peripheral tolerance perhaps was more important than central tolerance. In this paper, the authors compared the phenotype of *Foxp3/AIRE* double knockout with the *Foxp3* or *AIRE* single knockout mice and also *Foxp3*^{-/-} in NOD versus B6 background. They found that both *Foxp3/AIRE* double knockout mice exhibited more severe autoimmunity than *Foxp3*^{-/-} mice. Interestingly, autoimmune damages were limited mostly to the lungs and liver, and many other organs remained untouched. These data suggest that both central and peripheral tolerance mechanisms are important and point to existence of additional tissue-specific mechanisms of self-tolerance.
8. DeVoss J, Hou Y, Johannes K, Lu W, Liou GI, Rinn J, Chang H, Caspi RR, Fong L, Anderson MS: **Spontaneous autoimmunity prevented by thymic expression of a single self-antigen. ••** *J Exp Med* 2006, **203**:2727-2735.
- These authors showed that ablation of a single eye-restricted antigen can lead to an autoimmune eye disease even in the presence of regulatory T cells. Thus, central tolerance mediated by intrathymically expressed antigens can play a dominant role over peripheral tolerance in certain instances.
9. Zhu M, Chin RK, Christiansen PA, Lo JC, Liu X, Ware C, Siebenlist U, Fu YX: **NF-kappaB2 is required for the establishment of central tolerance through an AIRE-dependent pathway. •** *J Clin Invest* 2006, **116**:2964-2971.
- NF-kB2 (p52)-deficient mice exhibit autoimmunity. This paper shows that this is because of defects in thymic stromal cells and that p52 is a transcriptional regulator of AIRE.
10. Kuroda N, Mitani T, Takeda N, Ishimaru N, Arakaki R, Hayashi Y, Bando Y, Izumi K, Takahashi T, Nomura T *et al.*: **Development of autoimmunity against transcriptionally unexpressed target antigen in the thymus of AIRE-deficient mice.** *J Immunol* 2005, **174**:1862-1870.
 11. 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.

12. Gillard GO, Farr AG: **Contrasting models of promiscuous gene expression by thymic epithelium.** *J Exp Med* 2005, **202**:15-19.
13. Gillard GO, Dooley J, Erickson M, Peltonen L, Farr AG: **AIRE-dependent alterations in medullary thymic epithelium indicate a role for AIRE in thymic epithelial differentiation.** *J Immunol* 2007, **178**:3007-3015.
- AIRE is widely thought to act as a factor that de-represses tissue-specific genes. This paper provides an experimental evidence for an alternative and interesting model that AIRE plays a crucial role in thymic epithelial differentiation and that de-repression of tissue-specific genes is a secondary effect of the differentiation state of the epithelial cells. The authors found transcription factors important for 'stem cell'-ness, such as Oct4, Nanog and Sox2, are expressed in thymic epithelial cells in an AIRE-dependent manner. Organization and composition of thymic epithelial cells are also altered in AIRE-deficient mice.
14. Huang F, Kitaura Y, Jang I, Naramura M, Kole HH, Liu L, Qin H, Schliessel MS, Gu H: **Establishment of the major compatibility complex-dependent development of CD4+ and CD8+ T cells by the Cbl family proteins.** *Immunity* 2006, **25**:571-581.
- This paper shows that c-Cbl and Cbl-b double knockout mice possess fewer DP thymocytes and exhibit enhanced negative selection. NF- κ B is constitutively active in these cells, suggesting that Cbl is a negative regulator of TCR-induced NF- κ B activity. It is not clear, however, if Bim or Nur77 activities are elevated in the absence of the Cbl proteins.
15. 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.
- These authors generated c-Cbl RING finger mutant mice and found that thymic cellularity of these mice decreases with age. Most membrane-proximal TCR signal transduction events are normal, with the exception of hyperactivation of AKT in the mutant thymocytes. These data provide evidence that Cbl proteins can negatively regulate downstream TCR signals.
16. Kane LP, Weiss A: **The PI-3 kinase/Akt pathway and T cell activation: pleiotropic pathways downstream of PIP3.** *Immunol Rev* 2003, **192**:7-20.
17. Maehama T, Taylor GS, Dixon JE: **PTEN and myotubularin: novel phosphoinositide phosphatases.** *Annu Rev Biochem* 2001, **70**:247-279.
18. Malstrom S, Tili E, Kappes D, Ceci JD, Tschlis PN: **Tumor induction by an Lck-MyrAkt transgene is delayed by mechanisms controlling the size of the thymus.** *Proc Natl Acad Sci U S A* 2001, **98**:14967-14972.
19. Jones RG, Elford AR, Parsons MJ, Wu L, Krawczyk CM, Yeh WC, Hakem R, Rottapel R, Woodgett JR, Ohashi PS: **CD28-dependent activation of protein kinase B/Akt blocks Fas-mediated apoptosis by preventing death-inducing signaling complex assembly.** *J Exp Med* 2002, **196**:335-348.
20. Dan I, Watanabe NM, Kobayashi T, Yamashita-Suzuki K, Fukagaya Y, Kajikawa E, Kimura WK, Nakashima TM, Matsumoto K, Ninomiya-Tsuji J *et al.*: **Molecular cloning of MINK, a novel member of mammalian GCK family kinases, which is upregulated during postnatal mouse cerebral development.** *FEBS Lett* 2000, **469**:19-23.
21. 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.
- This paper demonstrated the role of MINK kinase in negative selection using several T-cell receptor transgenic mouse models.
22. Cante-Barrett K, Gallo EM, Winslow MM, Crabtree GR: **Thymocyte negative selection is mediated by protein kinase C- and Ca²⁺-dependent transcriptional induction of bim [corrected].** *J Immunol* 2006, **176**:2299-2306.
- One of the interesting findings in this paper is that Bim induction in thymocytes is inhibited by PKC inhibitors but not by JNK, p38 or ERK1/2 inhibitors.
23. Daniels MA, Teixeira E, Gill J, Hausmann B, Roubaty D, Holmberg K, Werlen K, Hollander GA, Gascoigne NR, Palmer E: **Thymic selection threshold defined by compartmentalization of Ras/MAPK signalling.** *Nature* 2006, **444**:724-729.
- Using various ovalbumin peptides with different affinities for TCR, this paper shows that a small increase in ligand affinity can shift the signal threshold that distinguishes positive from negative selection.
- Interestingly, membrane compartmentalization of ERK1/2, ZAP70, LAT and other signaling proteins can be seen in negatively but not positively selecting cells. Thus, appropriate compartmentalization of early signal transduction proteins is a key event that distinguishes positive from negative selection.
24. Risueno RM, van Santen HM, Alarcon B: **A conformational change senses the strength of T cell receptor-ligand interaction during thymic selection.** *Proc Natl Acad Sci U S A* 2006, **103**:9625-9630.
- This paper describes using antibodies to detect conformational changes at the TCR that correlates with negative selection *in vivo*. Their data suggest TCR conformations differentiate positive versus negative selection by inducing distinct signaling pathways.
25. Fischer AM, Katayama CD, Pages G, Pouyssegur J, Hedrick SM: **The role of ERK1 and ERK2 in multiple stages of T cell development.** *Immunity* 2005, **23**:431-443.
26. Sohn SJ, Rajpal A, Winoto A: **Apoptosis during lymphoid development.** *Curr Opin Immunol* 2003, **15**:209-216.
27. Kasler H, Victoria J, Duramad O, Winoto A: **ERK5 is a novel type of MAP kinase containing a transcriptional activation domain.** *Mol Cell Biol* 2000, **20**:8382-8389.
28. Liston A, Hardy K, Pittelkow Y, Wilson SR, Makaroff LE, Fahrner AM, Goodnow CC: **Impairment of organ-specific T cell negative selection by diabetes susceptibility genes: genomic analysis by mRNA profiling.** *Genome Biol* 2007, **8**:R12.
29. 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.
30. Liston A, Lesage S, Gray DH, O'Reilly LA, Strasser A, Fahrner AM, Boyd RL, Wilson J, Baxter AG, Gallo EM *et al.*: **Generalized resistance to thymic deletion in the NOD mouse; a polygenic trait characterized by defective induction of Bim.** *Immunity* 2004, **21**:817-830.
31. 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.
32. Cheng LE, Chan FK, Cado D, Winoto A: **Functional redundancy of the Nur77 and Nor-1 orphan steroid receptors in T cell apoptosis.** *EMBO J* 1997, **16**:1865-1875.
33. Zhou T, Cheng J, Yang P, Wang Z, Liu C, Su X, Bluethmann H, Mountz JD: **Inhibition of Nur77/Nurr1 leads to inefficient clonal deletion of self-reactive T cells.** *J Exp Med* 1996, **183**:1879-1892.
34. Calnan B, Szychowski S, Chan FKM, Cado D, Winoto A: **A role of the orphan steroid receptor Nur77 in antigen-induced negative selection.** *Immunity* 1995, **3**:273-282.
35. Li H, Kolluri SK, Gu J, Dawson MI, Cao XH, Hobbs PD, Lin BZ, Chen GQ, Lu LS, Lin F *et al.*: **Cytochrome c release and apoptosis induced by mitochondrial targeting of nuclear orphan receptor TR3.** *Science* 2000, **289**:1159-1164.
36. Lin B, Kolluri SK, Lin F, Liu W, Han YH, Cao X, Dawson MI, Reed JC, Zhang XK: **Conversion of Bcl-2 from protection to killer by interaction with nuclear orphan receptor Nur77/TR3.** *Cell* 2004, **116**:527-540.
37. Wu Q, Liu S, Ye XF, Huang ZW, Su WJ: **Dual roles of Nur77 in selective regulation of apoptosis and cell cycle by TPA and ATRA in gastric cancer cells.** *Carcinogenesis* 2002, **23**:1583-1592.
38. Wilson AJ, Arango D, Mariadason JM, Heerdt BG, Augenlicht LH: **TR3/Nur77 in colon cancer cell apoptosis.** *Cancer Res* 2003, **63**:5401-5407.
39. Lee KW, Ma L, Yan X, Liu B, Zhang XK, Cohen P: **Rapid apoptosis induction by IGFBP-3 involves an insulin-like growth factor-independent nucleomitochondrial translocation of RXRalpha/Nur77.** *J Biol Chem* 2005, **280**:16942-16948.
40. Luciano F, Krajewska M, Ortiz-Rubio P, Krajewski S, Zhai D, Faustin B, Bruey JM, Bailly-Maitre B, Lichtenstein A, Kumar Kolluri S *et al.*: **Nur77 converts phenotype of Bcl-B, an**

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- anti-apoptotic protein expressed in plasma cells and myeloma.** *Blood* 2007.
41. Tomayko MM, Punt JA, Bolcavage JM, Levy SL, Allman DM, Cancro MP: **Expression of the Bcl-2 family member A1 is developmentally regulated in T cells.** *Int Immunol* 1999, **11**:1753-1761.
42. Strasser A, Harris AW, von BH, Cory S: **Positive and negative selection of T cells in T-cell receptor transgenic mice expressing a bcl-2 transgene.** *Proc Natl Acad Sci U S A* 1994, **91**:1376-1380.
43. Sentman CL, Shutter JR, Hockenbery D, Kanagawa O, Korsmeyer SJ: **Bcl-2 inhibits multiple forms of apoptosis but not negative selection in thymocytes.** *Cell* 1991, **67**:879-888.
44. Strasser A, Harris AW, Cory S: **Bcl-2 transgene inhibits T cell death and perturbs thymic self-censorship.** *Cell* 1991, **67**:889-899.
45. Chen L, Willis SN, Wei A, Smith BJ, Fletcher JI, Hinds MG, Colman PM, Day CL, Adams JM, Huang DC: **Differential targeting of prosurvival Bcl-2 proteins by their BH3-only ligands allows complementary apoptotic function.** *Mol Cell* 2005, **17**:393-403.
46. Schmitz I, Clayton LK, Reinherz EL: **Gene expression analysis of thymocyte selection in vivo.** *Int Immunol* 2003, **15**:1237-1248.
47. Cunningham NR, Artim SC, Fornadel CM, Sellars MC, Edmonson SG, Scott G, Albino F, Mathur A, Punt JA: **Immature CD4+CD8+ Thymocytes and Mature T Cells Regulate Nur77 Distinctly in Response to TCR Stimulation.** *J Immunol* 2006, **177**:6660-6666.
48. Rajpal A, Cho YA, Yelent B, Koza-Taylor PH, Li D, Chen E, Whang M, Kang C, Turi TG, Winoto A: **Transcriptional activation of known and novel apoptotic pathways by Nur77 orphan steroid receptor.** *EMBO J* 2003, **22**:6526-6536.
49. Philips A, Lesage S, Gingras R, Maira M-H, Gauthier Y, Hugo P, Drouin J: **Novel dimeric nur77 signaling mechanism in endocrine and lymphoid cells.** *Mol Cell Biol* 1997, **17**:5946-5951.
50. Weih F, Ryseck R-P, Chen L, Bravo R: **Apoptosis of nur77/N10-transgenic thymocytes involves the Fas/Fas ligand pathway.** *Proc Natl Acad Sci U S A* 1996, **93**:5533-5538.