Bim-Mediated Apoptosis Is Not Necessary for Thymic Negative Selection to Ubiquitous Self-Antigens

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Bim-Mediated Apoptosis Is Not Necessary for Thymic Negative Selection to Ubiquitous Self-Antigens

Qian Hu, Alyssa Sader, Julia C. Parkman, and Troy A. Baldwin

T cell education in the thymus is critical for establishing a functional, yet self-tolerant, T cell repertoire. Negative selection is a key process in enforcing self-tolerance. There are many questions that surround the mechanism of negative selection, but it is currently held that apoptosis initiated by Bim and/or Nur77 is critical for negative selection. Recent studies, however, have questioned the necessity of Bim in maintaining both central and peripheral T cell tolerance. To reconcile these apparently contradictory findings, we examined the role of Bim in negative selection in the well-characterized, physiological HYcd4 mouse model. We found that while Bim expression was required for CD4+CD8+ double-positive thymocyte apoptosis, it was not required for negative selection. Furthermore, Bim deficiency did not alter the frequency or affinity of male reactive cells that escape negative selection in an oligoclonal repertoire. Collectively, these studies indicate that negative selection occurs efficiently in the absence of apoptosis and suggest that the current paradigm of negative selection requiring apoptosis be revisited. The Journal of Immunology, 2009, 183: 7761–7767.
In vitro stimulation

Thymocytes were harvested, labeled with CFSE, mixed with wild-type female splenocytes at a 4:1 ratio, and stimulated with the indicated concentration of s-myc peptide. The cultures were harvested following 24 or 48 h of stimulation, and CD69 induction (24 h) or CFSE dye dilution (48 h) was measured.

Results

Thymic profile of HY<sup>cd4</sup> Bim<sup>−/−</sup> mice

Given the existing controversy over the requirement for Bim in thymic negative selection, we wanted to examine the role of Bim in T cell development using the well-established HY<sup>cd4</sup> model. Previously, the role of Bim in thymocyte selection was studied in classical TCR transgenic mice where the TCR αβ heterodimer is expressed in the DN stage of development, resulting in premature negative selection at the DN to DP transition or in a superantigen-driven selection system where superantigen-MHC recognition by the TCR is mechanistically different than pMHC recognition by the TCR (20, 28). Neither of these model systems is reflective of pMHC-mediated negative selection at the DP stage (2, 30). The HY<sup>cd4</sup> model utilizes a Cre/loxP system to conditionally express the thymocyte with anti-Thy1.2, which mimics endogenous TCRα expression (29). We bred HY<sup>cd4</sup> mice to create HY<sup>cd4</sup> Bim<sup>−/−</sup> mice. This allowed us to specifically determine the role of Bim in pMHC-mediated positive and negative selection of DP thymocytes in female and male mice, respectively. Thymocytes expressing the male Ag-reactive TCR were identified using the T3.70 mAb. Bim deficiency did not alter the percentage of T3.70<sup>+</sup> thymocytes in HY<sup>cd4</sup> Bim<sup>−/−</sup> female mice compared with HY<sup>cd4</sup> female mice, but HY<sup>cd4</sup> Bim<sup>−/−</sup> male mice contained an elevated percentage of T3.70<sup>+</sup> cells compared with HY<sup>cd4</sup> male mice (data not shown). Additionally, by staining the thymocytes with anti-Thyl.2, we did not detect an increase in non-T cell populations (i.e., Thy1.2<sup>+</sup>) in Bim-deficient compared with Bim-sufficient mice (data not shown). We next examined the CD4/CD8 profiles of T3.70<sup>+</sup> thymocytes from the different mouse strains. Bim deficiency did not appear to dramatically alter the CD4/CD8 profile in either female or male mice (Fig. 1A). Both HY<sup>cd4</sup> and HY<sup>cd4</sup> Bim<sup>−/−</sup> female mice contained both DP thymocytes and a substantial population of CD8SP thymocytes, while both HY<sup>cd4</sup> and HY<sup>cd4</sup> Bim<sup>−/−</sup> male mice contained mostly DP thymocytes and lacked a substantial CD8SP population (Fig. 1A). We consistently observed an increase in CD4 and CD8 “dulling” in HY<sup>cd4</sup> Bim<sup>−/−</sup> male DP thymocytes compared with HY<sup>cd4</sup> male DP thymocytes, resulting in a higher percentage of DN phenotype cells (Fig. 1A). We hypothesize the exaggerated “DP dull” phenotype and higher percentage of DN phenotype thymocytes arises from prolonged high-affinity TCR signaling resulting from an increased lifespan due to an inhibition in apoptosis in the absence of Bim (see below). Alternatively, these DN cells could originate from DP thymocytes that truly failed negative selection.

To ensure that Bim deficiency did not alter pMHC recognition, we compared the levels of CD69, CD5, CD2, and PD-1 expression on T3.70<sup>+</sup> DP thymocytes. As previously observed, all of these markers were up-regulated in HY<sup>cd4</sup> male mice compared with B6 and HY<sup>cd4</sup> female mice (29, 31). We observed an increased proportion of DP thymocytes expressing CD69 from HY<sup>cd4</sup> Bim<sup>−/−</sup> female mice compared with HY<sup>cd4</sup> female mice, suggesting enhanced positive selection efficiency, but no difference in CD5 or CD2 expression levels (Fig. 1B and data not shown). No difference in expression of CD69, CD5 or CD2 on DP thymocytes was found in HY<sup>cd4</sup> Bim<sup>−/−</sup> male mice compared with HY<sup>cd4</sup> male mice (Fig. 1B and data not shown). Interestingly, the percentage of DP thymocytes expressing PD-1, but not the level of PD-1 expression, was increased in HY<sup>cd4</sup> Bim<sup>−/−</sup> male mice (Fig. 1C). This further supports an increased lifespan for DP thymocytes in the absence of
Bim since we know that PD-1 expression on DP thymocytes is induced maximally 48 h following TCR stimulation (31). Normally, PD-1 expression on DP thymocytes from wild-type mice is difficult to detect (32); however, in nontransgenic Bim−/− mice, a significant population of PD-1−/− DP thymocytes was observed (data not shown). Given that PD-1 is expressed on DP thymocytes following a high-affinity signal, the PD-1+ DP thymocytes from nontransgenic Bim−/− mice may have received a high-affinity signal in vivo.

FIGURE 1. The phenotype of thymocytes undergoing positive and negative selection in HYcd4 Bim−/− mice. A, CD4 by CD8 profile of total thymocytes from Vβ8 and Vβ8 Bim−/− mice and CD4 by CD8 profiles of T3.70+ thymocytes from HYcd4 and HYcd4 Bim−/− female and male mice. Data are representative of at least eight mice per strain. B, The expression of CD69 and CD5 on total (B6) or T3.70+ thymocytes from HYcd4 Bim−/− female and male mice. Data are representative of four independent experiments. C, The expression of PD-1 on total DP from B6 or T3.70+ DP from HYcd4 male and HYcd4 Bim−/− male mice. Data are representative of four independent experiments.

Bim is required for DP thymocyte apoptosis

Previous experiments examining the requirement for Bim in negative selection indicated that Bim performed an essential role in negative selection by inducing apoptosis in DP thymocytes (20, 21). Therefore, we examined the consequence of Bim deficiency on DP thymocyte apoptosis using an Ab that recognizes only the cleaved and thus activated form of caspase 3. T3.70+ DP thymocytes from the indicated mice were electronically gated and the percentage of cells containing activated caspase-3 was determined. As previously reported, we found that compared with B6 or HYcd4 female mice, HYcd4 male mice contained a 6-fold higher percentage of active caspase-3+ T3.70+ DP thymocytes (Fig. 2, A and B) (31). Compared with HYcd4 male mice, HYcd4 Bim−/− male mice displayed a 16-fold reduction in the percentage of active caspase-3+ T3.70+ DP thymocytes (Fig. 2, A and B). No statistical difference in the percentage of cells containing active caspase-3 was observed between HYcd4 Bim−/− female and male mice (Fig. 2, A and B), suggesting that the high-affinity signal required to induce apoptosis normally was unable to do so in the absence of Bim. These data support Bim as an essential mediator of high-affinity Ag-induced apoptosis. Additionally, the few T3.70+ CD8SP thymocytes from HYcd4 Bim−/− male mice contained virtually no active caspase-3+ cells, while the same thymocyte population in HYcd4 male mice contained a substantial fraction of active caspase-3+ cells (supplemental Fig. 1).5 Given the critical role of Bim in DP thymocyte apoptosis, we next determined the influence of Bim deficiency on DP thymocyte numbers. As previously published, we observed a 3-fold decrease in the number of T3.70+ DP thymocytes in HYcd4 male mice compared with HYcd4 female mice (Fig. 2C) (29). However, there was no difference in the number of T3.70+ DP thymocytes in HYcd4 Bim−/− male mice compared with HYcd4 Bim−/− female mice. We also

5 The online version of this article contains supplemental material.
suggest that Bim is a critical mediator of DP thymocyte apoptosis in DN thymocyte survival (34). Collectively, these data depict the means of least five mice per strain in at least five independent experiments. Data are compiled from at least five mice per strain in at least five independent experiments. Data depict the means ± SD. *p = 0.0065; **p ≤ 0.001; and ***p ≤ 0.0001.

observed a decrease in the number of T3.70+ DP thymocytes in HYcd4 Bim−/− female mice compared with HYcd4 female mice (Fig. 2C). This difference is likely explained by a reduction in proliferation at the β-selection checkpoint resulting from Bim deficiency as reported by Hutcheson and Perlman (33) and not impairment in DN thymocyte survival (34). Collectively, these data suggest that Bim is a critical mediator of DP thymocyte apoptosis during negative selection and that Bim-mediated apoptosis is responsible for the 3-fold decrease in T3.70+ DP thymocytes in HYcd4 male mice compared with HYcd4 female mice.

Negative selection occurs in the absence of Bim

If Bim deficiency truly impaired negative selection, one might predict this impairment to manifest itself as an increase in the mature CD8SP thymocyte population. Therefore, we performed a detailed analysis of the thymic CD8SP compartment in HYcd4 and HYcd4 Bim−/− mice. HYcd4 female and HYcd4 Bim−/− female mice both contained a substantial population of CD8SP thymocytes (Fig. 3A). Interestingly, there was a 2-fold increase in the percentage of T3.70+ CD8SP thymocytes in HYcd4 Bim−/− female mice compared with HYcd4 female mice, suggesting enhanced positive selection in the absence of Bim. In both HYcd4 male and HYcd4 Bim−/− male mice, there was a dramatic reduction in the percentage of T3.70+ CD8SP thymocytes (Fig. 3A). To determine whether Bim deficiency enhanced the development of mature (CD24low) T3.70+ CD8SP thymocytes, we examined CD24 expression on T3.70+ CD8SP thymocytes. In HYcd4 and HYcd4 Bim−/− female mice, most T3.70+ CD8SP thymocytes were CD24low while in HYcd4 and HYcd4 Bim−/− male mice, about half of the few T3.70+ CD8SP thymocytes were CD24low (Fig. 3B). Overall, there was a 40-fold reduction in the number of CD24low T3.70+ CD8SP thymocytes in HYcd4 male compared with female mice and a 34-fold reduction in HYcd4 Bim−/− male compared with female mice (Fig. 3C). Although not statistically significant, there was a 2-fold increase in the number of CD24low T3.70+ CD8SP thymocytes in HYcd4 Bim−/− female mice compared with HYcd4 female mice, again supporting enhanced positive selection or CD8SP survival in the absence of Bim. A compilation of the T3.70+ thymocyte numbers from the HYcd4 and HYcd4 Bim−/− female and male mice is presented in Table I. Therefore, it appears that in the HYcd4 mouse model, Bim deficiency does not impair thymocyte negative selection.

The fact that Bim−/− mice do develop autoimmune disorders suggests that these mice may contain functional peripheral T cells that escape negative selection. To determine the impact of Bim on the peripheral CD8 compartment in HYcd4 mice, we examined the lymph node and spleen of HYcd4 and HYcd4 Bim−/− female and male mice. As previously reported (29), HYcd4 female mice contain relatively few T3.70+ T cells in the lymph node and spleen (supplemental Fig. 2 and data not shown). These T3.70+ cells are mostly CD8+ and display a naive, CD44low phenotype (supplemental Fig. 2). Bim deficiency does not appear to influence this phenotype. HYcd4 male mice contain an elevated percentage and number of peripheral T3.70+ T cells in comparison to female mice (supplemental Fig. 2). Most of the T3.70+ cells in male mice are CD8+, albeit with lower CD8 levels than in female mice, with only a small percentage bearing a DN phenotype. This is in contrast to the peripheral T3.70+ cells in conventional HY male mice that mostly display a DN phenotype (data not shown). The reason for the high number of peripheral T3.70+ cells in the HYcd4 male mice given the low number of thymic T3.70+ CD8SP is currently unclear, but it is likely due to the expansion of the few T3.70+

### Table I. Bim is required for DP thymocyte clonal deletion but not negative selection

<table>
<thead>
<tr>
<th>Strain</th>
<th>HYcd4 F</th>
<th>HYcd4 Bim−/− F</th>
<th>HYcd4 M</th>
<th>HYcd4 Bim−/− M</th>
<th>HYcd4 Bim−/− M</th>
</tr>
</thead>
<tbody>
<tr>
<td>T3.70+ DP</td>
<td>58.7 ± 29.3</td>
<td>47.4 ± 25.8</td>
<td>19.6 ± 7.07</td>
<td>44.9 ± 38.4</td>
<td>25.5 ± 11.9</td>
</tr>
<tr>
<td>T3.70+ CD8SP</td>
<td>3.04 ± 1.13</td>
<td>4.93 ± 2.54</td>
<td>0.142 ± 0.054</td>
<td>0.383 ± 0.262</td>
<td>0.205 ± 0.046</td>
</tr>
<tr>
<td>T3.70+ CD24hi CD8SP</td>
<td>1.95 ± 0.91</td>
<td>4.19 ± 2.56</td>
<td>0.048 ± 0.046</td>
<td>0.123 ± 0.048</td>
<td>0.040 ± 0.024</td>
</tr>
</tbody>
</table>

* Absolute number of T3.70+ thymocytes from the indicated strains. Data were compiled from between 4 and 10 mice (mean ± SD × 10⁶).
CD8SP that are able to escape negative selection once in an environment expressing their cognate Ag. In support of this idea, the T3.70⁺ CD8⁺ cells from HYcd4 male mice are CD44high, suggesting Ag-driven expansion (supplemental Fig. 2). While Bim deficiency does not influence the percentage of peripheral T3.70⁺ cells in HYcd4 male mice, it does influence their phenotype since there are approximately equal percentages of CD8⁺ and DN phenotype cells in the absence of Bim (supplemental Fig. 2). Similar to T3.70⁺ CD8⁺ cells in HYcd4 male mice, T3.70⁺ CD8⁺ cells from HYcd4 Bim⁻/⁻ male mice are CD44high, while the T3.70⁺ DN phenotype cells from HYcd4 Bim⁻/⁻ male mice are CD44low (supplemental Fig. 2). The T3.70⁺ cells from the HYcd4 and HYcd4 Bim⁻/⁻ male mice are CD69⁺, suggesting that they are not overtly activated (data not shown). The reason for the change in phenotype in peripheral T3.70⁺ cells in HYcd4 Bim⁻/⁻ mice is currently unclear, but given the fact that HYcd4 male mice do not appear to develop autoimmune diseases despite high numbers of T3.70⁺ CD8⁺ peripheral cells, we do not think these or the HYcd4 Bim⁻/⁻ T3.70⁺ cells are pathogenic. Because of the complexities of the peripheral T cell population in HYcd4 and HYcd4 Bim⁻/⁻ male mice, we also examined the role of Bim in negative selection in Vβ8 TCR transgenic mice (see below).

**Bim deficiency does not affect negative selection in an oligoclonal repertoire**

To this point, we have examined the requirement for Bim in the negative selection of a monoclonal thymocyte population. We next wanted to determine whether Bim was required for negative selection in an oligoclonal repertoire. To do this, we utilized the Vβ8 TCR transgenic mouse that expresses the TCRβ-chain derived from the HY TCR. In female Vβ8 TCR transgenic mice, there is an elevated frequency of male Ag-reactive thymocytes and T cells with variable affinities for s-mcy peptide in the context of H-2Db, making it possible to examine selection events in an oligoclonal repertoire (35). By using pentamers of H-2Db/s-mcy complexes, we can specifically identify male Ag-reactive thymocytes and T cells. Compared with female B6 mice, we found an elevated percentage of CD8⁺ T cells and thymocytes specific for Dβ/s-mcy in the spleen, lymph node, and thymus of female Vβ8 mice (Fig. 4, A and B, and data not shown). We found a similar frequency of Dβ/s-mcy-specific T cells in Vβ8 Bim⁻/⁻ female mice compared with Vβ8 female mice (data not shown). As previously reported, negative selection mostly eliminates Dβ/s-mcy specificities in the peripheral CD8 T cell and CD8SP thymocyte compartment of Vβ8 male mice (29, 35), and Bim deficiency does not affect this elimination (Fig. 4, A and B, and data not shown). Since negative selection is not perfect and low-affinity clones are able to escape this process (36), we determined whether Bim deficiency influenced the affinity of the T cell clones that escape negative selection. Using a strategy similar to Zehn and Bevan (36), we CFSE-labeled bulk thymocytes from B6, Vβ8 female and male and Vβ8 Bim⁻/⁻ male mice to assess the response of the CD8SP thymocytes to increasing concentrations of s-mcy peptide. At 24 h following stimulation, a population of CD8SP thymocytes from Vβ8 female mice began to induce CD69 at a peptide concentration of 10⁻⁸ M, while CD8SP thymocytes from male Vβ8 and Vβ8 Bim⁻/⁻ mice did not begin to induce CD69 until an s-mcy concentration of 10⁻⁶ M (Fig. 4C). This indicates that the CD8SP thymocytes that escape negative selection in male mice have a 100-fold reduction in the affinity for s-mcy compared with female mice and that Bim deficiency does not influence the affinity of the escapees. Similar results were also obtained by measuring CFSE dilution 48 h poststimulation (data not shown). Therefore, it appears that mechanisms independent of Bim-mediated apoptosis can enforce negative selection in an oligoclonal repertoire.

**Discussion**

It is currently thought that apoptosis is the primary mechanism by which negative selection is achieved. The current paradigm states that a high-affinity TCR signal received by DP or semimature SP thymocytes induces the expression of proapoptotic molecules, namely Bim and Nur77, resulting in cell death. In the present study, we demonstrated that while Bim appears to be essential for DP thymocyte apoptosis, the loss of Bim does not impair negative selection. These data support the original findings of the Strasser group where they described Bim as an essential protein for thymocyte apoptosis (20) and the Marrack group’s data that demonstrated Bim was not required for negative selection (28). Based on our findings, it can be concluded that in addition to Bim-mediated apoptosis, other mechanisms exist for enforcing negative selection. We think our data also suggest that the current dogma surrounding negative selection be revisited.

If negative selection is still occurring in the absence of Bim-mediated apoptosis, then what mechanisms are responsible for enforcing negative selection independent of Bim? Two obvious candidate proteins are the death receptor Fas and the orphan nuclear
we find that most of the T3.70 while Nur77 expression is still elevated in HYcd4 Bim 
required for Nur77-mediated apoptosis, since the level of apoptosis 
Nur77 and Bim to operate in parallel pathways leading to clonal 
activation of caspase-3 is downstream of both the extrinsic pathway 
receptor Nur77. While the Fas pathway was recently shown to 
cooperate with Bim in preventing autoimmunity (37–39), evidence 
supporting a role for Fas (or any other death receptor) in negative 
selection to ubiquitous self-Ags is lacking (40). Furthermore, ac-
tivation of caspase-3 is downstream of both the extrinsic pathway 
(Fas) and intrinsic pathway (Bim) and we do not detect an increase 
in active caspase-3 in HY^cd4 Bim^−/− male over female mice as 
would be expected if the Fas pathway was compensating for the 
loss of Bim. Based on previous experiments, one might predict 
Nur77 and Bim to operate in parallel pathways leading to clonal 
deletion. However, all of these experiments were performed in 
Bim-expressing thymocytes. Our data suggests that Bim is re-
quired for Nur77-mediated apoptosis, since the level of apoptosis 
equivalent in HY^cd4 Bim^−/− female and male DP thymocytes 
while Nur77 expression is still elevated in HY^cd4 Bim^−/− male DP 
over female DP (data not shown). Therefore, without Bim expres-
ion, high-affinity Ag-mediated induction of Nur77 is unable to 
induce apoptosis over background levels, suggesting an apoptosis-
independent mechanism of negative selection in Bim-deficient 
mice. Data in the literature support the idea that Bim and Nur77 
cooperate to induce apoptosis during negative selection. Inhibition 
of Nur77 activity is able to inhibit apoptosis in F5 transgenic mice 
ijected with NP peptide even though Bim should still be ex-
pressed in these mice (23). Furthermore, thymocyte apoptosis is 
inhibited in Bim-deficient mice even though Nur77 expression 
should be unaffected (20). It is unclear at this point at what level 
Bim is required for Nur77-mediated apoptosis. Examining apo-
ptosis induction in Bim-deficient Nur77 transgenic thymocytes and 
negative selection in the absence of both Bim and Nur77 activity 
will be critical for determining the relationship between Bim and 
Nur77 in negative selection.

In addition to apoptosis, receptor editing and anergy have been 
described as mechanisms employed to induce negative selection 
(Fig. 5) (2). Since receptor editing does not appear to be a mech-
anism utilized to induce negative selection in male Ag-reactive 
thymocytes (41), we do not favor this as the alternative mech-
anism to apoptosis. Interestingly, in HY^cd4 Bim^−/− male mice, 
we find that most of the T3.70^+ DP thymocytes express PD-1 
and given the role of PD-1 expression in peripheral T cell an-
ergy (42), it is tempting to speculate that anergy induction is the 
apoptosis-independent mechanism of negative selection em-
ploved in this situation. Additional experiments examining the 
role of PD-1 in thymic negative selection in the presence and 
absence of Bim will be required to determine the consequence 
of PD-1 expression on DP thymocytes following high-affinity 
stimulation.

Two other scenarios that may explain negative selection in the 
absence of apoptosis both involve a block in positive selection of 
DP thymocytes (Fig. 5). In the first scenario, only a low-affinity 
signal would be able to induce a factor required for positive 
selection. We have ruled out Id3 and Runx3 as possible candidate 
proteins in this scenario because the expression level of Id3 and 
Runx3 is similar in HY^cd4 Bim^−/− female and male DP thymo-
cytes (supplemental Fig. 3). Since our molecular understanding of 
positive selection is incomplete, there could be other unidentified 
positive selection factors only induced by a low-affinity signal, 
leaving this model as a viable possibility. In the second scenario, 
a high-affinity signal could induce a protein that interferes with the 
positive selection process. We favor the second scenario over the 
first since negative selection is dominant over positive selection in 
the HY^cd4 model, and T3.70^+ DP thymocytes theoretically should 
encounter both the positively selecting and the negatively selecting 
ligand in male mice.

One of the other questions raised by our data relates to the re-
lative contributions of the apoptotic and nonapoptotic mechanisms 
of negative selection in wild-type mice. In HY^cd4 male mice, we 
find that Bim-dependent apoptosis is able to reduce the number of 
DP thymocytes by approximately two-thirds. This leaves approxi-
imately one-third of the DP thymocytes possibly subject to non-
apoptotic mechanisms of negative selection. Determining the rel-
ative importance of each “arm” of negative selection will require an 
understanding of the nonapoptotic arm and ways to inhibit this 
mechanism.

With respect to positive selection, we found that there was an 
increase in the percentage and absolute number of T3.70^+ CD8SP 
thymocytes in HY^cd4 Bim^−/− female mice compared with HY^cd4 
female mice. This could occur as a result of enhanced survival of 
positively selected Bim^−/− CD8SP thymocytes; however, we did 
not observe any differences in apoptosis in T3.70^+ CD8SP from 
HY^cd4 or HY^cd4 Bim^−/− female mice. Instead, a reduction in the 
number of T3.70^+ DP thymocytes, but an increase in the number of 
T3.70^+ CD8SP thymocytes, in HY^cd4 Bim^−/− female mice 
suggests that the efficiency of positive selection is enhanced in 
the absence of Bim. We hypothesize that in the absence of Bim, DP 
thymocytes could survive longer, thereby increasing the probabil-
ity of encountering their positively selecting ligand. Since we 
know that CD69 expression is gradually increased over time dur-
ing positive selection (31), increased levels of CD69 on T3.70^+ 
DP thymocytes from HY^cd4 Bim^−/− female mice supports this 
hyptosis.

Collectively, our data provide new insight into the molecular 
mechanism of thymocyte negative selection. They indicate that 
while apoptosis is a robust mechanism to induce negative selection 
and the proapoptotic protein Bim plays a crucial role in clonal 
deletion, other potent nonapoptotic mechanisms can also be em-
ploved. This study was limited to negative selection in response to a 
ubiquitous, MHC class I-presented peptide. However, we know 
that negative selection to TRAs is critical for self-tolerance. Since 
negative selection to TRAs requires positive selection and traffick-
ing to the medulla (43), it is possible that Bim deficiency can 
rescue TRA-specific thymocytes from negative selection. Based on 
our work, we hypothesize that the autoimmune disorders observed 
in Bim-deficient mice could result from incomplete negative se-
lection of TRA-specific T cells rather than ubiquitous self-Ag-
specific T cells. Understanding how negative selection to ubiqui-
tous self-Ags as well as TRAs is governed will provide insight into 
how central tolerance is enforced and autoimmunity is prevented.
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Disclosures

The authors have no financial conflicts of interest.

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