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Opening a Window on Thymic Positive Selection: Developmental Changes in the Influence of Cosignaling by Integrins and CD28 on Selection Events Induced by TCR Engagement

Bruno Lucas* and Ronald N. Germain†

How TCR and non-TCR signals are integrated by thymocytes to generate a decision to undergo either positive or negative selection remains incompletely understood. Recent evidence suggests that TCR signal transduction changes its quality during thymocyte maturation, but whether the contributions of various cosignaling or costimulatory pathways to thymocyte selection also are modified during development is unclear. Questions also remain about the possible selective roles of specific costimulatory pathways in induction of differentiation vs death among thymocytes at any given stage of maturity. To address these issues, a quantitative in vitro analysis of initiation of CD4+CD8+ thymocyte differentiation as measured by CD69 up-regulation/coreceptor down-modulation was conducted in parallel with an analysis of induction of death. Using transfected cells varying in their surface display of ICAM-1 or B7.1 along with antibody blocking experiments, we demonstrate here that ICAM-1 provides a selective boost to signaling for differentiation without substantially affecting induction of death among CD4+CD8+ cells, a property that is lost as thymocytes mature further. In contrast, B7 engagement enhances both cell activation and death in parallel. Based on these data, we propose that the high level of ICAM-1 on cortical epithelial cells plays a special role in opening a window between TCR signaling for differentiation vs death, permitting efficient initiation of positive selection on epithelial ligands. In contrast, late CD82-dependent cosignaling on hemopoietic cells in the medulla would help enforce negative selection by augmenting the effects of TCR engagement by low levels of high affinity ligands. The Journal of Immunology, 2000, 165: 1889–1895.

Following successful rearrangement of the gene segments encoding the component α- and β-chains, precursor T lymphocytes each express a unique receptor (TCR) that regulates mature Ag-induced effector function. Because of the unpredictable diversity in their binding specificity, the differentiation and survival of precursor T cells include a test of the recognition properties of these somatically generated receptors against the internal antigenic environment (1–4). A key aspect of this process involves the death of developing thymocytes with TCR whose interactions with self Ags (short peptides bound to MHC molecules) might result in full activation of the mature T cell bearing that TCR (5, 6). This creates an apparent paradox: how the same set of self peptide-associated MHC molecules can deliver both death-inducing signals that yield a repertoire depleted of potentially harmful self-reactive T cells and positive signals ensuring survival but not overt activation of other T cells also recognizing these ligands (7).

One hypothesis proposed to explain how this distinction between cell fates arises comes from the demonstration that full activation of mature T cells requires cosignals delivered by a variety of receptor/counter-receptor interactions, especially those involving CD28 on the T cell and CD80/86 (B7.1, B7.2) on the APC. This model postulates that thymocytes simultaneously signaled through the TCR and by this costimulatory pathway are induced to undergo apoptosis and that epithelial thymic stromal cells differ from the hemopoietic cells typically involved in activation of mature T cells in lacking expression of these key costimulatory molecules (8–15). Thus, only thymocytes with TCR recognizing peptide:MHC complexes uniquely expressed on the costimulatory-deficient stromal cells (16) would survive the selection process, preventing maturation of cells recognizing self ligands on activated, costimulatory hemopoietic presenting cells in the peripheral immune system.

The experiments examining this model have involved single ligand concentrations, one stage of T cell maturation, or failed to compare thymocyte differentiation vs death in the same experiments (8, 11). Differences in these parameters among experimental models may account for the discrepant results and also obscure the real role of these interactions in thymocyte development. In addition, this proposal implies that no peptides shared between epithelial cells and hemopoietic cells can be involved in positive selection, a conclusion for which no support currently exists and that is at odds with recent data from this laboratory on the ability of dendritic cells to support late stages of positive selection in reaggregate organ cultures (17).2

To re-examine the role of cosignaling during thymocyte development in a quantitative manner, we have used an in vitro model system in which thymocytes and mature T cells can be exposed to self-peptide:MHC molecule ligands or to graded concentrations of foreign Ag:MHC molecule ligands, each in the presence or the

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absence of various cosignaling molecules on MHC class II-expressing transfected L cells. While it has its own limitations, this in vitro approach allows direct analysis of the response of pure populations of thymocytes at discrete developmental stages to a homogenous accessory cell population. In vivo or organ culture models cannot provide such quantitative information, enforce such a limitation of the cell types involved in signal delivery or receipt, or restrict the surface molecule display as completely. The latter is an important point, as many studies using mice, whole organs, or isolated stromal cells are confounded by the operation of redundant pathways that obscure the capacity of the deleted or blocked molecule to perform a given function, while not being absolutely required for that function.

Our results using this model system suggest a key role for ICAM-1 in allowing immature double-positive (DP)3 thymocytes interacting with cortical epithelial cells to achieve a level of intracellular signaling suitable for initiation of positive selection without activation of a death program. This window of opportunity provided by ICAM-1 is lost as the thymocytes mature. B7:CD28 interactions, in contrast, work especially efficiently with maturing thymocytes to augment signaling that can lead to cell death, helping to enforce negative selection upon TCR engagement of even a low density of high affinity/high quality ligands on hemopoietic cells in the thymic medulla. Together with prior evidence for maturation-related changes in the nature of signal generation by the TCR complex itself (18–20), the present findings of differential, developmentally controlled modification of the outcome of this TCR signaling by integrin and CD28 cosignaling pathways provide additional insight into how the decision to mature or die is made by thymocytes.

Materials and Methods

Mice

The H-2k mice were generated by breeding and were maintained in a National Institute of Allergy and Infectious Diseases Research Animal Facility. They were provided by Dr. B. J. Fowlkes.

Cells

DAP.3 (23) is an MHC class II-negative fibroblast cell line derived from H-2k mice. DCEKhi7 and P13.9 L cells are daughter cell lines of DAP.3 stably transfected with cDNA expression constructs encoding I-Ed or I-Ek, B7.1, and ICAM-1, respectively (24) (Table I). FT7.1C6 and P12 are daughter cell lines of DAP.3 transfected with cDNA expression constructs encoding I-Ab or I-Ak, B7.1, and ICAM-1, respectively (24) (Table I). FT7.1C6 and P12 express IAk.

Monoclonal Abs

Abs were purchased from PharMingen (San Diego, CA). For inhibition of cell surface molecule function during cell culture, purified anti-B7.1 (16-4; 251), B7.1, and ICAM-1, respectively (24) (Table I). FT7.1C6 and P12 are daughter cell lines of DAP.3 transfected with cDNA expression constructs encoding I-Ak or I-Ed, B7.1, and ICAM-1 respectively. EKAM1.5 is a cell line derived from DCEKhi7 L cells by transfection with pβA-ICAM (25), a cDNA construct encoding the costimulatory molecule ICAM.1. EKAM2.5 L cells were obtained from the same transfection but do not express ICAM.1 and therefore are phenotypically identical with their parental cell line DCEKhi7 (18).

In vitro culture assay

Thymi or pooled mesenteric lymph node and spleen cells were cultured on a nylon cell strainer (Falcon, Franklin Lakes, NJ) in complete RPMI 1640 medium with 10% heat-inactivated FCS. Thymocytes (1 × 106) or 0.5 × 106 peripheral T cells and 1 × 106 or 0.5 × 106 fibroblasts of the indicated cell line, respectively, were centrifuged and then incubated in 6-ml polypropylene, round-bottom tubes with caps (Falcon) at 37°C in an atmosphere containing 5% CO2. Where indicated, peptides were added at 5 × 10−3 μM to 50 μM. The following peptides were used for these experiments. PCC88–104 is KAERADLIYLKQATAK; P99 and Q99 peptides are synthetic peptides in which the lysine in position 99 is changed to proline or glutamine, respectively. All peptides were synthesized and purified by the National Institute of Allergy and Infectious Diseases Peptide Synthesis Facility, National Institutes of Health (Bethesda, MD). For Ab-mediated inhibition studies, fibroblasts were preincubated for 4 h at 4°C with the indicated Ab. After 18–21 h of culture, thymocyte cell death, IL-2 production by peripheral T cells, and CD69 expression on thymic DP cells or on thymic and peripheral CD4+ cells were determined.

In vitro cell death assay

After overnight culture, thymocytes were harvested, and cell death was measured as previously described (27–29). These previous studies have shown that this method accurately assesses cell loss resulting from T cell apoptosis. Briefly, all recovered cells were stained for CD4, CD8, and CD69; washed; then resuspended in identical volumes. Data were acquired at 60 s at a constant flow rate using a FACScan equipped with CellQuest software (Becton Dickinson, Mountain View, CA). Total viable cells were enumerated using a restricted gate defined by forward/side scatter parameters and verified by the absence of propidium iodide incorporation. The numbers of viable DP thymocytes were quantified by setting a gate on the basis of CD4/CD8 fluorescence intensity (as shown in Fig. 1A). The results were converted to percentage of maximal cell death as explained below. No increase in cell death was observed among peripheral CD4+ cells during culture at any peptide concentration.

Calculations

Because reactivity against some fibroblast cell lines was observed without adding any source of peptide, the various measured parameters were expressed as percentages of maximal response after subtraction of the response obtained with the same APCs not exposed to peptide: this protocol allowed us to measure specific reactivities against the added peptide (PCC88–104) by subtracting the “natural” reactivity against the APCs. Because a significant percentage of DP thymocytes died during culture due to peptide exposure and because CD69 expression on DP thymocytes was routinely measured by flow cytometry only on viable cells, we have corrected for this by assuming that all DP cells induced to die by peptide exposure were also expressed CD69. Specifically, the percentages of CD69+ DP thymocytes obtained by cytometry analysis were modified as follows: % of CD69+ induced on DP cells = (% of viable DP cells × % of CD69 measured by cytometry) + (% of dead DP cells).

In Table I, cell surface phenotypes of L cells used as APCs are listed.

Table I. Cell surface phenotypes of L cells used as APC

<table>
<thead>
<tr>
<th></th>
<th>IEβ</th>
<th>ICAM.1</th>
<th>B7.1</th>
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<tbody>
<tr>
<td>DAP.3</td>
<td>ND</td>
<td>ND</td>
<td>Low</td>
</tr>
<tr>
<td>FT7.1C6b</td>
<td>ND</td>
<td>ND</td>
<td>Low</td>
</tr>
<tr>
<td>P12b</td>
<td>ND</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>DCEK</td>
<td>High</td>
<td>ND</td>
<td>Low</td>
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<tr>
<td>EKAM 2.5</td>
<td>High</td>
<td>ND</td>
<td>Low</td>
</tr>
<tr>
<td>EKAM 1.5</td>
<td>High</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>P12</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>P13.9</td>
<td>ND</td>
<td>ND</td>
<td>Low</td>
</tr>
</tbody>
</table>

*ND, Not detectable.

b FT7.1C6 and P12 express IAk.
Results

Developmental stage-specific contributions of ICAM-1- and B7.1-dependent cosignaling to self-ligand-induced thymocyte differentiation

To achieve a quantitative understanding at discrete developmental stages of the contributions of Ag-specific and unspecific signaling to T cell differentiation or death, we employed an in vitro cell-cell interaction system in which cell death, the expression of relevant marker genes for differentiation, or other outcomes of TCR-dependent signaling can be measured and in which the TCR ligands and cosignaling molecules available to the thymocyte can be carefully controlled. CD69 expression, which occurs on all thymocytes undergoing Ag-induced positive as well as negative selection (26, 30–32), was analyzed as an indicator of TCR signaling for differentiation, in parallel with induction of cell death. The thymocytes were from RAG-2<sup>2<sup>−/−</sup></sup> AND TCR transgenic H-2<sup>b</sup>b mice (21, 33), in which selection of mature CD<sup>4+</sup>T cells bearing this TCR occurs, but which do not express the I-E<sup>k</sup> MHC class II molecule able to present the cognate foreign Ag ligand for this TCR (pigeon cytochrome c, residues 88–104). All T-lineage cells from these mice express a single defined TCR.

Neither fibroblasts (DAP.3) lacking the I-E<sup>k</sup> MHC class II molecule that is involved in foreign Ag recognition by this TCR nor I-E<sup>k</sup><sup>+</sup> but ICAM-1<sup>−</sup>-, B7.1<sup>−/−</sup> fibroblasts (EKAM2.5) induced CD69 expression upon overnight coculture with AND thymocytes (Fig. 1A). Strikingly, without any exogenous source of peptide, a significant number of thymic DP and CD4<sup>+</sup> mature thymocytes expressed CD69 after overnight culture with I-E<sup>k</sup><sup>+</sup>, ICAM-1<sup>−</sup> (EKAM1.5) or I-E<sup>k</sup><sup>+</sup>, ICAM-1<sup>−</sup>, B7.1<sup>+</sup> (P13.9) fibroblasts. These responses were not due solely to interactions with ICAM-1 and/or B7.1, because ICAM-1<sup>−</sup>, B7.1<sup>+</sup>, I-A<sup>b</sup><sup>+</sup> fibroblasts (P12) did not induce any CD69 up-regulation (data not shown). These results suggest that the I-E<sup>k</sup><sup>+</sup> fibroblasts express a ligand capable of engaging the AND transgenic TCR, but that this TCR-ligand pair only produces a signal able to induce a visible functional response in the presence of ICAM-1 and/or B7.1. This ability of I-E<sup>k</sup><sup>+</sup>, ICAM-1<sup>−</sup> fibroblasts to induce CD69 up-regulation is consistent with the capacity of I-E<sup>k</sup> bound to self-peptides to negatively select many AND TCR transgenic thymocytes (34). Interestingly, the roles of ICAM-1 and B7.1 in this CD69 response appear to be different at distinct stages of T cell development. DP cells respond to coculture with ICAM-1-expressing I-E<sup>k</sup><sup>+</sup> fibroblasts (EKAM1.5), and this response is not markedly affected by the coexpression of B7.1 (Fig. 1A). In contrast, B7.1 overexpression considerably increases thymic (as well as peripheral) SP CD4<sup>+</sup>T cell responses, which are marginal using I-E<sup>k</sup><sup>+</sup> cells with only ICAM-1 but not B7.1 expressed at high levels. These results help explain why various analyses that do not discriminate among the effects of blocking these cosignaling pathways at different stages on T cell development yield different interpretations.

Differing roles of ICAM-1 and B7.1 costimulation in DP vs mature thymocyte responses to foreign agonist ligand

ICAM-1 expression is high on both cortical epithelial cells and dendritic cells (35), whereas CD80/86 expression is largely confined to the latter in the thymus (36). Because these two cell populations have been proposed to function almost exclusively in positive and negative selection, respectively, it was clearly of interest...
to examine more quantitatively ICAM-1 and B7.1 contributions to DP cell activation and death. This was done using different fibroblast cell lines (Table I) in the presence of different concentrations of PCC88-104, the full agonist peptide recognized by the AND transgenic TCR. Some investigators have claimed that low concentrations of such agonists can be effective in positive selection (37–39), whereas others could not obtain such agonist-driven maturation, but observed only induction of apoptosis when using thymic organ cultures (40–42). CD4 and CD8 coreceptor down-regulation was examined (Fig. 1B). The results obtained by measuring coreceptor loss were identical with those obtained studying the induction of CD69 expression, in agreement with earlier findings (26, 43–46). Thus, as a measure of TCR signaling potentially adequate for induction of differentiation, we examined CD69 levels, whose up-regulation is known to correlate with initiation of thymocyte selection (26, 30–32), and compared these to the extent of induced cell death under various stimulation conditions.

In the absence of ICAM-1 expression, all tested concentrations of ligand induced CD69 expression on and death of the same fraction of DP thymocytes, that is, no thymocyte was induced to differentiate without also being signaled to die (Fig. 2A). These data are superficially consistent with results indicating that potent agonists cannot induce positive selection without overriding induction of apoptotic cell death (42). Surprisingly, however, in the presence of ICAM-1 the induction of a given amount of cell death required the same or only slightly lower Ag concentrations, whereas the induction of a given number of high CD69-expressing cells occurred at significantly lower Ag concentrations, with a 5- to 10-fold concentration window opening up within which many cells could be induced to high CD69 expression without accompanying cell death (Fig. 2B). For example, at a concentration of 0.05 μM PCC88-104, 20.5, 27.6, and 24.3% of DP thymocytes were induced to die using EKAM 2.5-, EKAM 1.5-, and P13.9-presenting cells, respectively. However, 22.3, 75.1, and 74.2% of the remaining viable cells were induced to express CD69 under the same conditions. Because reactivity to some fibroblast lines was observed without adding any PCC peptide (Fig. 1A), the results shown in the figure have taken account of this and also of variations in the absolute levels of induced differentiation and death among different experiments by expressing the data as a percentage of the maximal response after subtraction of the values obtained with the same APCs not exposed to peptide (Fig. 2A). Results similar to those seen using APC with varying expression of ICAM-1 were obtained using Ab inhibition to interfere with ICAM-1 binding to its integrin receptor(s) (Fig. 3). Thus, ICAM-1 does not appear to substantially protect thymocytes against death induced by TCR signaling per se as previously concluded (11), but instead, it seems to selectively synergize with TCR signaling for thymocyte activation, permitting ligands in a specific concentration range to induce biologic responses associated with positive selection without apoptotic loss (37, 38).

The effects of B7.1 were very different from those of ICAM-1. This cosignaling ligand has been claimed to specifically facilitate thymocyte cell death upon TCR ligation (8, 9, 11, 12, 14, 15). However, when examined in a quantitative fashion in comparison to cell activation assessed by CD69 expression, it is clear that B7.1 acts to increase thymocyte sensitivity to agonist signaling by the TCR, without a selective change in either death or differentiation (CD69) responses (Figs. 2A and 3). Using assays for death only or analysis at only one ligand concentration, however, this effect would appear as a selective increase in cell loss, as these previous studies have reported.

It is also important to note that inhibition of B7.1 expression did not totally abrogate DP thymocyte CD69 responses or induction of death, whereas IL-2 production by peripheral CD4SP cells was reduced to nearly undetectable levels. These results suggest that B7/CD28 interaction is not required to induce immature thymocyte cell death (and therefore negative selection), and also that the signaling required for death in the thymus is not equivalent to the signaling cascade allowing IL-2 production at the peripheral level.

![FIGURE 2](http://www.jimmunol.org/)

**FIGURE 2.** Contributions of ICAM-1 and B7.1 to stimulation of CD69 and death responses of thymocytes or CD69 and IL-2 responses of mature peripheral CD4+ T cells. Thymocytes or peripheral lymph node CD4+ T cells from H-2b AND TCR transgenic RAG-2-/- mice were incubated overnight with the indicated L cell APCs with or without the indicated concentrations of antigenic peptide. CD69 expression, cell death, and/or IL-2 production were measured, and the responses are presented as a percentage of the maximal response calculated as described in Materials and Methods. A, Data derived from the use of transfectants differing in B7.1 and/or ICAM-1 expression as APCs. B, Quantitation of thymic positive selection window.
ICAM-1 and B7.1 show the same functionalities using variant ligands with partial agonist properties for mature AND T cells

The preceding data were obtained using a potent agonist ligand for the TCR. Although recent data (18, 47) indicate that variant peptides that create partial agonist or antagonist ligands for mature T cells act as weak agonists for DP, it was of interest to determine whether the roles of ICAM-1 and B7.1 revealed above were also true when these less potent ligands were employed as stimuli. Fig. 4 shows that although using these weak ligands results in a striking shift in the overall dose response to a requirement for higher peptide concentrations for equivalent responses, the effects of ICAM-1 and B7.1 on the relationship between CD69 induction and cell death is similar for the P99 and Q99 variant peptides and the full agonist PCC peptide. Thus, ligands that may more closely simulate those involved in thymic positive selection do not reveal a different role for these counter-receptors from that seen using a strong ligand expected to mediate negative selection.

Discussion

Here we have used an in vitro model to perform a quantitative analysis of the differentiation and/or death responses of individual cells at distinct stages of T cell maturation in the presence of changing cosignaling environments. Our data do not support proposals that the CD28 pathway is linked uniquely to thymocyte death (8, 9, 11, 12, 14, 15); rather, with strong agonist ligands this receptor/counter-receptor system appears to have the ability to synergize with multiple intracellular pathways, such as that involving Vav (48–50), and/or to augment TCR engagement of ligand, shifting the dose-response for many functional outcomes of TCR signaling. More interestingly, interaction with ICAM-1, which is very highly expressed by cortical epithelial cells contacting precursor CD4⁺CD8⁺ thymocytes (35), was shown to have the capacity to selectively amplify TCR signaling for differentiation in comparison to signaling for death. This opens a ligand concentration window within which the DP thymocyte can be signaled to differentiate without induction of apoptosis, even by strong agonist ligands, forming an initial pool of positively selected thymocytes capable of further maturation. These observations applied similarly to strong agonists and to weaker ligands with little or no capacity to trigger mature T cells with the AND TCR. The latter might be considered an approximation of the type of ligand involved in normal thymic selection, indicating that the effects of ICAM-1 and B7 that we observed are not peculiar to ligands that would only delete developing thymocytes.

Although many of these conclusions are based on analysis of CD69, CD4, and CD8 expression using an in vitro model and transfected cells for presentation, we have been able to show recently that the CD69⁺CD4coldCD8low thymocytes, generated either under the conditions described here with fibroblasts (unpublished observations) or using CD45-negative, nontransformed thymic stromal cells or dendritic cells (17) (see Footnote 2), show enhanced differentiation into functional CD4⁺T cells in reaggregate thymic organ culture. Such findings clearly indicate that thymocytes induced in vitro under our conditions to acquire the CD69⁺CD4coldCD8low phenotype have initiated intracellular changes involved in positive selection, supporting the physiological relevance of the above conclusions concerning the roles of cosignals and TCR ligands in the selection process.

It is more difficult to provide such confirmatory data using either animals or organ cultures for our in vitro analysis of cell death, and questions have been raised about the validity of such in vitro models of negative selection. Thymic selection is not grossly disturbed in mice with targeted deletions in ICAM-1 (51, 52) (our unpublished observations). Only specific exons were targeted in these animals, however, and the resulting mice are thus not null (53). In addition, these animals still express alternative LFA-1 ligands such as ICAM-2. Thymic development has not been carefully studied in LFA-1 (54) or in CD43 (55) targeted mice. Functional redundancy

![Graph](http://www.jimmunol.org/)

FIGURE 3. Differing roles of ICAM-1 and B7.1 costimulation in DP vs mature thymocyte responses to foreign agonist ligand. Thymocytes or peripheral lymph node CD4⁺ T cells from H-2b AND TCR transgenic RAG-2⁻/⁻ mice were incubated overnight with I-Ek, B7.1, ICAM-1-expressing P13.9 cells as presenting cells with or without inhibiting B7.1, ICAM-1, or both using mAbs.
nisms must exist that operate later in development to insure dele-
tivation of potentially autoreactive clones. Negative selection could
result from dramatic changes in the thymic environment (a change
possibly involving thymocyte migration from the thymic cortex to
the thymic medulla) and/or changes in the perception of self li-
gands by thymocytes. We and others have recently shown that
the signaling properties of TCR change during thymocyte matura-
tion (18–20). Weak agonists for DP thymocytes lose potency during
development, whereas sensitivity to strong agonists is maintained.
These changes together with the lower expression of ICAM.1 on
bone-marrow derived medullary stromal cells compared with ep-
thelial cortical cells (35) are precisely those required for deletion
of potentially autoreactive clones seeing potent self-ligands and for
generation of a mature T cell pool possessing a wide margin of
safety against activation by self ligands. This conclusion is con-
istent with and amplifies the findings of Page on the role of ac-
cessorry molecules in thymocyte selection in fetal thymic organ
culture (60), while agreeing with quantitative data on late negative
selection by hemopoietic cells in the thymus (61). It also suggests
that overt autoreactivity among peripheral T cells might be ob-
erved in animals with limited B7 expression on thymic presenting
cells but intact cosignaling by presenting cells in lymphoid organs.

Some previous attempts to explain how mature T cells can re-
cieve an essential differentiation-promoting signal through the
TCR upon self recognition without this leading to effector func-
tion, yet remain highly responsive to full activation upon recog-
nition of a slight variant in ligand structure (foreign peptide:MHC
molecule complexes), have treated the developmental process
leading to this state in a largely one-dimensional way. Signaling by
self-ligand recognition was presumed to occur at a specific stage
of thymocyte development and to have a particular quantity or quality
that defined its ability to promote differentiation vs death. Our
results indicate that a one-time, one-place model for decision-mak-
ing in the thymus is untenable, and that therefore a more multi-
dimensional view is required to explain ligand-dependent thymic
development and the properties of the mature T cell repertoire.

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We thank Dr. B. J. Fowlkes for mice and for discussion and Dr. Koji
Yasutomo for sharing unpublished data.

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FIGURE 4. ICAM-1 and B7.1 show the same functionalities using vari-
ant ligands with partial agonist properties for mature AND T cells. Thy-
mocytes from H-2b AND TCR transgenic RAG-2-/- mice were incubated
overnight with the indicated L cell APCs with the indicated peptide. CD69
expression and cell death were measured, and the responses are presented
as a percentage of the maximal response calculated as described in Ma-
terials and Methods.

in these pathways can complicate attempts to see the contribution
of a single pathway when others are still active. Thus, the in vivo
studies do not contradict our observations and might be considered
less informative about what a molecule is capable of as opposed to
what it is absolutely necessary for. Furthermore, our results are in
agreement with the recent data reported by Dautigny et al. (56).
Indeed, they found that although endogenous superantigen-driven
thymic negative selection could occur at different steps during
DP/SP cell transition, this event was never observed among
CD69lowCD8low thymocytes, i.e., within the first subset to
be generated upon TCR-mediated activation of immature DP cells.
Numerous other reports have also reported that thymocytes can
initiate selection and then be induced to undergo cell death at a
later stage of development (57–59).

All these data suggest that strong agonist ligands can initiate
thymic positive selection, and we have recently been able to di-
rectly demonstrate the generation of Ag-responsive mature T cells
using TCR transgenic thymocytes and full agonist ligand to start
the differentiation process (see Footnote 2). Thus, further mecha-
nisms must exist that operate later in development to insure dele-

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Negative selection of CD4+CD8+ thymocytes by T cell receptor-induced apo-
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