Characterization of Transcriptional Regulation During Negative Selection In Vivo
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Characterization of Transcriptional Regulation During Negative Selection In Vivo

Deborah DeRyckere, Derrick L. Mann, and James DeGregori

Negative selection is the process whereby immature thymocytes expressing TCRs with high affinity for self-peptide:MHC complexes are induced to undergo apoptosis. The transcriptional events that occur as a result of TCR signaling during negative selection are not well-characterized. Using oligonucleotide arrays, we have identified 33 genes that exhibit changes in RNA levels in CD4+CD8+ thymocytes during negative selection in vivo. Of 18 genes that have been further characterized, 13 are regulated in response to stimulation with Ag or anti-CD3 and anti-CD28 Abs ex vivo, indicating that these genes are regulated independently of activation of the peripheral immune system. These data also support the idea that anti-CD3/CD28-mediated thymocyte apoptosis is a valid model for negative selection in vivo. A detailed examination of the regulation of many of the identified genes in response to treatment with dexamethasone or gamma-radiation or in response to anti-CD3/anti-CD28 stimulation in the presence of pharmacological inhibitors of mitogen-activated protein kinase kinase kinase 1, p38 mitogen-activated protein kinase, phosphatidylinositol 3-kinase, calcineurin, and cyclin-dependent kinase 2 has facilitated the elucidation of a map of the transcriptional events that occur downstream of the TCR. These studies support a model whereby similar signal transduction pathways are activated by stimuli that induce positive and negative selection and are consistent with the idea that the balance between opposing proapoptotic and antiapoptotic pathways determines cell fate. The data presented in this study also suggest that calcineurin functions to amplify TCR signals by promoting sustained increases in the levels of specific transcripts. The Journal of Immunology, 2003, 171: 802–811.
Expression of dominant-negative forms of many of the components of this pathway, including p21<sup>Cip1</sup>, Raf, and MAP/ERK kinase (MEK)1, abrogated the generation of SP thymocytes in female HY transgenic mice. Pharmacological inhibition of mitogen-activated kinase kinase (MKK)1 also decreased positive selection of female HY transgenic thymocytes in fetal thymic organ cultures (FTOC). Similarly, expression of constitutively active forms of Raf and MEK1 enhanced positive selection in vivo and in FTOCs. In sharp contrast, negative selection of male HY transgenic DP thymocytes was not affected by pharmacological or genetic inhibition of the ERK MAPK pathway. The c-Jun N-terminal kinase (JNK) pathway also appears to play a differential role in positive and negative selection. Induction of DP thymocyte apoptosis in response to treatment with anti-CD3 Ab in vivo and in vitro was abrogated by deletion of JNK1 or JNK2 or by expression of a dominant-negative form of JNK1 (4–6). Similar results were observed using thymocytes isolated from mice in which the c-jun gene carried mutations that disrupt the N-terminal JNK phosphorylation sites (7). In addition, deletion of TCR transgenic thymocytes in response to antigenic peptide stimulation in vivo was decreased in mice expressing a dominant-negative form of JNK1 (4). Taken together, these observations indicate a role for JNK signaling in negative selection. In contrast, positive selection of TCR transgenic thymocytes by endogenous Ags in vivo was not affected by expression of dominant-negative JNK1 (4). Evidence suggests that p38 MAPK also functions specifically in negative selection. Pharmacological inhibition of p38 activity in FTOCs decreased the elimination of DP thymocytes induced by an endogenous Ag or by treatment with anti-CD3 Ab. Similarly, introduction of MKK6, an upstream activator of p38, into developing thymocytes was sufficient to reduce the number of DP cells, suggesting that activation of the p38 pathway is a critical step in the induction of apoptosis during negative selection (8). However, the interpretation of these experiments is complicated because p38 activity regulates development of thymocytes to the DP stage and, in particular, decreased p38 activity can promote the double-negative to DP transition (9). No defects in positive selection were observed in FTOCs in the presence of a pharmacological inhibitor of p38 MAPK (8) or in transgenic mice expressing a dominant-negative p38 or constitutively active MKK6 (10). Thus, the more distal TCR signaling pathways mediating positive and negative selection are distinct. The ERK MAPK pathway is specifically required for positive selection and the JNK and p38 MAPK pathways function in negative selection. These MAPK signaling pathways eventually impinge on the nucleus, where a variety of proteins that function in negative selection have been identified. The IFN-regulatory factor 1 transcription factor and ID3, an inhibitory helix-loop-helix protein, are required for both positive and negative selection of TCR transgenic thymocytes in vivo (11, 12). Similarly, transgenic mice expressing a constitutively active NF-κB inhibitor exhibited defects in the production of SP thymocytes and peripheral T cells, particularly in the CD8 compartment, indicating a role for NF-κB activity in positive selection (13–15) and thymocytes isolated from these mice exhibited defects in anti-CD3-mediated apoptosis, suggesting an additional role in negative selection (16). Thus, IRF1, ID3, and NF-κB appear to function in both positive and negative selection. In contrast, E2F1, nur77<sub>nor1</sub>, and Bim function specifically in negative selection. The E2F1 transcription factor is required for efficient induction of thymocyte apoptosis by Ag and by exogenous or retroviral superantigens (17–19). At the molecular level, E2F1 is required for increased expression of the p19ARF gene and increased levels of p53 protein during negative selection in vivo (18). nur77 and nor1 are members of the orphan steroid receptor family of ligand-dependent transcription factors. Expression of nur77 and nor1 is induced in thymocytes upon TCR stimulation and disruption of nur77/nor1 activity by expression of antisense RNA or a dominant-negative form of nur77 prevents TCR-mediated thymocyte apoptosis in vitro (20, 21) and in vivo (22, 23), indicating an important role for nur77/nor1 in negative selection. Surprisingly, despite its ascribed function as a transcription factor, neither the DNA binding nor transcriptional activation domains of nur77 are required for its apoptotic function. Instead, in response to apoptotic stimuli, nur77 is relocated from the nucleus to the outer mitochondrial membrane where it functions to mediate cytochrome c release and membrane depolarization (24). Another protein that functions specifically in negative selection and regulates mitochondrial membrane permeability is Bim, a proapoptotic Bcl2 family member. Disruption of the Bim gene blocked thymocyte apoptosis induced by exogenous and endogenous Ags and superantigens and by treatment with anti-CD3 in vivo and in vitro (25). Bim exerts its proapoptotic function by binding to and inhibiting Bcl2 (26), and presumably other Bcl2 family members. In addition, like nur77 and E2F1, Bim has no demonstrated role in positive selection. Thus, consistent with their known apoptotic functions, E2F1, nur77/nor1, and Bim function specifically to mediate TCR signals in DP thymocytes resulting in negative selection.

To identify additional components of the TCR signaling pathways mediating negative selection in DP thymocytes, we used DO11.10 TCR transgenic mice and Affymetrix oligonucleotide arrays to identify 33 transcripts that are differentially expressed following TCR stimulation by an antigenic peptide in vivo. Many of the identified genes play roles in the regulation or execution of apoptosis. Similar changes in gene expression were observed in DP thymocytes and peripheral T cells following TCR stimulation. In contrast, the pathways mediating glucocorticoid- and radiation-induced apoptosis are distinct from those activated by TCR signaling. Finally, the characterization of TCR-dependent transcriptional regulation in the presence of pharmacological inhibitors of known signal transduction pathways has facilitated the elucidation of a map of the transcriptional events that occur downstream of the TCR.

Materials and Methods

Mice

Rag2 mutant and DO transgenic mice were engineered by the F. Alt (Harvard Medical School, Boston, MA) and D. Loh (Nippon Roche Research Center, Kanagawa, Japan) Laboratories, respectively, and obtained from P. Marrack (National Jewish Hospital, Denver, CO). Mice were housed in the University of Colorado Health Sciences Center Animal Resource Center (Denver, CO). Rag2 mutant mice were maintained under sterile conditions. Rag2 and DO genotypes were determined by PCR analysis using genomic DNA isolated from tail biopsies. All experiments were performed using mice between 4 and 7 wk of age. OVA peptide (ISQAVHAAHAEINLUPA) was synthesized by Research Genetics (Huntsville, AL).

Flow cytometry and oligonucleotide microarrays

Single cell suspensions in PBS containing 5% FBS (FBS/PBS), 1 mM MgCl<sub>2</sub>, and 100 U/ml DNase I were obtained from thymuses and stained through nylon mesh. Cells (2 × 10<sup>6</sup>) were stained in 30 μl of CD4/CD8/CD69 Ab solution (1/100 PE-anti-CD4, 1/100 CyChrome-anti-CD8, 1/100 FITC-anti-CD69 (BD Pharmingen, San Diego, CA), and 1/100 goat serum (Life Technologies, Rockville, MD)), in FBS/PBS and analyzed by flow cytometry. The remaining cells (2.0 × 10<sup>5</sup>–2.5 × 10<sup>6</sup>) were stained in 750 μl of CD4/CD8 Ab solution (1/100 PE-anti-CD4, 1/100 allophycocyanin-anti-CD8 (BD Pharmingen), and 1/100 goat serum, in FBS/PBS) and subjected to single cell sorting. All reagents used during the isolation procedure were 4°C and cells were sorted into a receptacle on ice. Isolated DP thymocytes were resuspended in TRIzol reagent (Invitrogen, San Diego, CA), Biotin-labeled cRNA probes were prepared and hybridized to Affymetrix Murine 11KsubB oligonucleotide microarrays (Santa Clara, CA).
Abs were purified previously described (27). Real-time RT-PCR analysis was performed in the presence of 18S primer pair. Oligonucleotide probes were designed and optimization experiments were performed as per Affymetrix protocols. An adjustment to the scanner settings made Ab amplification unnecessary for the other two experiments. The data were analyzed using Affymetrix Microarray Suite 4.0 software and the default parameters specified by Affymetrix.

**Stimulation of thymocytes and peripheral lymphocytes in vitro**

Single cell suspensions obtained from thymuses or lymph nodes and spleen were strained through nylon mesh, washed in PBS, and resuspended at a final concentration of 6.25 × 10^6/ml or 8.2 × 10^6/ml, respectively, in DMEM containing 10% FBS (HyClone Laboratories, Logan, UT), 0.1 mM β-ME, and 1% penicillin-streptomycin (Life Technologies). Cells (2.5 × 10^6/dish) were cultured in 60-mm dishes that either contained an overnight culture of A20 P815 cells (5.5 × 10^4/ml) or had been coated overnight at 4°C with PBS or PBS containing 10 μg/ml anti-CD3 and 10 μg/ml anti-CD28.

OVA peptide was added to in vitro cultures of A20 cells and thymocytes at a final concentration of 5 μM where indicated. anti-CD3 and anti-CD28 Abs were purified from tissue culture supernatants of clones 2C11 and 37.51, respectively, using HITrap Protein G columns (Amersham, Arlington, TX) according to the manufacturer’s instructions. Where indicated, thymocytes were incubated with 50 μM PD908059 (Cell Signaling Technology, Beverly, MA), 20 μM SB203580 (Sigma-Aldrich, St. Louis, MO), 3 μM LY294002 (Sigma-Aldrich), 0.2 μM cyclosporin A (CSA; Sigma-Aldrich), or 50 μM roscovitine (Sigma-Aldrich) for 45 min before plating. During culture, Cells were harvested 2 or 8 h after plating and resuspended in TRIZol reagent (Invitrogen). Where indicated, DP thymocytes or CD4+ peripheral T cells isolated from DO transgenic mice were purified by MACS using CD8α (Ly-2) or CD4 (L3T4) MACS microbeads (Miltenyi Biotec, Auburn, CA), respectively, according to the manufacturer’s protocol. Stimulation of thymocyte apoptosis in the presence of OVA peptide or anti-CD3/anti-CD28 and inhibition of anti-CD3/anti-CD28-mediated apoptosis in the presence of roscovitine or SB203580 was confirmed by flow cytometric analysis of thymocyte cultures that had been harvested 24–28 h after plating and stained with propidium iodide (Nippon Roche Research Center). Stimulation of peripheral T cells in the presence of anti-CD3/anti-CD28 and 10 μM BrdU (Boehringer Mannheim, Mannheim, Germany) was determined by a flow cytometric analysis of lymphocyte cultures that had been harvested 48–52 h after plating and stained with FITC-anti-BrdU Ab (BD Pharmingen).

**Treatment of thymocytes with other apoptotic stimuli**

Single cell suspensions were obtained and cells were resuspended at a final concentration of 2.5 × 10^6/ml in culture medium as described above. Aliquots of cells were exposed to 1000 cGy gamma-radiation from a Cobalt 60 source or were incubated with dexamethasone (Sigma-Aldrich) at a final concentration of 0.2 μM. Control cultures were mock-irradiated or treated with ethanol. Thymocytes (2.5 × 10^6/dish) were cultured in 60-mm dishes for 2 or 8 h and were harvested, washed once with PBS, and resuspended in TRIZol. Stimulation of thymocyte apoptosis following gamma-irradiation or treatment with dexamethasone was confirmed by flow cytometric analysis of thymocyte cultures that had been harvested 5–7 h after treatment with the apoptotic stimulus and stained with propidium iodide.

**Real-time RT-PCR**

RNA was isolated from cells resuspended in TRIZol (Invitrogen) according to the manufacturer’s instructions. RNA samples were heated to 95°C for 3 min and quenched on ice to denature DNA/RNA hybrids. Samples were then treated with RNase-free DNase (Ambion, Austin, TX) and DNase was removed by purification over RNeasy columns (Qiagen). Samples were diluted to ~25 ng/μl in TE (10 mM Tris pH 8.0, 1 mM EDTA) containing 40 μg/ml polyadenyl acid (Sigma-Aldrich). This dilution was used as a template for real-time RT-PCR in the presence of all primer pairs except for 18S. A 1/50 serial dilution of each sample was used as a template for real-time RT-PCR in the presence of the 18S primer pair. Oligonucleotide primers were designed and optimization experiments were performed as previously described (27). Real-time RT-PCR analysis was performed in the presence of SYBR green dye or fluorogenic probes, also as previously described (27). Fluorogenic probe reactions were performed using TaqMan Universal PCR Master Mix, No AmpErase UNG (Applied Biosystems, Foster City, CA) and gene-specific TaqMan probes that hybridize to EF2 (5′-CTGATACCCCTCAGCAGTGTGGT) or 18S RNA (5′-TGGTGCCACGAGATGGCCTC) sequences were conjugated to 6FAM or VIC reporter dyes, respectively, and TAMRA quencher dye (Applied Biosystems). Fluorogenic probe reactions were performed in a final volume of 25 μl and contained 1× TaqMan Universal PCR Master Mix, 1 U Anti-RNase (Ambion), 6.25 U MMNLV-RT (Life Technologies), 0.1 μM probe, and optimized concentration of gene-specific primers. Reactions were performed in triplicate and mean cycle threshold values were used for all calculations. Cycle threshold values were normalized to the 18S RNA internal control. To confirm the absence of DNA contamination, control reactions were performed in duplicate for all samples in the absence of reverse transcriptase using primers that are known to amplify genomic sequences. Primer sequences and optimal primer concentrations are shown in supplementary Table I.

**Results and Discussion**

**Identification of genes that exhibit changes in RNA levels during negative selection in vivo**

To identify genes that function as components of the signal transduction pathways downstream of the TCR during negative selection of immature thymocytes, oligonucleotide arrays were used to identify transcripts that are differentially represented in DP thymocytes following TCR stimulation in vivo. DO11.10 TCR transgenic mice (28) were injected i.p. with 500 μg of antigenic OVA peptide or an equivalent volume of PBS. Under these conditions, a significant reduction in the number of cells in the thymus is observed between 10 and 12 h postinjection (p.i.) (data not shown).

Thymocytes were harvested and stained with fluorescent-linked anti-CD4, anti-CD8, and anti-CD69 Abs 2 and 8 h p.i. prior to any overt apoptosis. Flow cytometric analysis revealed that the CD69 activation marker was expressed at higher levels on the surface of thymocytes isolated from Ag-injected mice than on thymocytes from PBS-injected control mice, indicating effective antigenic stimulation of the TCR (data not shown). DP thymocytes were purified to >96% by single cell sorting and RNA was isolated. Equivalent amounts of RNA from two to three sex-matched littermates per experiment were combined for each sample. These RNAs were used as templates for the multistep synthesis of biotin-labeled cRNA, which was hybridized to Affymetrix Murine 11KsubB oligonucleotide arrays. The amount of cRNA hybridized to each oligonucleotide probe was then quantitated. Two independent experiments were performed as described. An additional experiment used RNA isolated from total thymocytes 8 h p.i. and these populations contained between 69.5 and 84.0% DP thymocytes.

The data were analyzed using Affymetrix Microarray Suite 4.0 software. Transcripts that were scored as increased, decreased, marginally increased, or marginally decreased and exhibited a ≥1.8-fold difference in RNA levels in thymocytes isolated from Ag-injected mice as compared with thymocytes from PBS-injected mice in two of two (2 h p.i.) or at least two of three (8 h p.i.) experiments were considered to be regulated. By these criteria, a total of 18 genes exhibited increased expression in thymocytes undergoing negative selection and 15 genes exhibited decreased expression (Table I). With one exception, decreased gene expression was not observed until 8 h poststimulation, either because the major mode of transcriptional regulation proximal to the TCR is activation, with repression being a secondary signaling event, or because these messages are relatively stable. Regulation of several of the identified genes in thymocytes following TCR stimulation, including early growth response (Egr2) (29), nur77 (20), and ID3 (30, 31), has been previously reported. In addition, previous work has demonstrated that both nur77 (20–23) and ID3 (12) play roles in negative selection, confirming that the approach taken in this

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**The on-line version of this article contains supplemental material.**
The complete data sets are available as a supplement to the on-line version of this article.

study is valid for the identification of TCR signaling components that function in this process. Functional bias testing revealed that genes that have been implicated in either the regulation or execution of apoptosis (NF-κB2, ID3, Stat1, myelination factor (MyD)118, χP2RX1, nur77, Ets2, CD98, glucocorticoid-induced leucine zipper (GILZ), A20, dynin, c-fos, and Bcl2) are significantly over-represented in the set of genes that were identified in this screen (39.4% as compared with the 3.2% incidence of apoptotic genes represented on the Affymetrix 11KSub B array; χ² test p < 0.05).

The criteria used to define genes that are differentially regulated in these experiments are relatively stringent and should therefore limit the incidence of false positives (i.e., unregulated genes that are scored as regulated). Nonetheless, these experiments used in vivo samples, rather than homogeneous cell populations stimulated to undergo negative selection in vitro, and the samples may therefore be expected to exhibit a relatively higher level of biological variation in gene expression patterns. For this reason, we used an independent method to confirm statistically significant differences in RNA levels of a subset of the genes that were scored as regulated based on the array experiments. Toward this end, total thymocytes were harvested from between 19 and 40 individual DO11.10 TCR transgenic B10.D2 mice 2 or 8 h after i.p. injection of 500 μg of OVA peptide or an equivalent volume of PBS. RNA was isolated and the levels of specific transcripts were determined by real-time RT-PCR. Using these methods, regulation of two control transcripts whose regulation upon activation of immature thymocytes has been previously reported (Bfl1 (32) and c-myc (33)), was confirmed (Table II). Transcript levels corresponding to 19 of the genes were scored as regulated based on the array experiments were determined by real-time RT-PCR. The regulation of 18 of these transcripts as indicated by the array experiments was confirmed (Table II). In five cases, regulation at both time points was indicated by RT-PCR whereas regulation at a single time point had been indicated by the array experiments, consistent with the increased sensitivity that a statistical approach allows. Regulation of a single transcript (IRG47) was not confirmed. Although IRG47 RNA levels were increased an average of 2.4-fold at 8 h p.i. as determined by RT-PCR, this induction was not statistically significant (p = 0.0656), most likely due to the relatively large variations in basal and activated levels of IRG47 RNA in samples isolated from individual mice (data not shown). These data indicate that the criteria used in the analysis of the oligonucleotide array experiments were sufficiently stringent for the identification of genes that are reproducibly regulated in a statistically significant manner following TCR stimulation in immature DP thymocytes in vivo.

The array experiments were performed using in vivo samples to maximize the identification of physiologically relevant changes in

### Table 1. Summary of genes exhibiting changes in RNA levels during negative selection in vivo

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Description/Function</th>
<th>Accession No.</th>
<th>Fold Change 2 h p.i.</th>
<th>Fold Change 8 h p.i.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MyD118/Gadd45β</td>
<td>Growth arrest and DNA damage-inducible ribosomal protein</td>
<td>NM_008655</td>
<td>6.2x</td>
<td>11.2x</td>
</tr>
<tr>
<td>Egr2/Krox20</td>
<td>Transcription factor</td>
<td>X06746</td>
<td>8.0x</td>
<td>4.7x</td>
</tr>
<tr>
<td>nur77</td>
<td>Orphan steroid hormone receptor</td>
<td>X16995</td>
<td>5.8x</td>
<td>6.0x</td>
</tr>
<tr>
<td>c-fos</td>
<td>Transcription factor and oncogene</td>
<td>V00727</td>
<td>5.8x</td>
<td>5.4x</td>
</tr>
<tr>
<td>NF-κB2</td>
<td>Transcription factor</td>
<td>NM_19408</td>
<td>4.3x</td>
<td>3.1x</td>
</tr>
<tr>
<td>GILZ</td>
<td>Glucocorticoid-inducible transcription factor</td>
<td>NM_100286</td>
<td>3.9x</td>
<td>NC</td>
</tr>
<tr>
<td>A20/TNF-αP3</td>
<td>TNF-α-inducible zinc finger protein</td>
<td>NM_000937</td>
<td>2.2x</td>
<td>NC</td>
</tr>
<tr>
<td>CD98/SLC3a2</td>
<td>Type II transmembrane glycoprotein and α amylase</td>
<td>NM_000857</td>
<td>2.0x</td>
<td>NC</td>
</tr>
<tr>
<td>Bcl2</td>
<td>Antiapoptotic mitochondrial membrane protein</td>
<td>MUSBC12</td>
<td>1.9x</td>
<td>NC</td>
</tr>
<tr>
<td>Ox2</td>
<td>Membrane glycoprotein</td>
<td>NM_100181</td>
<td>NC</td>
<td>4.6x</td>
</tr>
<tr>
<td>GBP2</td>
<td>IFN-inducible GTP-binding protein</td>
<td>NM_100260</td>
<td>NC</td>
<td>4.5x</td>
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<tr>
<td>IFN-induced p15</td>
<td>Ubiquitin-like protein</td>
<td>NM_015783</td>
<td>NC</td>
<td>4.1x</td>
</tr>
<tr>
<td>MAPKAP2</td>
<td>MAPK-activated Ser/Thr protein kinase</td>
<td>X76850</td>
<td>NC</td>
<td>3.3x</td>
</tr>
<tr>
<td>Pol polyprotein</td>
<td>Protease, reverse transcriptase, RNase H</td>
<td>L018395</td>
<td>NC</td>
<td>3.1x</td>
</tr>
<tr>
<td>Stat1</td>
<td>Signal transducer and transcriptional activator</td>
<td>NM_000283</td>
<td>NC</td>
<td>2.9x</td>
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<tr>
<td>IFN-induced 6-16 precursor</td>
<td>IFN-inducible protein</td>
<td>AK010014</td>
<td>NC</td>
<td>2.7x</td>
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<tr>
<td>ID1</td>
<td>Dominant-negative helix-loop-helix protein</td>
<td>W88041</td>
<td>NC</td>
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<tr>
<td>IRG47</td>
<td>IFN-regulated protein containing GTP-binding protein</td>
<td>M63630</td>
<td>NC</td>
<td>2.3x</td>
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<tr>
<td>Apolipoprotein E</td>
<td>Heparin-binding lipid transporter</td>
<td>NM_009696</td>
<td>D (2.1x)</td>
<td>NC</td>
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<tr>
<td>H2-T3</td>
<td>MHC class I; thymus leukemia Ag</td>
<td>NM_008208</td>
<td>NC</td>
<td>D (6.3x)</td>
</tr>
<tr>
<td>VAMP1</td>
<td>Vesicle-associated membrane protein</td>
<td>NM_000946</td>
<td>NC</td>
<td>D (4.0x)</td>
</tr>
<tr>
<td>TCRβ</td>
<td>T cell Ag receptor</td>
<td>M75875</td>
<td>NC</td>
<td>3.8x</td>
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<tr>
<td>CD4</td>
<td>Costimulatory T cell membrane glycoprotein</td>
<td>M36880</td>
<td>NC</td>
<td>D (2.9x)</td>
</tr>
<tr>
<td>ITPR1/P400</td>
<td>Inositol-1,4,5-triphosphate receptor</td>
<td>NM_010585</td>
<td>NC</td>
<td>D (3.7x)</td>
</tr>
<tr>
<td>Dynen</td>
<td>Axonemal H chain 8; microtubule movement</td>
<td>AF342999</td>
<td>NC</td>
<td>D (3.6x)</td>
</tr>
<tr>
<td>CAP1</td>
<td>Adenylyl cyclase-associated protein</td>
<td>NM_007598</td>
<td>NC</td>
<td>D (3.1x)</td>
</tr>
<tr>
<td>SERCA3b</td>
<td>ATPase and cation transporter</td>
<td>NM_016745</td>
<td>NC</td>
<td>D (3.1x)</td>
</tr>
<tr>
<td>E2F2</td>
<td>Transcription factor and cell cycle regulator</td>
<td>W83068</td>
<td>NC</td>
<td>D (2.9x)</td>
</tr>
<tr>
<td>Ets2</td>
<td>Transcription factor and oncogene</td>
<td>NM_011809</td>
<td>NC</td>
<td>D (2.7x)</td>
</tr>
<tr>
<td>Cyclin D3</td>
<td>Cyclin-dependent kinase subunit</td>
<td>U43844</td>
<td>NC</td>
<td>D (2.6x)</td>
</tr>
<tr>
<td>P2RX1</td>
<td>Ligand-gated ion channel; ATP receptor</td>
<td>NM_000771</td>
<td>NC</td>
<td>D (2.3x)</td>
</tr>
<tr>
<td>Smad5</td>
<td>Receptor-regulated transcriptional modulator</td>
<td>U77638</td>
<td>NC</td>
<td>D (2.1x)</td>
</tr>
<tr>
<td>Eef2</td>
<td>Eukaryotic translation elongation factor</td>
<td>BC007152</td>
<td>NC</td>
<td>D (1.9x)</td>
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</table>

* Summary of genes that exhibit changes in RNA levels during negative selection in vivo identified by oligonucleotide array analysis. I = increased, D = decreased, NC = no change in RNA levels in DO11.10 TCR transgenic thymocytes isolated from OVA-injected mice relative to PBS-injected control mice. The average fold change in RNA levels following OVA injection is shown in parentheses for genes that are regulated.

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The complete data sets are available as a supplement to the on-line version of this article.
gene expression patterns. However, this approach can complicate the interpretation of the experiments due to the possibility of non-cell autonomous changes in gene expression, which can occur as a result of activation of the peripheral immune system. Because the goal of these studies was to identify transcripts that are regulated by TCR signaling in a cell-autonomous manner, regulation of a subset of target genes was investigated using an ex vivo system, where TCR-mediated thymocyte apoptosis occurs in the absence of the peripheral immune system or SP thymocytes. Toward this end, the DO transgene was bred into the B10.BR background. B10.BR mice express the IAk allele, which encodes an MHC that is not recognized by the DO11.10 TCR, and therefore the majority of developing thymocytes are eliminated during negative selection. B10.BR mice were stimulated with OVA peptide in vivo or in vitro, or with immobilized anti-CD3 and anti-CD28 Abs in vitro and RNA levels were quantitated by real-time RT-PCR. I

### Table II. Regulation of RNA levels during negative selection in vivo and in vitro 

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Ag Stimulation In Vivo</th>
<th>Ag Stimulation In Vitro</th>
<th>α-CD3/CD28 Stimulation In Vitro</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>Fold change 2 h.p.i.</td>
<td>p Value</td>
<td>Fold change 8 h.p.i.</td>
</tr>
<tr>
<td>MyD118</td>
<td>1 (23.6×)</td>
<td>&lt;0.0001</td>
<td>1 (45.3×)</td>
</tr>
<tr>
<td>Egr2</td>
<td>1 (3.9×)</td>
<td>&lt;0.0001</td>
<td>1 (183.5×)</td>
</tr>
<tr>
<td>nur77</td>
<td>1 (19.8×)</td>
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<tr>
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<td>&lt;0.0001</td>
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</tr>
<tr>
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1 Regulation of RNA levels during negative selection in vivo and in vitro as determined by real-time RT-PCR analysis. DO11.10 transgenic thymocytes (B10.D2 or Rag2 mutant, B10.BR) were stimulated with OVA peptide in vivo or in vitro, or with immobilized anti-CD3 and anti-CD28 Abs in vitro and RNA levels were quantitated by real-time RT-PCR. I = increased, D = decreased, NC = no change in RNA levels in stimulated thymocytes relative to RNA levels in unstimulated thymocytes. The average fold change in RNA levels is shown in parentheses for genes that are regulated. Genes were scored as increased or decreased if a p value < 0.05 was assigned using the paired (in vitro samples) or unpaired (in vivo samples) Student t tests.

The transcriptional programs specified by TCR stimulation in thymocytes and peripheral T cells are conserved

Stimulation of the TCR can specify distinct cell fates at different stages of T cell development. Apoptosis or differentiation to the SP stage can be induced by TCR ligation in immature DP thymocytes, depending on the strength of the antigenic stimulus (reviewed in Ref. 38). In contrast, mature naive peripheral T cells proliferate in
response to antigenic stimulation. To identify differences in transcriptional programs that may function to mediate these distinct cell fates in immature thymocytes and peripheral T cells, regulation of a subset of the transcripts that are increased or decreased following TCR stimulation in DP thymocytes was investigated in primary naive peripheral T cells following stimulation with immobilized anti-CD3 and anti-CD28 Abs in vitro. Peripheral lymphocytes were harvested from the spleen and lymph nodes of DO11.10 transgenic B10.D2 mice and were cultured on uncoated plates or plates coated with anti-CD3 and anti-CD28 Abs. After 2 or 8 h, the cells were harvested and CD4+ T cells were purified to >95% by MACS. RNA was isolated and transcript levels were determined by real-time RT-PCR. Of the nine transcripts that were quantitated, eight were regulated upon TCR stimulation in peripheral T cells in a manner similar to that observed in DP thymocytes (Fig. 1). Of the transcripts that were not examined, regulation of Egr2 (39), nur77 (20, 21), Ets2 (40), c-fos (41), TCR B (42), CD4 (42), and cyclin D3 (43) in activated peripheral T cells has been previously demonstrated. While this manuscript was in preparation, two studies in which microarrays were used to quantitate transcript levels in primary human T cells stimulated with anti-CD3 and anti-CD28 Abs in vitro were published. One or both of these studies confirmed regulation of the MyD118, ID3, NF-kB2, Bcl2, CD98, and A20 transcripts in peripheral T cells as reported in this study and also described regulation of the Stat1, adenylcyclase-associated protein (CAP1), ApoE, GTP-binding protein (GBP)2, and elongation factor 2 transcripts (44, 45). Thus, of the 33 transcripts we identified by microarray analysis, at least 20 are regulated similarly in thymocytes and peripheral T cells. Despite the dramatically different cell fates that are specified by TCR signaling in thymocytes and peripheral T cells, these data indicate that the changes in gene expression induced by TCR signaling are well-conserved. The expression of four genes was differentially regulated in thymocytes and peripheral T cells. The ITTPR1 transcript was decreased an average of 6.6-fold in thymocytes after 8 h of stimulation with anti-CD3 and anti-CD28 Abs (Table II). In contrast, ITTPR1 RNA levels did not change following anti-CD3/CD28-mediated stimulation of peripheral T cells (Fig. 1). In addition, the timing and/or magnitude of the regulation of three transcripts was different in thymocytes and peripheral T cells. The dynein transcript was reduced more dramatically in peripheral T cells than in thymocytes and the Bcl2 transcript increased earlier and more dramatically in thymocytes than in T cells (Table II and Fig. 1). Similarly, although ID3 expression was induced to similar levels relative to untreated controls in thymocytes and peripheral T cells, induction was only transient in thymocytes, whereas increased expression of ID3 was more sustained in T cells (Table II and Fig. 1). Differential regulation of expression of the ITTPR1, Bcl2, dynein H chain, and ID3 transcripts may thus contribute to the induction of apoptosis and/or differentiation upon TCR signaling in DP thymocytes.

A subset of the genes that are regulated in thymocytes in response to TCR signaling are regulated in response to treatment with other apoptotic stimuli To begin to map the genes that are regulated downstream of the TCR in DP thymocytes to pathways, the regulation of these genes in response to other apoptotic stimuli was investigated. Thymocytes were harvested from DO11.10 TCR transgenic or nontransgenic B10.D2 mice and were cultured in the presence or absence of dexamethasone. Alternatively, thymocytes were exposed to gamma-radiation before culture. Cells were harvested 1, 2, and 3.5 (gamma-irradiation and control cultures) or 1, 2, and 4 (dexamethasone and control cultures) h after exposure to apoptotic stimuli. The latest time points are coincident with the onset of apoptosis in a small fraction of the cultures (data not shown). In addition, by 6 h after treatment with dexamethasone or gamma-irradiation, >75% of the thymocytes had a subG1 DNA content, confirming the effectiveness of the apoptotic stimuli (data not shown). RNA was isolated and transcript levels corresponding to 21 genes were determined by real-time RT-PCR. Transcripts were scored as regulated if they exhibited a >2-fold statistically significant difference (paired Student’s t test p value < 0.05) in RNA levels as compared with control cultures at one or more time points (supplementary Table II). By these criteria, the Ets2, A20, E2F2, and c-fos genes were regulated similarly in response to anti-CD3/anti-CD28 stimulation and treatment with dexamethasone, suggesting that they play similar roles during Ag- and glucocorticoid-induced thymocyte apoptosis (Fig. 2). The A20 gene is also induced in a variety of cell types in response to treatment with TNF (46) and, as for dexamethasone-induced and TCR-mediated apoptosis, increased expression of A20 correlates with TNF-induced apoptosis, despite its ascribed antiapoptotic role (47, 48). In contrast, upon treatment with dexamethasone or gamma-irradiation, several transcripts were regulated in a manner opposite to what was observed following TCR stimulation. ID3 and c-myc transcript levels decreased following dexamethasone treatment and Bcl2 transcript levels decreased following irradiation. Two of the four transcripts that were regulated during negative selection in vivo but were not regulated following TCR stimulation in vitro exhibited differences following treatment with other apoptotic stimuli. Smaa5 transcript levels were significantly reduced following gamma-irradiation and GILZ expression was efficiently induced following treatment with dexamethasone. Thus, regulated expression of these genes may be a more general response to cellular stress, rather than a specific consequence of TCR signaling. Significant differences in MyD118, Egr2, Stat1, nur77, IRG47, MAPKAP2, NF-kB, dynemin, CD98, ITTPR1, cyclin D3, and Bfl1 transcript levels following exposure to dexamethasone or gamma-radiation were not observed (supplementary Table II). Finally, because none of the transcripts investigated in this study were dramatically regulated following gamma-irradiation, p21 RNA levels, which are known to be induced as a consequence of gamma-irradiation (49), were determined. Indeed, following gamma-irradiation p21 transcript levels exhibited

![FIGURE 1. Regulation of RNA levels in peripheral T cells following TCR stimulation. Peripheral lymphocytes were isolated from lymph nodes and spleen of DO11.10, B10.D2 mice and were cultured on uncoated plates or plates coated with anti-CD3 and anti-CD28 Abs. After 2 or 8 h, the cells were harvested and CD4+ T cells were purified by MACS. RNA was isolated and the indicated transcript levels were determined by real-time RT-PCR. Mean transcript levels in cells treated with anti-CD3 and anti-CD28 Abs relative to those in unstimulated control cells are shown. Error bars represent the SEM. Values are derived from three to four independent experiments; *, paired Student’s t test p value < 0.05.](image-url)
a statistically significant 14.8-fold increase relative to untreated control samples (supplementary Table II). Thus, although treatment with dexamethasone results in increased levels of a subset of the transcripts that are regulated upon activation of TCR signaling in thymocytes, transcriptional changes that were common to thymocytes undergoing radiation-induced apoptosis and thymocytes stimulated with anti-CD3 and anti-CD28 Abs were not detected. These observations have been incorporated into the model shown in Fig. 5.

**Regulation of specific TCR signaling pathway components is abrogated in the presence of the pharmacological inhibitors PD98059 and CsA**

To determine whether any of the transcriptional changes that occur upon TCR signaling in immature thymocytes are dependent on known signaling pathways, levels of a subset of the transcripts that were regulated in a thymocyte autonomous manner were quantitated following stimulation of thymocytes with anti-CD3 and anti-CD28 Abs in the presence of pharmacological inhibitors. The following inhibitors were used: PD98059, a specific inhibitor of MEKK1, which functions to phosphorylate and activate the ERK MAPK (50); SB203580, a specific inhibitor of p38 MAPK (51); LY294002, which inhibits phosphatidylinositol 3-kinase (52); c-jun N-terminal kinase (JNK) inhibitor of p38 MAPK (51); CN also appears to play a role in the maintenance of high levels of CD98, c-myc, CD98, E2F2, NF-κB2, c-fos, c-myc, Etst2, dynein, CD98, E2F2, and ITPR1 were properly regulated in the presence of PD98059 (supplementary Table III) and NF-κB2, MyD118, ID3, nur77, c-fos, Bcl2, Etst2, dynein, and ITPR1 were properly regulated in the presence of CsA (supplementary Table IV). LY294002 (3 μM) and SB203580 (20 μM) did not have a significant effect on the transcriptional regulation of Egr2 (Fig. 3, ID3, MyD118, nur77, E2F2, c-myc, NF-κB2, c-fos, Etst2, dynein, Bcl2, or ITPR1 (supplementary Tables V and VI). These data suggest that the ERK MAPK signaling pathway functions downstream of the TCR to mediate increased expression of Egr2 and decreased expression of E2F2. CN also appears to play a role in the maintenance of high levels of CD98, c-myc and Bfl1 transcripts following TCR stimulation.

In contrast to the relatively specific effects of PD98059 and CsA, treatment with roscovitine resulted in reduced basal expression and/or complete abrogation of the transcriptional regulation of almost every gene examined (supplementary Table VIII). This observation is consistent with previous reports suggesting that roscovitine can inhibit phosphorylation of the C-terminal domain of RNA polymerase II and thereby inhibit RNA synthesis (57). In

**FIGURE 2.** Regulation of RNA levels in thymocytes in response to treatment with dexamethasone or gamma-irradiation. Thymocytes were harvested from DO11.10 TCR transgenic or nontransgenic B10.D2 mice and were cultured in the presence or absence of 0.2 μM dexamethasone. Alternatively, thymocytes were exposed to 1000 cGy gamma-radiation before culture. Cells were harvested 1, 2, and 3.5 (gamma-irradiation) or 1, 2, and 4 (dexamethasone) h after exposure to the apoptotic stimuli. Untreated control cultures were harvested at the same times. RNA was isolated and transcript levels corresponding to specific genes were determined by real-time RT-PCR. The following inhibitors were used: PD98059, a specific inhibitor of ERK MAPK; SB203580, a specific inhibitor of p38 MAPK; LY294002, which inhibits phosphatidylinositol 3-kinase; CN, which inhibits the calcium-dependent phosphatase CN; and roscovitine, an ATP analog that inhibits cyclin-dependent kinases 1, 2, and 5. Thymocytes were harvested from DO11.10 TCR transgenic B10.D2 or DO11.10 TCR transgenic, Rag2 mutant, B10.BR mice and treated with an inhibitor or DMSO (untreated control) for 45 min before culturing in the presence or absence of immobilized anti-CD3 and anti-CD28 Abs. Under these conditions, treatment with SB203580 significantly attenuated and treatment with roscovitine almost completely abrogated anti-CD3/anti-CD28-induced apoptosis (Fig. 3). These observations are consistent with previous reports (8, 55, 56). The other inhibitors had no significant effect on thymocyte apoptosis under the conditions used in this study. After 2 and 8 h, cells were harvested and DP thymocytes from B10.D2 cultures were purified to >80% by MACS. Purification of DP thymocytes from B10.BR cultures was not necessary as >90% of the thymocytes isolated from DO11.10 Rag2−/− B10.BR mice are DP (data not shown). RNA was isolated and the levels of 14 different transcripts were determined using real-time RT-PCR (Fig. 4 and supplementary Tables III-VII). Treatment with PD98059 abrogated the anti-CD3/CD28-induced increase in expression of the Egr2, ID3, MyD118, and nur77 transcripts (Fig. 4A). CsA disrupted regulation of the Egr2 and E2F2 transcripts (Fig. 4B). In addition, in the presence of CsA, several of the transcripts that were properly regulated after 2 h of stimulation were expressed at significantly lower levels as compared with control cultures after 8 h. These include CD98, c-myc, and Bfl1 (Fig. 4B). NF-κB2, c-fos, c-myc, Etst2, dynein, CD98, E2F2, and ITPR1 were properly regulated in the presence of PD98059 (supplementary Table III) and NF-κB2, MyD118, ID3, nur77, c-fos, Bcl2, Etst2, dynein, and ITPR1 were properly regulated in the presence of CsA (supplementary Table IV). LY294002 (3 μM) and SB203580 (20 μM) did not have a significant effect on the transcriptional regulation of Egr2, ID3, MyD118, nur77, E2F2, c-myc, NF-κB2, c-fos, Etst2, dynein, Bcl2, or ITPR1 (supplementary Tables V and VI). These data suggest that the ERK MAPK signaling pathway functions downstream of the TCR to mediate increased expression of Egr2 and decreased expression of E2F2. CN also appears to play a role in the maintenance of high levels of CD98, c-myc and Bfl1 transcripts following TCR stimulation.

In contrast to the relatively specific effects of PD98059 and CsA, treatment with roscovitine resulted in reduced basal expression and/or complete abrogation of the transcriptional regulation of almost every gene examined (supplementary Table VIII). This observation is consistent with previous reports suggesting that roscovitine can inhibit phosphorylation of the C-terminal domain of RNA polymerase II and thereby inhibit RNA synthesis (57). In

**FIGURE 3.** Anti-CD3/anti-CD28-induced apoptosis is attenuated in the presence of the pharmacological inhibitors SB203580 and roscovitine. Thymocytes were isolated from DO11.10 TCR transgenic B10.D2 or B10.BR mice and were incubated in the presence of the indicated pharmacological inhibitor or DMSO for 45 min before plating on anti-CD3/anti-CD28 coated or uncoated dishes. Cells were harvested after 24 h, stained with propidium iodide, and analyzed by flow cytometry. The percentage of cells with a subG1 DNA content in anti-CD3/anti-CD28-stimulated cultures relative to unstimulated control cultures is shown. Error bars indicate the SEM and p values were determined using the unpaired Student’s t test. Values are derived from three to eight independent experiments.
these experiments, roscovitine was used at the minimum concentration required for complete inhibition of anti-CD3/CD28 mediated apoptosis (data not shown). Thus, the abrogation of thymocyte apoptosis by roscovitine may result from a general inhibition of RNA Pol II-dependent transcription. These observations also demonstrate an important caveat to the interpretation of experiments using pharmacological inhibitors. Nonetheless, the results of the experiments described in this study provide a working model of the TCR signaling pathways that function in DP thymocytes and will be useful for the development of additional hypothesis-driven experiments that do not require the use of pharmacological inhibitors. In addition, CsA is used clinically as an immunosuppressant and thus, any insight into its mechanism of action may have important clinical implications.

The results reported in this study, along with previous reports, support the model shown in Fig. 5. The NFAT4, NFATx, and NFATc transcription factors are expressed in DP thymocytes (58, 59). NFAT DNA binding activity is induced upon activation and this increase in binding activity is inhibited by CsA, suggesting that NFAT functions downstream of CN in immature thymocytes (59). Similarly, the regulation of Egr2 following TCR stimulation is dependent on CN (Fig. 4B; Refs. 29 and 60). Consistent with the idea that Egr2 expression is regulated by NFAT activity in peripheral T cells, the expression of Egr2 is not induced upon stimulation of primary T cells isolated from NFATp/NFAT4 mutant mice with anti-CD3 and anti-CD28 Abs in vitro (39). Increased expression of Egr2 following TCR stimulation in immature thymocytes is also abrogated upon inhibition of the ERK MAPK pathway. The ERK MAPK pathway is activated downstream of Ras following TCR stimulation in immature thymocytes (61, 62). In the presence of PD98059 (Fig. 4A) or dominant-negative Ras (29), increased expression of Egr2 is not observed following stimulation with anti-CD3 Ab. The sensitivity of Egr2 induction to both CsA and PD98059 suggests that activation of both CN and the ERK MAPK pathways is essential for Egr2 regulation. The regulation of ID3 transcription also requires activation of the ERK MAPK pathway. Increased expression of ID3 is not observed in DP thymocytes following TCR stimulation in the presence of PD98059 (Fig. 4A; Ref. 30) or dominant-negative Ras and ID3 expression is induced upon ectopic expression of constitutively active MEK1, which functions downstream of Ras to mediate activation of the ERK MAPKs (30). In addition, ectopic expression of Egr1 is sufficient for the induction of ID3 transcript levels, indicating that Egr transcription factors can function either directly or indirectly to regulate ID3 expression (30). However, activation of Egr2 may not be the sole mechanism by which the ERK MAPK pathway functions to induce ID3 expression, as the attenuation of Egr2 induction in the presence of CsA is not sufficient to abrogate ID3 expression (supplementary Table IV). This work represents the first report of the CsA-sensitive transcriptional regulation of E2F2 in DP thymocytes in response to TCR stimulation. Similarly, regulation of nur77 and MyD118 induction by the ERK MAPK pathway has not been previously reported. In a T cell hybridoma line, MyD118 is regulated by NF-κB (63) and extensive characterization of the nur77 promoter has shown that its expression is regulated by the calcium-dependent myocyte enhancer factor (MEF)2 transcription factor following activation (64). In contrast, nur77 regulation in response to anti-CD3/anti-CD28 stimulation is not sensitive to CsA in primary DP thymocytes and may therefore occur by a distinct mechanism (supplementary Table IV).

The results described in this study also have implications for the mechanisms by which positive and negative selection are implemented in DP thymocytes. Several transcripts were misregulated in the presence of pharmacological inhibitors that did not disrupt anti-CD3/anti-CD28-induced apoptosis, suggesting that the regulation of these transcripts is not required for negative selection. These include E2F2, Egr2, MyD118, ID3, and nur77. In addition, it is interesting that expression of nur77, which is specifically required for negative selection, is regulated by the ERK MAPK pathway, which is specifically required for positive selection. Nonetheless, three of the four transcripts that are regulated by the ERK MAPK pathway have been implicated in positive selection or inhibition of apoptosis. ID3 is required for MHC class I- and class II-restricted positive selection of TCR transgenic thymocytes in vivo (12). Ectopic expression of Egr1 promotes (65), but is not required for (66, 109)
positive selection, suggesting that Egr2, which is expressed in a similar pattern, may function redundantly to mediate positive selection. MyD118 functions downstream of NF-κB to inhibit the JNK pathway and TNF-α-induced apoptosis in a T cell hybridoma line (63). Thus, the requirement for activation of signaling pathways that function specifically in positive selection for appropriate regulation of a particular transcript may generally correlate with, but does not necessarily indicate, a role for that transcript in positive selection. Similarly, the expression of several genes that have well-characterized antiapoptotic functions, including Bcl2, Bfl1, and NF-εB2, is increased in DP thymocytes following induction of apoptosis. These observations suggest that similar signal transduction pathways are activated by stimuli that induce positive and negative selection and are consistent with the idea that the balance between opposing proapoptotic and antiapoptotic pathways eventually determines cell fate. A final point of interest is the attenuation of CD98, c-myc, and Bfl1 levels following anti-CD3/CD28 stimulation in the presence of CsA, suggesting that CN functions to mediate the sustained transcriptional activation of a subset of TCR-mediated thymocyte apoptosis in the experiments described in this study, CsA can prevent negative selection in response to relatively weak TCR stimuli (68). Taken together, these observations suggest a molecular mechanism by which CN may function to amplify TCR signals and thereby promote negative selection.

Summary

Using Affymetrix oligonucleotide arrays, we have identified 33 genes that exhibit changes in RNA levels in DP thymocytes during negative selection in vivo. The statistically significant regulation of 18 of these genes in individual mice has been confirmed using real-time RT-PCR. Of these, 13 are regulated in response to stimulation with Ag or anti-CD3 and anti-CD28 Abs ex vivo, indicating that these genes are regulated independently of activation of the peripheral immune system. These data also support the idea that anti-CD3/CD28-mediated thymocyte apoptosis is a valid model for negative selection in vivo. In addition, most of the genes we identified are similarly regulated following TCR stimulation in DP thymocytes and peripheral T cells. However, expression of the A20, Bcl2, dynin H chain, and ID3 transcripts is differentially regulated in thymocytes and peripheral T cells and may thus contribute to the induction of differential cell fates upon TCR signaling. A detailed examination of the regulation of a subset of the identified genes in response to treatment with dexamethasone or gamma-irradiation or in response to TCR stimulation in vitro in the presence of pharmacological inhibitors of known signal transduction pathway components has facilitated the elucidation of a map of the transcriptional events that occur downstream of the TCR. These studies support a model whereby similar signal transduction pathways are activated by stimuli that induce positive and negative selection and are consistent with the idea that the balance between opposing proapoptotic and antiapoptotic pathways eventually determines cell fate. The data presented in this study also suggest that CN functions to amplify TCR signals by promoting sustained increases in the levels of specific transcripts.

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References


