E2A/HEB and Id3 Proteins Control the Sensitivity to Glucocorticoid-induced Apoptosis in Thymocytes by Regulating the SRG3 Expression*

Myunggon Ko‡§, Jeongeun Ahn‡§, Changjin Lee‡§, Heekyoung Chung‡, Sung H. Jeon‡§, Hee-Y. Chung†, and Rho H. Seong‡**

From the ‡School of Biological Sciences and Institute of Molecular Biology and Genetics, Seoul National University, Seoul 151-742 and the ¶Department of Microbiology and the §Department of Pathology, Hanyang University School of Medicine, Seoul 133-791, Korea

Received for publication, February 26, 2004
Published, JBC Papers in Press, March 11, 2004, DOI 10.1074/jbc.M402145200

The E protein family transcription factors encoded by the E2A and HEB genes are known to play critical roles in the coordinate regulation of lymphocyte development. Previous studies have shown that T cell receptor (TCR) signals rapidly induce Id3, a dominant negative antagonist of E2A activity and allow thymocytes to survive selection events in the thymus. Here we show that SRG3 acts as a novel downstream target of E2A/HeLa E box-binding (HEB) complex and modulates glucocorticoid (GC) susceptibility in thymocytes in response to TCR signals. We have identified a putative E box element in the SRG3 promoter that is required for optimal promoter activity. The transcription factors E2A and HEB specifically associate with the E box element. Moreover, E2A-HEB heterodimers cooperated to activate SRG3 transcription, which was inhibited by the expression of Id3 proteins. TCR-mediated signals rapidly induced Id3 via MEK/ERK activation and thereby kept E2A and HEB activity is required at multiple stages of thymocyte development. For example, E2A deficiency allowed the developmental progression and proliferation of DN thymocytes that have high susceptibility to glucocorticoid (HLH) proteins, were originally identified as factors that specifically bind with high affinity to the palindromic CANNTG sequence, referred to as the E box element in the Ig enhancer (12–14). The mammalian E proteins consist of E2A, E2-2, and HeLa E box-binding (HEB) proteins (15–17). Although HEB and E2-2 are encoded by distinct genes, E2A gene encodes two E proteins, E12 and E47, through alternative RNA splicing (13, 14, 17, 18). E proteins share a highly conserved domain called helix-loop-helix (HLH), which allows for dimerization between HLH family members (19). The conserved basic domain immediately NH2-terminal to HLH domain facilitates the binding of HILH dimers to the canonical E box element. All of the E proteins have the potential to interact with DNA in a dimeric configuration either as an E protein homodimer/heterodimer or, more commonly, as a heterodimer containing an E protein family member together with one of several cell type-specific bHLH proteins. Upon DNA binding, E proteins regulate the transcription of target genes through NH2-terminal transactivation domains (20–22).

Tissue-specific differential combinatorial associations between bHLH dimers play pivotal roles in imparting specificity and diversity in functional modulation of distinct E box transcriptional regulatory activities depending on the cell context. In thymocytes, the E2A/HEB heterodimer is known as a major basic HLH (bHLH), whereas E2A homodimer is predominant in B-lineage cells (23, 24). By analyzing various knockout mice deficient in E protein member(s), it is well characterized that E2A and HEB activity is required at multiple stages of thymocyte maturation. Mice deficient in E2A or E47 exhibited hypocellularity in the thymus and a partial block at the CD44+CD25+ (double-negative 1, DN1) stage (8, 9). Targeted inactivation of HEB also led to thymic hypocellularity and a strong developmental block at the immature single-positive stage (10). Mice expressing HEBΔN, a dominant negative HEB that inhibits both E2A and HEB activity, displayed a developmental arrest at the CD44+CD25+ (DN3) stage (11). However, for the proper progression at some checkpoints, E2A activity is negatively regulated by signals emanating from antigen receptors. For example, E2A deficiency allowed the developmental progression and proliferation of DN thymocytes that have...
failed β-selection (7). E2A or E47 deficiency also promoted the positive selection of CD4+CD8+ double-positive (DP) thymocytes expressing the relevant αβTCR (8, 9). In addition, E2A protein played positive regulatory roles in apoptosis and growth arrest, suggesting that inactivation of E2A may allow survival of thymocytes that would have otherwise died during thymic selection processes (25–28).

E protein activity can be controlled by heterodimerization with Id proteins referred to as class V HLH proteins (29). Id proteins act as functional antagonists of E protein activity in a dominant negative manner since they do not bind to DNA because of the lack of a basic DNA-binding domain while efficiently forming highly stable heterodimers with selected E proteins through the HLH domain (15, 16, 30). Acting as functional antagonists of bHLH transcription factors, Id proteins have been shown to play pivotal roles as positive regulators of cell growth and negative regulators of cell differentiation (27, 30–37). There are four Id protein members: Id1, Id2, Id3, and Id4 (30, 38–40). Among them, Id2 and Id3 are known to be abundantly expressed in B and T lymphocytes (41, 42). Genetic studies have demonstrated that Id3 is essential for proper thymocyte maturation in both class I- and class II-restricted TCR transgenic mice (42). Intriguingly, inactivation of both E47 and Id3 restored normal thymocyte maturation, which clearly indicates genetic interaction between them in the thymus (42). Upon pre-TCR-mediated signaling in DN thymocytes, Id3 proteins were rapidly induced and thereby down-regulated E protein DNA binding to promote β-selection (7). In addition, it has been suggested that Id3 expression by TCR activation of the Ras/MEK/ERK pathway also promotes the maturation of immature DP cells by inhibiting the E box binding activity of E2A (9, 43). Taken together, these results imply that only the DP thymocytes that repress E2A activity by Id3 upon TCR/CD3 engagement may survive thymic selection and are able to differentiate into SP thymocytes. However, the specific nuclear target that is regulated by this interaction between E2A and Id3 protein at this transition has yet to be identified.

Here we showed that SRG3 acts as a novel nuclear target whose expression is regulated by the E2A-Id3 interaction. E2A/HEB specifically bound to the E box element in the SRG3 promoter and activated its activity. Upon TCR-triggered signals, Id3 was rapidly induced and prevented the E2A/HEB activator complex from binding to the E box element in the SRG3 promoter, resulting in SRG3 down-regulation. Additionally, ectopic overexpression of Id3 by the retroviral transduction system decreased SRG3 expression by inhibiting E2A/HEB binding. It also rendered primary thymocytes and immature thymoma cells resistant to GC-triggered apoptosis by down-regulating the expression of SRG3. Taken together, these observations suggest that Id proteins may enhance the viability of DP thymocytes by rendering GC resistance by down-regulating SRG3 expression.

**EXPERIMENTAL PROCEDURES**

**Mice, Cells, Reagents, and Antibodies—**Transgenic mice overexpressing SRG3 in the FVB background were described previously (44). The murine DP thymoma, 16610D9, was provided by Dr. C. Murre (University of California, San Diego, La Jolla, CA) and cultured in Opti-MEM (Invitrogen) containing 10% FBS supplemented with 50 μg 2-mercaptoethanol. NIH3T3 was maintained in Dulbecco’s modified Eagle’s medium containing 10% bovine calf serum.

We purchased PD98059 from Calbiochem; phorbol 12-myristate 13-acetate (PMA), A23187 (ionomycin), cycloheximide (CHX), and dexamethasone (Dex) from Sigma; anti-actin (sc-1615) and anti-GR (M-20) antibodies from Santa Cruz Biotechnology; and fluorescein isothiocyanate (FITC), tetramethylrhodamine isothiocyanate (TRITC), and peroxidase (POD) from CP Biochemicals. Antibodies from Pharmingen. Antiserum against SRG3 was raised from rabbits in our laboratory as described previously (44).

**Northern Blot Analysis and Reverse Transcriptase (RT)-PCR—**Total RNA was prepared from cells by resuspension in TRIzol reagent (Invitrogen) according to the manufacturer’s instruction. Total RNA

**FIG. 1. Retroviral transduction of Id3 down-modulates the SRG3 protein expression.** A, schematic illustration of retroviral vectors. To construct the Id3-overexpressing retroviral vector, cDNA for FLAG-tagged Id3 was inserted into S-003 vector. LTR, long terminal repeat; IRES, internal ribosome entry site. **B**, flow cytometric analysis of transduction efficiency. EGFP expression in 16610D9 cells transduced by the indicated retrovirus was measured by flow cytometry. The percentage of EGFP+ transductants is indicated.

**C**, Western blot analysis showing that Id3 inhibits the SRG3 protein expression. Equal amounts of whole-cell extracts were prepared from vector- and Id3-transduced 16610D9 cells and immunoblotted with antibodies specific to SRG3, GR, or actin. The level of actin proteins served as a control.
vector- and Id3-transduced 16610D9 cells were resolved on a 1.2% formaldehyde gel and then blotted to Hybond™-N (Amersham Biosciences). The Northern blots were probed with specific SRG3 and FLAG-Id3 probes. 18 S and 28 S rRNAs are shown. Results RT-PCR analysis was performed as described previously (45).

**Plasmids, Mutagenesis, Transfection, and Reporter Assay**—The pSRG3-Luc reporter construct was described previously (46). pHBAneo-E47 and pHBAneo-E12 were from Dr. C. Murre (University of California, San Diego, La Jolla, CA). For construction of pCAGGS vector, the BamHI fragment of pHBAneo-E47 was cloned into pBluescript and then the Xhol/EcoRI fragment was inserted into pCAGGS vector. pCAGGS-E12 was constructed by ligating the BamHI fragment of pHBAneo-E12 with BglIII-digested pCAGGS vector. HEB cDNA was amplified by RT-PCR and cloned into pGEM®-T Easy vector, and then the XhoI fragment was inserted into pCAGGS vector. The PCR primers for construction of pCAGGS-HEB are as follows: 5'-CTGGCTCGAGATTCTGCTCAGATGAATCCCAGCA-3' and 5'-CTGGCTCGAGATTTTCTAGAGTATACCCCAT-3'. Mutagenesis of the E box sequences (CATCTG into CTGCAG) in the HEB cDNA was described previously (46). To join the putative E box element in the SRG3 promoter, transgenic transfection, and luciferase assay were performed as described previously (46). The PCR primers for the mutagenesis of E84 element are as follows: 5'-AGGAGTGGTCGCAAGCAGCGGCGGCGG-3' and 5'-GGGCGGCCTCTGACGACCATCT-3'.

**Retroviral Transduction**—The retroviral vector LZ8Stm-BMN-linker IRES-EGFP (NotI) (S-003) and Id3/S-003 was from Dr. C. Murre (University of California, San Diego, La Jolla, CA). The dNEX-eco packaging cell line was transfected with S-003 or Id3/S-003 vector by calcium phosphate precipitation and incubated in the presence of 2 μg/ml puromycin for 4 weeks. Retrovirus-containing supernatants were collected following incubation of the selected retroviral producer cells for 24 h and stored at −80 °C after filtering. For transduction, the retroviral supernatant (1 ml) was supplemented with Polybrene (8 μg/ml), incubated on ice for at least 10 min, and transferred to the standard 6-well tissue culture plates containing target cells (1 × 10^6 cells in 1 ml of fresh medium), followed by centrifugation at 2,800 rpm for 90 min at 32 °C. After centrifugation, growth medium was replaced with fresh medium. Cells were harvested and analyzed 36–48 h after infection.

For the transduction of primary thymocytes, cells were pre-treated with 3 μg/ml concanavalin A (Vector Laboratories) for 2 days and spin-inactivated as described.

**Cell Death Measurement**—Single cell suspensions of thymocytes derived from 4- to 5-week-old wild-type or hCD2-SRG3+ Tg FVB mice were maintained in RPMI 1640 medium containing 10% FBS and 50 μM 2-mercaptoethanol supplemented with glutamine, penicillin, and streptomycin. A portion of apoptotic cells was assessed by annexin V staining. At the end of each treatment, cells were harvested and resuspended in biotin-conjugated annexin V, incubated at room temperature for 15 min, washed, resuspended in quantum red-conjugated streptavidin, and analyzed by flow cytometry (20,000–30,000 events) with the CellQuest™ software using FACStar (BD Biosciences).

**RESULTS**

Overexpression of Id3 Proteins Represses the SRG3 Gene Expression—In thymocytes, E2A protein mostly binds to the E box element with the consensus CANNTG sequence as a complex with the HeLa E box-binding (HEB) protein, another bHLH family transcription factor highly expressed in developing T cells (23). We found that the putative E box element (designated E84) CATCTG exists at the −84 region in the SRG3 promoter and that it plays crucial roles in TCR signaling-mediated repression of the SRG3 gene expression to render thymocytes resistant to GC-mediated apoptosis (55). In addition, the E2A/HEB protein complex is specifically associated with the putative E box element in the SRG3 promoter. It was suggested that expression of the E-protein inhibitor Id3 interferes with E-protein DNA binding activity because of the lack of the basic DNA-binding domain (29). Therefore, it is conceivable that Id3 proteins negatively regulate SRG3 gene expression by antagonizing the binding of E2A to the E84 element in the SRG3 promoter. To examine the regulatory role of Id3 in modulating SRG3 expression, Id3 was retrovirally transduced into 16610D9 cells by using a retroviral vector, S-003. The Id3/S-003 retroviral vector allows translation of both FLAG-tagged mouse Id3 and enhanced green fluorescence protein (EGFP) from a bicistronic retroviral transcript (Fig. 1A). To transduce Id3 into 16610D9 cells, we used supernatants from dNEX-eco retroviral packaging line into which Id3/S-003 or S-003 empty vector had been stably transfected. The transduction level was determined by flow cytometric analysis of transductants expressing EGFP (Fig. 1B). Subsequently, we assessed the level of SRG3 proteins in the whole-cell extracts from control and Id3-infected cells by immunoblotting. As shown in Fig. 1C, ectopic overexpression of Id3 reduced SRG3 protein expression by more than half, whereas the expression of GR and actin remained unchanged, suggesting that SRG3 gene expression is negatively controlled by Id3 expression. To determine whether the decrease in SRG3 protein levels upon Id3 transduction resulted from suppression of SRG3 transcription, we next proceeded to analyze the level of various transcripts in control and Id3-transduced cells by Northern blotting. As shown in Fig. 2A, mRNAs for FLAG-tagged Id3 whose expression is driven by the Id3 retroviral construct were detectable only in Id3-infected cells. As a result, the level of SRG3 mRNA was reduced by approximately half (Fig. 2A), which
was comparable with the decrease in SRG3 proteins upon Id3 transduction (Fig. 1). The same result was reproduced by RT-PCR analysis showing repression of SRG3 transcription by Id3 overexpression, whereas the level of glyceraldehyde-3-phosphate dehydrogenase or hypoxanthine-guanine phosphoribosyltransferase control transcripts was not affected (Fig. 2B). Taken together, these results indicate that Id3 represses the SRG3 gene expression at the transcriptional level.

**E2A/HEB Complex Activates the SRG3 Promoter Activity through the E84 Element, Which Is Inhibited by Id Proteins**

It has been established that Id3 was well known to repress the E box binding activity of E proteins in a dominant negative manner and E protein family members such as E47, E12, and HEB directly associated with the putative E box element in the SRG3 promoter (55). It is possible that E2A and HEB proteins act to activate SRG3 expression, and Id3 protein represses SRG3 transcription by preventing E2A/HEB binding to the E box element in the SRG3 promoter, leading to a decrease in SRG3 protein level. To clarify the contribution of the E2A/HEB complex to the activation of SRG3 gene expression, we generated pSRG3-210-Luc, a luciferase reporter construct containing the proximal SRG3 promoter region encompassing the 210-bp nucleotides upstream of the SRG3 translation start site. This cell line is appropriate for this assay because the SRG3 gene was expressed at low levels in the absence of E2A/HEB binding to the E box element (E84) in the SRG3 promoter (55). As expected, ectopic expression of E2A or HEB significantly activated the SRG3 promoter activity 3-5-fold, which was further enforced when E2A and HEB were co-expressed (Fig. 3A). However, introduction of a mutation in the E84 element significantly inhibited the ability of E2A and HEB proteins to activate the SRG3 promoter activity (Fig. 3B). These results suggest that E2A/HEB activates the SRG3 transcription through the E84 element.

By using 16610D9 immature DP T cells, it has been proven that Id3 is rapidly induced by signals emanating from the
TCR/CD3 complex via activation of the Ras/MEK/ERK cascade and inhibits DNA binding activity of the E2A/HEB complex (43). Furthermore, retroviral overexpression of Id3 in this cell line also lowered E2A DNA binding activity and promoted the acquisition of phenotypes characteristic of cells undergoing positive selection (9). Intriguingly, TCR signals suppressed the SRG3 transcription through the E84 element by activating the Ras/MEK/ERK pathway, resulting in GC desensitization in thymocytes (55). Furthermore, we also observed that there was significant kinetic correlation between induction of Id3 transcripts and repression of SRG3 transcripts. Therefore, it is possible that Id3 negatively regulates the SRG3 expression by antagonizing the binding of E2A/HEB activator complexes to the E box element in the SRG3 promoter. Indeed, co-transfection of Id3 completely abolished E2A/HEB activation of the SRG3 promoter activity in NIH3T3 cells (Fig. 3A). Id1 proteins also exhibited similar repressive effects. This effect was not specific to NIH3T3 cells because ectopic expression of Id proteins in 16610D9 cells also reduced the SRG3 promoter activity (Fig. 3C). Taken together, these observations suggest that SRG3 is a novel direct target of E2A/HEB heterodimers whose expression is negatively modulated by Id proteins.

To investigate if TCR signals inhibit the binding of the E2A/HEB complex to the E84 element via the Ras/MEK/ERK cascade as suggested previously (43), we subsequently examined the effect of PD98059, a pharmacological drug that specifically inhibits MEK1 and MEK2, on the E2A/HEB binding to the E84 element in activated thymocytes. To this end, EMSA was undertaken by using nuclear extracts from primary thymocytes in C57BL/6 mice treated with PMA + ionomycin in the presence or absence of PD98059. Combined treatment of PMA, a protein kinase C activator, and ionomycin, a calcium ionophore, was shown to mimic the intracellular signaling triggered from proper cross-linking of the TCR/CD3 complex, resulting in thymocyte survival and differentiation (47–53). The labeled oligonucleotides encompassing the E84 element in the SRG3 promoter was used as a probe. Much stronger inhibition of the E2A/HEB complex binding to the E84 element was observed as the concentration of PMA increased (Fig. 3D, lanes 1, 3, and 5), whereas Oct-1 binding remained unchanged, suggesting that the E84 occupancy by E2A/HEB complex is differentially regulated depending on the strength of TCR signals. In contrast, addition of PD98059 partially restored E2A/HEB DNA binding activity in the stimulated thymocytes, implying that binding of this complex to the E84 element is negatively regulated by the TCR-activated Ras/MEK/ERK cascade. However, treatment of PD98059 alone could not completely block inhibition of interaction between E2A/HEB and E84 element in response to PMA + ionomycin treatment.

To assess directly the ability of Id3 to inhibit the binding of the E2A/HEB complex to the E84 element, we subsequently carried out EMSAs using nuclear extracts from control and Id3 transductants described in Fig. 1. As shown in Fig. 4, E2A/HEB binding to E84 and μE5 probes that contain functional E box sequences was similarly inhibited to a similar extent by Id3 expression, whereas binding of unrelated Oct-1 proteins remained unchanged. The rate of inhibition of E2A/HEB binding by Id3 overexpression (~50–60%) was similar to the transduction efficiency, which is also well reflected in the Id3-mediated reduction in the level of SRG3 protein and transcripts (Figs. 1 and 2). Taken together, these results, combined with our previous observations, suggest that Id3 protein is induced by TCR activation of the Ras/MEK/ERK pathway and represses the SRG3 transcription by acting as a dominant negative antagonist for E2A/HEB transactivation of the SRG3 gene by antagonizing the E box binding activity of this complex.

Id3 Antagonizes Dexamethasone-induced Apoptosis of Thymocytes by Repressing SRG3 Expression—We reported previously that SRG3 gene expression is differentially regulated during thymocyte maturation in the thymus and control of the SRG3 expression level is crucial in modulating GC sensitivity (44, 46, 54). SRG3 exacerbated the apoptotic role of GCs in T cells by physically associating with GR and thereby potentiating its transcriptional activity in vitro and in vivo. In addition, the susceptibility to GCs in T cells could be altered by modifying the SRG3 expression at different levels in transgenic mice, suggesting significant correlation between SRG3 expression level and GC sensitivity in T cells (44, 46). Specifically, transgenic overexpression of SRG3 in peripheral lymphocytes, which would express SRG3 at low levels and show relative resistance to GCs in wild-type mice, rendered these cells more susceptible to GC-induced apoptosis, whereas inhibition of SRG3 in immature thymocytes, which would highly express SRG3 and exhibit exquisite sensitivity to GCs, protected these cells from GC-mediated apoptosis. Because Id3 down-regulated the level of SRG3, a positive co-activator of GR, it is plausible that Id3 overexpression is sufficient to render T cells resistant to GC-mediated cell death. To address this possibility, apoptosis upon exposure to Dex, a synthetic GC, was measured in S49.1 thymoma cells transduced with control or Id3/S-003 retroviruses. When we measured the fraction of apoptotic cells after incubating each transductant in the presence of Dex or anti-Fas + CHX, Dex-induced apoptosis was significantly inhibited in cells highly overexpressing Id3, whereas Fas-induced apoptosis was only partially suppressed by Id3 expression (Fig. 5). Similar results were reproduced in primary thymocytes from wild-type FVB mice that were pre-stimulated with concanavalin A for 2 days and transduced with vector control or Id3/S-003 retroviral supernatants (Fig. 6). Approximately 65–70% of thymocytes transduced with vector control (EGFP-negative) underwent specific apoptosis, whereas 35–40% of Id3-infected thymocytes (EGFP-positive) were apoptotic. Subsequently, we tested if Id3 renders thymocytes resistant to Dex-induced apoptosis by modulating SRG3 expression. Thymocytes derived from wild-type or transgenic mice over-expressing SRG3 (hCD2-SRG3$^+$ Tg) were pre-stimulated
with concanavalin A (3 μg/ml) in the presence of interleukin-2 and transduced with vector or Id3/S-003 retroviruses. Thirty six hours post-transduction, Dex at 10^{-7} M was added, and a fraction of apoptotic cells was measured by staining with biotin-conjugated annexin V and quantum red-conjugated streptavidin. Percentages of cells in the individual subpopulations are shown in each quadrant.

**DISCUSSION**

The data we presented here showed that SRG3 is a direct target of interaction between helix-loop-helix proteins, E2A/HEB and Id proteins, in modulating the sensitivity of immature T cells to GC-triggered apoptosis. We identified a putative E box element in the SRG3 proximal promoter. EMSA analyses with concanavalin A (3 μg/ml) in the presence of interleukin-2 and transduced with vector or Id3/S-003 retroviruses. Thirty six hours post-transduction, Dex at 10^{-7} M was added, and a fraction of apoptotic cells was measured by staining with biotin-conjugated annexin V and quantum red-conjugated streptavidin. Percentages of cells in the individual subpopulations are shown in each quadrant.

**DISCUSSION**

The data we presented here showed that SRG3 is a direct target of interaction between helix-loop-helix proteins, E2A/HEB and Id proteins, in modulating the sensitivity of immature T cells to GC-triggered apoptosis. We identified a putative E box element in the SRG3 proximal promoter. EMSA analyses...
showed that the E2A/HEB heterocomplex specifically associates with the E84 element and that E2A and HEB were shown to function collaboratively to activate SRG3 promoter activity (Fig. 3A). However, Id3 proteins effectively disrupted the E2A/HEB transactivation of the SRG3 gene by preventing E2A/HEB heterodimers from interacting with the E box element in the SRG3 promoter (Figs. 3A and 4). This resulted in a decrease in the level of SRG3 proteins by transcriptionally repressing the SRG3 gene (Figs. 1 and 2). In addition, E2A/HEB could not up-regulate the SRG3 promoter activity when we introduced mutations in the E84 element (Fig. 3B). These observations imply that the SRG3 gene expression is regulated by the E2A/HEB complex through the functional E box element called E84 in the SRG3 promoter, and that Id3 proteins can modulate the level of SRG3 gene expression by acting as a functional antagonist for E2A/HEB transcriptional activities. Indeed, there is a similar gradient of the expression level of E47 and SRG3 proteins that is correlated well at different developmental stages in the thymus. Both protein levels remain high until DP stage and are down-regulated upon transition to SP stage (7).

Previously, we have shown that the expression level of SRG3 plays critical roles in modulating the sensitivity to GC-mediated apoptosis of developing thymocytes (44, 46, 54). Because overexpression of Id3 repressed the transcription of the SRG3 gene, which acts as a positive regulator of GC sensitivity, we attempted to assess if Id3 can directly prevent GC-mediated thymocyte apoptosis. Intriguingly, ectopic expression of Id3 partially protected immature thymoma cells and thymocytes from Dex-induced apoptosis (Figs. 5 and 6). However, Id3 alone could not completely inhibit the apoptotic actions of Dex in both cases, suggesting that other additional molecules are required for optimal protection against Dex-induced thymocyte death. Moreover, overexpression of SRG3 in transgenic mice prevented the protective role of Id3 against Dex-mediated thymocyte apoptosis (Fig. 7), suggesting that Id3 conferred GC resistance by down-regulating the SRG3 gene expression.

Studies employing mice with targeted inactivation of E protein family transcription factors have identified the important roles of E proteins in regulating various aspects of lymphocyte development. In E2A- or E47-deficient mice, increased proportion of DP thymocytes matured into CD4+ and CD8+ SP thymocytes (8). The absence of E47 facilitated the production rate of mature CD4+ and CD8+ SP T cells and markedly enhanced the positive selection of TCRs, which were both class I and class II major histocompatibility complex-restricted (9). Furthermore, E47 deficiency allowed the maturation of some TCR+CD8+ SP cells even in the absence of β2-microglobulin. Because it is thought that relatively weaker signals are required for CD8 lineage compared with CD4 lineage, these observations suggest that E2A or E47 deficiency might allow the maturation of T cells that would normally die by lowering the threshold of avidity required for positive selection. In contrast, positive selection of both class I and class II major histocompatibility complex-restricted TCRs was impaired in Id3-deficient mice (42). Remarkably, the deficiency of both E2A and Id3 restored normal thymocyte maturation, which indicates that the genetic interaction between Id3 and E2A is crucial for the proper thymocyte maturation. Indeed, recent studies (7, 43) have shown that Id3 induction, in response to signals emanating from surface antigen receptors such as pre-TCR or αβTCR through the Ras/MEK/ERK/EGFR cascade, plays critical roles in down-modulating E2A/HEB activity and promotes developmental progression at both the DN and DP stage. However, the ultimate nuclear effectors of these signaling pathways remain unknown. The present study suggests that blockade of E box binding activity of E2A/HEB upon TCR signaling promotes thymocyte maturation by at least down-regulating SRG3 expression, which contribute to thymocyte survival by resulting in GC resistance. It is conceivable that the down-regulation of SRG3 may be essential for proper thymocyte development and that enhanced thymocyte viability by GC desensitization may be just one result of it.

We also found that the SRG3 transcription is repressed in response to TCR or Ras activation and that this responsiveness was conferred in the −210-bp proximal SRG3 promoter region (55). Introduction of a mutation in the E box element (E84) in the SRG3 promoter diminished the basal SRG3 promoter activity by half in immature T cells and impaired the Ras or TCR activity to repress SRG3 expression, suggesting a crucial role of the E84 element in TCR/Ras modulation of the SRG3 gene expression. TCR/CD3 signals rapidly up-regulated Id3 expression through the Ras/MEK/ERK cascade which, in turn, repressed the SRG3 transcription by preventing the E2A/HEB complex from interacting with the E84 element, resulting in GC resistance (Fig. 3D) (55). Although pharmacological inhibition of the MEK/ERK pathway by using PD98059 significantly blocked the Ras activity to repress the SRG3 promoter activity, it could not completely restore the occupancy of E2A/HEB in the E84 element in the thymocytes activated by PM + ionomycin treatment (Fig. 3D), suggesting that additional signaling pathways other than Ras/MEK/ERK cascade may diverge from TCR stimulation and may also be implicated in preventing E2A/HEB from binding to the E84 element. Consistently, TCR signals were more potent than Ras activation in repressing the SRG3 promoter activity, and PD98059 partially restored the level of SRG3 transcripts in T cells stimulated with PM + ionomycin (55), suggesting that signals other than Ras/MEK/ERK cascade may emanate from TCR stimulation and be implicated in SRG3 suppression. Furthermore, analyses of cell type-specific E2A/HEB binding to the E84 element showed that the E2A/HEB complex mainly binds to the E84 element only in GC-sensitive immature T cells, which highly express SRG3, but not in mature T or non-lymphoid cells, which express low levels of SRG3 and are relatively resistant to GCs. Thus, it is proba-
ID3-mediated GC Desensitization through SRG3 Repression

ID3-mediated GC Desensitization through SRG3 Repression

...ble that the E2A/HEB complex plays a part in modulating GC sensitivity of various cell types by regulating the level of SRG3 expression through its binding activity toward the E box element in the SRG3 promoter. Taken together, these results suggest that SRG3 acts as a novel downstream target of E2A/HEB proteins and that ID3 enhances the viability of immature thymocytes and promotes their differentiation by at least rendering them resistant to GCs through SRG3 down-regulation.

Acknowledgment—We thank Dr. C. Murre for 16610D9 cell line, Id3/S-003 retroviral vector, pHBAneo-E47, and pHBACell.