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Peripheral T Cells Become Sensitive to Glucocorticoid- and Stress-Induced Apoptosis in Transgenic Mice Overexpressing SRG3

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Immature double-positive thymocytes are sensitive to glucocorticoid (GC)-induced apoptosis, whereas mature single-positive T cells are relatively resistant. Thymocytes seem to acquire resistance to GCs during differentiation into mature single-positive thymocytes. However, detailed knowledge concerning what determines the sensitivity of thymocytes to GCs and how GC sensitivity is regulated in thymocytes during development is lacking. We have previously reported that the murine SRG3 gene (for SWI3-related gene) is required for GC-induced apoptosis in a thymoma cell line. Herein, we provide results suggesting that the expression level of SRG3 protein determines the GC sensitivity of T cells in mice. SRG3 associates with the GC receptor in the thymus, but rarely in the periphery. Transgenic overexpression of the SRG3 protein in peripheral T cells induces the formation of the complex and renders the cells sensitive to GC-induced apoptosis. Our results also show that blocking the formation of the SRG3-GC receptor complex with a dominant negative mutant form of SRG3 decreases GC sensitivity in thymoma cells. In addition, mice overexpressing the SRG3 protein appear to be much more susceptible to stress-induced deletion of peripheral T cells than normal mice, which may result in an immunosuppressive state in an animal. The Journal of Immunology, 2001, 167: 805–810.

Glucocorticoids (GCs)4 are known to modulate the development, distribution, and function of immune cells. GCs are produced in the thymus as well as in the adrenal gland and play critical roles during thymocyte development (1, 2). Immature, double-positive thymocytes are sensitive to GC-induced apoptosis; in contrast, mature single-positive T cells are relatively resistant (3). Recently, expression of the intracellular domain of Notch1 was shown to inhibit GC-induced apoptosis in thymocytes (4). It appears that double-positive thymocytes acquire resistance to GCs during differentiation into mature single-positive thymocytes. However, it is not clear what factors determine the sensitivity of T cells to GCs and how GC sensitivity is regulated during T cell development. It was reported that DNA binding of the GC receptor (GR) is a prerequisite for GC-induced apoptosis in thymocytes. In GRtrans/dim mice, whose GR lost the ability to transactivate target genes through cooperative DNA binding, GC-induced apoptosis of thymocytes was clearly blocked (5). Therefore, regulation of the DNA-binding capability of GR appears to be important in controlling the GC sensitivity of developing thymocytes.

We have previously reported that GC-induced apoptosis requires the SRG3 protein in a thymoma cell line, S49.1 (6). SRG3, a mouse homologue of yeast SWI3 and human BAF155, was isolated as a gene expressed highly in immature thymocytes, but at basal level in mature T cells (6). It is a core component of the SWI-SNF protein complex, a chromatin-remodeling complex required for the regulation of transcriptional processes (7). However, we have found that the SRG3 protein may also exist as an independent form of the mouse SWI/SNF complex in GC-sensitive thymocytes (6). Interestingly, we found a correlation between the expression level of SRG3 and GC sensitivity. The protein is expressed at an approximately three times higher level in GC-sensitive thymocytes than in GC-resistant peripheral T cells. Furthermore, lowering the expression of the protein rendered the GC-sensitive S49.1 cells resistant (6). These results suggest that SRG3 may play a critical role in controlling GC-mediated apoptosis of developing thymocytes. Thus, to fully understand the nature of GC sensitivity of T cells, we investigated how the SRG3 protein controls GC sensitivity in T cells.

GCs are produced by activation of the hypothalamic-pituitary-adrenal (HPA) axis under stress conditions in animals (8). GCs in the periphery have been shown to exert multiple anti-inflammatory activities by regulating transcription factors involved in cytokine production, such as NF-kB and AP-1 (9, 10). The capabilities of GCs to suppress T cell proliferative responses to specific Ags as well as those to mitogens are also well documented (11). However, it is possible that GCs released by stress would induce apoptotic death of peripheral T cells under certain conditions. This would
also result in an immunosuppressive state in an animal. In this report we provide evidence for such a possibility.

Materials and Methods

Mice, cells, reagents, and Abs

FVB mice were supplied by B&K Universal (Sollentuna, Sweden) and maintained at the Institute of Molecular Biology and Genetics, Seoul National University (Seoul, Korea). The murine thymoma cell line S49.1 was maintained as previously described (6). Dexamethasone (DEX) and staurosporin were purchased from Sigma (St. Louis, MO). Biotin-conjugated H57.597, FITC-conjugated 53-6.72, PE-conjugated GK1.5, PE-conjugated streptavidin, quantum red-conjugated streptavidin, anti-Fas Ab (Jo2), and FITC-conjugated annexin V were purchased from Pharmingen (San Diego, CA). BuGr-2 (12) was purchased from Affinity Bioreagents (Golden, CO). Antiiserum against hBRG1, a human homologue of SW12, was donated by H. Kwon (Kyung Hee University, Seoul, Korea).

Transgenic (Tg) mice

Full-length cDNA of SRG3 was inserted into the Smal site of the VhCD2 expression vector (13) by blunt end ligation. The 15-kb KpnI/Ncol fragment containing the whole expression unit (hCD2 promoter, SRG3 cDNA, poly(A), and locus control region) was purified and microinjected into fertilized pronuclei of the FVB mouse. Transgene integration was determined by Southern blotting and PCR using primers (5'-GACTAGACCAAAAACTACCTC-3' and 5'-GTCAACTGGACGCTAGAT-3') with genomic DNA isolated from the tail.

Comimmunoprecipitation and immunoblotting

Thymocytes and lymph node (LN) cells from 4- to 6-wk-old mice or cultured cells were extracted and immunoprecipitated with BuGr-2 Ab as previously described (14). For immunoblot analysis, proteins were subjected to 7.5% SDS-PAGE, transferred to a nitrocellulose membrane, and immunoblotted with anti-hBRG1 antisera, anti-SRG3 antisera, BuGr-2, or anti-Xpress Ab. Specific bands were visualized by the ECL system (Pierce, Rockford, IL) and quantified by using Gel-Pro Analyzer software (Media Cybernetics, Silver Spring, MD).

Measurement of apoptosis and GC sensitivity

Mesenteric lymphocytes from hCD2-SRG3 Tg mice and littersate control mice were incubated at 1–3 × 10^6 cell/ml in RPMI 1640 medium, 10% FBS, and various concentrations of DEX. The cells were harvested at various time points and stained with CD45RC FITC and propidium iodide (PI). The 10,000–30,000 events were analyzed by flow cytometry. Two clones of S49.1 transfectants (no. 3 and 8) expressing the 89-aa fragment of SRG3 and vector transfectant were treated with the indicated concentrations of DEX at three time points and stained with annexin V-FITC and PI. Fas-mediated T cell apoptosis was performed as previously described (15). Freshly isolated mesenteric lymphocytes (2 × 10^6 cells/well) were treated with the indicated amounts of anti-Fas mAb (Jo2; Pharmingen) for 22 h in the presence of 30 µg/ml cycloheximide (Sigma) or were treated with the indicated concentrations of staurosporin (Sigma) for 12 h. The percentage of specific apoptosis was calculated by the formula previously reported (16).

In vitro translation of the 89-aa SRG3 fragment

The pcH260 plasmid was constructed by inserting a 260-bp Sac/Ilba fragment of the SRG3 (aa residues 854–922) into the pcDNA3.1/HisB vector containing the T7 promoter/primer site, CMV promoter, N-terminal polyhistidine Taq, anti-Xpress Ab epitope tag (DLYDDDDK), and the neomycin resistance gene (Invitrogen, San Diego, CA). In vitro translation of this construct was performed using the TNT T7 quick-coupled transcription/translation system (Promega, Madison, WI) and [35S]methionine (Amersham, Arlington Heights, IL) following the manufacturer’s protocol.

Luciferase assay

The two plasmid constructs used were pRcASRG3, expressing an antisense SRG3 transcript (6), and pcH260. The S49.1 cells were cotransfected with 5 µg pGRE-LUC, 1 µg RSV- luc2, along with 11 µg of one of the plasmids described above using DEAE-dextran sulfate as previously described (17). Transfected cells were incubated overnight in fresh medium and then treated with 10^{-6} M DEX for 24 h. Luciferase activity was assayed according to the manufacturer’s (Promega) protocol. β-Galactosidase activity was measured to normalize the transfection efficiencies.
the formation of SRG3-GR complex is dependent on the expression level of SRG3 protein in T cell. Overexpression of SRG3 slightly increased (1.2 ± 0.04 times compared with control) the expression of mBRG1 in thymocytes and peripheral T cells, a mouse homologue of SWI2, which is another core component of the SWI/SNF complex (Fig. 1B).

Peripheral T cells expressing Tg SRG3 become more sensitive to GC-induced apoptosis

We then examined whether the LN T cells, overexpressing SRG3 protein and now containing the SRG3-GR complex, became sensitive to GC-induced apoptosis. Cells from peripheral LNs of the Tg and littermate control mice were incubated in medium containing DEX. As shown in Fig. 2A, mature T cells become more sensitive to GC-induced apoptosis by increased expression of SRG3, whereas there were no significant differences in apoptosis produced by anti-Fas Ab or staurosporin treatment (Fig. 2, B and C). These results also suggest that the expression level of SRG3 is important for the formation of SRG3-GR and also in determining the sensitivity of T cells to GC-induced apoptosis.

Blocking the association of SRG3 with GR affects the GC-induced apoptosis

We have found that the SacI/XbaI fragment, an 89-aa fragment from aa 854–922 of SRG3, exhibits a dominant negative mutant effect against SRG3. The 89-aa fragment of SRG3 was in vitro translated using [35S]methionine. The product of the in vitro translation was confirmed by immunoprecipitation with SRG3 antisera (Fig. 3A, lane 1). The product was mixed with thymic extract and then immunoprecipitated with BuGR-2. As shown in Fig. 3A, the labeled 89-aa fragment of SRG3 was coimmunoprecipitated with GR (lane 2). This result indicates that the 89-aa fragment of SRG3 can form a protein complex with GR and suggests that the fragment may act as a dominant negative mutant of SRG3 by blocking the SRG3-GR association.

To test the above possibility, we examined whether this fragment would block GR-mediated transcription (Fig. 3B). The pGRE-LUC plasmid containing two GREs was transfected into S49.1 cells. When these cells were treated with DEX after transfection, luciferase activity showed up to an ∼30-fold increase compared with that of nontreated transfectants (Fig. 3, A and B). Cotransfection of this DNA together with the pRcASRG3 plasmid, expressing the antisense SRG3 gene under the control of a CMV promoter (6), blocked the enhancement of luciferase activity induced by DEX treatment (Fig. 3B, lane 2). When the pch260 plasmid DNA expressing the 89-aa fragment of SRG3 was co-transfected, the enhancement of luciferase activity by DEX treatment was also reduced (Fig. 3B, lane 3). These results suggest that SRG3 is required for GR-mediated transcription, and the 89-aa fragment can block this process.

To test whether the 89-aa fragment can disrupt the association between SRG3 and GR, pch260 DNA was stably transfected into GC-sensitive S49.1 cells. Two clones (3 and 8) expressing the 89-aa fragment at different levels were isolated by selection with G418. Western blot analysis with cell extracts showed that clone 8 expressed a significantly higher level (5 times higher) of the fragment compared with clone 3, whereas the expression levels of the SRG3 protein were similar (Fig. 4A). Using total cell extracts from

FIGURE 1. The SRG3-GR complex is formed in LN T cells from hCD2-SRG3 Tg mice. A, Schematic illustration of the hCD2-SRG3 transgene. B, Western blot analysis of SRG3 and mBRG1 with tissue extracts from WT and Tg mice. The mean ratios of intensity of bands from three experiments are as follows: 1 (WT-Thy):2 ± 0.12 (Tg-Thy):0.33 ± 0.02 (WT-LN):0.6 ± 0.06 (Tg-LN; SRG3, top) and 1 (WT-Thy):1.2 ± 0.05 (Tg-Thy):0.73 ± 0.06 (WT-LN):0.84 ± 0.09 (Tg-LN; BRG1, bottom). C, One milligram of total cell extracts of thymus (lane 1) and LNs (lanes 2 and 3) from WT or Tg mice was immunoprecipitated with the anti-GR mAb BuGR-2. The precipitates were analyzed by Western blot with anti-SRG3 antiserum (top) and BuGR-2 (bottom).

FIGURE 2. Increased GC-induced apoptosis in Tg LN cells. Mesenteric lymphocytes from hCD2-SRG3 Tg mice (■) and littermate control mice (□) were cultured in medium with the indicated concentrations of DEX (A), anti-Fas Ab (Jo2) and cycloheximide (B), or staurosporin (C). The cells were analyzed for apoptosis using flow cytometry at the indicated time points. To determine the amount of T cell apoptosis, cells were gated for the expression of αβTCR and analyzed for annexin V binding. The average percent specific apoptosis of cells from each mouse is indicated (SEM) on its value percentage (three separate experiments).
ENHANCED GC SENSITIVITY IN SRG3 Tg MICE

Peripheral T cells from the Tg mice are more sensitive to stress

It is well established that stress activates the HPA axis to produce GCs, and affects the homeostasis of various organs, especially the immune system (21). Because peripheral T cells from the Tg mice overexpressing SRG3 are more sensitive to GC-induced apoptosis, it is likely that these cells are more sensitive to stress. To test this possibility, 6- to 7-wk-old mice were subjected to RST, and total T cell numbers from the thymus and spleen of each mouse were counted using a Coulter counter and a flow cytometer. Total thymocyte numbers were reduced by about 18% in Tg mice (1.46 × 10^8) compared with littermate control mice (1.79 × 10^8). As the stress intensity increased, numbers in thymocytes decreased in a similar way in both Tg mice and littermate control mice (Fig. 5A). In the spleen, the total numbers of T cells were similar between Tg mice (3.84 × 10^7) and control littersmates (3.43 × 10^7) under normal conditions. However, after 1 day of the RST, the Tg mice showed a much greater reduction (~54.4%) in the total number of splenic T cells compared with littermate control mice (~23.3%). RST for 3 days resulted in a significantly greater reduction in Tg mice (~75.5%) compared with littermate controls (~41.5%; Fig. 5B). There was no significant difference in these results between male and female littermate mice. This reduction in the number of splenic T cells seems to be due to the death of cells induced by GCs released under stress conditions, which suggests that peripheral T cells overexpressing SRG3 are much more susceptible to stress-induced cell death.

Discussion

Thymocytes are exquisitely sensitive to GC-induced cell death, whereas resting peripheral T cells are comparatively resistant to it. However, it is not clear what causes the difference in the GC sensitivity between the two T cell populations and how the GC sensitivity is regulated during thymocyte development. Recently, it was shown that the activated form of Notch1 inhibited GC-induced apoptosis in thymocytes as well as in thymic lymphoma and T cell hybridoma lines (4). Bcl-2, an anti-apoptotic protein, is known to play a protective role against GC-induced apoptosis and is up-regulated by the expression of the activated form of Notch1. However, in a T cell hybridoma line the expression of the activated form of Notch1 induced resistance to GC-induced apoptosis without an up-regulation of Bcl-2 (4), indicating that some other protein(s) besides Bcl-2 may also be involved in regulating the GC sensitivity of thymocytes. We have previously shown that SRG3 is required for apoptosis induced by GCs in the S49.1 cell line (6). The protein is expressed highly in thymocytes, but at a low level in peripheral T cells. We have also found that the activated form of Notch1 down-regulates SRG3 expression by repressing its promoter activity.5

In this study we investigated whether SRG3 is involved in the regulation of GC sensitivity in vivo as well as in vitro. We provided evidence that the level of SRG3 expression is important for formation of the SRG3-GR complex and that the level of this complex is crucial in determining the GC sensitivity of T cells. Expression of a dominant negative form of SRG3 fragment efficiently blocked the formation of the complex and, correspondingly, reduced GC-mediated apoptosis in S49.1 cells. This complex appears to be required for the GR-mediated transcription, because the same SRG3 fragment blocked the GR-mediated reporter gene transcription. We have also found that the SRG3-GR complex binds to the GRE sequence motif when analyzed by chromatin immunoprecipitation assay (22) and gel mobility supershift assay (data not shown). Therefore, it is likely that the complex containing SRG3 and GR binds to GRE sequences on the promoter of a target gene(s) to induce GC-induced apoptosis in thymocytes.

Previously, it was reported that SWI/SNF protein complex is involved in GR-mediated transcriptional regulation as a chromatin-remodeling factor (7, 23). The rat GR, when expressed in yeast, requires SWI/SNF proteins for transcriptional activation of GR-responsive genes, and GR-SWI13 complexes were coimmunoprecipitated in yeast extract (22). In a human breast cancer cell line, interaction between the GR and SWI/SNF complex was detected in a ligand-dependent manner (14). It was also recently reported that the SWI/SNF complex could potentiate the activity of GR through r1 in both yeast and mammalian cells (24). However, we have previously found that SRG3 protein exists as an independent form of the mouse SWI/SNF complex as well as a subunit of the complex in GC-sensitive thymocytes (6). We also found that some SRG3-GR complexes do not contain the mBRG1 protein in the thymus (manuscript in preparation). These results suggest that the SRG3 protein may function as an independent form of the SWI/SNF complex in controlling the GC sensitivity of thymocytes.

Our results also showed that thymocytes contained GR associated with SRG3 even without pretreatment of exogenous GCs. This SRG3-GR complex was detected to a much lesser extent in peripheral T cells than in thymocytes. It is possible that endogenous GCs produced in the thymus may induce SRG3-GR complex formation. However, Tg overexpression of SRG3 resulted in the formation of the complex in LN T cells where such complexes are hardly detectable under normal conditions (Fig. 1C). In addition, when we expressed SRG3 cDNA in the NIH-3T3 cell line under control of a viral promoter, we could detect the SRG3-GR complex (data not shown). These results suggest that the formation of the SRG3-GR complex is dependent on the expression level of SRG3 in a cell, and GC may not be a prerequisite for it. However, SRG3 and GR may not interact directly, because both yeast two-hybrid assay and coimmunoprecipitation with in vitro translated products of the two proteins failed (data not shown).

Tg overexpression of SRG3 results in formation of the SRG3-GR complex and renders the GC-resistant LN T cells more sensitive to GCs. However, Tg LN T cells were still less sensitive to GC-induced apoptosis than normal thymocytes. Tg LN T cells showed ~20% specific apoptosis when treated with 10^{-8} M DEX for 12 h (Fig. 4A), whereas normal thymocytes showed about 40% specific apoptosis under the same conditions (data not shown). This discrepancy in GC sensitivity between the two populations may be due to a limited expression of SRG3 (see Fig. 1B) in Tg peripheral T cells. LN T cells from the Tg mice expressed SRG3 at half the level of normal thymocytes. We were not able to produce Tg mice with LN cells expressing SRG3 at the level of normal thymocytes. It seems that overexpression of SRG3 protein cause deleterious effects on cells, because overexpression of SRG3 in the EL-4 cell line disrupted normal progression of the cell cycle, resulting in aneuploidy and slow growth (data not shown). The discrepancy in GC sensitivity may also be explained by a protective mechanism provided by Bcl-2 expressed in mature T cells (25–27). It is also possible that some other factors may be necessary for functional SRG3-GR complex formation, and these may be limited in peripheral T cells.

Elevated GC sensitivity of peripheral T cells from hCD2-SRG3 Tg mice allowed us to investigate the effects of stress on the immune system. Stress is a cognitive stimulus that activates the HPA

![FIGURE 4](http://www.jimmunol.org/) Inhibition of the SRG3-GR complex formation reduces GC-induced apoptosis. The pcH260 plasmid, which expresses the 89-aa fragment of SRG3 tagged with anti-Xpress Ab epitope, was transfected into S49.1 cells by electroporation. Two clones (lanes 3 and 8) expressing the fragment were isolated. A, Cell extracts were analyzed by immunoblotting with anti-Xpress Ab and anti-SRG3 antiserum. The endogenous SRG3 expression was not altered significantly in these cell lines. B, The extracts from the cells transfected with vector alone, clone 3, and clone 8 were immunoprecipitated with BuGR-2 Ab, and then immunoblotted with anti-SRG3 antiserum. The SRG3-GR association was reduced to 86% of that in transfectants containing only the vector in clone 3 and to 42% of the control value in clone 8. C, The cells were treated with the indicated concentrations of DEX at three time points and stained with annexin V-FITC and PI. Three independent experiments were performed. * Vector transfectant; □, clone 3; △, clone 8.

![FIGURE 5](http://www.jimmunol.org/) Effects of RST on thymocyte and splenic T cell death from hCD2-SRG3 Tg mice. Littermate control (○) and hCD2-SRG3 Tg (■) mice were restrained for 16 h with one to three cycles (17). Single-cell suspensions of thymocytes (A) and splenocytes (B) were prepared from these animals and also from nonstressed littermate control animals (n = 3–17). Average total T cell counts are shown (±SE). Control, Nonstressed group; RST1d, group restrained for 16 h with one cycle; RST2d, group with two cycles; RST3d, group with three cycles.
axis, which leads to the secretion of GCs from the adrenal cortex (8). Because the expression level of SRG3 is important in GC-mediated responses, we speculated that the increased expression of SRG3 might change the immunomodulatory effects of GCs. Under stress conditions, splenic T cells were much more efficiently removed in Tg mice than in littermate control mice. These results imply that under conditions expressing SRG3 at levels higher than normal, T cells would become more susceptible to apoptosis by GCs released by stress. The expression level of the SRG3 protein is not regulated by GCs itself (data not shown). It appears to be strictly regulated along the developmental stages of thymocytes, and there is a possibility that this regulation may be disturbed by factors such as aging, pathological agents, and other related factors. Therefore, our results suggest a novel possibility of stress effects on the immune system, that is, an immunosuppression due to GC-induced apoptosis of peripheral T cells.

References