Some evidence has shown that TCR activation of Ras/MEK/ERK pathway plays a critical role in the inhibition of glucocorticoid (GC)-induced apoptosis. We have previously demonstrated the significant correlation between the expression level of SRG3 and the GC sensitivity of developing thymocytes. In this study, we examined the effect of TCR/CD3 signaling on the expression of the SRG3 gene using murine immature thymoma cell lines. TCR/CD3 signaling resulted in a dramatic decrease in SRG3 expression. Specifically, TCR/CD3 downregulation of the SRG3 gene was mediated by Ras/MEK/ERK and PI3K, but not Ral.GDS pathway. We also found that binding of E47/HEB complex to the E-box element in the SRG3 minimal promoter was inhibited by Id3 inhibitor of E proteins. Finally, introduction of mutations into the E-box element in the SRG3 promoter completely abrogated the TCR/Ras responsiveness of the SRG3 promoter.

G109 Notch1 confers a Resistance to Glucocorticoid Developing Thymocytes by Down-regulating SRG3 Expression

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We have previously reported that SRG3 is required for the glucocorticoid-induced apoptosis in the S49.1 thymoma cell line. Activation of Notch1 was shown to induce glucocorticoid resistance in thymocytes. However, the specific downstream target of Notch1 conferring thymocytes glucocorticoid resistance is currently unknown. We found that the expression level of SRG3 was critical in determining glucocorticoid sensitivity in developing thymocytes. The expression of SRG3 was also downregulated by the activated form of Notch1 (NotchIC). The promoter activity of the SRG3 gene was also downregulated by NotchIC. Expression of transgenic SRG3 resulted in the restoration of glucocorticoid sensitivity in thymocytes expressing transgenic Notch1. These results suggest that SRG3 is the downstream target of Notch1 in regulating glucocorticoid sensitivity of thymocytes.

G110 Peripheral T Cells Become Sensitive to Glucocorticoids- and Stress-induced Apoptosis in Transgenic Mice Overexpressing SRG3

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Immature double positive thymocytes are sensitive to glucocorticoid-induced apoptosis, while mature single positive T cells are relatively resistant. Thymocytes seem to acquire resistance to glucocorticoids during differentiation into mature single positive thymocytes. However, detailed knowledge concerning what determines the sensitivity of thymocytes to glucocorticoids and how glucocorticoid-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 the glucocorticoid-induced apoptosis in a thymoma cell line. Herein, we provide results suggesting that the expression level of SRG3 protein determines the glucocorticoid-sensitivity of T cells in mice. SRG3 associates with the GR 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 to become sensitive to glucocorticoid-induced apoptosis. Our results also show that blocking the formation of the SRG3-glucocorticoid receptor complex with a dominant negative mutant form of SRG3 decreases glucocorticoid-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.

G801 Screening of ligand for Ly-6A.2 antigen, a stem cell marker protein

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Ly-6A.2 is involved in activation, development of lymphocytes and differentiation of hematopoietic stem cells. The experimental results from the Ly-6A.2 cross-linking with antibody and the suppression of Ly-6A.2 expression with specific antisense oligonucleotides suggested that Ly-6A.2 is a lymphocyte costimulation molecule. These results are conflict with the result that Ly-6A.2 deficient mouse T cells have hyperproliferative response to some mitogenic stimuli. Ly-6A.2 ligand may be one of key molecule to understand the function of Ly-6A.2 in lymphocyte activation and hematopoiesis, but biological and biochemical information are very limited. To identify and characterize Ly-6A.2 ligand, we generated a cell line, A15 which secreting recombinant Ly-6A.2-hIgG protein. The Ly-6A.2-hIgG recombinant protein was purified from the culture media of SP2/0-Ag14 transfectant using affinity chromatography. We tried to immunoprecipitate Ly-6A.2 ligand using Ly-6A.2-hIgG recombinant protein from cell extracts of RAW264.7 and isolated a candidate protein (100 kDa), which share N-terminal first 10 amino acid sequence with Ly-6A.2 antigens.

G802 Protein Modification in the Activated Macrophage: Nitration and S-nitrosylation

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Nitric oxide (NO) can regulate the protein function with the modification of the tyrosine residue (nitration) and cysteine residue (S-nitrosylation). Although the some nitrated and S-nitrosylated proteins were reported and studied in the activated macrophage, the systematic functional analysis for nitration and S-nitrosylation were not tried so far. In this study, we analyse systematically these protein modification in the LPS and/or SNAP (NO donor) treated murine macrophage cell line RAW264.7 using several techniques: 2 dimentional gel elecrophoresis, in vitro nitrosylation assay, in vitro nitration assay and immunoprecipitation. We detected the several increased and decreased protein spots in the LPS stimulated and SNAP treated macrophage in the comparison with control RAW264.7 cells. We also analyse the protein modification with phosphorylation in the LPS stimulated and SNAP treated RAW264.7 cells. In the Western analysis after immunoprecipitation with 4G10, anti-phosphotyrosine antibodies, we could not detect the significant difference between control and stimulated RAW264.7 cells. The detailed results will be discussed.