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

Yong Chan Kim, Hayyoung Lee[1], kwang Il Kang[1], Sang-Gi, Paik[2], and Young Sang Kim
Department of Biochemistry, [2]Biology, [1]Institute of Biotechnology, College of Natural Sciences, Chungnam National University, Daejeon 305-764, Korea

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

Kum Jin Yang*, kwang Il Kang[1], Hayyoung Lee[1], Young Sang Kim[2], and Sang-Gi Paik
Department of Biology. [2]Biochemistry, [1]Institute of Biotechnology, College of Natural Sciences, Chungnam National University, Daejeon 305-764, Korea

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.