Ly-1 遺伝子のクローニングとそのタンパク構造について***

Molecular Cloning of Murine Lymphocyte Differentiation Antigen Ly-1
and Its Predicted Structure

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T細胞のみならず一部のB細胞にも表現されているLy-1は、リンフォカインリセプターとの関連でも注目されている分子である。筆者らは、そのLy-1をコードする遺伝子をクローニングし、分子遺伝学的背景からLy-1遺伝子の性状を解析した。

索引用語: Ly-1, DNA, IL-2, Leu-1

Key words: Ly-1, DNA, IL-2, Leu-1

Ly-1, a glycoprotein with a molecular weight of 67kDa, is expressed on murine thymocytes, mature T cells and a functionally distinct subpopulation of B cells named as Ly-1 B. Antibodies against Ly-1 molecules can augment mitogen or alloantigen induced lymphocyte proliferation suggesting a possible role for Ly-1 in regulating T cell proliferation. The enhancing effect of anti Ly-1 antibodies on T cell proliferation is associated with the increased secretion and expression of IL-2 and IL-2 receptor, respectively.

To further our understanding of the structure and function of Ly-1 and its differential expression on different subpopulations of lymphocyte, we undertook molecular cloning of the Ly-1 gene. Originally, we planned to isolate the human Ly-1 homologue, Leu-1 gene by a method as "Alu repeated sequence rescue". While this work was in progress, however, a

putative human Leu-1 cDNA clone was isolated by N. Jones in J. Strominger laboratory. Leu-1 amplified genomic transfectants we generated helped to confirm that this putative Leu-1 cDNA clone in fact partially encoded Leu-1 protein. Using this human Leu-1 cDNA as a probe, we isolated several mouse Ly-1 cDNA candidates including one of 1.4 kb length. Northern and southern analysis providing the evidence that this clone contains Ly-1 cDNA insert. It hybridized specifically with RNA from mouse thymocytes, Ly-1 B cell line NFS-5.3 and Ly-1 amplified transfectants. It also hybridized intensely with DNA from the Ly-1 amplified transfectants, but less intensely with that from mouse L cell. With the aid of this clone, we isolated a Ly-1 cDNA clone, MD-10, which contains a cDNA insert of 2.1 kb. It was proved to be full-length by DNA sequencing and transfection of Ly-1 into L cells with this clone. The fact that a molecule of 67 kDa in molecular weight can be immunoprecipitated from MD-10 cDNA transfectant by anti Ly-1 antibodies further confirmed MD-10

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being full-length.

Analysis of the predicted amino acid sequence indicated that the Ly-1 polypeptide is synthesized with a 23-amino acid leader and that the mature protein consists of an extracellular region of 347 amino acids, a transmembrane sequence of 30 residues and a cytoplasmic region of 94 amino acid. The extracellular region appears to be divided into two subregions by a threonine and proline rich sequence of 23 amino acids (amino acid 112-134) which is highly conserved between Ly-1 and Leu-1 in position and amino acid composition. The first extracellular subregion (amino acid 1-111) is predicted to be arranged in a beta-pleated sheet structure of 6 strands. The predicted secondary structure of this subregion and identities of certain conserved residues among most members of the immunoglobulin supergene family suggest that Ly-1 is (distant) members of this family. The significant homology in sequence between the second part (amino acid 243-347) of the second extracellular subregion and the first extracellular subregion suggests that these two sequences arose by a gene duplication event. Thus, it appears that the extracellular region of Ly-1 molecule is a

structure of three domains which are amino acid 1-111, amino acid 135-242 and amino acid 243-347. The entire extracellular region is rich in cysteine with all of its 22 cysteine residues conserved between Ly-1 and Leu-1. The cytoplasmic region has no cysteine.

Ly-1 and Leu-1 are 63% identical with a gradient of identical residues of 43% for the first extracellular domain to 52% for the second extracellular domain, 66% for the third extracellular domain and 90% for the cytoplasmic region. The strong homology of the cytoplasmic region of Ly-1 and Leu-1 suggests a functional importance of this region. We noted that there is a potential tyrosine phosphorylation site at amino acid position 429. There are also two potential threonine phosphorylation sites at positions 410 and 453. Having suggested that Ly-1 acts as a receptor in regulating T cell proliferation, and given that phosphorylation of tyrosine and threonine/serine may play important physiological roles for several growth factor receptors, we are examining whether anti Ly-1 antibody, which may mimic the ligand of Ly-1, could cause phosphorylation of cytoplasmic region of Ly-1.