53. *Molecular Structure of Interleukin 6 Receptor*^{†)}

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Interleukin 6 (IL-6/BSF-2) is a multifunctional cytokine produced by both lymphoid and nonlymphoid cells.¹⁾ It is established that IL-6 has many biological functions, which include growth and differentiation activities on B cells,¹⁾⁻⁵⁾ T cells,⁶⁾⁻⁸⁾ myeloma-plasmacytomas,⁹⁾⁻¹⁰⁾ hepatocytes,^{11),12)} hematopoietic stem cells,¹³⁾ and nerve cells.¹⁴⁾

To elucidate how one cytokine can mediate multiple functions, the structure of their receptor molecules must be determined. However, the low number of cytokine receptors on target cells (10^2 to 10^3 per cell) makes their isolation and characterization difficult. This study reports the cloning of the cDNA for IL-6 receptor (IL-6-R) utilizing a high efficiency COS7 cell expression system with the CDM8 vector.¹⁵⁾ The expressed receptors were detected with biotinylated recombinant IL-6 (B-rIL-6) and fluorescein conjugated avidin (FITC-A). The IL-6-R consists of 468 amino acids, including a signal peptide of ~ 19 amino acids and a domain of ~ 90 amino acids that is similar to a domain in the immunoglobulin (Ig) superfamily. The cytoplasmic domain of ~ 82 amino acids lacks a tyrosine-kinase domain, unlike other growth factor receptors.

A cDNA library was made from poly(A)⁺ RNA of a human NK-like cell line, YT. Plasmid DNA was transfected into monkey COS7 cells, the cells expressing IL-6-R were stained with B-rIL-6 and FITC-A and positively sorted out by fluorescein-activated cell sorter (FACS), resulting in the identification of a candidate plasmid clone, pBSF2R.236. It was found that more than 10% of pBSF2R.236-transfected murine COP cells expressed IL-6-R as measured by B-rIL-6 binding. The binding of B-rIL-6 was competitively inhibited by excess amounts of rIL-6 but not rIL-1 β or rIL-2.

In order to characterize IL-6-R encoded by pBSF2R.236 insert cDNA, a stable transfectant, JBSF2R expressing IL-6-R was established from a human T cell line, Jurkat. Scatchard plot analysis demonstrated that there are two classes of IL-6-R: a high-affinity binding site with a K_d value in the order of 10^{-11} M and a low-affinity binding site with a K_d value in the order of 10^{-9} M. The myeloma cell line, U266 was also demonstrated to have two classes of IL-6-R with approximately the same order of K_d values as compared to those expressed in JBSF2R transfectant cells. The results indicate that pBSF2R.236 cDNA has

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Fig. 1. a: Deduced amino acid sequence of the insert cDNAs of pBSF2R.236. Numbers at the left margin of the sequence show positions of amino acids. The asterisks, potential N-glycosylation sites (Asn-X-Ser/Thr); underline region, a presumed signal peptide; the box, a presumed transmembrane domain; the circles, cysteine residues. b: Alignment of IL-6-R domain to Ig superfamily protein domains. IL-6-R sequence was aligned to several proteins of Ig superfamily by inspection. Alignment of Ig superfamily protein domains is based on the data by Williams.¹⁷⁾ * represents conserved patterns common to the V, C1 and C2 sets; \$, common to the V and C2 sets; #, common to the C1 and C2 sets.¹⁷⁾ The known locations of β-strands in Ig V domains are marked with bars and capital letters above the bars. The numbers above the alignment represent positions of amino acids of IL-6-R sequence. The position of the putative disulfide bridge within IL-6-R domain is indicated by S---S.

a potential to express both high- and low-affinity binding sites.

Northern blot analysis showed that pBSF2R.236 cDNA hybridized to a single species of mRNA of approximately 5,000 nucleotides long extracted from the YT cell line. Similar length IL-6-R mRNA was also detected in RNA extracts of the myeloma cell line (U266), the histiocytic leukemia cell line (U937), and the Epstein-Barr virus transformed B cell line (CESS). However, the T cell line (Jurkat) and the Burkitt's lymphoma cell line (BL29) were found to be devoid of mRNA hybridizable with pBSF2R.236 cDNA. The results were in complete accordance with those obtained by Scatchard analysis.¹⁶⁾

The deduced amino acid sequence and its hydropathy plot analysis demonstrated that IL-6-R consists of 468 amino acids including a signal peptide (residues 1 and 19) and a putative transmembrane domain (residues 359 to 386) (Fig. 1a). There are six potential N-linked glycosylation sites (Asn-X-Ser/Thr). The sequence of IL-6-R was compared with proteins at the National Biomedical Research Foundation database and Genetic Sequence Data Bank (Blot Beranek and Newman, Inc., Los Alamos National Laboratory); homologies were found with several members of the Ig superfamily, including Ig light chain variable (V) region, rabbit poly-Ig receptor, CD4 molecule and alpha-1-B-glycoprotein. Furthermore, inspection of the IL-6-R sequence shows that the region spanning

between position ~ 20 and ~ 110 fulfills the criteria proposed by Williams for the constant 2 (C2) set of Ig superfamily¹⁷⁾ as shown in Fig. 1b. The C2 set includes several adhesion molecules, platelet derived growth factor (PDGF)-R, colony stimulating factor-1 (CSF-1)-R, Fc γ R and alpha-1-B-glycoprotein.¹⁷⁾ This is of particular interest since receptors for polypeptide growth factors, such as PDGF, CSF-1 and IL-6 could then be grouped in the C2 set.

It is interesting to note that IL-6-R does not have a tyrosine kinase domain unlike certain other growth factors, although IL-6 has been found to be a potent growth factor for myeloma/plasmacytoma cells.^{9),10)} The mechanism(s) of its signal transduction for growth and/or differentiation could be mediated through a biochemical pathway as yet to be discovered.

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