Soluble Cytokine Receptors as Immunomodulators

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Introduction

During the past few years it has become clear that many membrane proteins can be found in soluble forms in body fluids. Examples include histocompatibility antigens [1] and Fc receptors [2]. The extracellular portion of the membrane protein may be released by proteolysis, or by phospholipase action in the case of those molecules that are linked to the membrane by a phosphoinositol linkage. Alternatively, the soluble protein may be encoded by an alternatively spliced messenger ribonucleic acid (mRNA) species. Frequently the soluble, extracellular portion of the protein retains the same ligand-binding properties as the membrane-bound form.

Cytokine receptors also show the same behavior. Soluble receptors that retain ligand-binding properties have been found in urine and serum for the interleukin-2 (IL-2) receptor-alpha [3], tumor necrosis factor (TNF) receptor (two forms) [4], IL-6 receptor [5], gamma-interferon (γ-IFN) receptor [5], growth hormone receptor [6], and nerve growth factor receptor [7]. Recently we have identified alternatively spliced mRNA species that encode soluble forms of the IL-4 and IL-7 receptors [8, 9]. Here we discuss these results and describe how soluble cytokine receptors, either naturally occurring forms or generated by recombinant deoxyribonucleic acid (DNA) manipulation, can be used as immunomodulatory agents, both in vitro or in vivo.

Cytokine Receptor Families

Elucidation of the primary amino acid sequences of many cytokine receptors as a result of cDNA cloning has allowed the grouping of these receptors into families, based on similarities in their extracellular, ligand-binding domains. For those receptors whose ligands regulate hematopoiesis and immunity, three families have emerged. The first family is the well-known and very large immunoglobulin superfamily, most of whose members are not cytokine receptors. However, the receptors for IL-1, colony stimulating factor-1 (CSF-1), and PDGF belong to this group, with three, five, and five immunoglobulin-like domains respectively in their extracellular portions [10, 11]. CSF-1 and PDGF receptors have intracellular tyrosine kinase domains, the IL-1 receptor does not.

The second, more recently recognized, family consists solely of cytokine receptors. We have designated this as the hematopoietin receptor family as almost all of these receptors mediate effects on hematopoietic cells [12]. The members of this family currently consist of the receptors for IL-2 (β subunit) [13, 14], IL-3 [15], IL-4 [8, 12], IL-6 [16, 17], IL-7 [9], GM-CSF [18, 19], G-CSF [20], erythropoietin [21], prolactin (two forms of receptor) [22, 23], and growth hormone [6]. The common sequence element in the extracellular domains of these receptors is a stretch of about 200 amino acids that

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shows considerable sequence conservation between the different members of the family [8, 18, 24, 25]. When these sequences are compared using the ALIGN program to generate pairwise scores that measure the degree of amino acid sequence relatedness, scores are mostly in the range of 3–12 [24]. Any score greater than 3 is considered to indicate significant sequence relatedness [10].

Within these 200 amino acids there are certain features that show particular conservation (see Fig. 1). These include the positions of four N-terminal cysteines (although many family members have additional nonconserved cysteines) and a WSXWS motif located at the C-terminus of the conserved region, usually just outside the transmembrane domain. The C-terminal 90–100 amino acids of the conserved region show significant homology to type III fibronectin domains [26], and the G-CSF receptor is so far unique in having three additional fibronectin-like domains between the conserved region and the transmembrane domain [19, 20]. It can be speculated that the fibronectin-like domains play a role in interaction of the growth factor receptors with extracellular matrix components or other cell surface proteins.

The IL-3 receptor has a duplication of the 200 amino acid conserved region [15], and the IL-6 and G-CSF receptors have N-terminal immunoglobulin-like domains [16, 19, 20], showing that receptors can belong to more than one family.

In contrast to the striking degree of sequence relatedness between the extracellular domains of the receptors, the

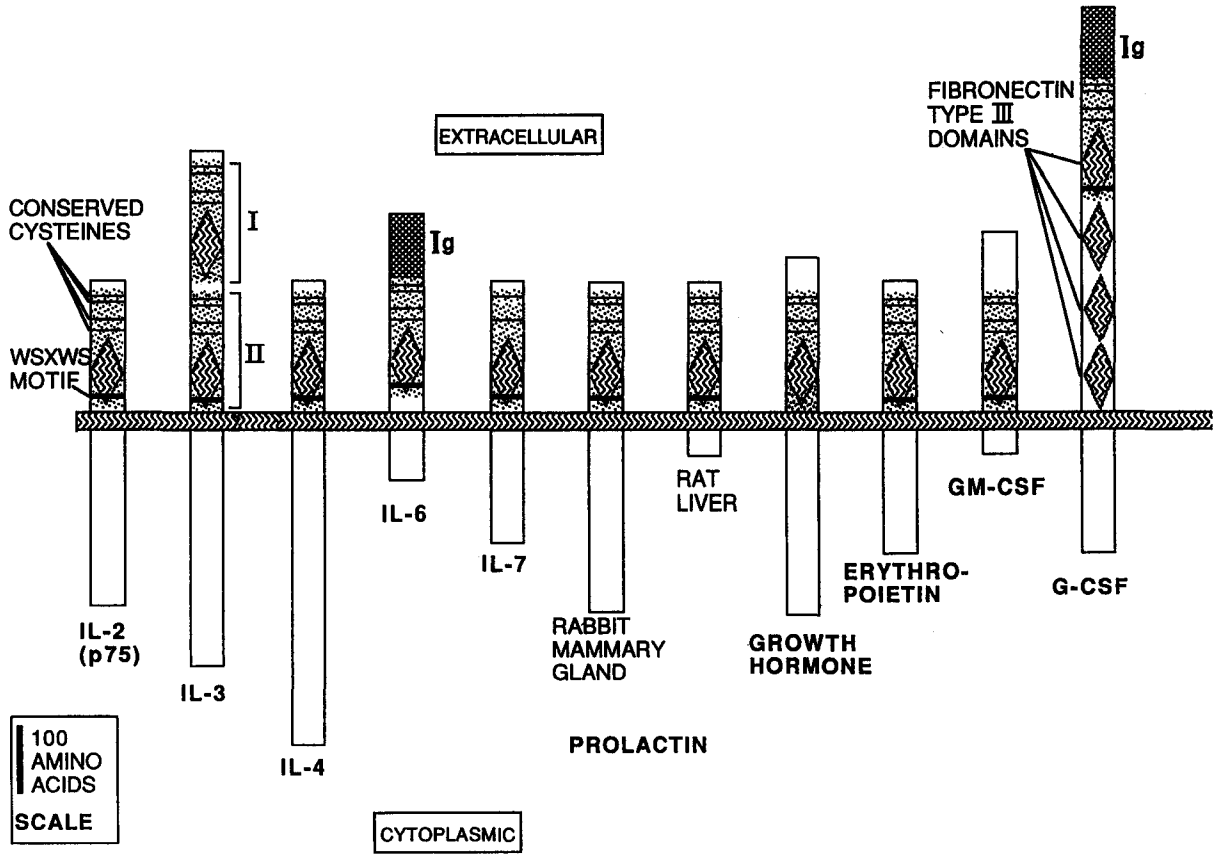

Fig. 1. The hematopoietin receptor superfamily. Schematic representations of the structures of all known members of the family are shown. *Thin horizontal bars* represent conserved cysteine residues. *Thick horizontal bars* represent the conserved Trp-Ser-X-Trp-Ser motif (WSXWS). Fibronectin type III domains are shown as *diamond shapes*, and the stretch of 200 amino acids characteristic of these receptors is *shaded*. The immunoglobulin-like domains at the N-termini of the IL-6 and G-CSF receptors are also indicated

cytoplasmic sequences show little if any similarity apart from a general tendency towards a high content of serine, proline, and acidic amino acids. This reflects our current ignorance as to the mechanisms of signal transduction by these receptors.

The third family centers around the newly cloned TNF receptors p80 and p60 [27–29]. Both these molecules have a cysteine-rich, extracellular, ligand-binding domain that can be subdivided into four internally homologous subdomains. Other members of the family sharing this structure are the nerve growth factor receptor [30]. CD40, a cell surface protein involved in B cell activation [31], 4-1BB, characterized as a mRNA species induced upon T cell activation [32], and OX40, a membrane protein present on rat $CD4^+$ T cells that can contribute to T cell proliferation [33]. The last three proteins may well be cytokine receptors with unknown ligands.

An additional member of this family, with particularly strong homology to TNF receptor p80, is the T2 open reading frame from Shope fibroma virus, a rabbit pox virus [34]. The predicted protein sequence has characteristics of a secreted TNF receptor, and we have shown that the T2 ORF can be expressed in mammalian cells. The protein is secreted and binds TNF [35]. It seems likely that the virus has acquired a rabbit TNF receptor during evolution and that it expresses a soluble TNF receptor as a defense against the portion of the host's immune response mediated by TNF.

Once again, the members of the TNF receptor family show little or no sequence relatedness in their cytoplasmic domains, nor do the IL-1 and TNF receptors, despite the fact that IL-1 and TNF share many biological activities.

Soluble Cytokine Receptors

The existence of soluble extracellular domains of cytokine receptors that retain their ligand-binding capabilities suggested that such molecules might be able to block interaction of their cognate ligands with cell surface receptors. This might have a normal immunoregulatory role in vivo, or could be exploited pharmacologically to down-modulate undesirable immune reactions, such as allergy, autoimmunity, or graft rejection.

In order to test this hypothesis, we have expressed soluble murine IL-1 and IL-4 receptors in mammalian cells and purified the recombinant proteins by affinity chromatography. The soluble IL-1 receptor was generated by inserting a translation termination codon immediately 5′ to the transmembrane domain [36], and the soluble IL-4 receptor used a cDNA from a naturally occurring, alternatively spliced mRNA species [8]. The purified receptors were tested for their ability to block specifically the biological activities of their respective ligands. IL-1 and IL-4 can each stimulate B cell proliferation when anti-immunoglobulin is used as a co-mitogen. IL-1 mediated B cell proliferation was completely inhibited by soluble IL-1 receptor, whereas soluble IL-4 receptor had no effect. Conversely, IL-4 mediated B cell proliferation was inhibited by soluble IL-4 receptor but not by soluble IL-1 receptor [37]. These results demonstrate not only that soluble IL-1 and IL-4 receptors have highly specific neutralizing capacity, but also that IL-1 and IL-4 mediate B cell proliferation by independent pathways.

Following the demonstration of in vitro biological activity, the soluble receptors were tested in vivo in two models that involve lymphocyte activation in response to alloantigenic challenge [38, 39]. In the first, Balb/c mice were injected in the footpad with irradiated allogeneic spleen cells from C57BL/6 mice. Over the course of 7 days there was a host-versus-graft response leading to lymphoproliferation and consequent swelling of the draining popliteal lymph nodes. The strength of this reaction could be quantitated by excision and weighing of the lymph nodes. As a control, each mouse was injected in the contralateral footpad with an equal number of syngeneic,

irradiated spleen cells, so that the specific response could be measured as the weight of the lymph nodes draining the site of allogeneic cell injection minus the weight of the lymph nodes draining the site of syngeneic spleen cell injection. Daily injections of soluble IL-1 receptor or soluble IL-4 receptor could completely block the lymphoproliferative response. Injections were given intraperitoneally or subcutaneously for 4 days, using mouse serum albumin as a negative control. As little as 100 ng–1 μg per dose of receptor showed significant inhibition, and the optimum time to commence treatment was 1 day prior to challenge with the allogeneic spleen cells [38, 39]. In each case, the inhibitory effect of the soluble receptor could be reversed by its cognate ligand.

In a second model system, hearts from newborn C57BL/6 mice were grafted into ear pinnae of Balb/c mice. The hearts continued to beat until rejected by the hosts at around 12 days after transplantation. Daily administration of soluble IL-1 receptor or soluble IL-4 receptor for 4–6 days, starting on the day of transplantation, significantly prolonged graft survival [38, 39].

These data implicate both IL-1 and IL-4 as being important in the initiation of an immune response to alloantigenic challenge in vivo, and suggest that both soluble receptors may be clinically useful in preventing graft rejection. Based on the known biological activities of IL-1 and IL-4, it might be predicted that the soluble receptors would be of therapeutic value in other disease states. IL-1 has many pro-inflammatory properties; examples include induction of prostaglandin release, stimulation of cartilage breakdown, and induction of cytokines with chemotactic activity for neutrophils and monocytes. Soluble IL-1 receptor might be a useful anti-inflammatory agent in diseases such as rheumatoid arthritis. IL-4 promotes synthesis of IgE by an isotype class-switching mechanism in B cells and is a growth factor for mast cells in conjunction with IL-3. It is thought to be a central mediator of allergic responses and consequently soluble IL-4 receptor may have therapeutic value in controlling allergy. The demonstrated efficacy of soluble cytokine receptors as immunomodulators opens up possibilities for clinical intervention in many disease states, and this promises to be an area of active investigation.

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