MINIREVIEW

Interleukin-6–Type Cytokines *In Vivo:* Regulated Bioavailability[1] (44055)

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Abstract. **Investigators have traditionally thought of the class of inflammation- and injury-associated cytokines in large part as "free" entities in the peripheral circulation. In the case of interleukin-6 (IL-6), the cytokine can be found in blood in complexes of molecular mass 400–500, 150–200, and 25–35 kDa in association with binding proteins that can include soluble IL-6 receptor (sIL-6R), anti–IL-6, and anti–sIL-6R IgG, and others. Sustained high levels of different particular IL-6 complexes are observed in the human circulation in cancer patients subjected to particular active anticancer immunotherapy regimens. In the "chaperoned" state, circulating IL-6 complexes display differential immunoreactivity in different ELISAs and possess differential biological activity as assayed *ex vivo.* The discovery of "chaperoned" circulating IL-6 in humans points to a new level of modulation of cytokine function, that of regulated bioavailability of IL-6 *in vivo.*** [P.S.E.B.M. 1996, Vol 213]

In the last decade, there has been impressive progress in the isolation and characterization of numerous cytokines that affect cellular functions in well-defined ways. A great deal of effort has gone into the molecular cloning of cytokine genes, investigating the regulation of cytokine gene expression, and characterizing the various cytokine gene products. At the same time, a great deal of effort has gone into investigating how these cytokines elicit their biological effects at the cellular level—great progress has been made in terms of identifying cell-surface receptors for cytokines, delineating the biochemical mechanisms involved in cytokine-receptor-triggered signal transduction and in elucidating the consequent molecular events involved at the transcriptional and post-transcriptional levels that lead to the cytokine-regulated expression of appropriate cellular genes. Nevertheless, there remains a critical gap between investigations of cytokine genes and their expression on the one hand, and investigations of the response of target cells and tissues to cytokines on the other: How do cytokines get from where they are induced to where they act *in vivo?* In many instances, the primary characteristic of cytokine action is local or "paracrine" and therefore this question is not that pressing. However, in the case of a cytokine such as interleukin-6 (IL-6), whose primary function is "long-distance" communication between local sites of infection or other "injury" and various systemic recovery mechanisms, this question is of critical importance (1–4). With the realization that "soluble" receptors for many cytokines are a regular presence in the peripheral circulation, the role of these and other circulating cytokine "binding" or "chaperone" proteins in modulating cytokine function *in vivo* requires attention (reviewed in Ref. 4). The unexpected and paradoxical enhancement of cytokine function *in*

[1] This manuscript is an update of a previously published minireview entitled, "Interleukin-6 in infection and cancer," Proc Soc Exp Biol Med **195:**183–191, 1990.

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Research in the author's laboratory described in this article was supported by Research Grant AI-16262 from the National Institute of Health and a contract from The National Foundation for Cancer Research.

0037-9727/96/2133-0238$10.50/0

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vivo (for example of IL-3, IL-4, IL-6, IL-7) by anti-cytokine antibodies that are otherwise "neutralizing" antibodies in cell culture assays (5–7), and the discovery of high-molecular mass circulating complexes of IL-6 in association with other "chaperone" proteins (7–9) in the last few years highlight this area of research. This minireview is focused on questions that deal with how cytokines, the IL-6–type cytokines in particular, produced at one site travel *via* the circulation and act on tissues and cells at another site (Fig. 1). From a physiological standpoint, these considerations define a new level of regulation of cytokine function, that of regulated bioavailability and of regulated tissue targeting *in vivo*.

The importance of this discussion, as well as its relevance to cytokines other than the IL-6–type cytokines, is illustrated by comments found throughout the immunological literature today. Proposals to use soluble receptors, decoy receptors, cytokine antagonists, receptor antagonist, neutralizing antibodies, and chimeric antibody/receptor molecules to interrupt or modulate cytokine function *in vivo* surface with regularity (7, 10–13). This immunological literature serves as a backdrop to the issues under discussion here: How is a cytokine brought from where it is induced to where it acts *in vivo* in the face of circulating binding proteins? In the case of IL-6, at a minimum, high levels of the cytokine-binding soluble IL-6 receptor α chain (sIL-6R; 15–150 ng/ml in serum) and the soluble gp130 β chain of the receptor (sgp130; 300–400 ng/ml in serum) are always present in the circulation (14, 15). Therefore, free IL-6 can, at best, have only a transient existence in the human blood stream. Figure 1 defines the focus of the issues under discussion in this review

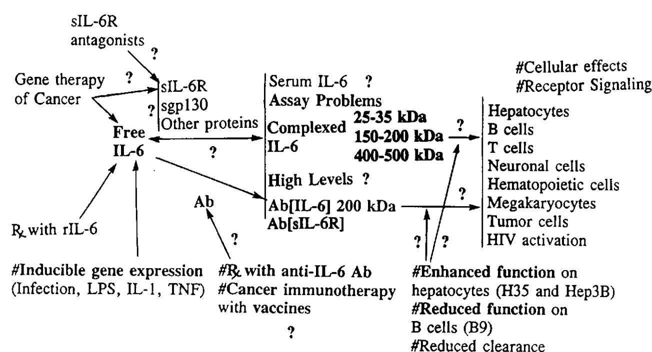

Figure 1. Modulation of IL-6 function: regulated bioavailability *in vivo*. IL-6 produced by cells in response to infection and inflammation-associated cytokines must travel through the blood stream in order to reach its target tissues. In the blood stream IL-6 encounters various binding proteins such as soluble IL-6R α and β receptors, antibodies, and others, which then modulate the ability of circulating IL-6 to gain access to its target tissues. Therapeutic intervention such as active immunotherapy with vaccines and administration of anti–IL-6 antibodies and of anti-IL-6 "antagonists" lead to altered bioavailability of circulating IL-6. Question marks in the figure refer to incompletely understood questions.

and some of the biological and clinical implications involved. The present discussion concerns the question of the regulated bioavailability of endogenously produced cytokines *in vivo*.

A separate question arises in patients administered exogenous cytokines. Are there clinical situations wherein the further administration of a particular cytokine is futile because these patients have a large excess of "binding proteins" and/or a large excess of that particular cytokine already in their circulation for other reasons? The identification of a class of patients (cancer patients subjected to active immunization protocols as part of specific immunotherapy of their cancer with vaccines) wherein the "chaperoning" of IL-6 leads to sustained high levels of circulating cytokine (up to 600 ng/ml) over several months and even years illustrates this phenomenon in a clinical setting of unusually broad therapeutic significance (9).

Considerations of cytokine-binding proteins in the peripheral circulation such as soluble receptors, anti-cytokine antibodies, and other cytokine-binding proteins raise critical questions about the ability of conventional cytokine assays (bioassays or immunoassays) to measure the true concentrations of cytokines in particular body fluids. These considerations become pivotal in reliably evaluating the diagnostic and prognostic significance of measurements of cytokine levels in human body fluids. With respect to IL-6, the occurrence of this cytokine in the peripheral circulation in high molecular mass complexes with other proteins imposes limitations on the numerical reliability of relevant clinical data (8, 9).

The underlying theme throughout this discussion is the following question: How does IL-6 exist and function in the human body?

Background: IL-6–type Cytokines and Their Receptors

Interleukin-6 is the designation for a cellular secreted protein that was originally referred to as β_2-interferon (16, 17). Biological activities attributable to IL-6 include stimulation of hepatocytes (hepatocyte stimulating factor [HSF]) to elicit major changes in the acute phase plasma protein secretion by the liver (18–20), stimulation of B cells to differentiate into immunoglobulin (Ig)-secreting plasma cells (21, 22), plasmacytoma or hybridoma growth factor activity (23, 24), cytotoxic T-cell activation (25–27), induction of myeloid differentiation (28), augmentation of natural killer cell activity (29), stimulation of keratinocyte proliferation (30), inhibition of proliferation of breast carcinoma cell lines (31, 32) with an increase in motility of these cells (32), enhancement of α1-antitrypsin secretion by monocytes (33), induction of complement factor C3 and B in human fibroblasts (34), enhancement

of differentiation of multipotent hematopoietic stem cells (35), induction of neurite outgrowth in PC-12 cells (36), and stimulation of adrenocorticotrophin release from the anterior pituitary (37).

In 1986 the single human IL-6 gene was assigned to chromosome 7p21 (38). Also in 1986, the human IL-6 mRNA sequence was independently reported by several investigators (39–42). The deduced amino acid sequence for human IL-6 contains an open reading frame of 212 amino acids. The mature IL-6 is secreted as 183– to 185–amino acid polypeptides with different N termini, polypeptides which are, in turn, subjected to differential *N*- and *O*-glycosylation and serine phosphorylation in a cell-type–specific manner leading to the secretion of proteins of molecular mass from 19 to 30 kDa from different cell types (43–45). The human IL-6 gene is transcriptionally inducible in numerous different cell-types in response to a variety of noxious agents that include other inflammation-associated cytokines such as tumor necrosis factor (TNF) and interleukin-1 (IL-1), acute virus infections, bacterial products such as endotoxin, and various second messenger agonists (46–49).

The human IL-6 promoter has been dissected in detail and mapped within it are two partially overlapping regions termed multiple response element I (MRE I) and MRE II in the region from −140 to −175 that represent the inducible enhancers responsive to a wide variety of cytokines, viruses, and second messengers (46–69). MRE II includes the C/EBP-binding site. Additionally, an NF-κB site that acts synergistically with the C/EBP element was also defined (reviewed in Refs. 48 and 49). A c-*fos* serum-response element–like region (MRE I) and a retinoblastoma protein (Rb)-control element–like (RCE) region has been identified in the IL-6 promoter (49). Indeed both Rb and p53, another "tumor supressor" gene product, can repress the IL-6 promoter (50–52). The overall conclusion derived from these molecular promoter dissection studies is that a combination of transcription factors (e.g., C/EBPβ and NF-κB p65) is the most efficient in the activation of the IL-6 promoter (53). Furthermore, there is strong cell-type–dependent variation in the particular transcription factors involved (e.g. IL-6 induction in monocytic cell lines appears to be more dependent on NF-κB than that in HeLa cells).

The mechanisms by which glucocorticoids and estrogens inhibit IL-6 gene expression have also been investigated in some detail (53). The glucocorticoid receptor (GR) binds across the MRE enhancer, the major TATA-box, and the major RNA start site (Inr site) in the human IL-6 promoter providing a basis for "occlusion" of the functional elements of the promoter (49). Additionally, and perhaps more importantly, GR also functionally represses the transcription factors p65 NF-κB and C/EBPβ by direct protein-protein interactions (53, 54). The estrogen receptor (ER) does not bind IL-6 DNA directly but appears to inhibit this promoter through inhibitory functional interactions with p65 NF-κB and C/EBPβ (alias NF-IL6) (55, 56).

The IL-6 cell surface receptor is a complex consisting of an α-chain (the gp80 or IL-6R) that binds the ligand and a β-chain consisting of the signal transducer protein gp130 (57–59). Signal transduction is thought to involve dimerization of the gp130 β-chain (3, 60). Soluble IL-6R (sIL-6R; approximately 55–60 kDa) and soluble gp130 (sgp130; approximately 95 kDa) are present in the peripheral circulation in humans at concentrations in the range of 15–150 ng/ml for sIL-6R and 300–400 ng/ml for sgp130 (14, 15). Given this insight, even in normal individuals "free" IL-6 at best can have only a transient existence in the circulation. IL-6 function is enhanced by association with sIL-6R (59, 60), but may then be inhibited when the IL-6/sIL-6R complex binds sgp130 (15). It is now firmly established that, from a functional standpoint, IL-6 first associates with its binding protein, IL-6R, with the latter either in the soluble sIL-6R form or as the cell membrane-bound α-chain (3, 58, 59). It is the formation of the latter dimer complex that then generates a transmembrane signal. It is the presentation of a complex of IL-6 with a binding protein to the β-chain of the cell-membrane receptor that generates a cellular response. Thus, it is well established from cell culture studies that the minimal functional unit of IL-6 is the binary complex of IL-6 with sIL-6R. Cells lacking the IL-6Rα-chain functionally respond to the soluble binary complex of IL-6/sIL-6R (the gp130 β-chain is ubiquitously present on cells). It is the presentation of IL-6 as a complex with a binding protein (sIL-6R) that converts cells (certain hepatoma lines and myeloid lines) unresponsive to "free" IL-6 into responders (58, 61).

It is now clear that IL-6 is only one of several apparently "unrelated" cytokines that possess the "four-α-helix bundle" structure that exert their biological effects *via* activation of the same signal transducing β-chain in their cell membrane receptor (the gp130 molecule). The cytokines IL-6, leukemia inhibitory factor (LIF), oncostatin M (OM), interleukin-11 (IL-11), ciliary neurotrophic factor (CNTF), and cardiotrophin-1 (CT-1) all use the gp130 β-chain as part of their signal transducing receptor complex (Table I) (3,

Table I. Interleukin-6–Type Cytokines

Interleukin-6 (IL-6)
Interleukin-11 (IL-11)
Leukemia inhibitory factor (LIF)
Oncostatin M (OM)
Ciliary neurotrophic factor (CNTF)
Cardiotrophin-1 (CT-1)

Note. Cell-surface receptor contains common gp130 β-chain.

4). As an approximation, the cell-surface receptor for each of these cytokines includes distinct α-chain components that determine the high-affinity access of the ligand to the signal transducing chain. It is this group of cytokines to which the collective term IL-6–type cytokines is applied (Table I). The ability of many of these cytokines to stimulate acute phase plasma protein synthesis in appropriate hepatoma cell lines in a qualitatively similar manner is a property ascribable to the fact that their receptors contain the same gp130 signal transducing chain. From among the IL-6–type cytokines listed in Table I, IL-6 serves as the major systemic long-distance circulating cytokine that alerts the body to the presence of tissue injury or damage. Additionally, in contrast to sIL-6R, sCNTFR-α and sIL-11R each of which can enhance the biological activity of the respective cytokine ligand, sLIFR-α inhibits the biological activity of LIF (reviewed in Refs. 3 and 4).

There has been spectacular progress in the dissection of the intracellular signal transduction pathways activated by IL-6–type cytokines through the gp130 chain of the receptor. Several Tyr-kinases (Jak-1, Jak-2, Tyk-2) and STAT protein family members (STAT-1, STAT-3, STAT-5) that function in the IL-6–type cytokine triggered signal transduction pathways in different cell types have been characterized (reviewed in Refs. 3, 60, 62, and 63). The generality of these signaling events is emphasized by the observations that several of the IL-6–type cytokines as well as other unrelated cytokines such as the interferons use the same or similar pathways in different cells (62).

Detailed descriptions of the structure and function of IL-6–type cytokines, their receptors, and the signal transduction pathways triggered by these ligand-receptor interactions can be obtained from recent reviews (1–4).

In the last 3–4 years, two separate lines of research have led to paradoxical observations that have served to highlight the modulation of cytokine function, in particular that of IL-6, when the cytokine is present in the peripheral circulating in association with binding proteins. The first was the discovery in preclinical and clinical studies that "neutralizing" antibodies can paradoxically enhance the biological function of particular cytokines, including IL-6, *in vivo*. The second was the recognition that IL-6 can exist in the peripheral circulation in humans at high concentrations (in the 10–1000 ng/ml range) in various complexes (400–500, 150–200, and 25–35 kDa) in association with binding proteins that then mask or alter IL-6 immunoreactivity in particular enzyme-linked immunosorbent assays (ELISAs) and alter IL-6 biological activity. This phenomenon is particularly dramatic in cancer patients who have been subjected to various active immunization protocols with cancer vaccines (see below).

"Neutralizing" Antibodies Chaperone IL-6 in Preclinical Models: The *in Vivo* Paradox

Shortly after the development of "neutralizing" murine monoclonal antibody (mAb) to human IL-6 and rat mAb to murine IL-6, various investigators attempted to use these reagents in preclinical models of infection or other diseases in the baboon (for anti–human IL-6 mAb) and in the mouse (for anti–murine IL-6 mAb) (5, 64, 65). In each experiment wherein the test animals had been challenged with endotoxin, bacterial infection (e.g., Listeria monocytogenes), cytokines such as TNF, or implantation of a transplantable tumor, accompanied by administration of anti–IL-6 mAb a paradoxical result was observed. Irrespective of whether the anti–IL-6 mAb did or did not inhibit a particular disease manifestation, in all such experiments anti–IL-6 mAb paradoxically markedly enhanced the levels of circulating IL-6 levels as assayed in various ELISAs after appropriate dilution of the serum/plasma or as assayed in the B9 hybridoma growth assay (Table II). The ability to inhibit an *in vivo* disease manifestation in such models was dependent upon the particular stoichiometry of the anti–IL-6 mAb used in relationship to IL-6 (see below).

Inhibition of clearance of IL-6 by anti–IL-6 mAb has been suggested as a mechanism for the paradoxical enhancement of circulating IL-6 levels by "neutralizing" antibodies *in vivo* (7). However, this cannot be the dominant explanation because the paradoxical enhancement of circulating IL-6 levels can be observed within 30 min of instilling preformed complexes of IL-6 and anti–IL-6 intraperitoneally in mice (5). Induction of endogenous IL-6 production in such animals by IL-6/anti–IL-6 complexes can be excluded by showing that when complexes of human IL-6 with anti–IL-6 mAb are instilled intraperitoneally, it is exclusively human IL-6 that is found in the murine circulation (5).

Biologically, stoichiometric ratios of IL-6 to anti–

Table II. Effect of Preinjection of Anti–IL-6 mAb on Serum IL-6 and Fibrinogen Levels in Mice Administered TNF

Experimental group	Inducer	mAb	IL-6 (B9 U/ml) at 13–14 hr	Fibrinogen (mg/dl) at 13–14 hr (mean ± SD)
1	Saline	—	<4	138 ± 43
2	Saline	GL113	4	229 ± 33
3	Saline	20F3	13	156 ± 20
4	TNF	—	5	350 ± 62
5	TNF	GL113	3	362 ± 11
6	TNF	20F3	9115	131 ± 5[a]

Note. Anti–MuIL-6 mAb (20F3) or control mAb (GL113), 600 μg/mouse administered ip before the inducers; TNF, 5 μg/mouse administered ip. (From Ref. 5.)

[a] $P < 0.02$ comparing Group 5 and 6 using the Student's t test and analysis of variance.

IL-6 mAb in the range of 1:1 to 1:5 enhance the *in vivo* biological function of IL-6, whereas ratios in the range of 1:125 in favor of anti–IL-6 mAb are needed to observe *in vivo* inhibition of IL-6 function (e.g., inhibition of IL-6 induction of elevated fibrinogen levels) even though the levels of *circulating* IL-6 as measured in *ex vivo* assays are elevated at stoichiometric ratios of IL-6 to anti–IL-6 mAb in the entire range, from 1:1 to 1:125 (Fig. 2), suggesting functional sequestration of IL-6 in the intravascular compartment (5). Selective sequestration of IL-6 by mAb is suggested even in cell culture by the observation that not all biological functions of IL-6 are affected equivalently by particular "neutralizing" mAb: a mixture of IL-6 at different molar ratios of anti–IL-6 mAb can inhibit IL-6 function in the B9 hybridoma growth assay but may not do so in the hepatocyte stimulation assay (5). Thus, the manner in which IL-6 is presented to different target tissues from within the context of a complex with a particular anti–IL-6 mAb can modulate IL-6 function.

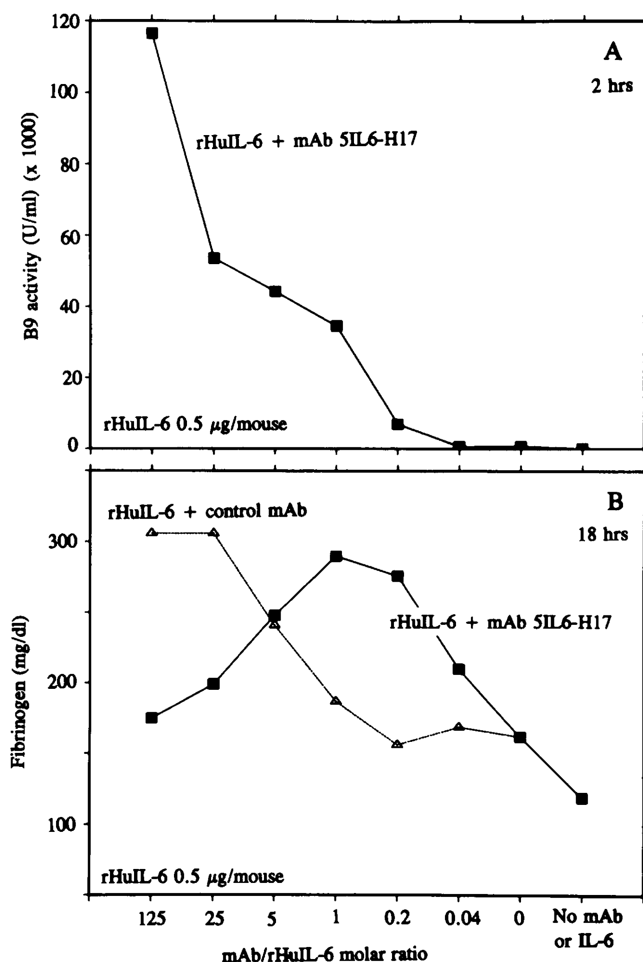

Figure 2. Consequences of administering rHuIL-6/anti–HuIL-6 mAb complexes ip into mice. Mice received varying amounts (different molar ratios) of anti–HuIL-6 mAb (5IL6-H17) or a control mAb mixed with a constant amount (0.5 μg) of rHuIL-6. (A) Circulating IL-6 titers as measured in the B9 bioassay at 2 hr after administering the IL-6/anti–IL-6 complexes. (B) Fibrinogen levels in serum at 18 hr after administering either the IL-6/anti–IL-6 complexes (■) or the IL-6/control mAb (△) mixtures. At 18 hr, the IL-6 tiers (B9 bioassay in U/ml) in sera of mice given IL-6/anti–IL-6 complexes (molar ratio in parentheses) were 7634 (125), 9713 (25), 2734 (5), 175 (1), 18 (0.2), and 8 (0.04); the corresponding titers in animals administered rHuIL-6 and control mAb were 9 (125), <4 (25), < 4 (5), <4 (5), <4 (0.2), <4 (0.04), <4 (0), and <4 (no mAb or IL-6). (From Ref. 5.)

Biochemically, circulating IL-6 in preclinical animal models in which anti–IL-6 mAb has been administered is of molecular mass 150–200 kDa as determined by Sephadex G-200 gel filtration chromatography (5). That this IL-6 is present in a physical complex with anti–IL-6 mAb can be verified in ELISAs that specifically detect IL-6 bound to IgG1 (the injected mAb) (5). Additionally, the 150- to 200-kDa IL-6 complexes can include sIL-6R (5). When present in this 150- to 200-kDa complex with anti–IL-6 mAb, the IL-6 can register differently in different ELISAs, even to the extent that the IL-6 can be completely masked. From a technical standpoint, extensive dilution of the plasma and the use of several different ELISAs based upon different anti–IL-6 mAb pairs may be necessary to detect these 150- to 200-kDa IL-6 complexes.

"Neutralizing" mAb Chaperone IL-6 in Clinical Testing: The *in Vivo* Paradox

Shortly after the development of "neutralizing" murine mAb to human IL-6 and the recognition that at least in some patients with multiple myeloma the tumor cells are driven to proliferate as a result of the endogenous production of IL-6 (whether paracrine or autocrine remains controversial), murine anti-human IL-6 mAb was administered to patients with multiple myeloma (7). The clinical outcome in these trials can be summarized as follows: there was an initial decrease in serum levels of C-reactive protein (CRP) (a marker for IL-6 activity *in vivo*), and a decrease in the mitotic index in myeloma cells which lasted several weeks. However despite continued therapy, the myeloma cells resumed their proliferation and the disease progressed.

A dramatic observation in these patients was that following administration of the anti–IL-6 mAb, there was a marked increase in the levels of IL-6 in the circulation (7). By gel filtration analysis, the bulk of this IL-6 was observed to be in complexes of molecular mass 150–200 kDa consistent with what would be expected for IL-6/anti–IL-6 IgG complexes. The IL-6 in these circulating complexes was biologically active in the B9 hybridoma growth assay (7). Murine anti–IL-6 mAb has also been used in a patient with plasma cell leukemia and one with Castleman's diseases with similar results (66, 67).

IL-6 Complexes in Human Plasma/Sera

Purification of human serum-derived IL-6 from patients with systemic infection by immunoaffinity

chromatography and the characterization of the purified IL-6 species by Western blotting under reducing denaturing conditions revealed that this cytokine, in serum, consisted of differentially modified polypeptides in the molecular mass range of 19–28 kDa (68). This was consistent with an early determination using the B9 hybridoma growth factor assay for IL-6, that serum IL-6 B9 bioactivity eluted at approximately 25 kDa off a Sephadex gel-filtration column (69).

The first hint that native IL-6 in human plasma/serum occurred in high–molecular mass complexes in normal volunteers and other patients came from experiments in which plasma/serum fractionated through a Sephadex G-200 gel filtration column was assayed using several different ELISAs for human IL-6. Each of these ELISAs (Ig61/5IL6 and 4IL6/5IL6 or 7IL6/5IL6) was a two-mAb sandwich ELISA in which the same biotinylated "readout" mAb ("5IL6-H17") was used in combination with different "capture" mAbs ("Ig61," "4IL6-H12," or "7IL6-H11") (8, 9). As with the earlier description of B9-bioactive IL-6 fractionating at 25 kDa, the Ig61 and 7IL6 ELISAs displayed an IL-6 peak at approximately 25 kDa (Fig. 3). In contrast, the "4IL6" mAb-based ELISA displayed the high molecular mass IL-6 complexes 400–500 and 150–200 kDa in size (Fig. 3). The 400- to 500- and 150- to 200-kDa IL-6 complexes from sera from normal, infected, or psoriatic patients were devoid of B9 bioactivity or reactivity in the Ig61 ELISA (8).

The identification of polypeptides in the 400- to 500- and 150- to 200-kDa as IL-6 was verified by further immunoaffinity purification of these proteins through a 5IL6-H17 anti-IL-6 mAb column and characterization of the purified proteins as IL-6 by (i) unmasking of B9 bioactivity, (ii) Western blotting and (iii) amino-terminal amino acid sequencing (8). The data suggested that the bulk of IL-6 in blood may be present in complexes with other proteins, which then masked IL-6 immunoreactivity in particular ELISAs and in particular bioassays. Proteins "operationally" associated with IL-6 (i.e., those that co-purified with IL-6 through anti-IL-6 mAb immunoaffinity chromatography) included sIL-6R, complement C3c and C4b (γ-chain), and fragments of C-reactive protein (8).

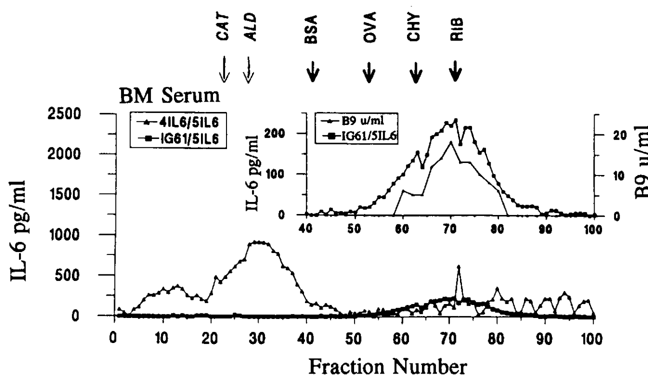

Figure 3. Sephadex G-200 column fractionation of IL-6 in serum. A 0.8-ml aliquot of serum from a bone marrow transplant patient was fractionated through Sephadex G-200, and each of the eluted fractions was analyzed for IL-6 content in the 4IL6/5IL6 (▲) and Ig61/5IL6 (■) ELISAs and the B9 hybridoma growth factor assay (●). Each of these assays was calibrated using the 88/514 *Escherichia coli*–derived IL-6 interim reference standard. The inset shows a comparison of the B9 bioassay and the Ig61/5IL6 ELISA. BM, bone marrow; CAT, chloramphenicol acetyltransferase; ALD, adolase; BSA, bovine serum albumin; OVA, ovalbumin; CHY, chymotrypsin; RIB, ribonuclease A. (From Ref. 8.)

Very High Sustained Levels of Endogenous 200-kDa IL-6 Complexes in Cancer Patients

An unexpected observation helped place high–molecular mass IL-6 complexes in an unusual biological context—that of immunotherapy of human cancer. During the course of a Phase 1 trial of IL-6 in cancer patients ostensibly for its platelet enhancing properties immediately following marrow-toxic chemotherapy, it was observed that melanoma patients who had been previously administered active specific immunotherapy for their cancer (consisting of initial immunization with the anti–anti-idiotypic mAb MK2-23 that carries the internal image of a high–molecular weight melanoma-specific antigen coupled to keyhole limpet hemocyanin [KLH] together with a preparation of Bacillus Calmette Guerin (BCG) as adjuvant followed by repeated boosters with MK2-23 coupled to KLH) had levels of circulating IL-6 in the 100- to 1000-ng/ml range, whereas other cancer patients had IL-6 levels in the 20- to 200-pg/ml range (9). The marked elevation of IL-6 levels in MK2-23–treated melanoma patients was observed using either the 4IL6 or the 7IL6 ELISAs for IL-6, although the former assay provided estimates approximately 10-fold higher than the latter (9). An evaluation of serum IL-6 levels in these melanoma patients during the course of their immunotherapy showed that there occurred a marked increase in serum IL-6 levels coincident with the immunization—the more aggressive the immunization, the higher the IL-6 levels (Fig. 4). The high levels of circulating IL-6 were sustained for over several months and even years (Fig. 4); newer data indicate that these high levels can persist for over 2–3 years after cessation of the MK2-23 immunotherapy. Although these patients displayed elevations in serum C-reactive protein levels that were correlated with increased serum IL-6 levels, these patients remained normothermic indicating the absence of an overt "acute phase" response.

Serum IL-6 in melanoma patients administered MK2-23 was biologically inactive in the B9 hybridoma growth assay (8). G-200 Sephadex-gel filtration studies showed that the circulating IL-6 in these MK2-23–treated melanoma patients was of molecular mass 150–200 kDa, which was detectable using either the 4IL6 and the 7IL6 ELISAs (Fig. 5). This IL-6 could be pu-

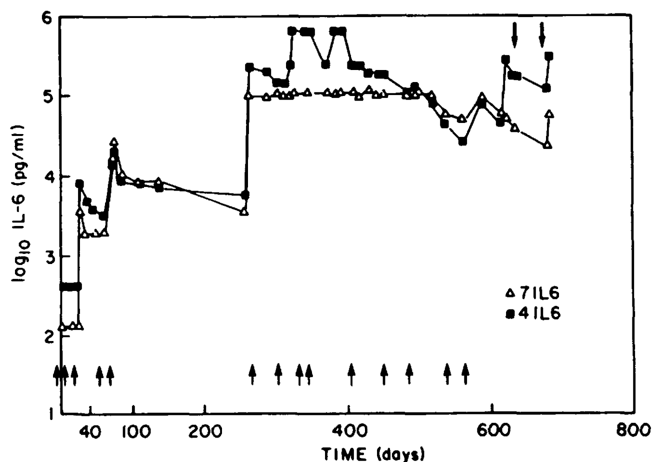

Figure 4. Longitudinal study of IL-6 levels in plasma samples from a patient (JS) during active specific immunotherapy. Upward pointing arrows represent the time of injection of (mAb MK2-23 + KLH + BCG). Downward pointing arrows represent time of subcutaneous injection of rIL-6 (5 μg/kg body mass). Measurements of IL-6 by 4IL6/5IL6 EILSA (■) and 7IL6/5IL6 ELISA (△) are illustrated. (From ref. 9.)

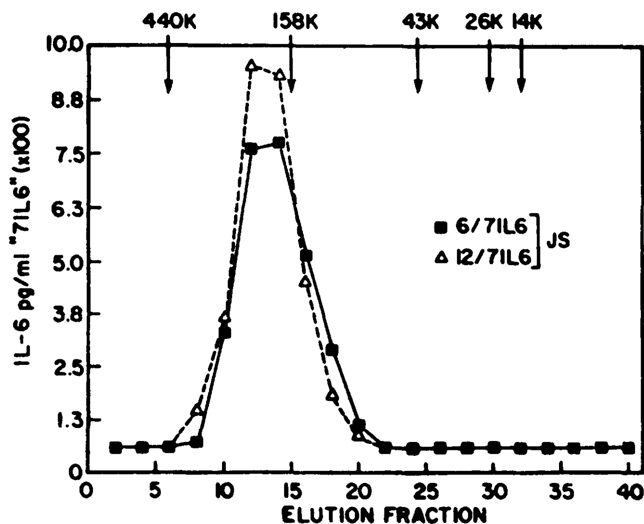

Figure 5. Sephadex G-200 column fractionation of plasma IL-6 from a patient (JS) with "high" endogenous levels of IL-6 before (6/7IL6; ■) and 30 min after (12/7IL6; △) subcutaneous injection of rIL-6. A 0.5-ml sample of plasma was fractionated through Sephadex G-200 column and each of the eluted fractions was analyzed for IL-6 content in the 7IL6/5IL6 ELISA. (From Ref. 9.)

rified by immunoaffinity chromatography on an 5IL6-H17 mAb column and displayed by Western blotting (8). While the 200-kDa IL-6 complexes contained sIL-6R, the latter was not a basis for the generation of the 200-kDa IL-6 complexes because (i) sIL-6R was present in insufficient amounts to stoichiometrically contribute to the formation of the complex and (ii) sIL-6R levels in serum did not increase during the course of immunotherapy commensurate with the 100- to 1000-fold increase in 200-kDa IL-6 levels (unpublished data). Evidence has been obtained recently for the presence of anti–IL-6 IgG1 and anti–sIL-6R IgG1 in these 200-kDa IL-6 complexes. The available data suggest that the 200-kDa IL-6 complexes observed in melanoma patients subjected to the MK2-23 regimen consist of IL-6, sIL-6R, anti–IL-6 IgG, and anti–sIL-6R IgG in appropriate combinations in a manner reminiscent of the 200-kDa complexes in animals and patients administered anti–IL-6 mAb.

It is noteworthy that the 200-kDa IL-6, which reacts in both the 4L6 and the 7IL6 ELISAs in melanoma patients subjected to immunization using an aggressive polyclonal B cell adjuvant regimen (BCG and KLH), is devoid of B9 hybridoma growth bioactivity. We hypothesize that these 200-kDa IL-6 complexes result from production of auto anti–IL-6 Ig and of auto anti–IL-6R Ig as a result of repeated aggressive polyclonal B cell stimulation by adjuvants such as BCG and KLH.

Bendtsen and colleagues reported the detection of polyclonal anti–IL-6 IgG in sera of a small proportion of patients with rheumatoid arthritis and other collagen diseases. In these instances the endogenous anti–IL-6 IgG was detected by its ability (i) to interfere with a particular ELISA for IL-6 and (ii) to immunoprecipitate ^{125}I-IL-6 (70, 71). Complexes of IL-6 with sIL-6R have also been detected in sera of patients with juvenile rheumatoid arthritis using an ELISA that uses an anti–IL-6 mAb for "capture" and an anti–sIL-6R mAb for "readout" (72).

The detection of sustained high levels of circulating 200-kDa IL-6 complexes in melanoma patients subjected to active specific immunotherapy with MK2-23 mAb (plus BCG plus KLH) prompted two questions: (i) is the appearance of high levels of "chaperoned" IL-6 in serum a general underlying event during the course of active immunotherapy for cancer (using other regimens for melanoma and for other cancers), and (ii) is there a relationship between development of high serum IL-6 levels and clinical outcome of the immunotherapy? The second of these questions subsumes within it a discussion of the function of such IL-6 complexes *in vivo* as well as in biological assays carried out *ex vivo*.

An Additional Level of Regulation of IL-6 Function by Binding Proteins in Cancer Patients

The characterization of circulating IL-6 in cancer patients subjected to active immunotherapy other than anti-idiotypic mAb (plus BCG and KLH) described above revealed yet further complexity. Cancer patients (melanoma, renal cancer, lung cancer) who had been vaccinated previously with an autologous anticancer preparation (AAAP) (prepared from their tumor tissue obtained at surgery) constituted a group of patients who also displayed sustained high levels of circulating IL-6 levels (median 10.6 ng/ml; range 0.02–158.9 ng/ml; n = 14) compared with cancer patients not subjected to immunotherapy (median 0.05 ng/ml;

range 0.02–15.1 ng/ml; $n = 19$) (data based upon the 7IL6 ELISA) (73). When IL-6 from AAAP-treated patients was characterized by Sephadex G-200 gel filtration chromatography, two major peaks of mass 400–500 and 30 kDa were observed using the 7IL6 ELISA. Neither the 400- to 500- nor the 30-kDa IL-6 from these patients was immunoreactive in the 4IL6 ELISA for IL-6 depicted in Figures 3 and 4. Both the 400- to 500- and 30-kDa 7IL6 ELISA–reactive and 4IL6 ELISA–nonreactive IL-6 displayed biological activity in the B9 hybridoma growth assay and in the Hep3B hepatocyte stimulation assay. In contrast to patients treated with the anti-idiotypic Ab MK2-23 protocol, no 200-kDa IL-6 complexes were detectable using these four assays for IL-6 (two ELISAs and two bioassays) in sera of patients administered only AAAP as immunotherapy (unpublished data).

Despite the presence of circulating IL-6 that displayed biological activity in *ex vivo* assays, these AAAP-treated patients were normothermic indicating the absence of an overt acute phase response.

A small group of cancer patients were identified who had been subjected to MK2-23 therapy followed later by AAAP, and those who had first received AAAP and then MK2-23. In the latter instance (first AAAP and then MK2-23), these patients showed high levels of IL-6, which was detectable largely as 200-kDa complexes following Sephadex G-200 chromatography. These 200-kDa complexes were preferentially 4IL6 ELISA reactive, less so in the 7IL6 ELISA, and had little B9 or Hep3B bioactivity in *ex vivo* assays (unpublished data).

A melanoma patient who had first received MK2-23 and was now receiving AAAP displayed three IL-6 complexes in serum upon Sephadex G-200 gel filtration analysis: (i) a 400- to 500-kDa IL-6 complex, which was preferentially reactive in the 7IL6 ELISA compared with the 4IL6 EILSA, (ii) a 200-kDa IL-6 complex, which was preferentially reactive in the 4IL6 ELISA but less so in the 7IL6 ELISA, and (iii) 30-kDa IL-6, which was reactive in the 7IL6 ELISA, and nonreactive in the 4IL6 ELISA. Biologically, the 7IL6 ELISA–reactive 400- to 500-kDa and 30-kDa IL-6 was active in the B9 hybridoma and Hep3B hepatocyte assays, but the 4IL6 ELISA–reactive 20-kDa IL-6 was inactive in both the B9 and Hep3B bioassays (unpublished data).

The current working hypothesis is that the 200-kDa IL-6 complexes contain anti–IL-6 IgG (and anti–sIL-6R IgG), whereas the 400- to 500-kDa complexes contain IL-6 together with sIL-6R and other proteins (?sgp130). The 30-kDa IL-6 may or may not represent "free" monomeric IL-6; in light of its longevity in the circulation, this 30-kDa IL-6 may well represent IL-6 in association with other smaller binding proteins that serve as chaperones.

The new data greatly increase our appreciation of the structural and functional complexity of IL-6 as it exists in the human circulation. The new data, although they help resolve conflicts in prior IL-6 literature with respect to the nature of circulating IL-6 in humans (54), point to severe problems with IL-6 assays in deriving "clinical"-quality estimates of IL-6 in body fluids. The relationship between the sustained elevations in IL-6 levels in cancer patients subjected to active immunotherapy protocols and clinical outcome remains to be investigated.

A great deal of effort is currently being invested in the derivation of inhibitors or antagonists of each of the IL-6–type cytokines (10–13). The strategies include the synthesis of fragments of receptors or mutants of receptors (e.g., of sIL-6R that bind IL-6 but do not present it to the cell-surface signal transducing chain gp130 or of fragments or mutants of gp130 or mAb to gp 130 that inhibit interactions between IL-6/sIL-6R complexes with the gp130 on the target cell surface). It is immediately apparent that in each of these instances the inhibition strategy entails the generation of a complex with the cytokine *in vivo*. The new results show that investigations of such antagonists in cell culture assays may provide little reliable information about how such antagonists behave *in vivo*. Additionally, the new data indicate that the underlying chemistry of circulating human IL-6 can differ markedly in different clinical situations in different individuals further confounding any simple application of IL-6 or IL-6R antagonists. It is critical for the evolution of these strategies into the clinic that the origins, biochemistry and clinical implications of chaperoned IL-6 as it exists in high-molecular mass complex in the human circulation be fully understood.

This article is dedicated to Elyse S. Goldweber, Josephine Lauriello, Suzanne Andrews, Larry Jainchill, Benjamin Z. Holczer, and Kimberly A. Sorrentino, without whose help this work would not have been possible.

1. Sehgal PB, Tosato G, Grieninger G, Eds. Regulation of the acute phase and immune responses: interleukin-6. Ann NY Acad Sci **557:**1–583, 1989.
2. Van Snick J. Interleukin-6: An overview. Annu Rev Immunol **8:**253–278, 1990.
3. Kishimoto T, Akira S, Narazaki M, Taga T. Interleukin-6 family of cytokines and gp130. Blood **86:**1243–1254, 1995.
4. Mackiewicz A, Koj A, Sehgal PB, Eds. Interleukin-6–type cytokines. Ann NY Acad Sci **762:**1–513, 1995.
5. May LT, Neta R, Moldawer LL, Kenney JS, Patel K, Sehgal PB. Antibodies chaperone circulating IL-6: Paradoxical effects of anti-IL-6 "neutralizing" antibodies *in vivo*. J Immunol **151:** 3225–3236, 1993.
6. Finkelman FD, Madden KB, Morris SC, Holmes JM, Boiani N, Katona IM, Maliszeski CR. Anti-cytokine antibodies as carrier proteins: prolongation of in vivo effects of exogenous cytokines by injection of cytokine-anti-cytokine antibody complexes. J Immunol **151:**1235–1244, 1993.

7. Lu ZY, Brochier J, Wijdenes J, Brailly H, Bataille R, Klein B. High amounts of circulating interleukin (IL)–6 in the form of monomeric immune complexes during anti–IL-6 therapy. Towards a new methodology for measuring overall cytokine production in human *in vivo*. Eur J Immunol **22**:2819–2824, 1992.
8. May LT, Viguet H, Kenny JS, Ida N, Allison AC, Sehgal PB. High levels of "complexed" IL-6 in human blood. J Biol Chem **267**:19698–19704, 1992.
9. May LT, Patel K, Garcia D, Ndubuisi MI, Ferrone S, Mittelman A, Mackiewicz A, Sehgal PB. Sustained high levels of circulating chaperoned interleukin-6 after active specific cancer immunotherapy. Blood **84**:1887–1895, 1994.
10. Lahm A, Savino R, Salvati AL, Cabibbo A, Ciapponi L, DeMartis A, Toniatti C, Paonessa G, Altamura S, Ciliberto G. The molecular design of human IL-6 receptor antagonists. Ann NY Acad Sci **762**:136–151, 1995.
11. Brakenhoff JPJ, DeHon FD, Aarden LA. Development of human IL-6 receptor antagonists. Ann NY Acad Sci **762**:129–135, 1995.
12. Mackiewicz A, Wiznerowicz M, Roeb E, Nowak J, Pawlowski T, Baumann H, Heinrich PC, Rose-John S. Interleukin-6–type Cytokines and their receptors for gene therapy of melanoma. Ann NY Acad Sci **762**:361–374, 1995.
13. Economides AN, Ravetch JV, Yancopoulos GD, Stahl N. Designer cytokines: Targeting actions to cells of choice. Science **270**:1351–1353, 1995.
14. Gailard J-P, Bataille R, Brailly H, Zuber C, Yasukawa K, Attal M, Maruo N, Taga T, Kishimoto T, Klein B. Increased and highly stable levels of functional soluble interleukin-6 receptor in sera of patients with monoclonal gammopathy. Eur J Immunol **23**:820–824, 1993.
15. Narazaki M, Yasukawa K, Saito T, Ohsugi Y, Fukui H, Koishihara Y, Yancopoulos GD, Taga T, Kishimoto T. Soluble forms of the interleukin-6 signal-transducing receptor component gp130 in human serum possessing a potential to inhibit signals through membrane-anchored gp130. Blood **82**:1120–1126, 1993.
16. Weissenbach J, Chernajovsky Y, Zeevi M, Shulman L, Soreq M, Nir U, Wallach D, Perricaudet M, Tiollais P, Revel M. Two interferon mRNAs in human fibroblasts: *In vitro* translation and *E. coli* cloning studies. Proc Natl Acad Sci USA **77**:7152–7156, 1980.
17. Sehgal PB, Sagar AD. Heterogeneity of poly(I)poly(C)-induced human fibroblast interferon mRNA species. Nature **287**:95–97, 1980.
18. Gauldie J, Richards C, Harnish D, Lansdorp P, Baumann H. Interferon β_2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte stimulating factor and regulates the major acute phase protein response in liver cells. Proc Natl Acad Sci USA **84**:7251–7255, 1987.
19. Ritchie DG, Fuller GM. Hepatocyte stimulating factor: a monocyte-derived acute phase regulatory protein. Ann NY Acad Sci **408**:490–502, 1983.
20. Ritchie DG, Levy BA, Adams MA, Fuller GM. Regulation of fibrinogen synthesis by plasmin-derived fragments of fribinogen and fibrin: An indirect feedback pathway. Proc Natl Acad Sci USA **79**:175–179, 1982.
21. Hirano T, Taga T, Nakano N, Yasukawa K, Kashiwamura S, Shimizu K, Nakajima K, Pyun KH, Kishimoto T. Purification to homogeneity and characterization of human B-cell differentiation factor (BCDF or BSFp-2). Proc Natl Acad Sci USA **82**:5490–5494, 1985.
22. Hirano T, Taga T, Yasukawa K, Nakajima K, Nakano N, Takatsuki F, Shimizu M, Murashima A, Tsunasawa S, Sakiyama F, Kishimoto T. Human B-cell differentiation factor defined by an anti-peptide antibody and its possible role in autoantibody production. Proc Natl Acad Sci USA **84**:228–231, 1987.
23. Aarden LA, Lansdorp PM, de Groot ER. A growth factor for B-cell hybridomas produced by human monocytes. Lymphokines **10**:175–185, 1985.
24. Nordan R, Potter M. A macrophage-derived factor required by plasmacytomas for survival and proliferation *in vitro*. Science **233**:566–569, 1986.
25. Cernetti C, Steinman RM, Piperno-Granelli A. Identification of a 24-kDa cytokine that is required for development of cytolytic T-lymphocytes. Proc Natl Acad Sci USA **85**:1605–1609, 1988.
26. Takai Y, Wong GG, Clark SC, Burakoff SJ, Hermann SH. B cell stimulatory factor 2 is involved in the differentiation of cytotoxic T lymphocytes. J Immunol **140**:508–512, 1988.
27. Garman RD, Jacobs KA, Clark SC, Raulet DH. B-cell stimulatory factor 2 (β_2 interferon) functions as a second signal for interleukin-2 production by mature murine T cells. Proc Natl Acad Sci USA **84**:7629–7633, 1987.
28. Shabo Y, Lotem J, Rubinstein M, Revel M, Clark SC, Wolf SF, Kamen R, Sachs L. The myeloid blood cell differentiation-inducing protein MGI-2A is interleukin-6. Blood **72**:2070–2073, 1988.
29. Luger T, Krutman J, Kirnbauer A, Urbanski A, Schwarz T, Klappacher G, Kock A, Micksche M, Malejczyk J, Schauer E, May LT, Sehgal PB. IFN-β_2/IL-6 augments the activity of human natural killer cells. J Immunol **143**:1206–1209, 1989.
30. Grossman RM, Krueger J, Yourish D, Granelli-Piperno A, Murphy DP, May LT, Kupper TS, Sehgal PB, Gottlieb A. Interleukin-6 (IL-6) is expressed in high levels in psoriatic skin and stimulates proliferation of cultured keratinocytes. Proc Natl Acad Sci USA **86**:6367–6371, 1989.
31. Chen L, Mory Y, Zilberstein A, Revel M. Growth inhibition of human breast carcinoma and leukemia/lymphoma cell lines by recombinant interferon β_2. Proc Natl Acad Sci USA **85**:8037–8041, 1989.
32. Tamm I, Cardinale I, Krueger J, Murphy JS, May LT, Sehgal PB. Interleukin-6 decreases cell-cell association and increases motility of ductal breast carcinoma cells. J Exp Med **170**:1649–1669, 1989.
33. Perlmutter DH, May LT, Sehgal PB. Interferon-β_2/Interleukin-6 modulates synthesis of α_1-antitrypsin in human mononuclear phagocytes and in human hepatoma lines. J Clin Invest **84**:138–144, 1989.
34. Katz Y, Revel M, Strunk RC. Interferon-β_2 (Interleukin-6) stimulates synthesis of complement proteins factor B and C3 in human skin fibroblasts. Eur J Immunol **19**:983–988, 1989.
35. Ikebuchi K, Wong GG, Clark SC, Ihle JN, Hirai Y, Ogawa M. Interleukin-6 enhancement of interleukin-3–dependent proliferation of multipotential hemopoietic progenitors. Proc Natl Acad Sci USA **84**:9035–9039, 1987.
36. Satoh T, Nakamura S, Taga T, Matsuda T, Hirano T, Kishimoto T, Kaziro Y. Induction of neuronal differentiation in PC12 cells by B-cell stimulatory factor 2/Interleukin-6. Mol Cell Biol **8**:3546–3549, 1988.
37. Naitoh Y, Fukuta J, Tominaga T, Nagai Y, Tamai S, Mori K, Imura H. IL-6 stimulates the secretion of adrenocorticotropic hormone in conscious freely-moving rats. Biochem Biophys Res Commun **155**:1459–1465, 1988.
38. Sehgal PB, Zilberstein A, Ruggieri M-R, May LT, Ferguson-Smith A, Slate DL, Revel M, Ruddle FH. Human chromosome 7 carries the β_2-interferon gene. Proc Natl Acad Sci USA **83**:5219–5222, 1986.
39. Hirano T, Yasukawa K, Harada H, Taga T, Watanabe Y, Matsuda T, Kashiwamura S, Nakajima K, Koyama K, Iwamatsu A, Tsunasawa S, Sakiyama F, Matsui H, Takahara Y, Taniguchi T, Kishimoto T. Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. Nature **324**:73–76, 1986.
40. Haegeman C, Content J, Volckaert G, Derynck R, Tavernier J,

Fiers W. Structure analysis of the sequence coding for an inducible 26 kDa protein in human fibroblasts. Eur J Biochem **159**:625–632, 1986.

41. May LT, Helfgott DC, Sehgal PB. Anti–β-interferon antibodies inhibit the increased expression of HLA-B7 mRNA in tumor necrosis factor-treated human fibroblasts: Structural studies of the β_2 interferon involved. Proc Natl Acad Sci USA **83**:8957–8961, 1986.
42. Zilberstein A, Ruggieri M-R, Korn JH, Revel M. Structure and expression of cDNA and gene for human interferon-β_2, a distinct species inducible by growth stimulatory cytokines. EMBO J **5**:2529–2537, 1986.
43. May LT, Ghrayeb J, Santhanam U, Tatter SB, Sthoeger Z, Sehgal PB. Synthesis and secretion of multiple forms of β_2-interferon/B-cell differentiation factor/hepatocyte stimulating factor by human fibroblasts and monocytes. J Biol Chem **262**:7760–7766, 1988.
44. May LT, Shaw JE, Khanna AK, Zabriskie JB, Sehgal PB. Marked cell-type–specific differences in glycosylation of human interleukin-6. Cytokine **3**:204–211, 1991.
45. May LT, Santhanam U, Tatter SB, Bhardwaj N, Ghrayeb J, Sehgal PB. Phosphorylation of secreted forms of IL-6. Biochem Biophys Res Commun **152**:1144–1150, 1988.
46. Ray A, Tatter SB, May LT, Sehgal PB. Activation of the β_2 interferon/hepatocyte stimulating factor/IL-6 promoter by cytokines, viruses and second messenger agonists. Proc Natl Acad Sci USA **85**:6701–6705, 1988.
47. Ray A, Sassone-Corsi P, Sehgal PB. A multiple cytokine- and second-messenger-responsive element in the enhancer of the human interleukin-6 gene: Similarities with *c-fos* gene regulation. Mol Cell Biol **9**:5537–5547, 1989.
48. Ray A, Tatter SB, Santhanam U, Helfgott DC, May LT, Sehgal PB. Regulation of expression of IL-6: Molecular and clinical studies. Ann NY Acad Sci **557**:353–362, 1989.
49. Ray A, LaForge KS, Sehgal PB. On the mechanism for efficient repression of the interleukin-6 promoter by glucocorticoids: Enhancer, TATA box, and RNA start site (Inr motif) occlusion. Mol Cell Biol **10**:5736–5746, 1990.
50. Santhanam U, Ray A, Sehgal PB. Repression of the interleukin-6 promoter by p53 and the retinoblastoma susceptibility gene product. Proc Natl Acad Sci USA **88**:7605–7609, 1991.
51. Margulies L, Sehgal PB. Modulation of the human interleukin-6 (IL-6) promoter and transcription factor C/EBPβ (NF-IL6) activity by p53 species. J Biol Chem **268**:15096–15100, 1993.
52. Sehgal PB, Margulies L. Cell-type and promoter-dependent ts phenotype of p53 Val-135. Oncogene **8**:3417–3419, 1993.
53. Ray A, Prefontaine KE. Physical association and functional antagonism between the p65 subunit of transcription factor NF-κB and the glucocorticoid receptor. Proc Natl Acad Sci USA **91**:752–756, 1994.
54. Ray A, Zhang DH, Siegel MD, Ray P. Regulation of interleukin-6 gene expression by steroids. Ann NY Acad Sci **762**:79–88, 1995.
55. Ray A, Prefontaine KE, Ray P. Down-modulation of interleukin-6 gene expression by 17β-estradiol in the absence of high affinity DNA binding by the estrogen receptor. J Biol Chem **259**:12940–12946, 1994.
56. Stein B, Yang MX. Repression of the interleukin-6 promoter by estrogen receptor is mediated by NF-κB and C/EBPβ. Mol Cell Biol **15**:4971–4979, 1995.
57. Yamasaki K, Taga T, Hirata Y, Yawata H, Kawanishi Y, Seed B, Taniguchi T, Hirano T, Kishimoto T. Cloning and expression of human IL-6 receptor. Science **241**:825–828, 1988.
58. Taga T, Hibi M, Hirata Y, Yamasaki K, Yasukawa K, Matsuda T, Hirano T, Kishimoto T. Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. Cell **58**:573–581, 1989.
59. Novick D, Engelman H, Wallach D, Rubinstein M. Soluble cytokine receptors are present in normal human urine. J Exp Med **170**:1409–1414, 1989.
60. Akira S, Nishio Y, Tanaka T, Inoue M, Matsuka T, Wang XJ, Wei S, Kishimoto T. Transcription factors NF-IL6 and APRF involved in gp130-mediated signaling pathway. Ann NY Acad Sci **762**:15–28, 1995.
61. Mackiewicz A, Schooltink H, Heinrich PC, Rose-John S. The complex of soluble human interleukin-6 receptor/interleukin-6 upregulates expression of acute phase proteins. J Immunol **149**:2021–2027, 1992.
62. Ihle JN, Witthuhn QFW, Yamamoto K, Thierfelder WE, Krieder B, Silvennoinen O. Signalling by the cytokine receptor superfamily: JAKs and STATs. TIBS **19**:222–227, 1994.
63. Nakajima K, Matsuda T, Fujitani Y, Kojima H, Yamanaka Y, Nakae K, Takeda T, Hirano T. Signal transduction through IL-6 receptor: Involvement of multiple protein kinases, stat Factors, and a novel H7-sensitive pathway. Ann NY Acad Sci **762**:55–70, 1995.
64. Oldenberg HSA, Rogy MA, Lazarus DD, Van Zee K, Keeler BP, Chizzonite RA, Lowry SF, Moldawer LL. Cachexia and the acute-phase protein response in inflammation are regulated by interleukin-6. Eur J Immunol **23**:1889–1894, 1993.
65. Heremans H, Dillen C, Put W, Van Damme J, Billiau A. Protective effect of anti–IL-6 antibody against endotoxin, associated with paradoxically increased IL-6 levels. Eur J Immunol **22**:2395–2400, 1992.
66. Klein B, Wijdenes J, Zhang X-G. Murine anti–interleukin-6 monoclonal antibody therapy for a patient with plasma cell leukemia. Blood **78**:1198–1204, 1991.
67. Beck JT, Hsu S-M, Wijdenes J, Bataille R, Klein B, Vesole D, Hayden K, Jagannath S, Barlogie B. Brief report: Alleviation of systemic manifestations of Castleman's disease by monoclonal anti–interleukin-6 antibody. N Engl J Med **330**:602–605, 1994.
68. Helfgott DC, Tatter SB, Santhanam U, Clarick RH, Bhardwaj N, May LT, Sehgal PB. Interferon-β_2/interleukin-6 in plasma and body fluids acute bacterial infection. J Immunol **142**:948–953, 1989.
69. Van Oers MHJ, Van Der Heyden AAPAM, Aarden L. Interleukin-6 in serum and urine of renal transplant recipients. Clin Exp Immunol **71**:314–320, 1988.
70. Hansen MB, Svenson M, Diamant M, Bendtzen K. Anti–interleukin-6 antibodies in normal human serum. Scand J Immunol **33**:777–781, 1991.
71. Hansen MB, Svenson M, Diamant M, Bendtzen K. High affinity IgG autoantibodies to IL-6 in sera of normal individuals are competitive inhibitors of IL-6 in vitro. Cytokine **5**:72–80, 1993.
72. DeBenedetti F, Massa M, Pignatti P, Albani S, Novick D, Martini A. Serum soluble interleukin 6 (IL-6) receptor and IL-6/soluble IL-6 receptor complex in systemic juvenile rheumatoid arthritis. J Clin Invest **93**:2114–2119, 1994.
73. Ndubuisi M, Patel K, May LT, Mittelman A, Sehgal PB. Sustained high levels of circulating interleukin-6 in patients subjected to active anti-cancer immunization protocols: Anti–IL-6 and anti–sIL-6R IgG. (abstract) Ann Meeting, Am Assoc Imm, 1996.