Interleukin-6: Molecular Pathophysiology

Pravinkumar B. Sehgal, M.B.B.S., Ph.D.
The Rockefeller University, New York, New York, U.S.A.

The cytokine interleukin-6 (IL-6) has emerged as a major systemic alarm signal which appears to be produced by essentially every injured tissue. Recent evidence points to the skin, particularly the injured skin, as one of the major sites of IL-6 production. The hallmark of IL-6 gene regulation is its induction by inflammation-associated cytokines, bacterial products, virus infection, and activation of any of the three major signal transduction pathways (diacylglycerol-, cAMP-, and Ca^{++}-activated). Many of these inducers act largely through a 23-bp "multiple-response element" in the IL-6 promoter. Different cell types, including keratinocytes, secrete multiple post-translationally modified forms of IL-6. This cytokine, in turn, plays a key role in activating a variety of local and systemic host defense mechanisms that are aimed at limiting tissue injury. Thus, IL-6 elicits major changes in the biochemical, physiologic, and immunologic status of the host (e.g., the "acute phase" plasma protein response; activation of B, T, and NK-cell function). IL-6 enhances the proliferation of human keratinocytes and of many B-cell lines but inhibits that of certain carcinoma cell lines; nevertheless, IL-6 can enhance the motility of these carcinoma cells. Elevated levels of IL-6 are observed in human body fluids during acute and chronic infections, neoplasia, autoimmune diseases, and psoriasis and following third-degree burns. It is likely that IL-6 produced by cellular elements in the skin represents an important means of communication between the external environment and the *millieu interieur. J Invest Dermatol 94:2S–6S, 1990*

F igure 1 illustrates an immunohistochemical test for the presence of interleukin-6 (IL-6) in a section through a psoriatic plaque [1]. The active plaque shows intense cytoplasmic immunoperoxidase staining by the anti-rIL-6 antibody of most keratinocytes, the dermal infiltrate, and endothelial cells (Fig 1*A,D*). Staining of the same plaque with preimmune serum shows only trace reactivity (Fig 1*B*). Uninvolved skin shows little IL-6 immunoreactivity (Fig 1*C*). Additionally, Fig 2 confirms that preincubation of the anti-rIL-6 antibody with IL-6 reduces the immunostaining observed in psoriatic plaques. These data, reported recently by Grossman et al [1], demonstrate the presence of IL-6 in cutaneous lesions in a rather graphic manner. Elevated levels of IL-6 mRNA are also found in psoriatic plaques by in situ nucleic acid hybridization, and elevated levels of biologically active IL-6 are observed in the peripheral circulation of patients with active psoriasis [1]. It is the purpose of this brief review to highlight key aspects of the genetics, molecular biology, and biochemistry of IL-6 that are of help in understanding the context within which IL-6 plays a role in a variety of different clinical situations. The historical aspects of developments in the IL-6 field have already been considered at length [2–4].

It is important to recognize at the very outset that cytokines do not function in isolation. They appear in human tissues within the context of other cytokines that can, in turn, strongly influence the biologic consequences observed. Thus, discussions about the function of any particular cytokine include a consideration of other cytokines that induce it, other cytokines that are induced by it, and the interactions, agonistic or antagonistic, of the various cytokines present in the tissue environment. The induction of IL-6 by cytokines such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), interferons (IFN), tumor necrosis factor (TNF), the colony stimulating factors (CSF), IL-1, IL-2, IL-4, and IL-6 itself under different experimental conditions in different cell types is one example of this complexity [2–4]. Nevertheless, as a practical matter, it is in the nature of an overview like this one to seek to focus attention on a particular cytokine and its role in cutaneous biology.

Human IL-6 consists of a series of phosphoglycoproteins of molecular mass in the range from 21 to 45 kDa [5–10] which affect the proliferation, differentiation, or specialized functions of hepatocytes, fibroblasts, keratinocytes, other epithelial cells, monocytes, B cells, T cells, NK cells, megakaryocytes, and neuronal cells (reviewed in [3,4]). IL-6 is readily detected in the peripheral circulation in patients with infection or injury at levels higher than those necessary to observe the effects of this cytokine on cells in culture [8–13]. Indeed, bacterial products such as endotoxin and acute virus infections (with several different RNA or DNA-containing viruses) greatly enhance IL-6 production in different cell types [14–17]. The ability of cytokines like IL-1 and TNF to also readily enhance IL-6 gene expression in keratinocytes, fibroblasts, and endothelial cells as well as numerous other cell types [3,5,18–21] may represent a local amplifying mechanism which leads to the appearance of high systemic levels of IL-6. Thus, IL-1 and TNF, which are difficult to detect in the peripheral circulation, may be viewed essentially as locally acting cytokines, whereas IL-6 may be viewed as not only a locally acting cytokine but also as one which appears at high concentrations in the peripheral circulation and thus plays a key role in orchestrating the systemic response of the host to local injury.
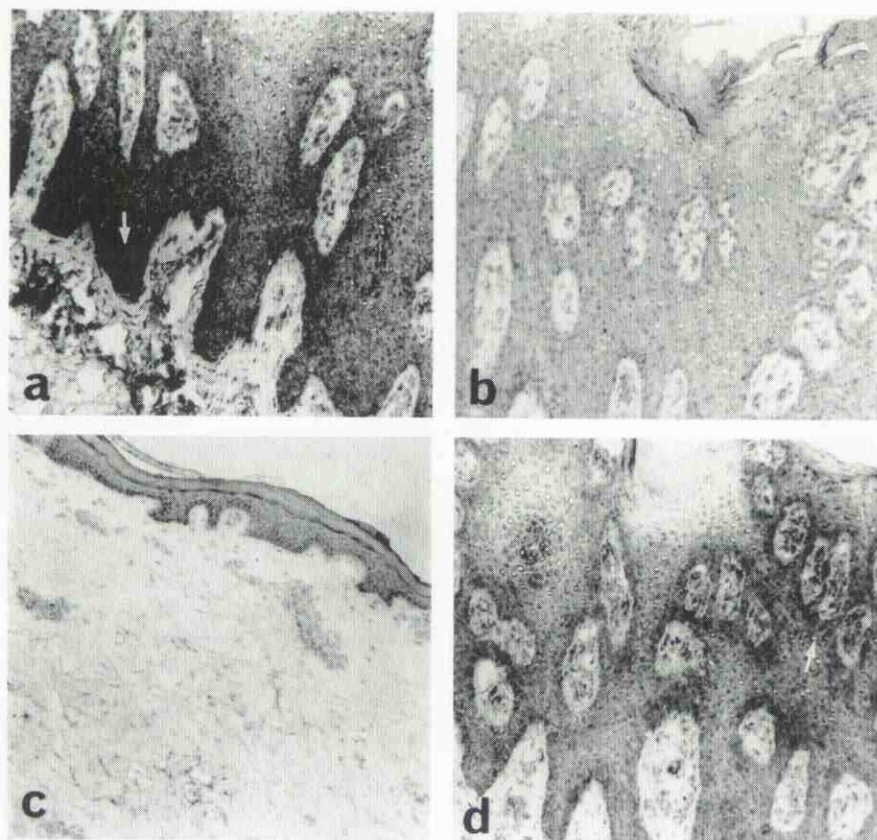
Figure 1. Increased immunoreactive IL-6 in psoriatic plaques. *(a)* Active plaque shows cytoplasmic immunoperoxidase staining by the anti-rIL-6 antibody of most keratinocytes *(white arrow)*, the dermal infiltrate, and endothelial cells. *(b)* Staining of the same plaque with preimmune serum shows only trace reactivity. *(c)* Uninvolved skin from the same patient demonstrates little reactivity. *(d)* Staining of the same active plaque with a different anti-rIL-6 antibody also shows cytoplasmic staining of most keratinocyte *(white arrow)*, the dermal infiltrate, and endothelial cells. (Magnification ✕85.) Reproduced from [1].

MOLECULAR GENETICS OF HUMAN IL-6

IL-6 cDNA was originally cloned 10 years ago by Weissenbach and collegues as the product of a poly(I).poly(C)-inducible gene ("interferon-β_2") in human fibroblasts [22,23]. Since then, IL-6 cDNA has also been independently cloned by numerous other investigators in the process of isolating the genes of different protein "factors" that were defined based on different biologic activities (B-cell differentiation factor-2, hybridoma growth factor, T-cell activating factor, cytolytic T-cell differentiation factor, monocyte-granulocyte inducer type-2, hepatocyte stimulating factor, and 26-kDa protein) (reviewed in [2–4]). Human IL-6 cDNA is derived from an mRNA of length 1.3 kb which contains an open reading frame of 212 amino acids from within which the 184–186 C-terminal amino acids are found in the major mature IL-6 species [3]. This mature polypeptide contains two potential N-glycosylation sites and several potential O-glycosylation sites. The IL-6 gene consists of five exons [23,24] and is located at 7p21 in the human genome [25–28].

The human IL-6 gene is polymorphic [27,28]. Three separately segregating allele systems have been detected in restriction fragment length polymorphism studies [27]. There are three alleles in the MspI system, two in the BglI system, and at least four in the BstNI system [3,27]. It appears that the MspI and BglI alleles are likely to represent point mutations in introns within the IL-6 gene [27]. However, the BstNI alleles represent high-frequency insertion/deletion events in a 0.5-kb AT-rich region on the 3' side of the IL-6 transcription unit [27]. Direct sequencing of this heterogeneous region in individual genomes using polymerase-chain-reaction procedures confirms the high frequency of nucleotide insertion/deletion events occuring on the 3' side of the IL-6 gene [28]. None of the polymorphisms described to date appear to affect the structure of IL-6 mRNA or its translation product. The relation-

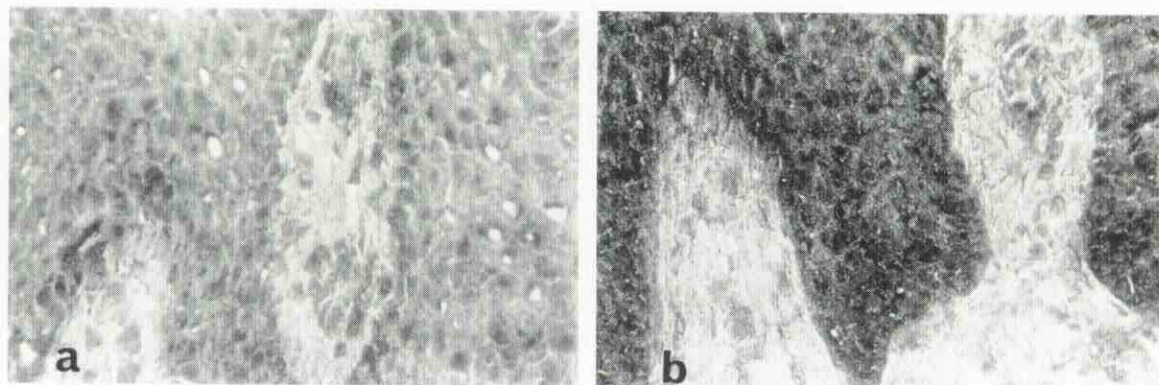**Figure 2.** Preincubation of the anti-rIL-6 antibody with IL-6 reduces staining activity. *(a)* Plaque treated with the anti-rIL-6 antibody absorbed with human IL-6 shows diminished immunoperoxidase staining of both the epidermis and dermis. *(b)* Plaque treated with the anti-rIL-6 antibody absorbed with bovine serum albumin shows cytoplasmic staining of both the epidermis and dermis. (Magnification ✕360). Reproduced from [1].

ships between particular IL-6 haplotypes and particular disease states or the severity of particular disease states are questions that remain open for further exploration.

REGULATION OF IL-6 GENE EXPRESSION

The nucleotide sequence 5' to the major inducible RNA start site in the human IL-6 gene (there is a second minor inducible RNA start site at −21; [23,29]) has been recognized to contain several DNA motifs that can be recognized as similar to those involved in the transcriptional regulation of a variety of other cellular genes [23,24,29–32]. These include areas similar in sequence to the c-fos serum response element, the AP-1/Jun binding site, the AP-2 binding site, the CREB-binding site, the steroid binding site, the NF-κB binding site, etc. There is an overall functional similarity between IL-6 and c-fos promoters as transfection of excess amounts of either promoter DNA into intact HeLa cells modulates the function of the heterologous promoter construct [29,32]. Furthermore, the transcription regulatory factor Fos transrepresses both the IL-6 and c-fos promoters [29,32]. Functional promoter dissection experiments carried out in HeLa cells reveal that the 115-bp region from −225 to −111 in the IL-6 promoter, which shares nucleotide sequence similarity with the c-fos serum response (SRE) and adjacent AP-1-like (the CGTCA motif) elements, confers responsiveness to several inducers including serum, forskolin, phorbol ester, IL-1, TNF, and RNA- (Sendai) and DNA-containing (pseudorabies) viruses [29,31,32]. This 115-bp IL-6 enhancer linked to the heterologous herpes virus thymidine kinase (TK) promoter renders it responsive to these inducers [29,31,32]. A 23-bp oligonucleotide designated AR1 from within this enhancer region (−173 to −151) contains a CGTCA motif and binds nuclear proteins which also associate with c-fos oligonucleotides containing either an intact SRE or AP-1-like site [32]. A single copy of AR1 inserted upstream of the herpes TK promoter renders this heterologous construct inducible by IL-1, TNF, and serum as well as by activators of the protein kinase A (forskolin) and protein kinase C (phorbol ester) signal transduction pathways ([32]; Fig 3). Mutations in the AP-1-like site in AR1 decrease the inducibility of the chimeric gene by phorbol esters and by forskolin but not by serum, IL-1, or TNF [32]. These data show not only that the AR1 segment from within the IL-6 enhancer binds nuclear proteins that also bind to c-fos regulatory elements but they also demonstrate that a single copy of this 23-bp element is functionally sufficient to confer responsiveness to several different cytokines and second messenger agonists [31,32] and thus help define a "multiple-response element" (MRE) in the IL-6 promoter.

While the major enhancer elements in the IL-6 promoter reside in the region −225 to −111 [31,32], it is already clear that DNA

elements upstream of −225 and downstream of −111 also participate in IL-6 gene expression in response to particular inducers. As examples, a deletion construct lacking the region from −225 to −111 is inducible to a small extent by phorbol ester [31,32] and a construct lacking the entire region upstream of −111 is inducible in HeLa cells to a small extent by pseudorabies virus (herpesviridae) [31]. The DNA elements involved in the upregulation of IL-6 transcription in monocytic cells in response to bacterial endotoxin [3,14,15] and those involved in the downregulation of IL-6 transcription in response to steroid hormones [33,34] remain to be delineated. The nature of the "constitutive" IL-6 promoter which functions in certain B and T cell lines and in certain tumor cell lines also remains to be delineated [2–4]. It will be of great interest to compare the functional elements in the inducible IL-6 promoter with those in the constitutive IL-6 promoter.

To summarize, the available experimental evidence demonstrates that serum, cytokines, and activation of both protein kinase A- and protein kinase C-signal transduction pathways can enhance IL-6 gene expression through the 23-bp MRE located in the IL-6 promoter [29,32]. The IL-6 MRE constitutes an excellent model system for detailed studies of how multiple different signal transduction pathways can converge onto the same DNA regulatory element. These studies essentially deal with one of the more important aspects of IL-6 biology, namely, that of the induction of a cytokine which then orchestrates the host response to tissue injury.

POST-TRANSLATIONAL MODIFICATIONS OF THE IL-6 POLYPEPTIDE

The single polypeptide derived from the 212-residue open reading frame in IL-6 mRNA is secreted in the form of mature IL-6 proteins with heterogeneous N-termini corresponding to residues 28–30 as numbered within this open reading frame [3]. A subset of these polypeptides are O-glycosylated and have a molecular mass of 23–25 kDa [5,7]. A further subset is both N- and O-glycosylated and is of molecular mass 28–30 kDa [5,7]. Certain B- and T-cell lines secrete IL-6 as a protein of molecular mass 21 kDa ([35], our unpublished data). Additionally, IL-6 complexes of molecular mass 43–45 kDa are also observed in the medium of fibroblasts, endothelial cells, and transformed B cells as well as in human plasma and synovial fluid ([7–10,21,36]; Dr. Giovanna Tosato, personal communication). Certain Chinese hamster cell clones expressing recombinant human IL-6 secrete this cytokine as a complex of molecular mass 60–70 kDa [37].

IL-6 species in the size range 21–30 kDa secreted by fibroblasts, monocytes, and endometrial stromal cells are serine-phosphorylated in a cell-type specific manner [6,34]. For example, whereas both the 23–25 and 28–30 kDa species secreted by fibroblasts are phosphorylated, only the lower molecular mass species secreted by monocytes are phosphorylated; monocytic 28–30 kDa IL-6 is devoid of detectable phosphorylation [6]. The role of serine-phosphorylation in the function of IL-6 remains to be explored. The biologic significance of the different forms of IL-6 seen in human tissues and the technical problems that these modifications may present in immunoassays for the measurement of IL-6 levels in human body fluids also remain to be explored. The relative ability of the different forms of IL-6 to react with cell surface receptors is also unexplored.

IL-6 RECEPTORS

High affinity IL-6 receptors with dissociation constants in the range 10^{-10} to 10^{-11} M have been detected in a variety of different cell lines and cell strains [38–40]. Most cells responsive to IL-6 (such as the hepatoma lines HepG2 and Hep3B, monocytic cell lines, and EBV-transformed B cell lines) have approximately 500–2000 high-affinity IL-6 receptors per cell [38–40]. Kreuger et al [40] report the detection of high-affinity IL-6 receptors in normal human keratinocytes.

The number of IL-6 receptors on the cell surface is largely unaffected by other cytokines such as IL-1 but can be upregulated by exposure to steroids such as dexamethasone [39].
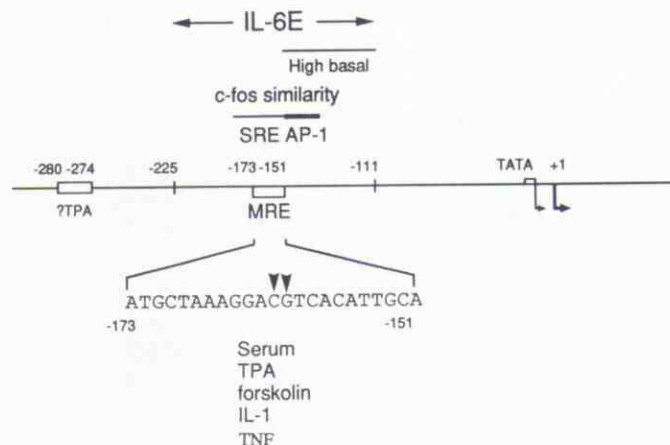

Figure 3. Transcriptional regulatory regions in the 5'-flanking region of the human IL-6 gene delineated using functional assays. MRE, multiple response element; *arrowheads*, locations at which mutations block responsiveness to TPA and forskolin but not to serum, IL-1, or TNF. Adapted from [32].

The cDNA for a cell-surface glycoprotein with molecular mass 80 kDa which binds IL-6 with a dissociation constant of 10^{-9} has been recently cloned from a cDNA expression library prepared from a human NK-like cell line [41]. This p80 molecule has the overall structural motif of a member of the immunoglobulin family and associates with another membrane protein of molecular mass 130 kDa [42]. It is believed that the p80.p130 complex together may represent the IL-6 signal transducing receptor [42]. The possibilities that other cell lines may possess different IL-6 receptors or that the IL-6-binding p80 may associate with different proteins in different cells to form the signal-transducing complex remain to be explored. The nature of the IL-6 receptor in keratinocytes [40] and the regulation of receptor number and affinity in keratinocytes also remain to be explored.

IL-6 AND THE KERATINOCYTE

Keratinocytic cell lines can be induced to express high levels of IL-6 mRNA and protein [43,44]. Normal human keratinocytes also express IL-6 following exposure to cytokines such as IL-1 [43,44]. In contrast to other cell types that have been tested, IL-4 induces IL-6 in only the keratinocyte [44]. Thus there are inducer-specific distinctions in keratinocytic expression of IL-6. IL-6 secreted by induced keratinocytes consists of differentially N- and O-glycosylated and phosphorylated species of molecular mass 23–30 kDa [44]. In addition, cell-associated IL-6-immunoreactive proteins of a higher molecular mass have also been detected in normal keratinocytes [44]. These cell-associated IL-6-immunoreactive proteins remain to be characterized structurally and functionally.

Keratinocytes isolated from normal skin show nuclear and cytoplasmic IL-6 immunofluorescence reactivity [1]. However, the intensity of IL-6 immunofluorescence in keratinocytes isolated from psoriatic lesions far exceeds that observed in keratinocytes isolated from normal skin [1].

IL-6 stimulates proliferation of normal human keratinocytes when these are grown in basal medium [1,40]. When keratinocytes are grown in medium supplemented with other growth factors (epidermal growth factor, insulin, hydrocortisone, and bovine pituitary extract), the addition of IL-6 has little further effect. However, in basal medium the addition of rIL-6 (10 units/ml) causes a seven fold increase in DNA synthesis and cell proliferation. The overexpression of IL-6 in hyperplastic psoriatic tissue may explain features of psoriasis that link keratinocyte proliferation with immune activation and tissue inflammation.

DISCUSSION: IL-6 IN CUTANEOUS BIOLOGY

A major theme to emerge from recent data in the IL-6 field is the ability of this cytokine to mediate the systemic consequences of local pathologic processes. These systemic effects include alterations in the biochemical, physiologic and immunologic status of the host. As examples, elevated IL-6 levels in the peripheral circulation correlate with fever, increases in C-reactive protein levels, increased erythrocyte sedimentation rate, and decreased albumin/globulin ratios (reviewed in [3,4]). Many of these effects can be readily recognized as the systemic manifestations of cutaneous disease states such as viral and bacterial exanthems, psoriasis, and cutaneous burns. It is noteworthy that infection by several different viruses (*adenoviridae, herpesviridae, myxoviridae, paramyxoviridae, rhabdoviridae*, etc.) as well as bacterial products (endotoxin, muramyl-peptide derivatives) enhance IL-6 production by fibroblasts and monocytes. Thus it can be expected that IL-6 will be part of the pathologic processes in dermatoses caused by microbial agents. Increased production of IL-6 following thermal injury has already been documented [11]. It also appears that ultraviolet irradiation leads to increased production of IL-6 by the skin; increased systemic levels of IL-6 can be detected in human volunteers and in experimental animals following UV-irradiation (T. Luger, personal communication). It can be expected that IL-6 will also come into play during the process of wound healing; preliminary immunohistochemical data reveal IL-6 immunostaining in sections through healing wounds (J. Kreuger et al, personal communication). Fi-

nally, we have already reported the increased levels of IL-6 immunostaining in active psoriatic plaques accompanied by elevations of circulating IL-6 levels in patients with systemic symptoms [1]. It is thus clear that cutaneous production of IL-6 is likely to represent a mechanism that then elicits the systemic manifestations of the local disease process.

IL-6 is also likely to participate, directly or indirectly, in the local cutaneous manifestations of systemic disease processes. It can be anticipated that systemic elevations of IL-6 levels such as observed in a variety of autoimmune disorders characterized by polyclonal activation of B cells and autoantibody production (systemic lupus erythematosus, scleroderma, Reiter's syndrome, rheumatoid arthritis, Sjogren's disease, etc.) are likely to play a role in the appearance of the cutaneous manifestations of these disorders.

The ability of IL-6 to enhance proliferation of keratinocytes and also to activate T-cell, NK-cell, monocytic, and B-cell functions suggests that this cytokine may play a crucial role in cutaneous lesions characterized by hyperkeratosis and immune activation. Psoriasis is an example in which IL-6 has already been identified as a candidate for this linkage between increased proliferation of keratinocytes and immune activation [1]. The ability of IL-6 to decrease junction formation between epithelial cells (e.g., in the T-47D or ZR-75-1 breast ductal carcinoma cells; [45]) and increase the motility of these cells suggests that the role of IL-6 in enhancing the local invasiveness of cutaneous neoplastic lesions needs to be carefully evaluated.

To summarize, IL-6 is clearly a participant in cutaneous physiologic and pathologic processes. IL-6 participates in these events as part of a network of cytokines which include IL-1, TNF, EGF, PDGF, IFN-γ, TGF-α, and TGF-β [3,40]. IL-6 is likely to synergize with cytokines like IL-1 in activating cells of the immune system in the local cutaneous environment. However, the feature that sets IL-6 apart from the rest of these cytokines is that IL-6 is produced in local lesions at high levels and can then be readily found in the peripheral circulation and is thus in a position to mediate the systemic response of the host.

REFERENCES

1. Grossman RM, Kreuger J, Yourish D, Granelli-Piperno A, Murphy DP, May LT, Kupper TS, Sehgal PB, Gottlieb AB: Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. Proc Natl Acad Sci USA 86:3667–6371, 1989

2. Sehgal PB, May LT, Tamm I, Vilček J: Human β_2 interferon and B-cell differentiation factor BSF-2 are identical. Science 235:731–732, 1987

3. Sehgal PB, Grieninger G, Tosato G, eds.: Regulation of the acute phase and immune responses: interleukin-6. Ann NY Acad Sci 557:1–583, 1989

4. Sehgal PB: Interleukin-6: a regulator of plasma protein gene expression in hepatic and non-hepatic tissues. Mol Biol Med (in press)

5. May LT, Ghrayeb J, Santhanam U, Tatter SB, Sthoeger Z, Helfgott DC, Chiorazzi N, Grieninger G, Sehgal PB: Synthesis and secretion of multiple forms of "β_2-interferon/B-cell differentiation factor BSF-2/hepatocyte stimulating factor" by human fibroblasts and monocytes. J Biol Chem 263:7760–7766, 1988

6. May LT, Santhanam U, Tatter SB, Bhardwaj N, Ghrayeb J, Sehgal PB: Phosphorylation of secreted forms of β_2-interferon/hepatocyte stimulating factor/interleukin-6. Biochem Biophys Res Commun 152:1144–1150, 1988

7. Santhanam U, Ghrayeb J, Sehgal PB, May LT: Post-translational modifications of human interferon-β_2/interleukin-6. Arch Biochem Biophys 274:161–171, 1989

8. Helfgott DC, Tatter SB, Santhanam U, Clarick RH, Bhardwaj N, May LT, Sehgal PB: Multiple forms of IFN-β_2/IL-6 in serum and body fluids during acute bacterial infections. J Immunol 142:948–953, 1989

9. Jablons DM, Mulé JJ, McIntosh JK, Sehgal PB, May LT, Huang CM, Rosenberg SA, Lotze MT: Interleukin-6/interferon-β_2 as a circulating hormone: induction by cytokine administration in man. J Immunol 142:1542–1547, 1989

10. Fong Y, Moldawer LL, Marano M, Wei H, Tatter SB, Clarick RH, Santhanam U, May LT, Sehgal PB, Lowry SF: Endotoxemia elicits increased circulating β_2-interferon/interleukin-6 in man. J Immunol 142:2321–2324, 1989

11. Nijsten MW, DeGroot ER, TenDuis HJ, Klesen HJ, Hack CE, Aarden LA: Serum levels of interleukin-6 and acute phase responses. Lancet II:921, 1989

12. VanOers MHJ, Van der Heyden AAPAM, Aarden LA: Interleukin-6 (IL-6) in serum and urine of renal transplant recipients. Clin Exp Immunol 71:314–319, 1988

13. Waage A, Brandtzaeg P, Halstensen A, Kierulf P, Espevik T: The complex pattern of cytokines in serum from patients with meningococcal septic shock. J Exp Med 169:333–338, 1989

14. Ritchie DG, Fuller GM: Hepatocyte-stimulating factor: a monocyte-derived acute-phase regulatory protein. Ann NY Acad Sci 408:490–502, 1983

15. Gauldie J, Richards C, Harnish D, Lansdorp P, Baumann H: Interferon-β_2/B cell stimulatory factor 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase response in liver cells. Proc Natl Acad Sci USA 84:7251–7255, 1987

16. Helfgott DC, May LT, Sthoeger Z, Tamm I, Sehgal PB: Bacterial lipopolysaccharide (endotoxin) enhances expression and secretion of β_2 interferon in human fibroblasts. J Exp Med 166:1300–1309, 1987

17. Sehgal PB, Helfgott DC, Santhanam U, Tatter SB, Clarick RH, Ghrayeb J, May LT: Regulation of the acute phase and immune responses in viral disease: enchanced expression of the "β_2-interferon/hepatocyte stimulating factor/interleukin-6" gene in virus-infected human fibroblasts. J Exp Med 167:1951–1956, 1988

18. Content J, DeWit L, Poupart P, Opdenakker G, Van Damme J, Billiau A: Induction of a 26-kDa-protein mRNA in human cells treated with interleukin-1-related, leukocyte-derived factor. Eur J Biochem 152:253–257, 1985

19. Kohase M, Henriksen-Distefano D, May LT, Vilček J, Sehgal PB: Induction of β_2-interferon by tumor necrosis factor: a homeostatic mechanism in the control of cell proliferation. Cell 45:659–666, 1986

20. Kohase M, May LT, Tamm I, Vilček J, Sehgal PB: A cytokine network in human diploid fibroblasts: interactions of β-interferons, tumor necrosis factor, platelet-derived growth factor and interleukin-1. Mol Cell Biol 7:273–280, 1987

21. May LT, Torcia, G, Cozzolino F, Ray A, Tatter SB, Santhanam U, Sehgal PB, Stern D: Interleukin-6 gene expression in human endothelial cels: RNA start sites, multiple IL-6 proteins and inhibition of proliferation. Biochem Biophys Res Commun 159:991–998, 1989

22. 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 *Escherichia coli* cloning studies. Proc Natl Acad Sci USA 77:7152–7156, 1980

23. Zilberstein A, Ruggieri R, Korn JH, Revel M: Structure and expression of cDNA and genes for human interferon-beta-2: a distinct species inducible by growth-stimulatory cytokines. EMBO J 5:2529–2537, 1986

24. Yasukawa K, Hirano T, Watanabe Y, Muratani K, Matsuda T, Nakai S, Kishimoto T: Structure and expression of human B cell stimulatory factory-2 (BSF-2/IL-6) gene. EMBO J 6:2939–2945, 1987

25. Sehgal PB, Zilberstein A, Ruggieri MR, 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 83:8957–8961, 1986

26. Ferguson-Smith A, Chen Y-F, Newman MS, May LT, Sehgal PB, Ruddle FH: Regional localization of the "β_2-interferon/B-cell stimulatory factor 2/hepatocyte stimulating factor" gene to human chromosome 7p15–p21. Genomics 2:203–208, 1988

27. Bowcock AM, Kidd JR, Lathrop M, Daneshvar L, May LT, Ray A, Sehgal PB, Kidd KK, Cavalli-Sforza LL: The human "beta-2 interferon/hepatocyte stimulating factor/interleukin-6" gene: DNA polymorphism studies and localization to chromosome 7p21. Genomics 3:8–16, 1988

28. Bowcock AM, Ray A, Erlich H, Sehgal PB: Rapid detection and sequencing of alleles in the 3' flanking region of the interleukin-6 gene. Nucl Acid Res 17:6855–6864, 1989

29. Ray A, Tatter SB, Santhanam U, Helfgott DC, May LT, Sehgal PB: Regulation of expression of interleukin-6: molecular and clinical studies. Ann NY Acad Sci 557:353–362, 1989

30. Walther Z, May LT, Sehgal PB: Transcriptional regulation of the "β_2-interferon/B-cell differentiation factor BSF-2/hepatocyte stimulating factor HSF" gene in human fibroblasts by other cytokines. J Immunol 140:974–977, 1988

31. Ray A, Tatter SB, May LT, Sehgal PB: Activation of the human "β_2-interferon/hepatocyte stimulating factor/interleukin-6" promoter by cytokines, viruses and second messengers. Proc Natl Acad Sci USA 85:6701–6705, 1988

32. 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

33. Kohase M, Henriksen-DeStefano D, Sehgal PB, Vilček J: Dexamethasone inhibits feedback regulation of the mitogenic activity of tumor necrosis factor, interleukin-1 and epidermal growth factor in human fibroblasts. J Cell Physiol 132:271–278, 1987

34. Tabibzadeh S, Santhanam U, Sehgal PB, May LT: Cytokine-induced production of interferon-β_2/interleukin-6 by freshly-explanted human endometrial stromal cells: modulation by estradiol 17β. J Immunol 142:3134–3139, 1989

35. 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

36. Bhardwaj N, Santhanam U, Lau LL, Tatter SB, Ghrayeb J, Rivelis M, Steinman RM, Sehgal PB, May LT: Interleukin-6/interferon-β_2 in synovial effusions of patients with rheumatoid arthritis and other arthritides: identification of several isoforms and studies of cellular sources. J Immunol 143:2153–2159, 1989

37. Revel M, Zilberstein A, Chen L, Gothelf Y, Barash I, Novick D, Rubinstein M, Michaelevicz R: Biological activities of recombinant human IFN-β_2/IL-6 (E. coli). Ann NY Acad Sci USA 557:144–156, 1989

38. Hirano T, Taga T, Yamasaki K, Matsuda T, Yasukawa K, Hirata Y, Yawata H, Tanabe O, Akira S, Kishimoto T: Molecular cloning of the cDNAs for interleukin-6/B cell stimulatory factor 2 and its receptor. Ann NY Acad Sci 557:167–180, 1989

39. Snyers L, Fontaine V, Content J: Modulation of interleukin-6 receptors in human cells. Ann NY Acad Sci 557:388–393, 1989

40. Kreuger JG, Krane JF, Carter DM, Gottlieb AB: Role of growth factors, cytokines, and their receptors in the pathogenesis of psoriasis. J Invest Dermatol 94(suppl):135S–140S

41. Yamasaki K, Taga T, Hirata Y, Yawata H, Kawanishi Y, Seed B, Taniguchi T, Hirano T, Kishimoto T: Cloning and expression of the human interleukin-6 (BSF-2/IFNβ2) receptor. Science 241:825–828, 1988

42. 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

43. Luger TA, Schwarz T, Krutman J, Kirnbauer R, Neuner P, Kock A, Urbanski A, Borth W, Schauer E. Interleukin-6 is produced by epidermal cells and plays an important role in the activation of human T-lymphocytes and natural killer cells. Ann NY Acad Sci 557:405–414, 1989

44. Kupper TS, Min K, Sehgal PB, Mizutani H, Birchall N, Ray A, May L: Production of IL-6 by keratinocytes: implications for epidermal inflammation and immunity. Ann NY Acad Sci 557:454–464, 1989

45. Tamm I, Cardinale I, Kreuger 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