68 Pettipher, E. R., Higgs, G. A., and Henderson, B., Interleukin 1 induces leukocyte infiltration and cartilage proteoglycan degradation in the synovial joint. Proc. natl Acad. Sci. USA *83* (1986) 8749–8753.
69 Phadke, K., Fibroblast growth factor enhances the interleukin-1-mediated chondrocytic protease release. Biochem. biophys. Res. Commun. *142* (1987) 448–453.
70 Postlethwaite, A. E., Lachman, L. B., Mainardi, C. L., and Kang, A. H., Interleukin 1 stimulation of collagenase production by cultured fibroblasts. J. exp. Med. *157* (1983) 801–806.
71 Postlethwaite, A. E., Raghow, R., Stricklin, G. P., Poppleton, H., Seyer, J. M., and Kang, A. H., Modulation of fibroblast functions by interleukin 1 : increased steady-state accumulation of type I procollagen messenger RNAs and stimulation of other functions but not chemotaxis by human recombinant interleukin 1α and β. J. Cell Biol. *106* (1988) 311–318.
72 Rainsford, K. D., Inhibition by cartilage and bone marrow fractions of cartilage resorption induced by interleukin-1 and tumor necrosis factor. Agents Actions *23* (1988) 67–68.
73 Reich, R., Thompson, E. W., Iwamoto, Y., Martin, G. R., Deason, J. R., Fuller, G. C., and Miskin, R., Effects of inhibitors of plasminogen activator, serine proteinases, and collagenase IV on the invasion of basement membranes by metastatic cells. Cancer Res. *48* (1988) 3307–3312.
74 Saklatvala, J., Tumor necrosis factor α stimulates resorption and inhibits synthesis of proteoglycan in cartilage. Nature *322* (1986) 547–549.
75 Schleef, R. R., Bevilacqua, M. P., Sawdey, M., Gimbrone, M. A., and Loskutoff, D. J., Cytokine activation of vascular endothelium. J. biol. Chem. *263* (1988) 5797–5803.
76 Schleuning, W.-D., Medcalf, R. L., Hession, C., Rothenbühler, R., Shaw, A., and Kruithof, E. K. O., Plasminogen activator inhibitor 2: Regulation of gene transcription during phorbol ester-mediated differentiation of U-937 human histiocytic lymphoma cells. Molec. cell. Biol. *7* (1987) 4564–4567.
77 Schnyder, J., and Payne, T., Effect of interleukin-1 and 2 on enzyme secretion and prostaglandin formation by chondrocytes. Br. J. Rheumat. *24*, suppl. 1 (1985) 128–132.
78 Schnyder, J., Payne, T., and Dinarello, C. A., Human monocyte or recombinant interleukin 1's are specific for the secretion of a metalloproteinase from chondrocytes. J. Immun. *138* (1987) 496–503.
79 Stanley, K., and Luzio, P., Perforin. A Family of killer proteins. Nature *334* (1988) 475–476.
80 Stoppelli, M. P., Tacchetti, C., Cubellis, M. V., Corti, A., Hearing, V. J., Cassani, G., Appella, E., and Blasi, F., Autocrine saturation of pro-urokinase receptors on human A 431 cells. Cell *45* (1986) 675–684.
81 Tryggvason, K., Höyhtyä, M., and Salo, T., Proteolytic degradation of extracellular matrix in tumor invasion. Biochim. biophys. Acta *907* (1987) 191–217.
82 Vaes, G., and Eeckhout, Y., The precursor of bone collagenase and its activation, in: Protides of the Biological Fluids. 22 nd Colloquium., pp. 391–397. Ed. H. Peeters. Pergamon Press, Oxford and New York 1975.
83 Werb, Z., and Gordon, S., Secretion of a specific collagenase by stimulated macrophages. J. exp. Med. *142* (1975) 346–360.
84 Werb, Z., and Gordon, S., Elastase secretion by stimulated macrophages. J. exp. Med. *142* (1975) 361–377.
85 Werb, Z., Mainardi, C. L., Vater, C. A., and Harris, E. D., Endogenous activation of latent collagenase by rheumatoid synovial cells. Evidence for a role of plasminogen activator. N. Engl. J. Med. *296* (1977) 1017–1023.

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Host defense against infections and inflammations: Role of the multifunctional IL-6/IFN-β2 cytokine

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Summary. IL-6/IFN-β2 appears to be one of the important mediators of the response to viral and bacterial infections and to shock. The biological effects now associated with IL-6/IFN-β2 include: stimulation of immunoglobulin secretion by mature B lymphocytes (BSF-2 activity), growth stimulation of plasmacytomas and hybridomas (HGF activity), activation of T cells, stimulation of hepatic acute phase protein synthesis (HSF activity), stimulation of hematopoiesis, cell differentiation (DIF activity), inhibition of tumor cell growth (AP activity) and other IFN-like effects. As a typical cytokine, IL-6/IFN-β2 is secreted by many cell types and acts in various combinations with other interleukins and interferons.
Key words. Interferon-β2; interleukin-6; B-lymphocytes; T-lymphocytes; acute phase response; hematopoeisis; differentiation; growth inhibition.

The IL-6/IFN-β2 cytokine: an important mediator of host defense

Since their discovery in virus-infected egg allantoic membranes by Isaac and Lindenman, a major motivation for the study of interferons (IFNs) has been their involvement in the defense of the organism against the infectious process. IFNs are typical cytokines, i.e. cell-secreted proteins carrying messages between cells, such as a warning message against the spread of infection. The type I IFN α and β genes do indeed appear to be principally activated by exposure of cells to viruses and virus-derived inducers such as double-stranded (ds) RNA [1, 2]. The multiple α IFN genes are mainly active in lymphoid and myeloid leukocytes, whereas fibroblastic, epithelial and other cells in solid tissues express essentially the IFN-β gene. It was of considerable interest when another cytokine was discovered, which is similarly induced in fibroblasts by viruses and dsRNA. This cytokine, originally named IFN-β2 [3, 4], but now classified as interleukin-6 (IL-6), appears to be one of the major mediators of the reaction to viral and bacterial infections, inflammation and shock [5, 6]. Although the full extent of its activities on various cells is still not completely settled, the major

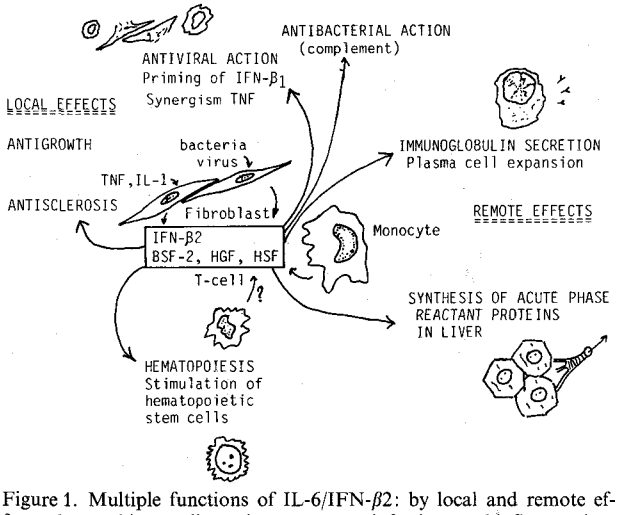

Figure 1. Multiple functions of IL-6/IFN-β2: by local and remote effects, the cytokine mediates the response to infections and inflammation.

biological effects now associated with IL-6/IFN-β2 are: stimulation of immuoglobulin secretion by mature B lymphocytes (BSF-2 activity), growth stimulation of plasmacytomas and hybridomas (HGF activity), activation of T-cells, stimulation of hepatic acute phase protein synthesis (HSF activity), stimulation of hematopoiesis, cell differentiation (DIF activity), inhibition of tumor cell growth (AP activity), and IFN-like effects (fig. 1). These activities will be detailed below.

Induction of IL-6/IFN-β2 production by various cells

IFN-β2/IL-6 is encoded by polymorphic gene alleles located in the human chromosome 7 p21 region[6,7]. As the IFN-γ gene on chromosome 12, it is separate from the type I IFN-α,β gene cluster on chromosome 9[2]. The IFN-β2/IL-6 gene is activated by viruses or ds RNA[3,4,10-12] but also by bacteria and bacterial lipopolysaccharides (LPS, endotoxin). LPS strongly induces IFN-β2/IL-6 expression in cultures of fibroblasts and monocytes[13]. Muramyl dipeptides induce the gene[14]. Moreover, in vivo administration of LPS to volunteers results in high serum levels of IL-6/IFN-β2[15], which are found also in patients with acute bacterial infections[16-18]. The gene is activated by the inflammatory cytokines IL-1 and TNF in a variety of cells[10,11,19-21]. Cancer patients treated by TNF, or IL-2 show an increase in circulating IL-6[22]. Anti-inflammatory steroids, such as dexamethasone, inhibit IL-6 induction[13,23]. Cell growth stimulants activate the synthesis of IL-6/IFN-β2; induction in fibroblasts was seen in response to PDGF, and to activators of protein kinase C, like diacylglycerol, phorbol esters (PMA), calcium ionophore and cAMP[11,19]. GM-CSF induces IL-6/IFN-β2 expression in monocytes (A. Mantovani, pers. commun.) and in bone marrow cells (M. Stephen and M. Revel, unpublished). IL-6/IFN-β2 can be induced merely by inhibiting protein synthesis by cycloheximide[3,24] suggesting that the cytokine serves as a common alarm signal for many types of cell stress. There is an autoinduction of the gene by IL-6/IFN-β2 which in fibroblasts is strongly potentiated by IFN-γ, and could serve to amplify the signal[25]. IL-6/IFN-β2 is produced by many cell types, although these may respond differently to various inducers (LPS, viruses, IL-1, TNF, IFNs, PMA). Fibroblasts, epidermal keratinocytes and other epithelial cells produce it in response to most inducers. Monocytes are an important source of IL-6/IFN-β2[26,27]; they respond well to LPS and PMA but appear to lack response to IL-1β. Production was shown in T-cells and autoimmune cardiac myxoma cells[28], and it is also made by endothelial cells[29]. Synovial cells produce IL-6, which is found to be elevated in rheumatoid arthritis[30]. Production by keratinocytes is increased in psoriasis and by liver cells in sarcoidosis (P. B. Sehgal and M. Tovey, pers. commun.). Skin burns induce high levels of circulating IL-6/IFN-β2, which correlate with the acute phase response[31]. Interestingly, serum levels of IL-6/IFN-β2 are elevated in tumor-bearing mice (D. M. Jablons, pers. commun.). Thus, viral and bacterial infections, inflammatory processes and malignant tumor growth all induce IL-6/IFN-β2 production in vivo.

Various molecular forms of IL-6/IFN-β2

The primary translation product of IL-6/IFN-β2 is a chain of 212 amino acids[10,28,32] with a hydrophobic leader which in the cell-secreted product is processed with some microheterogeneity at ALA[28], PRO[29] or VAL[30,33]. Recombinant IFN-β2/IL-6 produced in *E. coli* and purified for example by immunoaffinity on monoclonal antibodies and sulfonyl Sepharose, migrates as a 21 Kda protein[34,35]. The cytokine as produced by mammalian cells appears, however, to be rather heterogenous as a result of heavy post-translational modifications. In dsRNA-induced fibroblasts and in monocytes, the major products show triplet band at 23/25 Kda and 26/30 Kda in various denaturating gel electrophoretic systems[26,36]. The 23/25 Kda forms are O-glycosylated, and in the presence of monensin the product is 21 Kda like the *E. coli* product. Mild acid conditions remove O-linked sialic acid residues; this may explain the existence of some fast-migrating (19/21 Kda) natural IL-6, as is found in an HTLV-1 transformed T cell line[28]. The 26/30 Kda species are both O and N-glycosylated and do not appear when tunicamycin is added. These species are retained on Concanavalin A (ConA) columns and their formation is increased if cycloheximide treatment is used to induce the cells[36].

Another post-translational modification is serine phosphorylation[37]. In fibroblasts induced by LPS or IL-1, both 23/25 and 26/30 Kda forms are phosphorylated, but in monocytes induced by LPS only the 23/25 Kda species are phosphorylated. Dephosphorylation by alkaline phosphatase somewhat increases the electrophoretic mobility suggesting that the triplet bands may differ in the

extent of this modification. Sulfuration probably occurs on the carbohydrates.

In addition to the 19/21 Kda (unglycosylated processed forms) and the 23/25 and 26/30 Kda glycophosphoproteins, larger isoforms of 45 and 65/70 Kda are observed which are resistant to SDS gel electrophoresis and are readily detected by immunoblotting with various anti-IL-6/IFN-β2 antibodies[16, 22, 36, 38]. The 45 Kda species is the main form detected in the synovial fluid of rheumatoid arthritis patients, and circulating in the serum of TNF-or LPS-injected volunteers[15, 22]. Some 45 Kda protein is found in TNF, IL-1-induced fibroblasts and endothelial cells, and is active in inducing acute phase protein synthesis in liver cells and in stimulating growth of hybridomas[15, 22]. The 65/70 Kda form is found in the serum of patients with bacterial infections[16]. It is minor in dsRNA-induced fibroblasts but abounds in preparations from certain Chinese hamster ovary (CHO) cells expressing human IFN-β2/IL-6 cDNA, and selected for antiviral activity[38] (fig. 2). The heavier forms appear to be dissociatable complexes[15, 38], and immunoprecipitation releases 23/25 Kda monomers (fig. 2). In keratinocytes induced by PMA or IL-1 the 45 and 65 Kda forms appear as membrane-bound phosphoproteins (T. Kupper, pers. commun.). In summary, cells show a significant flexibility in the processing of the IL-6/IFN-β2 gene product, which may have some bearing on the regulation of the multiple biological activities of the cytokine. It is not excluded that the various forms relate to genetic heterogeneity[9, 10].

Do all the IL-6/IFN-β2 forms have the same biological activity?

The variability of IL-6/IFN-β2 forms which may be produced by human and animal cells can be illustrated in the CHO cell clones that we produced by transfection, using IL-6/IFN-β2 cDNA fused to a constitutive SV 40 promoter and coamplification of the transgenome with a marker dihydrofolate reductase gene[10]. In a first series of experiments, we selected CHO clones by their ability to secrete antiviral (AV) activity which inhibited vesicular stomatitis virus growth in human fibroblasts or induced (2′-5′) A synthetase in various cells[10]. The amounts of IL-6/IFN-β2 protein secreted were low in these clones, but immunoblots revealed[38] that the proteins purifying with the AV activity are enriched in the 65/70 Kda forms, the 45 and 23/25 Kda being also present but the 26/30 Kda form being completely absent (fig. 2). In dsRNA-superinduced fibroblasts, the AV activity which accompanies the IFN-β2 proteins also migrates at 70 Kda in SDS-gel electrophoresis (A. Zilberstein, unpublished). In contrast, other clones of transfected CHO cells, selected by immunoblotting for high IL-6/IFN-β2 secretion, pro-

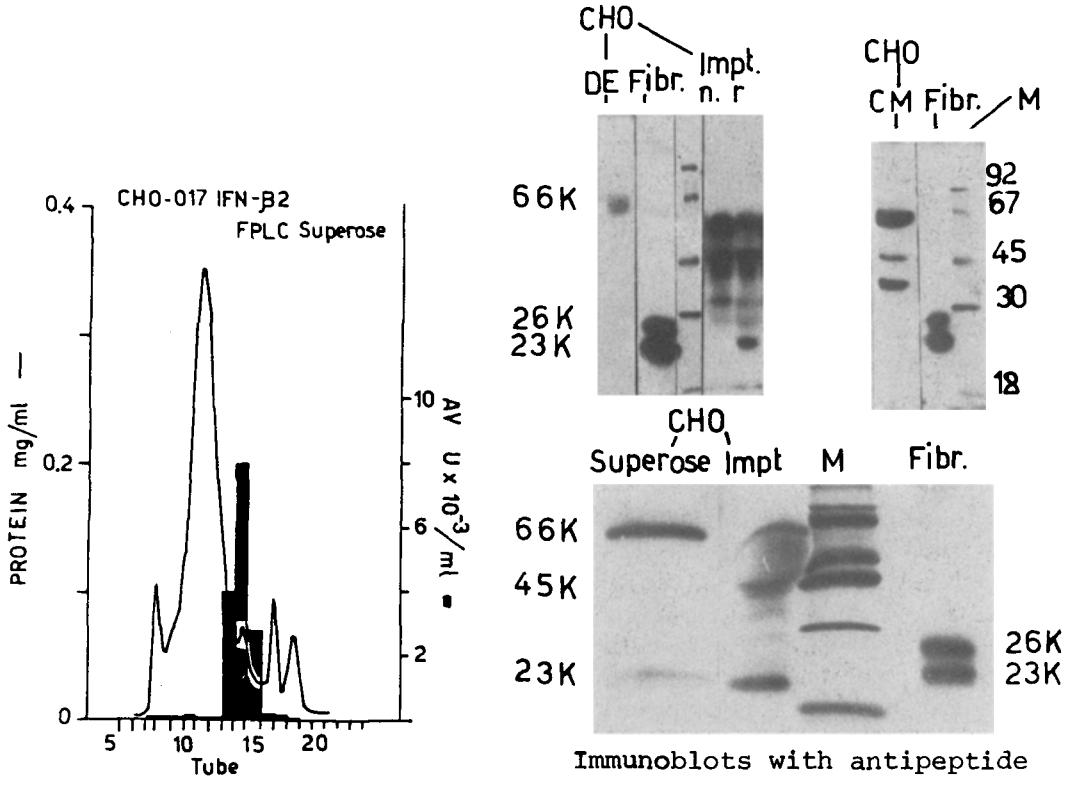

Figure 2. Various forms of IL-6/IFN-β2. Protein secreted by CHO clone B134-017 selected for IFN-β type antiviral activity[10] and purified as described[85]. A final step on FPLC-Superose is shown in the left panel (black blocks: inhibition of VSV cytopathic effect on FS11 fibroblasts). On the right are immunoblots with antibodies to an *E. coli* IL-6/IFN-β2 (gift of Dr P. B. Sehgal) or to an N-terminal peptide[46]. CHO-DE, CHO-CM, CHO-superose are proteins from CHO-B134-017 clone after DEAE and CM-sepharose or FPLC-superose[85]. Impt is the same but first precipitated by anti-IFN-β2 serum R[3] or normal serum (n). Fibr indicates poly (rI) (rC) superinduced human diploid fibroblasts product[3].

duced the 23/25 and 26/30 Kda forms only and AV activity was below detection. Several groups reported that IL-6/IFN-β2 purified by other biological activities have no AV effect[28,33]. Possibly only certain modified (oligomeric?) forms have AV activity. Nevertheless, AV activity and (2'-5') A synthetase induction was reported also for *E. coli* produced rIL-6/IFN-β2 alone or in synergy[34,36,39]. IL-6/IFN-β2 primes cells to produce IFN-β1[40] and AV effects or (2'-5') A synthetase induction may result from synergism with low levels of IFN-β1 as proposed for TNF[41] and IL-1[24].

Heterogeneity in IL-6/IFN-β2 preparations for other typical IL-6 activities has been mentioned. For example, differences in neutralization or binding of natural IL-6/IFN-β2 preparations (e.g. from synovial cells) by antibodies to the *E. coli* product were observed; the in vivo pyrogenic activity of natural IL-6/IFN-β2 was found to be 100 times higher than that of the protein produced by *E. coli* (P. B. Sehgal, pers. commun.). Hence, the possibility exists that post-translational modifications exert a control on the activity of the protein as it is secreted by different cells and in response to different inducers.

Main biological functions of IL-6/IFN-β2

Inflammatory processes initiated by bacterial and viral infections, or by various forms of physical injuries (mechanical shock, irradiations), involve local responses in the affected tissue as well as systemic responses of the organism. It is now believed that IL-6/IFN-β2 plays major roles in both local and systemic processes, with many of its activities overlapping in part the functions of the other monocytic inflammatory cytokines such as IL-1 and TNF. In this respect, it may be relevant that mRNAs from the TNF, IL-1 and IL-6 genes are readily detected in spleens collected from car accident casualties[42]. Effects of IL-6/IFN-β2 may be classified by considering various specialized cell type targets; lymphocytes, hepatocytes, hematopoietic stem cells or myeloid percursors. Some of these effects may reflect more general modulation of cell behavior which can be produced in fibroblasts and epithelial cells.

Effects on B-lymphocytes

IL-6/BSF-2 does not cause growth of normal B-lymphocytes, as does IL-4/BCGF[5,28]. However, Epstein-Barr virus (EBV)-transformed B-lymphocytes can respond to IL-6 by an increase in DNA synthesis and growth, and the efficiency of B-cell immortalization by EBV is enhanced[43,44]. IL-6/BSF-2 stimulates 10-fold the secretion of immunoglobulin (IgG) in the lymphoblastoid cell line CESS or of IgM in a subclone of Daudi cells[28,36,45,46]. The effect is accompanied by a shift from membrane-type to secreted-type IgM mRNA which involves a differential splicing in the last exon[47]. IL-6/IFN-β2 probably also plays a major role in vivo in terminal differentiation of normal B-cells[45,48]. In normal B-lymphocytes activated by anti-IgM or pokeweed mitogen, immunoglobulin secretion (but not growth) is dependent on IL-6[45], but full activation of B-cells requires the combination of both IL-6 and IL-1[49].

In contrast, IL-6 is a growth-stimulating factor for plasmacytoma, myeloma cells and derived hybridomas[20,45,50]. This hybridoma growth factor (HGF) activity often results only in stimulation of DNA synthesis. Certain hydridomas are 100 times more sensitive to the IL-6/HGF activity than the plasmacytoma cells, and may be dependent on IL-6 for survival. Some myelomas spontaneously secrete IL-6 which could serve as an autocrine growth factor promoting the growth of these tumors[51].

Since the growth effect of IL-6 is seen in EBV transformed cells or in tumors, it is possible that IL-6 works in synergism with other growth factors secreted by the transformed B-cells. In normal B-cells, synergism with IL-1 may also be very important for growth stimulation[49]. It should be recalled that IFN-α, β1 (and γ) can also stimulate growth of normal and malignant B-lymphocytes and induce maturation and immunoglobulin secretion[52,53].

Effects on T-lymphocytes

IL-6 participates in the mitogenic activation of T-cells by lectins, and presumably by the normal signals originating from antigen-presenting cells[54–59]. The requirement for monocytes in the activation of purified T cells by phytohemagglutinin (PHA) or by ConA can be in a large part replaced by IL-6. By itself IL-1β is not sufficient, but potentiation of the mitogenic stimulus is seen when IL-1 and IL-6 are added together. Both $CD8^+$ and $CD4^+$ cells respond to IL-6 but $CD4^+$ T cells could have a higher requirement for IL-1. The effect of IL-6 appears often to be independent of IL-2. To reconcile all the observations, it is proposed that IL-6 acts on several steps of T-cell activation: in an early step which would be IL-2-independent (seen in human and murine T cells), and in a later step which is IL-2-dependent (predominantly seen in murine T cells)[60]. In ConA stimulated $CD4^+$ cells, IL-6 markedly increases IL-2 synthesis and the response to IL-2 through its specific receptors[55]. When anti-CD 28 is used as stimulus for T cell mitogenesis, IL-6 is needed for the response to IL-2[57]. Some effect of IL-6 can be seen also after stimulation with anti-CD3[61].

Generation of cytotoxic T-lymphocytes (CTL) is stimulated by IL-6[62,63]. The effect is marked in conjunction with IL-2 and IFN-γ, IL-2 and an antigenic stimulus or with IL-1. Like type I IFNs, IL-6/IFN-β2 enhances natural killer cell activity[64]. Thus, IL-6/IFN-β2 plays a major role in activation of various T-lymphocyte functions, often in synergism with IL-1.

Effects on hepatocytes: induction of acute phase proteins

The typical response of the organism to acute infections and tissue injury includes fever, leukocytosis and increased secretion by the liver of several plasma proteins, mainly of the α-globulin group, grouped as acute phase proteins (APP)[65]. Among the APP are protease inhibitors, blood-clotting proteins, activators of the complement system, and protein carriers for circulating cytokines. Conditioned medium from LPS-activated monocytes mimicks APP induction in cultured hepatocytes such as human Hep3B and HepG2 hepatomas or rat hepatocytes, and this APP-inducing activity has been called hepatocyte stimulating factor (HSF)[66,67]. HSF activates the expression of the APP genes and the secretion of these proteins, while conversely decreasing albumin synthesis. IL-6/IFN-β2 can account for nearly all the monocyte-derived HSF activity (table 1)[68–71], especially when glucocorticoids are added to the hepatocyte cultures. Whereas it decreases IL-6/IFN-β2 synthesis by monocytes and other cells[13,23], dexamethasone increases the induction of several APP (e.g. CRP and SAA) by IL-6 in vitro. Although hepatocytes can produce IL-6/IFN-β2, this is not LPS-induced and addition of LPS to hepatocyte cultures does not induce the APP. The in vivo effect of LPS is probably mediated by IL-6/IFN-β2 induction in monocytes and other cells. In vivo injection of IL-6 in rats induces the APP[72]. The extent of individual APP induction may vary between species and liver cell cultures used. The other inflammatory monokines, IL-1 as well as TNF, can induce some, but not all, of the APP set (table 1). Full induction of some APP (e.g. CRP and SAA) may require IL-1 and IL-6 together[73] but IL-1 also antagonizes the effects of IL-6 on some APP (table 1). IFN-γ also induces some APP, for instance α2-macroglobulin in HepG2 cells[65], and complement proteins in many cell types[74].

Table 1. Acute phase plasma proteins synthesis in liver cells

Inducer IL-6/IFN-β2 or IL-1, TNF	IL-6/IFN-β2 only
α1-acid glycoprotein (AGP)	Fibrinogen (FBG)
Complement C3	α2 macroglobulin (α2M)
Complement factor B	α1 protease inhibitor (α1PI)
Serum amyloid A protein (SAA)	α1 antichymotrypsin (α1ACH)
Serum amyloid P protein (SAP)	α1 antitrypsin (α1AT)
Haptoglobin (HPT) (rat)	Cysteine protease inhibitor (CPI)
	Ceruloplasmin (CER)
	Hemopexin (HPX)
	C1 esterase inhibitor
	C-reactive protein (CRP)*
IL-1 + IL-6/IFN-β2 synergism	IL-1 inhibits IL-6/IFN-β2 effect
C-reactive protein	Fibrinogen
Serum amyloid A protein	Cysteine protease inhibitor
α1 acid glycoprotein	
Haptoglobin	
Complement C3	

CRP, SAA, SAP, factor B are APP in man. CPI and α2M are APP in rat. * in some human hepatoma as NPLC/PRF-5[73]. Data from references 68–71.

Table 2. DNA enhancer sequences responding to IL-6/IFN-β2

Human hemopexin gene	TGCAGTGATGTAATCAG
Human haptoglobin, element A	AAGTGTGAAGCAAGA
Human haptoglobin, element B	TCCATTTCGTAATT
Human CRP gene	AGCAATGTTGGAAAATT
Rat α2 macroglobulin gene	ATCCTTCTGGGAATTCT
consensus	C..Tgt.G.AAt
Human complement factor B gene	AGGAAACAGAAACT

for details see text.

The APP genes in hepatocytes are activated by IL-6/IFN-β2 at the transcription level[75]. The upstream regions of APP genes contain DNA elements which respond to IL-1 and others which serve as binding sites for the glucocorticoid receptor proteins. Specific short DNA elements upstream of the human HPT, HPX and CRP genes as well as of the rat α2M gene were identified as responsible for conferring IL-6-dependent transcriptional activity to heterologous promoters and genes. Although sequence homology is limited (table 2) these DNA elements have functional homology. Treatment of cells by IL-6/IFN-β2 triggers the binding to these sequences of specific proteins which may function as trans-acting transcriptional factors (V. Poli, and R. Cortese, pers. commun.).

Induction of complement in fibroblasts

IL-6/IFN-β2 induces in human diploid fibroblasts the synthesis of complement Factor B and C3, which are the principal components of the alternative pathway of complement activation[25,38]. The action of IL-6/IFN-β2 differs from that of IFN-γ which induces synthesis of C2, H or C1 components. However, IFN-γ strongly enhances the effect of IL-6/IFN-β2 on the synthesis of Factor B and C3 mRNA and proteins in fibroblasts. The upstream region of the human complement Factor B gene has a DNA element very similar to the interferon α, β and γ inducible-enhancer[76] but its role in the response to IL-6/IFN-β2 is not known (table 2).

LPS-dependent activation of the alternative pathway of complement and increased synthesis of Factor B and C3[77] may be in a large part mediated by IL-6/IFN-β2, itself induced by LPS in fibroblasts. Human fetal fibroblasts do not respond to LPS and no IL-6 is produced in these cells, but exogenously added IL-6 induces Factor B and C3 in fetal fibroblasts[25,38]. The effect of LPS on adult or neonatal fibroblasts is stimulated by addition of IFN-γ, as is the effect of IL-6/IFN-β2 on these cells; there is increased synthesis of Factor B and C3 mRNA and proteins, which correlates with an increase in the extent of IL-6/IFN-β2 induction[25,77]. This activity of IL-6/IFN-β2 on fibroblasts may participate in the local reaction against infection at the site of tissue injury.

Effects on hematopoietic cells

IL-6/IFN-β2 promotes several steps in the development of hematopoietic colony forming cells. An increase by

IL-6 of blast cell colonies, which may represent the transition of resting stem cells to colony progenitor cells at an early stage of differentiation, was documented in murine and human systems [78, 79]. IL-3 produces a strong stimulation of blast cell colony formation, but the combination of IL-3 and IL-6 reduces the time required for these colonies to grow. IL-1 (hemopoietin) is less effective. The blast colonies, when picked out and replated with CSF, differentiate into typical hematopoietic colonies. When these blast colonies are replated with IL-6, mainly GM colonies are formed (suggesting a direct differentiation effect) but IL-6 clearly has some effect on all lineages [80]. We first reported that IL-6/IFN-β2 stimulates hematopoeitic progenitors of all lineages in blood cell cultures from Hairy Cell Leukemia patients [81]. Further studies showed that in normal human bone marrow cells (depleted of monocytes and T cells and treated by 4-hydroperoxy cyclophosphamide {4HC} to eliminate committed colony-forming progenitors), IL-6/IFN-β2 increases 3–4-fold the ability of IL-3 to promote formation of colonies with mixed (CFU-GEMM), erythroid (CFU-E) and granulocytic monocytic (CFU-GM) phenotypes [38, 39]. In 4HC-treated bone marrow cells, IL-6/IFN-β2 added by itself at day 0 of plating in methylcellulose is not sufficient to support colony formation, which indicates that this cytokine does not function as a growth-promoting CSF. However, an effect of IL-6 by itself is observed in an assay which measures the formation of committed progenitors: the 4HC-treated bone marrow cells are first kept for one week in liquid cultures and the number of progenitors is then evaluated by plating in methyl cellulose with a full complement of CSF (provided as conditioned medium). Addition of IL-6/IFN-β2 during the first liquid culture step increases the number of colonies of the mixed, erythroid and granulocytic monocytic lineages, to about half of the level seen with IL-3 [38]. In this first step, the synergism of IL-3 and IL-6 is not seen and IL-6 appears to be more active than IL-1.

IL-6/IFN-β2 may, therefore, have several effects on hematopoietic cells: 1) an effect on the early stages of development of stem cells into colony progenitors and 2) an effect on myelomonocytic differentiation (see below). The effects observed in vitro may vary with the experimental conditions: if all committed cells are removed by 4HC, only the first of the two above actions is observed and colony formation requires IL-3 or GM-CSF. With IL-3, IL-6/IFN-β2 potentiates the growth of multilineage colonies and with GM-CSF the growth of GM colonies only [82]. Without 4HC treatment, some GM colonies may be formed with IL-6 alone. The mechanisms by which IL-6/IFN-β2 affects hematopoietic cell development could involve a direct effect on differentiation commitment of stem cells; for example IL-6 could induce receptors for IL-3 and GM-CSF, as was shown on leukemic cells for MGI-2 [83], which has now been identified as murine IL-6 [34]. However, some indirect effects like stimulation of CSF production are not excluded. In any event, the increase in hematopoiesis by IL-6/IFNβ2 can be viewed as part of the response to infection/ inflammation, contributing to the leukocytosis observed.

Differentiation effects on myeloleukemic cells

IL-6/IFN-β2 is a potent inducer of monocytic differentiation in the myeloleukemic cell line M1 [34, 84]. The M1 cells undergo complete growth arrest within 24 h following IL-6 addition. Cytoplasmic enlargement is observed at day 1 and morphological differentiation is complete in 3 days. The cells acquire lysozyme, phagocytic activity and monocytic surface antigens [38, 84]. A characteristic of differentiation in various leukemic cells is the increase in the IFN-induced enzyme (2′–5′) A synthetase, which can be blocked by addition of antibodies to type I IFNs, most often anti-IFN-β [85], indicating an autocrine IFN which seems to be involved in the growth-arrest and the down-regulation of c-myc expression occurring 12–24 h after induction of differentiation [86]. This typical increase in (2′–5′) A synthetase is observed in M1 cells from 24 to 48 h after IL-6/IFN-β2 addition [38]. If an endogenous murine IFN is involved, its action must be potentiated by the human IL-6/IFN-β2, since one does not detect the amounts of murine IFN that would be required to reach the high levels of (2′–5′) A synthetase induced in the M1 cells differentiating in response to IL-6/IFN-β2. Mouse IFN-β does not by itself cause differentiation of M1 cells but stimulates it in the presence of IL-1 or TNF [87]. Thus, IL-6/IFN-β2 acts on the one hand as a differentiation factor and on the other as a co-factor or inducer of autocrine IFN activity.

IL-6/IFN-β2 appears itself to be an autocrine mediator in the processes of differentiation and growth arrest. In human cells, IL-6/IFN-β2 is inducible by most agents triggering leukemic cell differentiation, such as LPS, PMA, TNF and IL-1 or GM- CSF (see above). As reviewed by Sachs [88], the differentiation factor MGI-2/IL-6 is induced by IL-3 and the other CSF in normal hematopoietic cells, and chemical treatments or culture conditions that induce differentiation of myeloleukemic cells often cause production of MGI-2/IL-6 by these cells.

Some myeloid leukemia cell lines appear to be resistant to the differentiation effect of IL-6/IFN-β2/MGI-2 [88] and can even show growth stimulation [89]. Some cell lines require a combination of IL-6/IFN-β2 with other cytokines to differentiate. For example, IL-6/IFN-β2 and IFN-γ synergize to induce differentiation, growth-arrest and (2′–5′) A synthetase in U937 cells [34, 38]. Synergism with IL-1 has been reported for differentiation of U937 and THP-1 cells [90]. Screening of patients with acute myelogenous leukemia (AML) showed that in a majority of cases, their peripheral blood leukemic cells respond to incubation with IL-6/IFN-β2 by a decrease in blast cell number and an increase in myelomonocytic forms [38].

Effect on epithelial tumor cells

Growth of human breast carcinoma cell colonies is inhibited by pure human recombinant IL-6/IFN-β2 [38, 46]. The clonogenic potential of the ductal carcinoma cell line T47D is strongly reduced by IL-6/IFN-β2 and the remnant colonies show only a few sparse cells, with increased spreading and loss of epithelial morphology [38]. Growth of the T47D cell colonies is less sensitive to IFN-β1 than to IL-6/IFN-β2 [85]. The clonogenic activity of adenocarcinoma MCF-7 cells is similarly inhibited by IL-6/IFN-β2 but these cells are also sensitive to IFN-β1 [46]. Addition of the anti-estrogen tamoxifen to IL-6/IFN-β2 completely abolishes colony growth in these cell lines [85]. IL-6/IFN-β2 reduces thymidine incorporation in semiconfluent T47D cell cultures, an effect which is counteracted by the same monoclonal antibodies to IL-6/IFN-β2 as those which neutralize the M1 cell growth arrest and the growth stimulation of plasmacytoma [38]. The rat pheocytochroma cell line PC-12 was reported to respond to IL-6 by morphological changes similar to neuronal differentiation [91]. Cells became elongated, with extended cytoplasmic projections. Pituitary cells respond to IL-6 by ACTH release [92]. These growth regulatory and differentiation effects of IL-6/IFN-β2, combined with the stimulation of T cells as well as of antibody and complement production, suggest that IL-6/IFN-β2 may have antitumor applications.

Molecular mechanisms of action

IL-6/IFN-β2 appears to be a truly multifunctional cytokine which affects a variety of cell types and controls their growth, gene expression and differentiation (fig. 1). It is still debatable whether all these effects are mediated by the same molecular mechanisms. Recently, an IL-6 receptor protein has been identified on the surface of myeloma and lymphoblastoid cells, myeloleukemic cells, various tumor cells, and amniotic cells [93, 94]. A cDNA encoding an 80 Kda protein was cloned from U266 and shown to confer high affinity binding of IL-6 to transfected cells [95]. The binding protein belongs to the immunoglobulin superfamily. The affinity of human IL-6 for the human receptor is much higher than for the murine receptor, although human IL-6 is active on mouse cells. In addition to the binding protein, biological activity such as M1 differentiation appears to require another membrane protein of 130 Kda which can be cross-linked with the 80 Kda binding proteins when radiolabeled IL-6 is allowed to interact with cells at physiological temperatures (T. Hirano, pers. commun.).

It is likely that this receptor complex is involved in many biological effects of IL-6, but other pathways are not excluded. It is also likely that the major functions of IL-6/IFN-β2 result from the up or down regulation of specific genes, as shown already for the induction of acute phase proteins in liver cells. However, in view of the varied cell targets and the complexity of the molecular forms of IL-6 secreted by cells or found in biological fluids, it will take some time until researchers can produce a general model for IL-6 action and an understanding of how the different activities are integrated in a meaningful circuit which fits into the overall cytokine network.

1 Baron, S., Stanton, J., Fleishman, W. R., and Dianzani, F., General considerations of the interferon system, in: The Interferon System; a Current Review to 1987, p. 1–17. Eds S. Baron, F. Dianzani, G. J. Stanton and W. R. Fleishman. University of Texas Press, Austin 1987.

2 Weissmann C., and Weber, H., The interferon genes. Progr. nucl. Acid Res. *33* (1986) 251–300.

3 Weissenbach, J., Chernajovsky, Y., Zeevi, M., Shulman, L., Soreq, H., Nir, U., Wallach, D., Perricaudet, M., Tiollais, P., and Revel, M., Two interferon mRNAs in human fibroblasts: In vitro translation and *Escherichia coli* cloning studies. Proc. natl Acad. Sci. USA *77* (1980) 7152–7156.

4 Sehgal, P. B., and Sagar, A. D., Heterogeneity of poly(I):poly(C)-induced human fibroblast mRNA species. Nature (London) *288* (1908) 95–97.

5 Wong, G. G., and Clark, S. C., Multiple actions of interleukin 6 within a cytokine network. Immun. Today *9* (1988) 137–139.

6 Marx, J. L., Orphan interferon finds a new home. Science *239* (1988) 25–26.

7 Sehgal, P. B., Zilberstein, A., Ruggieri, R., May, L. T., Ferguson-Smith, A., Slate, D. L., Revel, M., and Ruddle, F. H., Human chromosome 7 carries the β2 interferon gene. Proc. natl Acad. Sci. USA *88* (1986) 5219–5222.

8 Ferguson-Smith, A. C., Chen, Y.-F., Newman, M. S., May, L. T., Sehgal, P. B., and Ruddle, F. H., Regional localization of the interferon-β2/B cell stimulatory factor 2/hepatocyte stimulating factor gene to human chromosome 7p15-p21. Genomics *2* (1988) 203–208.

9 Bowcock, A. M., Kidd, J. R., Lathrop, G. M., Daneshvar, L., May, L. T., Ray, A., Sehgal, P. B., Kidd, K. K., and Cavalli-Sforza, L. L., The human interferon-β2/hepatocyte stimulating factor/ interleukin-6 gene: DNA polymorphism studies and localization to chromosome 7p21. Genomics *3* (1988) 8–16.

10 Zilberstein, A., Ruggieri, R., Korn, H. J., and 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* (1986) 2529–2537.

11 Ray, A., Tatter, S. B., May, L. T., and Sehgal, P. B., Activation of the human "beta2-interferon/ hepatocyte-stimulating factor/interleukin" promoter by cytokine, viruses and second messenger agonists. Proc. natl Acad. Sci. USA *85* (1988) 6701–6706.

12 Sehgal, P. B., Helfgott, D. C., Santhanam, U., Tatter, S. B., Clarick, R. H., Ghrayeb, J., and May, L. T., Regulation of the acute phase and immune responses in viral diseases. Enhanced expression of the beta2 interferon/ hepatocyte stimulating factor/ interleukin-6 gene in virus-infected human fibroblasts. J. exp. Med. *167* (1988) 1951–1956.

13 Helfgott, D. C., May, L. T., Shtoeger, Z., Tamm, I., and Sehgal, P. B., Bacterial lipopolysaccharide (endotoxin) enhances expression and secretion of beta-2 interferon by human fibroblasts. J. exp. Med. *166* (1987) 1300–1308.

14 Sanceau, J., Falcoff, R., Beranger, F., Gaudelet, C., and Wietzerbin, J., Muramyl dipeptide and tumor necrosis factor-α enhance interferon beta-2/BSF-2 gene expression in human monocytes. Lymph. Res. *7* (1988) 307.

15 Fong, Y., Moldawer, L. L., Marano, M., Wei, H., Tatter, S. B., Clarick, R. H., Santhanam, U., Sherris, D., May, L. T., Sehgal, P. B., and Lowry, S. F., Endotoxemia elicits increased circulating β2-interferon/ interleukin-6 in Man. J. Immun. *142* (1989) in press.

16 Helfgott, D. C., Tatter, S. B., Santhanam, U., Clarick, R. H., Bhardwaj, N., May, L. T., and Sehgal, P. B., Multiple forms of IFN-β2/IL-6 in plasma and body fluids during acute bacterial infection. J. Immun. *142* (1989) in press.

17 Houssiau, F. A., Bukasa, K., Sindic, C. J. M., VanDamme, J., and Van Snick, J., Elevated levels of the 26 K human hybridoma growth factor (interleukin-6) in cerebrospinal fluid of patients with acute infection of the central nervous system. Clin. exp. Immun. *71* (1988) 320–327.

18 Frei, K., Leist, T. P., Meager, A., Gallo, P., Leppert, D., Zinkernagel, R. M., and Fontana, A., Production of B cell stimulatory factor 2 and

interferon in the central nervous system during viral meningitis and encephalitis. Evaluation in a murine model infection and in patients. J. exp. Med. *168* (1988) 449–455.

19 Kohase, M., May, L. T., Tamm, I., Vilcek, J., and Sehgal, P. B., A cytokine network in human diploid fibroblasts: Interaction of beta interferons, tumor necrosis factor, platelet-derived growth factor and interleukin-1. Molec. cell. Biol. *7* (1987) 273–280.

20 VanDamme, J., Opdenakker, G. D., Simpson, R. J., Rubira, M. R., Cayphas, S., Vink A., Billiau, A., and VanSnick, J., Identification of the human 26-kD protein interferon-beta-2 as a B-cell hybridoma/plasmacytoma growth factor induced by interleukin-1 and tumor necrosis factor. J. exp. Med. *165* (1987) 914–919.

21 Walther, Z., May, L. T., and Sehgal, P. B., Transcriptional regulation of the interferon-beta2/BSF-2/hepatocyte stimulating factor gene in human fibroblasts by other cytokines. J. Immun. *140* (1988) 974–977.

22 Jablons, D. M., Mule, J. J., McIntosh, J. K., Sehgal, P. B., May, L. T., Huang, C. M., Rosenberg, S. A., and Lotze, M. T., Interleukin-6/interferon $\beta 2$ as a circulating hormone: Induction by cytokine administration in humans. J. Immun. *142* (1989) in press.

23 Kohase, M., Henriksen-DeStefano, D., Sehgal, P. B., and Vilcek, J., Dexamethasone inhibits the feedback regulation of the mitogenic activity of tumor necrosis factor, interleukin-1 and epidermal growth factor in human fibroblasts. J. Cell. Physiol. *132* (1987) 271–278.

24 Content, J., De Wit, L., Poupart, P., Opdenakker, G., van Damme, J., and Billiau, A., Induction of a 26 kDa-protein mRNA in human cells treated with an interleukin-1 related leucocyte-derived factor. Eur. J. Biochem. *152* (1985) 253–257.

25 Katz, Y., Revel, M., and Strunk, R. C., Interferon-$\beta 2$ (Interleukin-6) stimulates synthesis of complement proteins factor B and C3 in human skin fibroblasts. Eur. J. Immun. *19* (1989) in press.

26 Sanceau, J., Falcoff, R., Zilberstein, A., Beranger, F., Lebeau, J., Revel, M., and Vaquero, C., Interferon-beta2 (BSF-2) mRNA is expressed in human monocytes. J. Interferon Res. *8* (1988) 473–481.

27 Aarden, L. A., deGroot, E. R., Schaap, O. L., and Lansdorp, P. M., Production of hybridoma growth factor by human monocytes. Eur. J. Immun. *17* (1987) 1411–1416.

28 Hirano, T., Yasukawa, K., Harada, H., Taga, T., Watanabe, Y., Matsuda, T., Kashimura, S., Nakajima, K., Koyama, K., Iwamatsu, K., Tsunasawa, S., Sakiyama, F., Matsui, H., Takahara, Y., Taniguchi, T, and Kishimoto, T., Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulins. Nature (London) *234* (1986) 73–76.

29 Norioka, K., Hara, M., Harigai, M., Kitani, A., Hirose, T., Suzuki, K., Kawakami, M., Tabata, H., Kawagoe, M., and Nakamura, H., Production of B cell stimulatory factor 2/interleukin-6 activity by human endothelial cells. Biochem. biophys. Res. Commun. *153* (1988) 1045–1052.

30 Houssiau, F. A., Devogelaer, J. P., VanDamme, J., Dedeuxchaisnes, C. N., and VanSnick, J., Interleukin-6 in synovial fluid and serum of patients with rheumatoid arthritis and other inflammatory arthritides. Arthr. Rheum. *31* (1988) 784–790.

31 Nijsten, M. W. N., deGroot, E. R., Duis, H. J., Klasen, H. J., Hack, C. E., and Aarden, L. A., Serum levels of interleukin-6 and acute phase responses. Lancet *ii* (1987) 921.

32 Haegeman, G., Content, J., Volckaert, G., Derynck, R., Tavernier, J., and Fiers, W., Structural analysis of the sequence coding for an inducible 26-Kd protein in human fibroblasts. Eur. J. Biochem. *159* (1986) 625–632.

33 VanDamme, J., Cayphas, S., Van Snick, J., Conings, R., Put, W., Lenaerts, J. P., Simpson, R. J., and Billiau, A., Purification and characterization of human fibroblast-derived hybridoma growth factor identical to T-cell derived B-cell stimulatory factor 2 (interleukin-6) Eur. J. Biochem. *168* (1987) 543–550.

34 Chen, L., Novick, D., Rubinstein, M., and Reve, M., Recombinant interferon-$\beta 2$ (interleukin-6) induces myeloid differentiation. FEBS Letters *239* (1988) 299–304.

35 Asagoe, Y., Yasugawa, K., Saito, T., Maruo, N., Miyata, K., Kono, T., Mikaye, T., Kato, T., Kakidani, H., and Mitani, M., Human B-cell stimulatory factor 2 expressed in *E. coli.* Bio-Technology *6* (1988) 806–809.

36 May, L. T., Ghrayeb, J., Santhanam, U., Tatter, S., Shtoeger, D., Helfgott, D. C., Chiorazzi, N., Grieninger, G., and Sehgal, P. B., Synthesis and secretion of multiple forms of $\beta 2$-interferon/B cell differentiation factor 2/hepatocyte-stimulating factor by human fibroblasts and monocytes. J. biol. Chem. *263* (1988) 7760–7768.

37 May, L. T., Santhanam, U., Tatter, S. B., Bhardwaj, N., Ghrayeb, J., and Sehgal, P. B., Phosphorylation of secreted forms of human $\beta 2$-interferon/hepatocyte stimulating factor/interleukin-6. Biophys. biochem. Res. Commun. *152* (1988) 1144–1148.

38 Revel, M., Zilberstein, A., Chen, L., Gothelf, Y., Barash, I., Novick, D., Rubinstein, M., and Michalevicz, R., Biological activities of recombinant human IFN-$\beta 2$/IL-6 (*E. coli*), in: Regulation of the Acute Phase and Immune Responses: a New Cytokine. Eds P. B. Sehgal, G. Grieninger and G. Tosato. Annls N. Y. Acad. Sci. (*1989*) in press.

39 Revel, M., Chen, L, Mory, Y., Zilberstein, A., and Michalevicz, R., Interferon-type and other activities of IFN-$\beta 2$/BSF-2/HSF, in: Proceedings 4th Congress on Immunopharmacology 1988, Osaka, Japan. Ed. R. Hadden. Pergamon Press, in press 1989.

40 Zilberstein, A., Nissim, A., Ruggieri, R., Shulman, L., Chen, L., and Revel, M., The mode of action of interferon-$\beta 2$, in: The Biology of the Interferon System. Eds W. E. Stewart and H. Schellekens. pp. 119–124. Elsevier, New York 1986.

41 Reis, L. F. L., Le, J., Hirano, T., Kishimoto, T., and Vilcek, J., Antiviral action of tumor necrosis factor in human fibroblasts is not mediated by B cell stimulatory factor 2 (BSF-2)/ interferon-beta-2 and is inhibited by specific antibodies to IFN-beta. J. Immun. *140* (1988) 1566–1570.

42 Tovey, M. G., Content, J., Gresser, I., Gugenheim, J., Blanchard, B., Guymarho, J., Poupart, P., Gigou, M., Shaw, A., and Fiers, W., Genes for IFN-beta2 (IL-6), tumor necrosis factor, and IL-1 are expressed at high levels in the organs of normal individuals. J. Immun. *141* (1988) 3106–3110.

43 Tosato, G., Seamon, K. B. Goldman, N. D., Sehgal, P. B., May, L. T., Washington, G. C., Jones, K. D., and Pike, S. E., Monocyte-derived human B-cell growth factor identified as interferon-$\beta 2$ (BSF-2, IL-6). Science *239* (1988) 502–504.

44 Tosato, G., Gerrard, T. L., Goldmann, G., and Pike, S. E., Stimulation of EBV-activated human B cells by monocytes and monocyte products: role of IFN-$\beta 2$ /B cell stimulatory factor 2/IL-6. J. Immun. *140* (1988) 4329–4336.

45 Muraguchi A., Hirano T., Tang B., Matsuda T., Horii Y., Nakajima K., Kishimoto T., The essential role of B cell stimulatory factor 2 (BSF-2/IL-6) for the terminal differentiation of B cells. J. exp. Med. *167* (1988) 332–344.

46 Chen, L., Mory, Y., Zilberstein, A., and Revel, M., Growth inhibition of human breast carcinoma and leukemia/lymphoma cell lines by recombinant interferon-$\beta 2$. Proc. natl Acad. Sci. USA *85* (1988) 8037–8041.

47 Kikutani, H., Taga, T., Akira, S., Kishi, H., Miki, Y., Saiki, O., Yamamura, Y., and Kishimoto, T., Effect of B cell differentiation factor (BCDF) on biosynthesis and secretion of immunoglobulin molecules in human B cell lines. J. Immun. *134* (1985) 990–995.

48 Takatsuki, F., Okano, A., Suzuki, C., Chieda, R., Takahara, Y., Hirano, T., Kishimoto, T., Hamuro, J., and Akiyama, Y., Human recombinant IL-6/B cell stimulatory factor 2 augments murine antigen-specific antibody responses in vitro and in vivo. J. Immun. *141* (1988) 3072–3077.

49 Vink, A., Coulie, P. G., Wanters, P., Nordan, R. P., and VanSnick, J., B cell growth and differentiation activity of interleukin-HP1 and related plasmacytoma growth factors. Synergy with interleukin-1. Eur. J. Immun. *19* (1989) in press.

50 Aarden L.A., DeGroot, E., Schaap, O. L., and Lansdorp, P. M., Production of hybridoma growth factor by human monocytes. Eur. J. Immun. *17* (1987) 1411–1416.

51 Kawano M., Hirano T., Matusda T., Taga T., Horii Y., Iwato K., Asaoku H., Tang B., Tanabe O., Tanaka H., Kuramoto A., and Kishimoto T., Autocrine generation and requirement of BSF-2/IL-6 for human multiple myelomas. Nature *322* (1988) 83–84.

52 Ostlund, L., Einhorn, S., Robert, K. H., Juliusson, G., and Biberfeld, P., Chronic B-lymphocytic leukemia cells proliferate and differentiate following exposure to interferon in vitro. Blood *67* (1986) 152–159.

53 Siegel, D. S., Le, J., and Vilcek, J., Modulation of lymphocyte proliferation and immunoglobulin synthesis by interferon-γ and type I interferons. Cell. Immun. *101* (1986) 380–390.

54 Uyttenhove C., Coulie, P. G., and VanSnick J., T-Cell growth and differentiation induced by interleukin-HP1/IL-6 the murine hybridoma/plasmacytoma growth factor. J. exp. Med. *167* (1988) 1417–1427.

55 Garman, R. D., Jacobs, K. A., Clark, S. C., and Raulet, D. H., B-cell stimulatory factor 2 ($\beta 2$ interferon) functions as a second signal for interleukin 2 production by mature murine T cells. Proc. natl Acad. Sci. USA *84* (1987) 7629–7633.

56 Tosato, G., and Pike, S. E., Interferon-beta2/interleukin-6 is a costimulant for human T-lymphocytes. J. Immun. *141* (1988) 1556–1562.

57 Baroja, M. L., Ceuppens, J. L., Van Damme, J., and Billiau, A., Cooperation between an anti-T cell (anti CD28) monoclonal antibody and monocyte-produced interleukin-6 in the induction of T cell responsiveness to interleukin-2. J. Immun. *141* (1988) 1502–1507.

58 Lotz, M., Jirik, F., Kabouridis, P., Tsoukas, C., Hirano, T., Kishimoto, T., and Carson, D. A., B cell stimulatory factor 2/ interleukin-6 is a costimulant for human thymocytes and T lymphocytes. J. exp. Med. *167* (1988) 1253–1258.

59 Helle, M., Brakenhoff, J. P. J., Degroot, E. R., and Aarden, L. A., Interleukin-6 is involved in interleukin-1 induced activities. Eur. J. Immun. *18* (1988) 957–965.

60 VanSnick, J., and Uyttenhove, C., Role and mode of action of IL-6 in T cell activation. Lymphokine Res. *7* (1988) 324.

61 Aarden, L. A., deGroot, E. R., Helle, M., and VanLier, R. A. W., Role of interleukin-6 in T cell proliferation. Lymphokine Res. *7* (1988) 329.

62 Takai, Y., Wong, G. G., Clark, S. C., Burakoff, S. J., and Herrmann, S., B cell stimulatory factor-2 is involved in the differentiation of cytotoxic T lymphocytes. J. Immun. *140* (1988) 508–512.

63 Okada, M., Kitahara, M., Kishimoto, S., Matsuda, T., Hirano, T., and Kishimoto, T., IL-6/BSF-2 functions as a killer helper factor in the in vitro induction of cytotoxic T cells. J. Immun. *141* (1988) 1543–1549.

64 Luger, A., Krutman, J., Kirnbauer, R., Urbanski, A., Schwarz, T., Klappacher, G., Micksche, M., May, L. T., and Sehgal, P. B., Interleukin-6 is a potent activating signal for non MHC-restricted killer cells. Lymphokine Res. *7* (1988) 326.

65 Koj, A., in: The Acute Phase Response to Injury and Infection. Eds A. H. Gordon and A. Koj. pp. 139–144. Elsevier, New York 1985.

66 Darlington, G. J., Wilson, D. R., and Lachman, L. B., Monocyte conditioned medium, interleukin-1, and tumor necrosis factor stimulate the acute phase response in human hepatoma cells in vitro. J. Cell Biol. *103* (1986) 787–799.

67 Ritchie, D. G., and Fuller G. M., Hepatocyte-stimulating Factor: a monocyte-derived acute-phase regulatory protein. Annls NY Acad. Sci. *408* (1983) 490–496.

68 Gauldie, J., Richards, C., Harnish, D., Lansdorp, P., and Baumann, H., Interferon $\beta 2$/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* (1987) 7251–7255.

69 Baumann, H., Richards, C., and Gauldie, J., Interaction between hepatocyte stimulating factors, IL-1 and glucocorticoids for regulation of acute phase plasma proteins in human HepG2 cells. J. Immun. *139* (1987) 4122–4128.

70 Andus, T., Geiger, T., Hirano, T., Northoff, H., Ganter, U., Bauer, J., Kishimoto, T., and Heinrich, P. C., Recombinant human B cell stimulatory factor 2 (BSF-2/IFN-$\beta 2$) regulates β-fibrinogen and albumin mRNA levels in Fao-9 cells. FEBS Lett. *221* (1987) 18–22.

71 Castell, J. V., Gomez-Lecho, M. J., David, M., Hirano, T., Kishimoto, T., and Heinrich, P. C., Recombinant human interleukin-6 (IL-6/ BSF-2 /HSF) regulates the synthesis of acute phase proteins in human hepatocytes. FEBS Lett. *232* (1988) 347–350.

72 Geiger, T., Andus, T., Klapproth, J., Hirano, T., Kishimoto, T., and Heinrich, P. C., Induction of rat acute-phase proteins by interleukin-6 in vivo. Eur. J. Immun. *18* (1988) 717–721.

73 Ganapathi, M. K., May, L. T., Schultz, D., Brabenec, A., Weinstein, J., Sehgal, P. B., and Kushner, I., Role of interleukin-6 in regulating synthesis of C-reactive protein and serum amyloid A in human hepatoma cell lines. Biochem. biophys. Res. Commun. *157* (1988) 271–277.

74 Strunk, R., Cole, F. S., Perlmutter, D. H., and Colten, H. R., Gamma-interferon increases expression of class III complement genes C2 and factor B in human monocytes and in murine fibroblasts transfected with human C2 and factor B genes. J. biol. Chem. *260* (1985) 15280–15285.

75 Morrone, G., Ciliberto, G., Oliviero, S., Arcone, R., Dente, L., Content, J., and Cortese, R., Recombinant interleukin 6 regulates the transcriptional activation of a set of human acute phase genes. J. biol. Chem. *263* (1988) 12554–12558.

76 Cohen, B., Vaiman, D., and Chebath, J., Enhancer functions and in vitro protein binding of native and mutated interferon-responsive sequences. Nucl. Acids Res. *17* (1989) in press.

77 Katz, Y., Cole, F. S., and Strunk, R. C., Synergism between IFN-γ and lipopolysaccharide for synthesis of Factor B but not C2 in human fibroblasts. J. exp. Med. *167* (1988) 1–14.

78 Ikebuchi, K., Wong, G. G., Clark, S. C., Ihle, J. N., Hirai, Y., and Ogawa, M., Interleukin 6 enhancement of interleukin 3-dependent proliferation of multipotential hemopoietic progenitors. Proc. natl Acad. Sci. USA *84* (1987) 9035–9039.

79 Leary, A. G., Ikebuchi, K., Hirai, Y., Wong, G. G., Yang, Y. C., Clark, S. C., and Ogawa, M., Synergism between interleukin-6 and interleukin-3 in supporting proliferation of human hematopoietic stem cells – comparison with interleukin-1-α. Blood *71* (1988) 1759–1765.

80 Wong, G. G., Witek-Gianotti J. S., Temple P. A., Kriz R., Ferenz C., Hewick R. M., Clark S. C., Ikebuchi K., and Ogawa M., Stimulation of murine hemopoietic colony formation by human IL-6. J. Immun. *140* (1988) 3040–3044.

81 Revel, M., Zilberstein, A., Ruggieri, R., Chen, L., Mory, Y., Rubinstein, M., and Michalevicz, R., Human IFN-beta-2: A cytokine with multiple functions in infections and inflammation, in: Monokines and Other Non-Lymphocytic Cytokines. Progress in Leucocyte Biology, vol. 8, pp. 21–27. Eds M. C. Powanda, J. J. Oppenheim, M. J. Kluger and C. A. Dinarello. Alan R. Liss, New York 1988.

82 Hoang, T., Haman, A., Goncalves, O., Wong, G. G., and Clark, S. C., Interleukin-6 enhances growth factor dependent proliferation of the blast cells of acute myeloblastic leukemia. Blood *72* (1988) 823–826.

83 Lotem, J., and Sachs, L., Regulation of cell-surface receptors for different hematopoeitic growth factors on myeloid leukemic cells. EMBO J. *5* (1986) 2163–2170.

84 Miyaura, C., Onozaki, K., Akiyama, Y., Taniyama, T., Hirano, T., Kishimoto, T., and Suda, T., Recombinant human interleukin 6 (B cell stimulatory factor 2) is a potent inducer of differentiation of mouse myelodid leukemia cells (M1). FEBS Lett. *234* (1988) 17–21.

85 Revel, M., Zilberstein, A., Ruggieri, R., Rubinstein, M., and Chen, L., Autocrine interferons and interferon-$\beta 2$. J. Interferon Res. *7* (1987) 529–536.

86 Onozaki, K., Urawa, H., Tamanati, T., Iwamura, Y., Hashimoto, T., Baba, T., Suzuki, H., Yamada, M., Yamamoto, S., Oppenheim, J. J., and Matsushima, K., Synergistic interactions of interleukin-1, interferon-β and tumor necrosis factor in terminally differentiating a mouse myeloid leukemic cell line (M1). J. Immun. *140* (1988) 112–119.

87 Sachs, L., The molecular control of blood cell development. Science *238* (1987) 1374–1379.

88 Venturelli, D., Caracciolo, D., and Rovera, G., Leukemic growth factor-dependent cell lines. Hematologica *73* (1988) 255–258.

89 Onozaki, K., Akiyama, Y., Okano, A., Hirano, T., Kishimoto, T., Hashimoto, T., Yoshizawa, K., and Taniyama, T., Synergistic regulatory effects of BSF-2/IL-6 and IL-1 on the growth and differentiation of human and mouse myeloid leukemic cell lines into macrophage-like cells. Lymphokine Res. *7* (1988) 304.

90 Satoh, T., Nakamura, S., Taga, T., Matsuda, T., Hirano, T., Kishimoto, T., and Kaziro, Y., Induction of neuronal differentiation in PC12 cells by B-cell stimulatory factor 2/interleukin 6. Molec. cell. Biol. *8* (1988) 3546–3549.

91 Naitoh, Y., Fukata, J., Tominaga, T., Nagai, Y., Tamai, S., Mori, K., and Imura, H., Interleukin-6 stimulates the secretion of adrenocorticotropic hormone in conscious, freely moving rats. Biochem. biophys. Res. Commun. *155* (1988) 1459–1465.

92 Coulie, P. G., Vanhecke, A., VanDamme, J., Cayphas, S., Poupart, P., Dewit, L., and Content, J., High-affinity binding sites of human 26-Kda protein (interleukin 6, B cell stimulatory factor 2, human hybridoma plasmacytoma growth factor, interferon-beta2) different from those of type-1 interferon (alpha, beta) on lymphoblastoid cells. Eur. J. Immun. *17* (1987) 1435–1440.

93 Taga, T., Kawanishi, Y., Hardy, R. R., Hirano, T., and Kishimoto, T., Receptors for B cell stimulatory factor 2. J. exp. Med. *166* (1987) 967–981.

94 Yamasaki, K., Taga, T., Hirata, Y., Yawata, H., Kawanishi, Y., Seed B., Taniguchi, T., Hirano, T., and Kishimoto, T., Cloning and expression of the human interleukin-6 (BSF-2/IFN-beta2) receptor. Science *241* (1988) 825–828.

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