

REGULATION OF INTERLEUKIN 6 RECEPTOR EXPRESSION  
IN HUMAN MONOCYTES AND MONOCYTE-  
DERIVED MACROPHAGES

Comparison with the Expression in Human Hepatocytes

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IL-6 has been investigated under numerous designations by several laboratories independently since 1980. Its multiple biological activities were identified through these efforts (reviewed in reference 1). IL-6 was first described as a secretory product of fibroblasts after induction for IFN- $\beta$ , and was therefore designated IFN- $\beta$ 2 (2, 3) and 26-kD protein (4). Other investigators later identified a factor involved in terminal B cell differentiation synthesized by a virus-transformed T cell line (5). This factor stimulated Ig synthesis in activated B cells and was called B cell differentiation factor/B cell stimulatory factor 2 (BCDF/BSF-2)<sup>1</sup> (5). At the same time, a monocyte- and fibroblast-derived factor required for the growth of B cell hybridoma lines was described and designated as hybridoma/plasmocytoma growth factor (HPGF) (6-8).

After IFN- $\beta$ 2, 26-kD protein, BSF-2 and HPGF were each cloned by recombinant technology (3, 9-13), it became clear that they were in fact identical (14-17). Finally, hepatocyte stimulating factor, a monocyte-derived product that induces the hepatic acute phase response, was also discovered to be identical to IFN- $\beta$ 2/BSF-2 (18). To unify the large collection of names for the same molecule, the designation IL-6 was proposed (19, 20) and agreed upon (21).

IL-6 is synthesized under inflammatory conditions primarily in peripheral blood monocytes (6, 22-24), endothelial cells (25-27), and fibroblasts (2-4, 8, 10, 12, 28-34). Under certain nonphysiological stimulatory conditions (e.g., leukemia virus transformation or stimulation with phorbol esters), T cells have also been reported to secrete IL-6 (5, 11, 20, 24). Two reports described synthesis in stimulated B cells

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<sup>1</sup> *Abbreviations used in this paper:* BCDF, B cell differentiation factor; BSF, B cell stimulatory factor; CRP, C-reactive protein; HPGF, hybridoma/plasmocytoma growth factor; IMDM, Iscove's modified Dulbecco's medium; LAF, lymphocyte-activating factor; PE, phycoerythrin.

(24, 35). Furthermore, a large number of tumor cells have been found to secrete IL-6 (20, 28, 32, 36).

The main physiological functions of IL-6 are the differentiation of activated B cells to terminally matured, Ig-secreting plasma cells (5, 11, 37, 38) and the induction of the hepatic acute phase response (18, 39-47). The latter is characterized by increased expression of acute phase proteins, such as C-reactive protein (CRP) and decreased synthesis of albumin. Additionally, IL-6 has been shown to play an accessory role in T cell activation (48, 49) and induction of hematopoiesis (50-52).

Although a large body of information has emerged on IL-6, the interaction of this molecule with its target cells is still poorly understood. Recently, an IL-6-R has been cloned (53). It is now possible to study the tissue distribution of this receptor and the regulation of its expression in order to gain a better understanding of the role of IL-6 within the network of inflammatory mediators. Previously, expression of IL-6-R mRNA has only been reported for nonphysiological or transformed cell lines (53). A recent study in the rat showed that 80% of intravenously administered, radiolabeled, human rIL-6 binds to hepatocyte membranes (54). This suggests the presence of an IL-6-R on these cells.

In this communication we report on the expression of the IL-6-R in normal human blood monocytes. It is shown how IL-6-R expression is regulated in these cells, which are known to be potent IL-6 producers. By comparing IL-6-R expression in human monocytes and human hepatocytes, we will demonstrate that the IL-6-R is subjected to tissue-specific regulation.

### Materials and Methods

*Endotoxin, Cytokines, and cDNA Probes.* Endotoxin derived from *Salmonella abortus equi* was a generous gift of C. Galanos, Max-Planck-Institut für Immunbiologie (Freiburg, FRG). Human rIL-1 $\beta$  ( $5 \times 10^7$  U/mg LAF; purity 98.6%) was a generous gift of Biogen (Geneva, Switzerland). Human rIL-6 used in the experiments was either IL-6/BSF-2 ( $5 \times 10^3$  U/ $\mu$ g) or IL-6/HPGF ( $10^6$  U/ $\mu$ g) (13), the latter being a generous gift of L. Aarden (Central Laboratory of the Netherlands Red Cross, Amsterdam). We compared IL-6/BSF-2 and IL-6/HPGF with respect to activity in the B9 hybridoma cell assay and found 1 U BSF-2 to be equivalent to  $\sim$ 200 U HPGF. The endotoxin content of all cytokines was  $<0.1$  ng/ml stock solution, resulting in final concentrations of at least three magnitudes below that. cDNAs used in this study were a cloned BSF-2/IL-6 cDNA (pBSF2-38.1) (11) and a cDNA encoding human IL-6-R pBSF2R.236 (53). A cDNA for human  $\alpha$ 2-macroglobulin was a generous gift of A. Lundwall and L. Sottrup-Jensen (University of Aarhus, Denmark). CRP cDNA and albumin cDNA were gifts of G. Ciliberto (University of Naples, Italy) and R. Cortese (EMBL, Heidelberg, FRG).

*IL-6.* Biological activity of IL-6 was measured by its proliferative action on the murine hybridoma cell line B9 (22; generously provided by L. Aarden, Central Laboratory of the Netherlands Red Cross). Proliferation was measured by means of a colometric assay (55). Cells were maintained in Iscove's modified Dulbecco's medium (IMDM) supplemented with 5% FCS,  $5 \times 10^{-5}$  M 2-ME, penicillin, streptomycin, and 0.1% supernatant of a 24-h culture of human monocytes stimulated with endotoxin (100 ng/ml). For the assay, cells were harvested by centrifugation and washed four times in PBS with 5% FCS.  $10^4$  cells were seeded in flat-bottomed microtitre plates (Costar, Cambridge, MA) containing 100  $\mu$ l of a serial twofold dilution in IMDM of the material to be tested. After incubation for 4 d, 50  $\mu$ l of 1 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma Chemical Co., St. Louis, MD) was added to each well. After another 4-h incubation period, the supernatant was removed and blue formazan crystals were dissolved in 0.01 N HCl/ethanol. The absorbance at 570 nm was measured by a multiwell scanning spectrophotometer (Titertek

multiskan; Flow Laboratories, Inc., McLean, VA). 1 U/ml is defined as the concentration of IL-6 leading to half-maximal stimulation of the B9 cell line and equals  $\sim 1$  pg/ml IL-6.

**Human Monocyte-Macrophage Cultures.** Human blood monocytes were isolated and cultivated as described (56) with certain modifications (23). Briefly, buffy coat cells from healthy blood donors were diluted in PBS and centrifuged over Ficoll-Hypaque to separate mononuclear cells from granulocytes and red cells. These mononuclear cells were used for FACS analysis (as indicated, either directly or after a 24-h cultivation period). For the other experiments, monocytes were separated from lymphocytes by adherence.  $3 \times 10^6$  mononuclear cells/ml RPMI 1640 medium (supplemented with L-glutamine, antibiotics,  $5 \times 10^{-5}$  mol/liter 2-ME, and vitamins) plus 10% human AB serum were incubated for 60 min in plastic Petri dishes. The nonadherent cells were removed by gentle washings with warm ( $37^\circ\text{C}$ ) medium. More than 90% of the adherent cells were monocytes as revealed by morphology and phenotype analysis. For in vitro maturation, monocytes were incubated overnight in supplemented RPMI 1640 medium with 10% human AB serum, then removed from the dishes at  $4^\circ\text{C}$  by vigorous pipetting with a viability of 85–90% (as determined by exclusion of trypan blue) and placed into rectangular teflon bags (Biofolie 25; Heraeus, Hanau, FRG) at a concentration of  $5 \times 10^5$  cells/ml in supplemented RPMI 1640 medium containing 10% human AB serum, and cultured for different times. At days 3 and 5, fresh medium was added to the bags. For the experiments, macrophages were harvested from the bags and plated in 1640 RPMI medium as above. Between 50 and 80% of the cells that had been placed into the bags could be recovered. More than 90% of the cells recovered from the bags were subsequently adherent. After sedimentation, the few remaining nonadherent cells were removed by washing with the medium, resulting in 100% pure macrophage cultures. These cells were mature by virtue of strong staining for the maturation-specific surface antigen max1 (56) (not shown). Complete absence of endotoxin has recently been discovered to be crucial for unimpaired and reliable monocyte-macrophage in vitro maturation (23, 57, 58). Therefore, all media and sera used were tested for trace amounts of endotoxins according to Northoff et al. (59). Strict observation of this requirement resulted in reproducible experiments with cells from different donors.

**RNA Extraction and Northern Analysis.** RNA was extracted according to the protocol of Chomczynski and Sacchi (60). For Northern blot hybridizations, 20  $\mu\text{g}$  of total RNA was subjected to electrophoresis on a 1.2% agarose-formaldehyde gel for 6 h at 100 V and transferred to nitrocellulose filters (Schleicher & Schuell, Inc., Keene, NH) according to Maniatis et al. (61). Standardization was done with respect to 28 S and 18 S rRNA as well as by control hybridizations with a cDNA clone coding for the housekeeping protein glyceraldehyde phosphate dehydrogenase (62). Hybridizations were performed for 24 h with  $^{32}\text{P}$ -labeled specific cDNAs (63) using  $2 \times 10^6$  cpm of labeled probe per milliliter hybridization buffer ( $6\times$  SSC, 50% formamide, 0.02% Denhardt's solution). Filters were exposed to Kodak XAR5 films at  $-70^\circ\text{C}$ . The bands were scanned with a densitometer (E-C Apparatus Corp., St. Petersburg, FL).

**Fluorescent Antibody Detection by FACS Analysis.** Phycoerythrin (PE)-conjugated murine mAb Leu-M3, and FITC-conjugated HLe 1 were obtained from Becton Dickinson & Co. (Mountain View, CA). PE- and FITC-conjugated murine IgG2 myeloma proteins (Becton Dickinson & Co.) were used as control antibodies. The murine mAb MT18 (class IgG2b) (Hirata, Y., T. Taga, M. Hibi, N. Nakano, T. Hirano, and T. Kishimoto, manuscript submitted for publication) was used to detect the 80-kD IL-6-R. An unconjugated IgG2 murine anti-DNP mAb (DHK10.12, kindly provided by J. Unkeless, Mt. Sinai Medical Center, New York) served as control stain. Fluorescein-conjugated goat anti-mouse Ig was obtained from Cappel Laboratories (Malvern, PA). Mononuclear cells were separated by Ficoll-Hypaque centrifugation, washed three times in PBS-BSA (1%) with 0.01% sodium azide, and stained as previously described (64). Cells were fixed in formalin (1%) before FACS analysis. Staining was determined by analysis on an Epics C cytofluorograph (Coulter Electronics, Hialeah, FL), individually gating on the two major cell populations by lateral and forward light scatter: a Leu-M3<sup>+</sup>/HLe1<sup>+</sup> population (predominantly monocytes), and a Leu-M3<sup>-</sup>/HLe1<sup>+</sup> population (predominantly lymphocytes). At least 2,000 cells were counted per sample. Isotype controls were performed in parallel for both direct and indirect test antibodies. Results are displayed graphically as two-dimensional log fluorescence, and numerically as percent fluorescence.

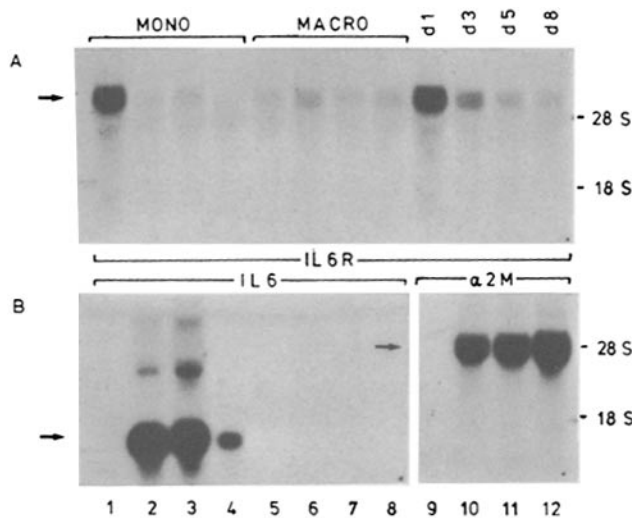
**Hepatocyte Cultures.** Primary hepatocyte cultures were prepared from fetal livers of human fetuses after legal abortion at 20 wk of gestation. The cultivation procedure has already been described (65). Hepatocytes used in this study were kept in Hams F 12 medium/2% human AB serum containing  $10^{-6}$  M dexamethasone,  $10^{-7}$  M insulin, and  $10^{-8}$  M glucagone. Absolutely endotoxin-free conditions were provided.

### Results

Expression of the IL-6-R was studied in cultured human blood monocytes and monocyte-derived macrophages. All experiments were carried out at least three times with monocytes from different donors. Representative experiments are shown. Freshly prepared, unstimulated human monocytes express high levels of IL-6-R mRNA (Fig. 1 A, lanes 1 and 9). Upon stimulation with endotoxin, IL-6-R mRNA decreased markedly within 4 h (Fig. 1 A, lane 2) and remained at nearly undetectable levels for at least 16 h (Fig. 1 A, lanes 3 and 4). The effect of endotoxin was dose dependent, with only a slight repression at a concentration of 0.1 ng/ml and maximal effects between 10 and 100 ng/ml. Repression of IL-6-R mRNA was specific, as shown by a concomitant increase of IL-6 mRNA (Fig. 1 B, lanes 1-4), resulting in a reverse pattern of regulation of the receptor and its ligand in the same cell population.

When unstimulated monocytes were allowed to undergo *in vitro* maturation into macrophages, a gradual spontaneous decline of IL-6-R mRNA was observed (Fig. 1 A, lanes 9-12), whereas expression of  $\alpha 2$ -macroglobulin, serving as a marker of macrophage maturation (23, 57, 58), increased steadily (Fig. 1 B, lanes 9-12). When *in vitro* matured macrophages were treated with endotoxin, no significant changes in IL-6-R mRNA could be observed (Fig. 1 A, lanes 5-8). In addition, endotoxin was unable to induce strong IL-6 expression in mononuclear phagocytes after terminal maturation (Fig. 1 B, lanes 5-8).

Endotoxin causes monocytes to release a large number of inflammatory medi-



**FIGURE 1.** Expression of IL-6-R mRNA, IL-6 mRNA, and  $\alpha 2$ -macroglobulin mRNA in monocytes and monocyte-derived macrophages. RNA was extracted from monocytes (lanes 1-4) and monocyte-derived macrophages after 8 d of cultivation (lanes 5-8). Cells were either unstimulated (lanes 1 and 5) or stimulated with 100 ng/ml LPS for 4 h (lanes 2 and 6), 8 h (lanes 3 and 7), or 16 h (lanes 4 and 8) before RNA extraction. In addition, RNA from unstimulated cells immediately after preparation (d1, lane 9), at day 3 (d3, lane 10), day 5 (d5, lane 11), and day 8 (d8, lane 12) of cultivation was analyzed. 20  $\mu$ g of total RNA/lane was subjected to 1.2% agarose gel electrophoresis, blotted onto nitro-

cellulose membranes, and hybridized with  $^{32}$ P-labeled IL-6-R cDNA (A). The same filters were rehybridized to radiolabeled IL-6 cDNA (B, lanes 1-8), and  $\alpha 2$ -macroglobulin cDNA (B, lanes 9-12). The autoradiographic exposure time was 3 d for A and 15 h for B).

ators (for review, see reference 66), which in turn may act back on the monocyte. We therefore investigated whether substances produced by monocytes upon endotoxin treatment could replace its effect on IL-6-R expression. As shown in Fig. 2 *A*, treatment of freshly prepared human monocytes with human rIL-1 was able to suppress IL-6-R expression (Fig. 2 *A*, lanes 1-5), whereas the expression of IL-6 was concomitantly induced (Fig. 2 *B*, lanes 1-5). The time kinetics of IL-6-R mRNA down regulation were comparable with those seen after endotoxin treatment. The effect of IL-1 was dose dependent, showing only a slight repression with 10 U/ml and a maximal effect with 100-200 U IL-1/ml. In contrast to monocytes, in vitro matured macrophages were hardly responsive to IL-1 treatment with respect to both IL-6-R mRNA and IL-6 mRNA regulation (Fig. 2, *A* and *B*, lanes 6-9).

Like IL-1, human rIL-6 was also able to induce a reduction in IL-6-R mRNA levels in monocytes. As shown in Fig. 3, treatment of monocytes with  $10^4$  U/ml HPGF (equivalent to 50 U/ml BSF-2) induced a marked decrease in IL-6-R mRNA.

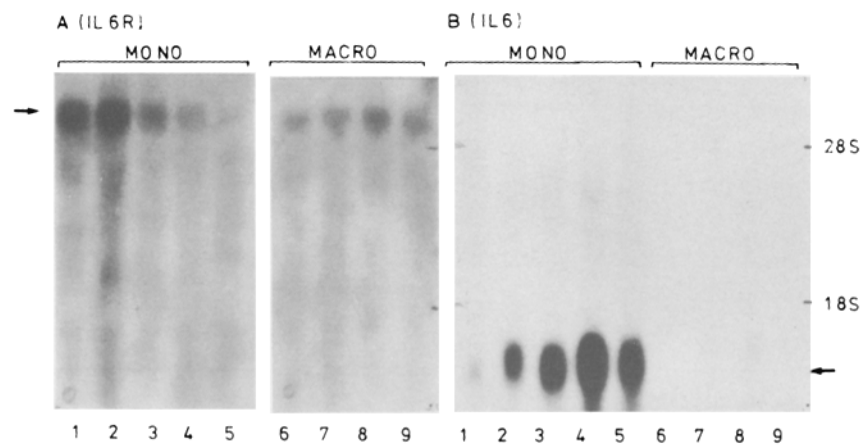


FIGURE 2. Effect of IL-1 on IL-6-R and IL-6 mRNA levels in monocytes and monocyte-derived macrophages. RNA was extracted from monocytes (lanes 1-5) and monocyte-derived macrophages after 7 d of cultivation (lanes 6-9). Cells were either unstimulated (lanes 1, 6), or stimulated with 200 U/ml human rIL-1 for 1 h (lane 2), 3 h (lanes 3 and 7), 6 h (lane 8), 9 h (lanes 4 and 9), or 18 h (lane 5). 20  $\mu$ g of total RNA/lane was subjected to Northern analysis as described in the legend of Fig. 1 and hybridized with  $^{32}$ P-labeled IL-6-R cDNA (*A*) or IL-6 cDNA (*B*). Autoradiographic exposure time was 3 d for *A* and 1 d for *B*.

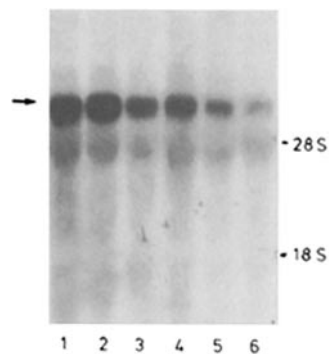


FIGURE 3. Regulation of IL-6-R mRNA in monocytes by IL-6. RNA was extracted from unstimulated monocytes (lane 1) or cells after various times of IL-6 treatment ( $10^4$  U/ml HPGF): 1 h (lane 2), 3 h (lane 3), 6 h (lane 4), 9 h (lane 5), and 18 h (lane 6). 20  $\mu$ g of total RNA/lane was subjected to Northern analysis as described in the legend of Fig. 1 and hybridized to  $^{32}$ P-labeled IL-6-R cDNA. The filter was autoradiographed for 5 d.

Slight effects of IL-6 were seen at  $10^3$  U/ml HPGF (equivalent to 5 U/ml BSF-2). Incubation of monocytes with IL-6 did not increase IL-6 mRNA levels (not shown).

Since the monocyte cultures used in our studies contained some 5–10% lymphocytes, we applied FACS analysis in order to see whether the IL-6-R mRNA detected in our experiments could be attributed to the monocytic population. For FACS analysis, mononuclear cells were examined either immediately after Ficoll centrifugation (Fig. 4, A–D) or after a 24-h incubation period (Fig. 4, E). Both lymphocytes and monocytes are recognized by the mAb HLe. The mAb Leu-M3 is directed against the monocyte-specific CD14 epitope. The total mononuclear cell population is shown in Fig. 4 A, and reveals a large granular (monocytes, Leu-M3<sup>+</sup>/HLe<sup>+</sup>), and a small, less granular subpopulation (lymphocytes, Leu-M3<sup>-</sup>/HLe<sup>+</sup>). The surface expression of IL-6-R was demonstrated on freshly isolated monocytes. 78% of Leu-M3<sup>+</sup>/HLe<sup>+</sup> cells (monocytes) costained with the MT18 mAb (Fig. 4 C), whereas

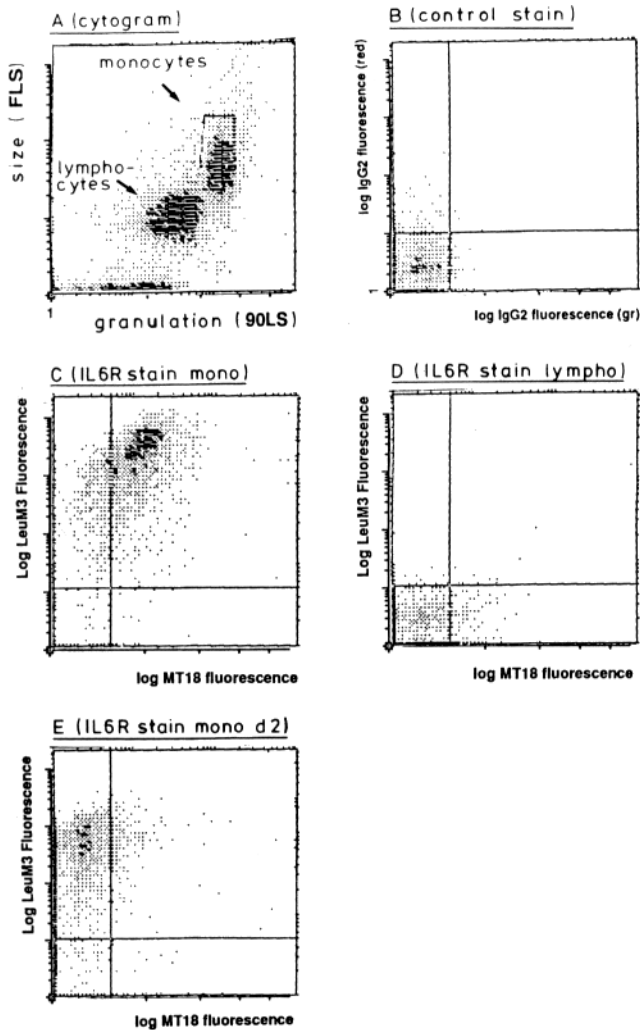


FIGURE 4. Detection of the IL-6-R on monocytes by FACS analysis using an anti-IL-6-R mAb (MT18). Freshly isolated PBMC after Ficoll centrifugation are shown in a cytogram gating of mononuclear cells (A). Granulation of the cells is indicated by the light scatter (LS); cell size by forward light scatter (FLS). Monocytes represent the larger and more granulated population shown in A. In C, cells identified as monocytes by staining for the monocyte marker Leu-M3 show costaining with the anti-IL-6-R mAb MT18 (78% specific fluorescence), whereas control immunofluorescence with isotype (IgG2b) PE- and FITC-labeled antibodies revealed <3.5% and <4.5% fluorescence, respectively. In D, HLe<sup>+</sup> cells identified as lymphocytes by the absence of Leu-M3 staining show only weak staining (9% specific fluorescence) with the MT18 anti-IL-6-R antibody. Mononuclear cells cultivated for 24 h (day 2 of culture) were analyzed in E. LeuM3<sup>+</sup> cells (monocytes) show markedly diminished staining with the MT18 anti-IL-6-R antibody (9% specific fluorescence) as compared with C.

only 9% of Leu-M3<sup>-</sup>/HLE1<sup>+</sup> cells (lymphocytes) revealed MT18 surface staining (Fig. 4 D). After incubation in endotoxin-free culture medium for 24 h, surface MT18 immunofluorescence for Leu-M3<sup>+</sup>/HLE1<sup>+</sup> cells (monocytes) dropped to only 9% (Fig. 4 E), reflecting the observed decrease of IL-6-R mRNA along in vitro maturation. These data were confirmed when IL-6-R mRNA was demonstrated in monocyte and lymphocyte populations after further purification (Fig. 5). IL-6-R mRNA signals in the lymphocyte preparation were several times weaker (as related to total cellular RNA) and could not be regulated by endotoxin.

Glucocorticoids are known to influence many inflammatory responses. We therefore examined the effect of glucocorticoid treatment on IL-6-R mRNA expression in monocytes (Fig. 6). Dexamethasone caused a transient and marked drop of IL-6-R mRNA within 3 h. After 7 h, mRNA levels increased slightly but were still below unstimulated control levels.

In contrast to monocytes, IL-6-R mRNA in hepatocytes is stimulated both by IL-1 and by IL-6 (Fig. 7 A). Nearly no IL-6-R mRNA can be detected in unstimulated primary hepatocytes. However, the presence of an intact IL-6-R molecule on

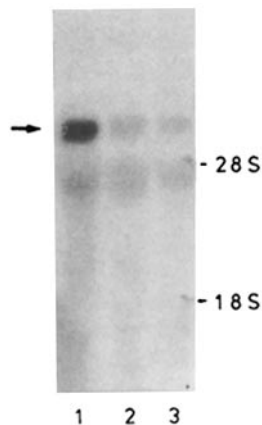


FIGURE 5. Comparison of IL-6-R mRNA levels in monocyte-enriched and lymphocyte-enriched cultures. RNA was extracted from unstimulated monocytes after repeated washing steps during 24 h of their initial adherence to plastic, resulting in further removal of lymphocytes (lane 1). These cells were therefore at day 2 in culture when harvested. Nonadherent mononuclear cells of the first adherence step were replated to plastic in order to achieve further purification from adhering monocytes. Nonadherent cells of the second adherence step represented a lymphocyte-enriched population from which RNA was extracted either directly (lane 3) or after 6 h of endotoxin stimulation (100 ng/ml) (lane 2). 20  $\mu$ g of total RNA/lane was subjected to Northern analysis as described in the legend of Fig. 1 and hybridized with <sup>32</sup>P-labeled IL-6-R cDNA. Autoradiographic exposure time was 5 d.

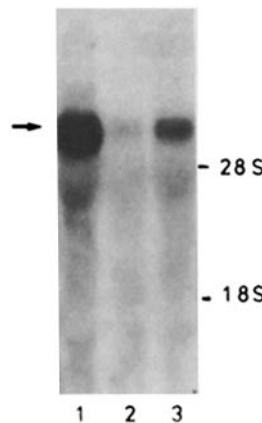


FIGURE 6. Decrease of monocytic IL-6-R mRNA levels upon treatment by dexamethasone. RNA was extracted from unstimulated monocytes (lane 1) or from cells after 3 h (lane 2) and 7 h (lane 3) of treatment by dexamethasone ( $10^{-7}$  M). 20  $\mu$ g of total RNA/lane was subjected to Northern analysis as described in the legend of Fig. 1 and hybridized with <sup>32</sup>P-labeled IL-6-R cDNA. Autoradiographic exposure time was 5 d.

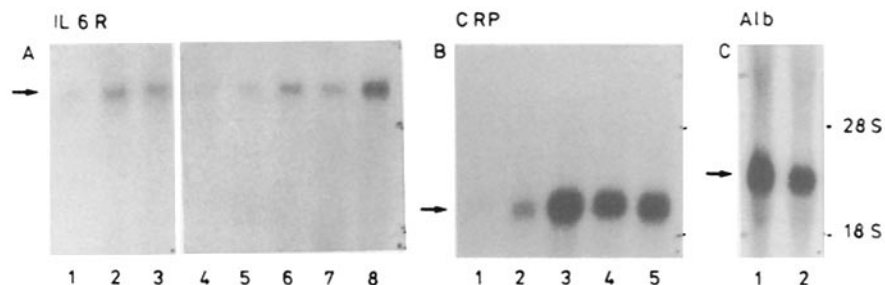


FIGURE 7. Expression of IL-6-R mRNA in human primary hepatocytes. Poly(A) RNA was prepared from fetal hepatocytes at day 4 in culture, subjected to Northern analysis (2.5  $\mu$ g/lane), and hybridized with radiolabeled IL-6-R cDNA (A), CRP cDNA (B), and albumin cDNA (C). (A) Lanes 1-3, a time kinetics of IL-6-R mRNA induction by IL-6 compared with unstimulated cells (A, lane 1), IL-6-R mRNA is induced in cells stimulated with 100 U/ml of BSF-2/IL-6 for 12 h (lane 2), or 24 h (lane 3). (A) Lanes 4-6, a concentration kinetics compared with unstimulated cells (lane 4), 24-h stimulation with 10 U/ml of BSF-2/IL-6 (lane 5), or with 100 U/ml of BSF-2/IL-6 (lane 6). (A) Lane 7, conditions as in lane 6 except that cells were kept in the absence of glucocorticoids. (A) Lane 8, stimulation for 24 h with 100 U/ml of IL-1 $\beta$ . (B) Lanes 1-5 represent the same experimental conditions as in A, lanes 4-8. (C) Lane 1, unstimulated cells; lane 2, stimulation with 100 U/ml of BSF-2/IL-6 for 24 h.

the membrane of unstimulated hepatocytes was shown by IL-6-dependent induction of CRP (Fig. 7 B). The specificity of this response is shown by concomitant downregulation of albumin (Fig. 7 C). If glucocorticoids were omitted from the hepatocyte culture medium, IL-6-dependent induction of the IL-6-R was slightly less pronounced, indicating an opposite effect of glucocorticoids on IL-6-R expression in monocytes and in hepatocytes.

### Discussion

In the past years, much has been learned about the pleiotropic biological activities of IL-6. Binding studies predicted the presence of an IL-6-R on resting T cells, activated B cells, and a number of cell lines, including lymphoblastoid cell lines, plasma cell lines, the histiocytic cell line U937, the promyelocytic line HL60, an astrocytoma, and a glioblastoma cell line (67, 68). However, detailed studies of the IL-6 target cells required the characterization of the IL-6-R. Recently, the IL-6-R has been cloned (53), and antibodies directed against this receptor have been developed (Y. Hirata, manuscript submitted for publication). The IL-6-R has been shown to be a single 80-kD molecule (69). If the ligand is bound to the 80-kD protein, the latter associates with a second membrane-bound protein of 130 kD, resulting in the signal transduction into the cell (69).

We have shown that unstimulated monocytes of healthy individuals express IL-6-R mRNA and that these cells carry the 80-kD receptor molecule on their surface. Upon stimulation with endotoxin, IL-1, or IL-6, the expression of IL-6-R mRNA is downregulated. These data suggest that the IL-6 secretion by monocytes under inflammatory conditions is accompanied by a decrease of their IL-6-R mRNA levels.

Until now, expression of IL-6-R mRNA has only been shown for a number of leukemic cell lines (53). It has also been reported that simultaneous expression of IL-6 and its receptor in plasmocytomas may constitute an autocrine stimulatory



loop resulting in uncontrolled proliferation (36, 70). We can now present an example of IL-6-R expression in a nonproliferating cell type that is known to be a potent IL-6 producer. However, downregulation of the monocytic IL-6-R at a time when IL-6 expression is induced may represent a protective mechanism against autocrine stimulation.

The physiological function of the monocytic IL-6-R remains to be elucidated. We found that treatment of monocytes with IL-6 results in an induction of  $\alpha$ 1-antitrypsin expression, revealing one of the possible functions of the monocytic IL-6-R. Induction of  $\alpha$ 1-antitrypsin in monocytes by IL-6 has recently been confirmed (D. H. Perlmutter in reference 21). In addition to the regulation of IL-6-dependent genes, expression of the IL-6-R on monocytes during noninflammatory homeostasis could indicate that monocytes may play a role in binding trace amounts of circulating IL-6.

Downregulation of the IL-6-R upon stimulation is a tissue-specific event. The differential regulation of the IL-6-R in human monocytes and hepatocytes may shed some light on an interesting biological mechanism: monocytes could bind IL-6 on their surface during noninflammatory homeostasis, thereby protecting other target cells from IL-6 activity. As soon as an inflammatory situation develops, the target for IL-6 is shifted from the monocyte to the hepatocyte and presumably to activated lymphocytes.

### Summary

IL-6 is a cytokine with pleiotropic biological functions, including induction of the hepatic acute phase response and differentiation of activated B cells into Ig-secreting plasma cells. We found that human peripheral blood monocytes express the IL-6-R, which is undetectable on the large majority of lymphocytes of healthy individuals. Stimulation of monocytes by endotoxin or IL-1 causes a rapid downregulation of IL-6-R mRNA levels and a concomitant enhancement of IL-6 mRNA expression. IL-6 itself was found to suppress the IL-6-R at high concentrations. A gradual decrease of IL-6-R mRNA levels was observed along in vitro maturation of monocytes into macrophages. We show that downregulation of IL-6-R mRNA levels by IL-1 and IL-6 is monocyte specific, since IL-6-R expression is stimulated by both IL-1 and IL-6 in cultured human primary hepatocytes. Our data indicate that under noninflammatory conditions, monocytes may play a role in binding of trace amounts of circulating IL-6. Repression of monocytic IL-6-R and stimulation of hepatocytic IL-6-R synthesis may represent a shift of the IL-6 tissue targets under inflammatory conditions.

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### References

1. Bauer, J. 1989. Interleukin-6 and its receptor during homeostasis, inflammation, and tumor growth (review). *Klin. Wochenschr.* 67:697.
2. Sehgal, P. B., and A. D. Sagar. 1980. Heterogeneity of poly(I)·poly(C)-induced human fibroblast interferon mRNA species. *Nature (Lond.)* 288:95.
3. Weissenbach, J., Y. Chernajovsky, M. Zeev, L. Shulman, H. Soreq, U. Nir, D. Wallach,

- M. Perricaudet, P. Tiollais, and M. Revel. 1980. Two interferon mRNAs in human fibroblasts: In vitro translation and Escherichia coli cloning studies. *Proc. Natl. Acad. Sci. USA.* 77:7152.
4. Content, J., L. De Wit, D. Pierard, R. Derynck, E. De Clercq, and W. Fiers. 1982. Secretory proteins induced in human fibroblasts under conditions used for the production of interferon  $\beta$ . *Proc. Natl. Acad. Sci. USA.* 79:2768.
  5. Hirano, T., T. Taga, N. Nakano, K. Yasukawa, S. Kashiwamura, K. Shimizu, K. Nakajima, K. H. Pyun, and T. Kishimoto. 1985. Purification to homogeneity and characterization of human B-cell differentiation factor (BCDF or BSFp-2). *Proc. Natl. Acad. Sci. USA.* 82:5490.
  6. Aarden, L., P. Lansdorp, and E. De Groot. 1985. A growth factor for B cell hybridomas produced by human monocytes. *Lymphokines.* 10:175.
  7. Van Damme, J., S. Cayphas, J. Van Snick, R. Conings, W. Put, J. P. Lenaerts, R. J. Simpson, and A. Billiau. 1987. 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.* 168:543.
  8. Van Damme, J., S. Cayphas, G. Opendakker, A. Billiau, and J. Van Snick. 1987. Interleukin 1 and poly(rI)·poly(rC) induce production of a hybridoma growth factor by human fibroblasts. *Eur. J. Immunol.* 17:1.
  9. May, L. T., D. C. Helfgott, and P. B. Sehgal. 1986. Anti- $\beta$ -interferon antibodies inhibit the increased expression of HLA-B7 mRNA in tumor necrosis factor-treated human fibroblasts: structural studies of the  $\beta$ 2 interferon involved. *Proc. Natl. Acad. Sci. USA.* 83:8957.
  10. Haegeman, G., J. Content, G. Volckaert, R. Derynck, J. Tavernier, and W. Fiers. 1986. Structural analysis of the sequence coding for an inducible 26-kDa protein in human fibroblasts. *Eur. J. Biochem.* 159:625.
  11. Hirano, T., K. Yasukawa, H. Harada, T. Taga, Y. Watanabe, T. Matsuda, S. Kashiwamura, K. Nakajima, K. Koyama, A. Iwamatsu, S. Tsunasawa, F. Sakiyama, H. Matsui, Y. Takahara, T. Taniguchi, and T. Kishimoto. 1986. Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. *Nature (Lond.)* 324:73.
  12. Zilberstein, A., R. Ruggieri, J. H. Korn, and M. Revel. 1986. Structure and expression of cDNA and genes for human interferon-beta-2, a distinct species inducible by growth-stimulatory cytokines. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:2529.
  13. Brakenhoff, J. P. J., E. R. De Groot, R. F. Evers, H. Pannekoek, and L. Aarden. 1987. Molecular cloning and expression of hybridoma growth factor in Escherichia coli. *J. Immunol.* 139:4116.
  14. Billiau, A. 1986. BSF-2 is not just a differentiation factor. *Nature (Lond.)* 324:415.
  15. Sehgal, P. B., L. T. May, I. Tamm, and J. Vilcek. 1987. Human  $\beta$ 2 interferon and B cell differentiation factor BSF-2 are identical. *Science (Wash. DC)* 235:731.
  16. Poupart, P., P. Vandenaebale, S. Cayphas, J. Van Snick, G. Haegeman, V. Kruys, W. Fiers, and J. Content. 1987. B cell growth modulating and differentiating activity of recombinant human 26-kD protein (BSF-2, HulFN- $\beta$ 2, HPGF). *EMBO (Eur. Mol. Biol. Organ.) J.* 6:1219.
  17. Van Damme, J., G. Opendakker, R. J. Simpson, M. R. Rubira, S. Cayphas, A. Vink, A. Billiau, and J. Van Snick. 1987. 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.* 165:914.
  18. Gauldie, J., C. Richards, D. Harnish, P. Lansdorp, and H. Baumann. 1987. 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.* 84:7251.

19. Poupart, P., P. Vandenabeele, S. Cayphas, J. Van Snick, G. Haegeman, V. Kruys, W. Fiers, and J. Content. 1987. B cell growth modulating and differentiating activity of recombinant human 26-kD protein (BSF-2, HulFN- $\beta$ 2, HPGF). *EMBO (Eur. Mol. Biol. Organ.) J.* 6:1219.
20. Yasukawa, K., T. Hirano, Y. Watanabe, K. Muratani, T. Matsuda, S. Nakai, and T. Kishimoto. 1987. Structure and expression of human B cell stimulatory factor-2 (BSF-2/IL-6) gene. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:2939.
21. Sehgal, P. B., G. Grieninger, and G. Tosato. 1989. Regulation of the acute phase and immune responses: a new cytokine. Conference of The New York Academy of Sciences. Dec. 12-14, 1988. *Ann. NY Acad. Sci.*
22. Aarden, L. A., E. R. De Groot, O. L. Schaap, and P. M. Lansdorp. 1987. Production of hybridoma growth factor by human monocytes. *Eur. J. Immunol.* 17:1411.
23. Bauer, J., U. Ganter, T. Geiger, U. Jacobshagen, T. Hirani, T. Matsuda, T. Kishimoto, T. Andus, G. Acs, W. Gerok, and G. Ciliberto. 1988. Regulation of interleukin-6 expression in cultured human blood monocytes and monocyte-derived macrophages. *Blood.* 72:1134.
24. Horii, Y., A. Maraguchi, S. Suematsu, T. Matsuda, K. Yoshizaki, T. Hirano, and T. Kishimoto. 1988. Regulation of BSF-2/IL-6 production by human mononuclear cells. *J. Immunol.* 141:1529.
25. Norioka, K., M. Hara, M. Harigai, A. Kitani, T. Hirose, K. Suzuki, M. Kawakami, H. Tabata, M. Kawagoe, and H. Nakamura. 1988. Production of B cell stimulatory factor-2/interleukin-6 activity by human endothelial cells. *Biochem. Biophys. Res. Commun.* 153:1045.
26. Sironi, M., F. Breviario, P. Proserpio, A. Biondi, A. Vecchi, J. Van Damme, E. Dejana, and A. Mantovani. 1989. IL-1 stimulates IL-6 production in endothelial cells. *J. Immunol.* 142:549.
27. Jirik, F. R., T. J. Podor, T. Hirano, T. Kishimoto, D. J. Loskutoff, D. A. Carson, and M. Lotz. 1989. Bacterial lipopolysaccharide and inflammatory mediators augment IL-6 secretion by human endothelial cells. *J. Immunol.* 142:144.
28. Poupart, P., L. De Wit, and J. Content. 1984. Induction and regulation of the 26-kDa protein in the absence of synthesis of  $\beta$ -interferon mRNA in human cells. *Eur. J. Biochem.* 143:15.
29. Content, J., L. De Wit, P. Poupart, G. Opendakker, J. Van Damme, and A. Billiau. 1985. Induction of a 26-kDa-protein mRNA in human cells treated with an interleukin-1-related, leukocyte-derived factor. *Eur. J. Biochem.* 152:253.
30. Kohase, M., D. Henriksen-DeStefano, L. T. May, J. Vilcek, and P. B. Sehgal. 1986. Induction of  $\beta$ 2-interferon by tumor necrosis factor: a homeostatic mechanism in the control of cell proliferation. *Cell.* 45:659.
31. Kohase, M., L. T. May, I. Tamm, J. Vilcek, and P. B. Sehgal. 1987. A cytokine network in human diploid fibroblasts: interactions of  $\beta$ -interferons, tumor necrosis factor, platelet-derived growth factor, and interleukin-1. *Mol. Cell. Biol.* 7:273.
32. Defilippi, P., P. Poupart, J. Tavernier, W. Fiers, and J. Content. 1987. Induction and regulation of mRNA encoding 26-kDa protein in human cell lines treated with recombinant human tumor necrosis factor. *Proc. Natl. Acad. Sci. USA.* 84:4557.
33. Van Damme, J., M. De Ley, J. Van Snick, C. A. Dinarello, and A. Billiau. 1987. The role of interferon  $\beta$ 1 and the 26-kDa protein (interferon  $\beta$ 2) as mediators of the antiviral effects of interleukin 1 and tumor necrosis factor. *J. Immunol.* 139:1867.
34. Van Damme, J., M. R. Schaafsma, W. E. Fibbe, J. H. F. Falkenburg, G. Opendakker, and A. Billiau. 1989. Simultaneous production of interleukin 6, interferon- $\beta$  and colony-stimulating activity by fibroblasts after viral and bacterial infection. *Eur. J. Immunol.* 19:163.
35. Freeman, G. J., A. S. Freedman, S. N. Rabinowe, J. M. Segil, J. Horowitz, K. Rosen, J. F. Whitman, and L. M. Nadler. 1989. Interleukin 6 gene expression in normal and

- neoplastic B cells. *J. Clin. Invest.* 83:1512.
36. Kawano, M., T. Hirano, T. Matsuda, T. Taga, Y. Horii, K. Iwato, H. Asaoku, B. Tang, O. Tanabe, H. Tanaka, A. Kuramoto, and T. Kishimoto. 1988. Autocrine generation and requirement of BSF-2/IL-6 for human multiple myeloma. *Nature (Lond.)* 332:83.
  37. Muraguchi, A., T. Hirano, B. Tang, T. Matsuda, Y. Horii, K. Nakajima, and T. Kishimoto. 1988. The essential role of B cell stimulatory factor 2 (BSF-2/IL-6) for the terminal differentiation of B cells. *J. Exp. Med.* 167:332.
  38. Takatsuki, F., A. Okano, C. Suzuki, R. Chieda, Y. Takahara, T. Hirano, T. Kishimoto, J. Hamuro, and Y. Akiyama. 1988. Human recombinant IL-6/B cell stimulatory factor 2 augments murine antigen-specific antibody responses in vitro and in vivo. *J. Immunol.* 141:3072.
  39. Baumann, H., and U. Müller-Eberhard. 1987. Synthesis of hemopexin and cysteine proteinase inhibitor is coordinately regulated by HSF-II and interferon  $\beta$ 2 in rat hepatoma cells. *Biochem. Biophys. Res. Commun.* 146:1218.
  40. Andus, T., T. Geiger, T. Hirano, H. Northoff, U. Ganter, J. Bauer, T. Kishimoto, and P. C. Heinrich. 1987. Recombinant human B cell stimulatory factor 2 (BSF-2/IFN- $\beta$ 2) regulates  $\beta$ -fibrinogen and albumin mRNA levels in Fao-9 cells. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 221:18.
  41. Geiger, T., T. Andus, J. Klapproth, T. Hirano, T. Kishimoto, and P. C. Heinrich. 1988. Induction of rat acute-phase proteins by interleukin 6 in vivo. *Eur. J. Immunol.* 18:717.
  42. Andus, T., T. Geiger, T. Hirano, T. Kishimoto, and P. C. Heinrich. 1988. Action of recombinant interleukin 6, interleukin 1 $\beta$  and tumor necrose factor  $\alpha$  on the mRNA induction of acute-phase proteins. *Eur. J. Immunol.* 18:739.
  43. Andus, T., T. Geiger, T. Hirano, T. Kishimoto, T. A. Tran-Thi, K. Decker, and P. C. Heinrich. 1988. Regulation of synthesis and secretion of major rat acute-phase proteins by recombinant human interleukin-6 in hepatocyte primary cultures. *Eur. J. Biochem.* 173:287.
  44. Ramadori, G., J. Van Damme, H. Rieder, and K. H. Meyer zum Büschenfelde. 1988. Interleukin-6, the third mediator of acute-phase reaction, modulates hepatic protein synthesis in human and mouse. Comparison with interleukin 1  $\beta$  and tumor necrosis factor- $\alpha$ . *Eur. J. Immunol.* 18:1259.
  45. Moshage, H. J., H. M. J. Roelofs, J. F. Van Pelt, B. P. C. Hazenberg, M. A. Van Leeuwen, P. C. Limburg, L. A. Aarden, and S. H. Yap. 1988. The effect of interleukin-1, interleukin-6 and its interrelationship on the synthesis of serum amyloid A and C-reactive protein in primary cultures of adult human hepatocytes. *Biochem. Biophys. Res. Commun.* 155:112.
  46. Castell, J. V., M. J. Gomez-Lechon, M. David, T. Hirano, T. Kishimoto, and P. C. Heinrich. 1988. Recombinant human interleukin-6 (IL-6/BSF-2/HSF) regulates the synthesis of acute phase proteins in human hepatocytes. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 232:347.
  47. Castell, J. V., M. J. Gomez-Lechon, M. David, T. Andus, T. Geiger, R. Trullenque, R. Fabra, and P. C. Heinrich. 1989. Interleukin-6 is the major regulator of the acute phase protein synthesis in adult human hepatocytes. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 242:237.
  48. Garman, R. D., K. A. Jacobs, S. C. Clark, and D. H. Raulet. 1987. B-cell-stimulatory factor 2 ( $\beta$ 2 interferon) functions as a second signal for interleukin 2 production by mature T cells. *Proc. Natl. Acad. Sci. USA.* 84:7629.
  49. Houssiau, F. A., P. C. Coulie, D. Olive, and J. Van Snick. 1988. Synergistic activation of human T cells by interleukin 1 and interleukin 6. *Eur. J. Immunol.* 18:653.
  50. Ikebuchi, K., G. G. Wong, S. C. Clark, J. N. Ihle, Y. Hirai, and M. Ogawa. 1987. Interleukin 6 enhancement of interleukin-3 dependent proliferation of multipotential hemopoietic progenitors. *Proc. Natl. Acad. Sci. USA.* 84:9035.
  51. Wong, G. G., J. A. S. Witek, P. A. Temple, R. Kriz, C. Ferenz, R. M. Hewick, S. C. Clark, K. Ikebuchi, and M. Ogawa. 1988. Stimulation of murine hematopoietic colony

- formation by human interleukin-6. *J. Immunol.* 140:3040.
52. Leary, A. G., K. Ikebuchi, Y. Hirai, G. G. Wong, Y. C. Yang, S. C. Clark, and M. Ogawa. 1988. Synergism between interleukin-6 and interleukin-3 in supporting proliferation of human hematopoietic stem cells: comparison with interleukin-1 alpha. *Blood.* 71:1759.
  53. Yamasaki, K., T. Taga, Y. Hirata, H. Yawata, Y. Kawanishi, B. Seed, T. Taniguchi, T. Hirano, and T. Kishimoto. 1988. Cloning and expression of the human interleukin-6 (BSF-2/IFN $\beta$ 2) receptor. *Science (Wash. DC).* 241:825.
  54. Castell, J. V., T. Geiger, V. Gross, T. Andus, E. Walter, T. Hirano, T. Kishimoto, and P. C. Heinrich. 1988. Plasma clearance, organ distribution and target cells of interleukin-6/hepatocyte-stimulating factor in the rat. *Eur. J. Biochem.* 177:357.
  55. Mosmann, T. 1984. Rapid colorimetric assay for cellular growth and survival: application to proliferative and cytotoxic assays. *J. Immunol. Methods.* 65:55.
  56. Andreesen, R., K. J. Bross, J. Osterholz, and F. Emmrich. 1986. Human macrophage maturation and heterogeneity: analysis with a newly generated set of monoclonal antibodies to differentiation antigens. *Blood.* 67:1257.
  57. Ganter, U., J. Bauer, C. Schulz-Huotari, P. J. Gebicke-Haerter, H. Beeser, and W. Gerok. 1987. Repression of alpha2 macroglobulin and stimulation of alpha1 proteinase inhibitor synthesis in cultured human mononuclear phagocytes by endotoxin. *Eur. J. Biochem.* 169:13.
  58. Bauer, J., U. Ganter, and W. Gerok. 1988. Endotoxin abolishes the induction of alpha2-macroglobulin synthesis in cultured human monocytes indicating inhibition of the terminal monocyte maturation into macrophages. In *Proteases: Potential Role in Health and Disease*. W. H. Hörl and A. Heidland, editors. Plenum Publishing Corp., New York. 425-433.
  59. Northoff, H., D. Kabelitz, and C. Galanos. 1986. Interleukin 1 production for detection of bacterial polysaccharide in fetal calf sera and other solutions. *Immunol. Today.* 7:126.
  60. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156.
  61. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. *Molecular Cloning: A Laboratory Model*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
  62. Fort, P., L. Marty, M. Piechaczyk, S. El Sabrouy, C. Dani, P. Jeanteur, and J. M. Blanchard. 1985. Various rat adult tissues express only one major mRNA species from the glyceraldehyde-3-phosphate-dehydrogenase multigenic family. *Nucleic Acids Res.* 13:1431.
  63. Feinberg, A. P., and B. Vogelstein. 1984. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 137:266.
  64. Mayer, L., S. M. Fu, C. Cunningham-Rundles, and H. G. Kunkel. 1984. Polyclonal immunoglobulin secretion in patients with common variable immunodeficiency using monoclonal B cell differentiation factors. *J. Clin. Invest.* 74:2115.
  65. Sells, M. A., J. Chernoff, A. Cerda, C. Bowers, D. A. Shafritz, N. Kase, J. K. Christman, and G. Acs. 1984. Long term culture and passage of human fetal liver cells that synthesize albumin. *In Vitro Cell Devel. Biol.* 21:216.
  66. Nathan, C. F. 1987. Secretory products of macrophages. *J. Clin. Invest.* 79:319.
  67. Taga, T., Y. Kawanishi, R. R. Hardy, T. Hirano, and T. Kishimoto. 1987. Receptors for B cell stimulatory factor 2. *J. Exp. Med.* 166:967.
  68. Coulie, P. G., A. Vanhecke, J. Van Damme, S. Cayphas, P. Poupart, L. De Wit, and J. Content. 1987. High-affinity binding sites for human 26-kDa protein, different from those of type I interferon, on lymphoblastoid cells. *Eur. J. Immunol.* 17:1435.
  69. Taga, T., M. Hibi, Y. Hirata, K. Yamasaki, K. Yasukawa, T. Matsuda, T. Hirano, and T. Kishimoto. 1989. Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp 130. *Cell.* 58:573.
  70. Kishimoto, T. 1987. B-cell stimulatory factors (BSFs): molecular structure, biological function, and regulation of expression. *J. Clin. Immunol.* 7:343.