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# Recombinant interferon-β2 (interleukin-6) induces myeloid differentiation

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Human IFN-β2 cytokine produced in *E. coli* was purified to homogeneity by immunoaffinity and ion-exchange chromatography. The cytokine inhibits the growth of myeloleukemic M1 cells and induces their morphological and functional differentiation into macrophages. Differentiation was also observed in the histiocytic lymphoma U937 cells. The effect on U937 was synergized by IFN-γ and under these conditions IFN-β2 produced the induction of (2′-5′) oligo(A) synthetase typical to IFN action and to differentiation.

Growth control; Leukemia; Differentiation; Interferon β2; Interleukin 6

## 1. INTRODUCTION

Leukemic cells proliferate without limit and without undergoing normal hematopoietic terminal differentiation, often having lost their requirements for exogenous growth factors or colony-stimulating factors (CSFs), presumably because of epigenetic alterations imposed by overexpression of oncogenes [1–3]. An important feature of this epigenetic defect is that it can be reversible, several chemical agents being able to push the cells toward differentiation and concomitant growth arrest [3,4]. Moreover, a number of naturally occurring cell-secreted protein factors (cytokines), produced physiologically in the organism, have now been found to restore the differentiation and growth arrest of leukemic cell lines in vitro, suggesting that these proteins may have antitumor regulatory functions in vivo [3,4]. For example, myeloid differentiation is induced in murine myeloleukemic cells such as WEHI-3B $D^+$ and M1 by hematopoietic colony-stimulating factors, e.g. G-CSF [5–7], and by other factors devoid of growth-stimulatory activity such as MGI-2 [4,8] and LIF [9]. Among the immunoregulatory cytokines, tumor necrosis factor (TNF-α) was found to induce differentiation of human myeloblastic ML-1 cells [10]. Interleukin-1 (IL-1α) acts in synergism with TNF-α or interferon (IFN-β) to induce monocytic differentiation of the M1 cells [11]. Since both TNF and IL-1 are strong inducers of the cytokine IFN-β2/IL-6 [IFN-β2 [13] is also referred to as B-cell stimulatory factor 2 (BSF-2), hybridoma plasmacytoma growth factor (HGF), hepatocyte stimulatory factor, 26 kDa protein and interleukin 6 (IL-6)] [12,13] and since this cytokine inhibits growth of carcinoma, lymphoma and leukemia cells [14,15], we investigated its effect on the differentiation of M1 cells. We show here that recombinant, *E. coli*-produced, unglycosylated IFN-β2 induces mass cultures of M1 myeloblasts to differentiate into growth-arrested macrophage-like cells, and causes partial differentiation of human histiocytic lymphoma U937 cells.

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## 2. MATERIALS AND METHODS

Human recombinant IFN-β2 was purified from *E. coli* JM101 $i^q$ harboring the direct expression plasmid pkk-β2/7 [15], which was grown to an $A_{600nm}$ value of 30 in a 16 l

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Microgen Fermentor SF-116 (New Brunswick Sci., NJ), harvested and disrupted by glass beads in a Dyno-Mill type KDL as detailed [16]. The extract was freed of nucleic acids by polyethyleneimine precipitation [16] and passage through DEAE-cellulose in 25 mM Tris-HCl (pH 7.5), 25 mM NaCl. Purification was achieved by chromatography on sulfonyl (S)-Sepharose (Pharmacia, NJ) in 10 mM Na acetate buffer (pH 5.0) with a 0–0.3 M NaCl gradient, followed by fast protein liquid chromatography (FPLC) on Mono-S (Pharmacia) under the same conditions. Whereas mammalian cell-produced IFN-$\beta$2 has 23 and 26 kDa forms [13], the *E. coli* preparation showed a single band at 21 kDa by SDS-polyacrylamide gel electrophoresis (fig.1). The first amino acid was identified as $Pro^{29}$ of the IFN-$\beta 2_a$ sequence [13]. Measured by stimulation of [$^3$H]thymidine incorporation in plasmacytoma T1165 cells (Dr R. Nordan, NIH) the specific activity was $2 \times 10^6$ U/mg (BSF-2/HGF activity). An alternative purification based on immunoaffinity chromatography was carried out as in [16] with immobilized anti-IFN-$\beta$2 monoclonal antibodies (hybridoma clone 34), the protein being eluted by citrate buffer (pH 2) and immediately neutralized. The specific activity of the immunopurified recombinant protein was the same as that obtained by chemical purification. The IFN-$\beta$2 preparation titrating 350000 U/ml contained an amount of lipolysaccharide of 6 pg/ml as measured by the limulus test. Human recombinant IFN-$\gamma$ ($10^7$ U/mg) was obtained from hamster cells as described [17].

Murine myeloleukemic M1 cells [18] and human lymphoma U937 cells [19] were grown in RPMI 1640 with 10% fetal calf serum (FCS). The cells were seeded at $10^5$/ml in wells of 12-well Costar plates. Pure rIFN-$\beta$2 was added at 0.1–75 ng/ml and the cultures were observed for 4–6 days. Cells were counted and stained for Giemsa and for non-specific esterase using the $\alpha$-naphthyl acetate esterase kit 91-A of Sigma (St. Louis, MO). Lysozyme activity was measured in 0.5% Triton X-100 cell extracts by a turbimetric assay of the lysis of *Micrococcus lysodeikticus* (Sigma), the assay being calibrated with egg-white lysozyme as described [8]. The (2′-5′)oligo(A) synthetase activity was assayed in Nonidet-P40 cell extracts as described [20].

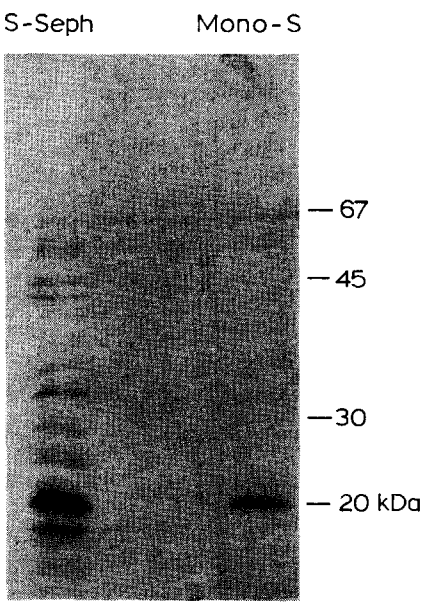


Fig.1. Polyacrylamide gel electrophoresis in SDS of rIFN-$\beta$2, after the sulfonyl-Sepharose step (2.4 $\mu$g, left lane) and after the Mono-S FPLC step (0.4 $\mu$g, right lane). The middle slot contained sample buffer only. Silver stain.

## 3. RESULTS

Without addition, the M1 cells grew without adhering to the dish and showed typical myeloblastic morphology. In contrast, after 4 days of culture with IFN-$\beta$2, the cells were adherent and showed dramatic morphological changes (fig.2). About 60% of the cells acquired macrophage-like morphology, the rest showing various degrees of maturation. Cytoplasms were enlarged, contained vacuoles and acquired a typical foamy appearance. Nuclei were eccentric, less round and contrasted, and had less prominent nucleoli. Viable cell counting showed that while the control culture grew for 6 days, the M1 cells treated with 50 U/ml IFN-$\beta$2 underwent 2–3 divisions and growth was arrested (table 1). At day 4 after seeding, less than 1 U/ml IFN-$\beta$2 (expressed in plasmacytoma growth units, see section 2) was sufficient to cause a 50% decrease in M1 cell number (fig.3). The growth-arrest effect was maximal above 30 U/ml IFN-$\beta$2 (15 ng/ml). Even with the chemically purified rIFN-$\beta$2, this concentration corresponds to no more than 2.5 pg/ml LPS which had no effect on the M1 cells. Growth inhibition and differentiation of M1 cells were observed when IFN-$\beta$2 was added with 5 $\mu$g/ml polymyxin B, further excluding any role of LPS traces. As a biochemical marker of differentiation we measured lysozyme activity [8] in extracts of $5 \times 10^6$ M1 cells cultured for 4 days with 30 U/ml IFN-$\beta$2. Lysosyme was undetectable in the control M1 cultures. Treatment with IFN-$\beta$2 induced lysozyme to levels of 0.85 $\mu$g lysozyme equivalent per $5 \times 10^6$ cells. Phagocytic activity on latex beads was also observed in the differentiated M1 cells. Thus IFN-$\beta$2 inhibits growth of M1 cells and induces their monocytic differentiation.

Human histiocytic lymphoma U937 cells can be induced to differentiate by phorbol esters and vitamin D3, and partially by IFN-$\gamma$ and other yet unidentified cytokines [21,22]. We examined the

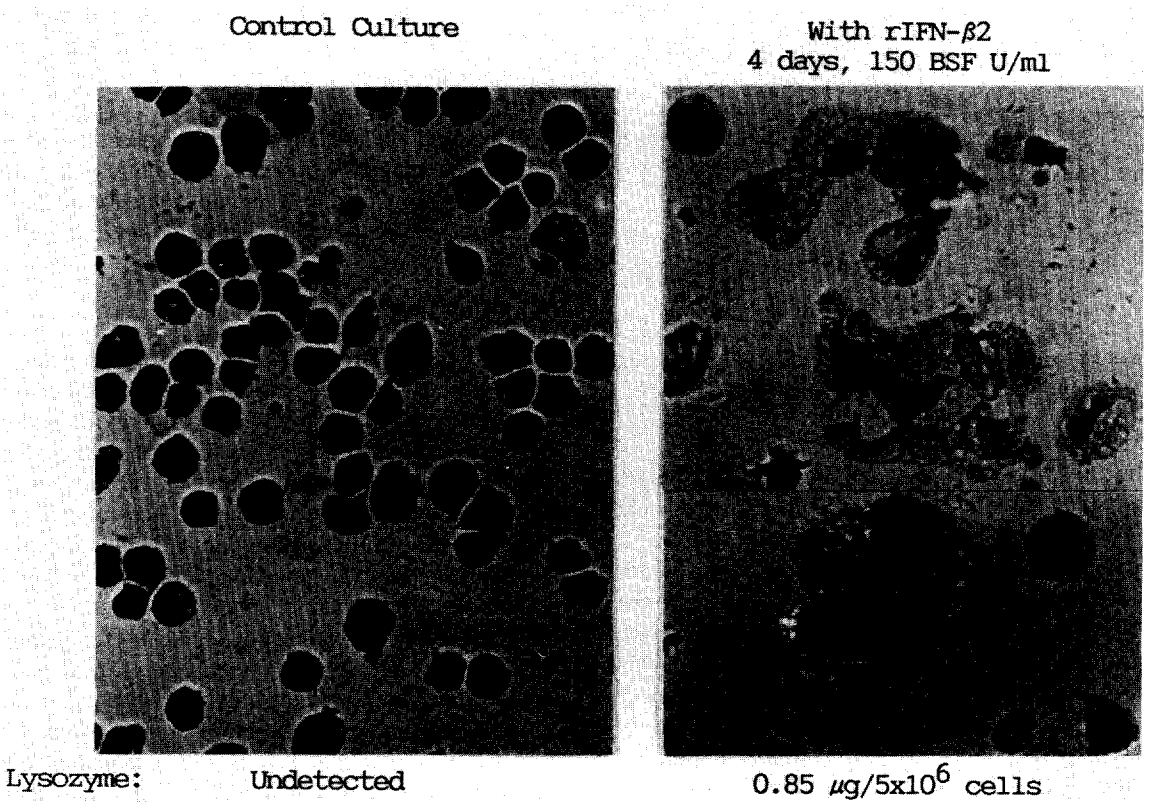


Fig.2. Morphological differentiation of M1 myeloblasts induced by rIFN-β2/IL-6. The culture was initiated by seeding $10^5$ cells/ml in a 12-well Costar plate in the absence (left) and presence of rIFN-β2 (FPLC purified, 150 BSF U/ml). After 4 days cells were Giemsa stained. Representative fields are shown (magnification × 400).

effect of 100 U/ml IFN-β2 addition on U937 cultures. After 4–5 days, about 25% of the cells showed monocytic/macrophage morphology and there was a 30% reduction in cell growth (table 2). The cells were stained for α-naphthyl acetate esterase, as a biochemical marker of differentia-

Table 1

Effect of IFN-β2/IL-6 on myeloleukemic M1 cell growth

| IFN-β2 (BSF U/ml) | Cell number ($\times 10^{-5}$) | | | | |
|---|---|---|---|---|---|
| | Day 0 | Day 1 | Day 4 | Day 5 | Day 6 |
| 0 | 1 | 2.3 | 19.0 | 25.0 | 35.0 |
| 25 | 1 | 2.0 | 3.5 | 0.9 | 0.7 |
| 50 | 1 | 1.4 | 2.3 | 0.8 | 1.2 |

Recombinant *E. coli* IFN-β2 purified by Mono-S FPLC

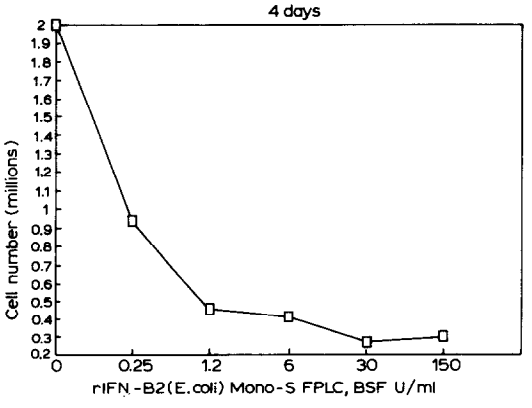


Fig.3. Growth inhibition of M1 myeloblasts by rIFN-β2/IL-6. Same experiment as in fig.1 with various concentrations of the cytokine.

Table 2

Effect of IFN-β2/IL-6 on histiocytic lymphoma U937 cell growth and differentiation

| Expt | IFN-β2 (BSF U/ml) | Cell number ($\times 10^{-5}$) | Esterase-positive cells (%) |
|---|---|---|---|
| 1 | 0 | 14.0 (100) | 4 |
| | 100 | 10.0 (71) | 24 |
| 2 | 0 | 26.7 (100) | ND |
| | 150 | 23.5 (88) | ND |
| | 1500 | 14.5 (54) | ND |

Cells treated for 5 days with or without rIFN-β2 purified on Mono-S FPLC. Staining for non-specific esterase as in section 2

tion not induced by IFN-γ [22]. About one quarter of the cells in the IFN-β2-treated culture were strongly positive for the non-specific esterase, whereas few positive cells were observed in the untreated (table 2) or mock-treated culture (not shown). With higher amounts of the pure rIFN-β2 preparations, growth inhibition (table 2) and partial morphological changes, such as cytoplasmic enlargement and nucleus indentation, were more pronounced. However, we found that when added together with IFN-γ, the effect of low-dose IFN-β2 was significantly potentiated (table 3). Under these conditions, cell growth was reduced and most of the cells showed cytoplasmic enlargement, changes in nuclear shape and nucleolus reduction, although monocytic differentiation was still incomplete. We previously reported that early during differentiation of U937 cells in response to phorbol esters, there was a marked rise in (2′-5′)-A synthetase activity of the cells [23]. Induction of (2′-5′)-A synthetase is mediated by an autocrine IFN and was related to the growth arrest associated with terminal differentiation [20,22,23]. The addition of IFN-γ strongly potentiated the induction of (2′-5′)-A synthetase by IFN-β2 (table 3), suggesting that the two cytokines cooperate to initiate the differentiation process, although other additions may be required to observe complete differentiation of the type seen with the M1 cells.

Table 3

Synergistic effects of IFN-β2/IL-6 and IFN-γ on histiocytic lymphoma U937 cells

| IFN-β2 (BSF U/ml) | IFN-γ (U/ml) | Cell number ($\times 10^{-5}$) | (2′-5′)A synthetase activity ([$^{32}$P]A2′pA cpm) |
|---|---|---|---|
| 0 | 0 | 20 (100) | 110 |
| 15 | 0 | 14 (70) | 310 |
| 0 | 100 | 12 (60) | 940 |
| 15 | 100 | 8.5 (42) | 4000 |

Cells treated for 6 days with or without rIFN-β2 immunoaffinity purified

## 4. DISCUSSION

Induction of terminal differentiation in myelomonocytic leukemic cell lines is a novel additional activity of the multifunctional cytokine IFN-β2/IL-6. This protein, first identified and cloned from human diploid fibroblasts induced by viruses or poly(rI)(rC) to produce IFN-β activities [24,25], was later found to be identical to B-cell stimulatory factor 2 (BSF-2 or IL-6) produced by an HTLV1-transformed T cell clone [26]. This IL-6 activity enhances immunoglobulin secretion in mature B lymphocytes and the lymphoblastoid cell line CESS, and may contribute to the differentiation of plasma cells. The cytokine has growth factor activity for Epstein-Barr virus-transformed human B lymphocytes [27] and for several plasmacytomas and hybridomas [28]. Activation of T-cell functions was also reported [29]. Another important activity is expressed in hepatocytes where IFN-β2 activates the expression of a battery of genes encoding the acute phase proteins, such as fibrinogen, antiproteases and components of the complement system [30]. The cytokine also induces complement factor B and C3 synthesis in fibroblasts, which may provide a local activation of the alternative complement pathway during infections [31].

The different activities found associated with IFN-β2/IL-6 suggest that this cytokine plays important functions in the defense against infections and in inflammatory processes [14,31]. The protein is induced in various cells by viruses, by bacterial products such as LPS [31,32] as well as by the inflammatory cytokines TNF and IL-1 [12,13,31]. Monocytes produce IFN-β2 either spontaneously or after stimulation by phytohemagglutinin [33]. A role in infections/inflammation is further supported by the fact that IFN-β2 stimulates hematopoiesis and more specifically myelopoiesis, a process characteristic of inflamma-

tions. Thus, in bone marrow and blood cultures in vitro, IFN-$\beta$2 synergizes the effect of multipoietin IL-3 on formation of blast colonies [34] and of differentiating hematopoietic colonies of the myelomonocytic, erythroid and mixed lineages [35]. IFN-$\beta$2 does not work as a colony-stimulating factor (CSF) by itself but stimulates hematopoietic progenitor cells in liquid cultures to respond to CSF and form colonies when plated on agar [35]. The differentiation of myeloblastic leukemic cells described here may, therefore, reflect the effect on normal hematopoiesis, rather than just overcoming the differentiation block due to the leukemic state.

During terminal differentiation, leukemic as well as normal cells undergo growth-arrest in $G_0/G_1$ and down-regulation of proto-oncogene expression [20]. This is accompanied by production of an autocrine interferon activity usually of the $\beta$-type, as attested by the increase in (2′-5′) oligo(A) synthetase and blockage with anti-IFN-$\beta$ antibodies [20,22,23]. In the U937 cells, phorbol esters induce IFN-$\beta$2 RNA [36] although other studies suggest an RNA related to IFN-$\beta$1 is made [20]. Here we show that IFN-$\beta$2 induces the typical (2′-5′) oligo(A) synthetase increase in U937 particularly in synergism with IFN-$\gamma$, conditions which enhance differentiation. The M1 cells respond to lung-conditioned medium [20] as well as to TNF and IL-1 [11] by production of IFN activity and increase in (2′-5′) oligo(A) synthetase. Neutralization of this IFN activity prevents the growth arrest [11,20,22]. Mouse IFN-$\beta_1$ did not induce M1 cell differentiation alone [11]. The present evidence that IFN-$\beta$2 inhibits M1 cell growth and causes their differentiation suggests that IFN-$\beta$2 could play this autocrine IFN function in cell differentiation. Recent results indicate that the differentiation factor MGI-2 [4,8] is identical to murine IL-6 (Sachs, L., personal communication). MGI-2 is made as an autocrine factor in response to several CSFs [4] and we have observed that IFN-$\beta$2 RNA is induced in bone marrow cells treated for 15 days with GM-CSF (Stephen, M. and Revel, M., unpublished). Although natural IFN-$\beta$2 is a complex glycoprotein [37], the unglycosylated recombinant protein used in this work appears very active as a growth inhibitor and differentiation factor. Whether IFN-$\beta$2/IL-6 acts directly to induce growth arrest and differentiation or induces still other cellular factors is not yet clear. However, some direct effect would be in line with the growth-inhibitory action of IFN-$\beta$2 on a number of cell lines [15].

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## REFERENCES

[1] Moore, M.A.S., Gabrilove, J.L., Welte, K. and Platzer, E. (1985) in: Immunity to Cancer (Reif, A.E. and Mitchell, M.S. eds) pp.513–529, Academic Press, New York.
[2] Weinberg, R.A. (1985) Science 230, 770–776.
[3] Ostertag, W., Stocking, C., Johnson, G.R., Kluge, N., Kollek, R., Franz, T. and Hess, N. (1987) Adv. Cancer Res. 48, 193–355.
[4] Sachs, L. (1987) Science 238, 1374–1379.
[5] Nicola, N.A., Metcalf, D., Matsumoto, M. and Johnson, G.R. (1983) J. Biol. Chem. 258, 9017–9023.
[6] Tomida, M., Yamamoto-Yamaguchi, Y., Hozumi, M., Okabe, T. and Takaka, F. (1986) FEBS Lett. 207, 271–275.
[7] Souza, L.M., Boone, T.C., Gabrilove, J., Lai, P.H., Zsebo, K.M., Murdock, D.C., Chazin, V.R., Bruszewski, J., Lu, H., Chen, K.K., Barendt, J., Platzer, E., Moore, M.A.S., Mertelsmann, R. and Welte, K. (1986) Science 232, 61–65.
[8] Weisinger, G. and Sachs, L. (1983) EMBO J. 2, 2103–2107.
[9] Gearing, D.P., Gough, N.M., King, J.A., Hilton, D.J., Nicola, N.A., Simpson, R.J., Nice, E.C., Kelso, A. and Metcalf, D. (1987) EMBO J. 6, 3995–4002.
[10] Takeda, K., Iwamoto, S., Sugimoto, H., Takuma, T., Kawatani, N., Noda, M., Masaki, A., Morise, H., Arimura, H. and Konno, K. (1986) Nature 323, 338–340.
[11] Onozaki, K., Urawa, H., Tamatani, T., Iwamura, Y., Hashimoto, T., Baba, T., Suzuki, H., Yamada, M., Yamamoto, S., Oppenheim, J.J. and Matsushima, K. (1988) J. Immunol. 140, 112–119.
[12] Kohase, M., Henrikson-Destefano, D., May, L.T., Vilcek, J. and Sehgal, P.B. (1986) Cell 45, 659–666.
[13] Zilberstein, A., Ruggieri, M., Korn, J.H. and Revel, M. (1986) EMBO J. 5, 2529–2537.
[14] Revel, M., Zilberstein, A., Ruggieri, R., Rubinstein, M. and Chen, L. (1987) J. Interferon Res. 7, 529–536.
[15] Chen, L., Mory, Y., Zilberstein, A. and Revel, M. (1988) Proc. Natl. Acad. Sci. USA 85, in press.
[16] Vaks, B., Mory, Y., Pederson, J.U. and Horovitz, O. (1984) Biotechnology Lett. 6, 621–626.
[17] Mory, Y., Ben-Barak, J., Segev, D., Cohen, B., Novick, D., Fischer, D., Rubinstein, M., Kargman, S., Zilberstein, A., Vigneron, M. and Revel, M. (1986) DNA 5, 181–193.
[18] Ishikawa, Y. (1969) J. Cell. Physiol. 74, 223–234.

[19] Sundstrom, C. and Nilsson, K. (1976) Int. J. Cancer 17, 565–577.
[20] Resnitzky, D., Yarden, A., Zipori, D. and Kimchi, A. (1986) Cell 46, 31–40.
[21] Harris, P.E., Ralph, P., Litcofsky, P. and Moore, M.A.S. (1985) Cancer Res. 45, 9–13.
[22] Testa, U., Ferbus, D., Gabbianelli, M., Pasucci, B., Boccolo, G., Louache, F. and Thang, M.N. (1988) Cancer Res. 48, 82–88.
[23] Yarden, A., Shure-Gottlieb, H., Chebath, J., Revel, M. and Kimchi, A. (1984) EMBO J. 3, 969–973.
[24] Weissenbach, J., Chernajovsky, Y., Zeevi, M., Shulman, L., Soreq, H., Nir, U., Wallach, D., Perricaudet, M., Tiollais, P. and Revel, M. (1980) Proc. Natl. Acad. Sci. USA 77, 7152–7156.
[25] Sehgal, P.B. and Sagar, A.D. (1980) Nature 288, 95–97.
[26] 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. (1986) Nature 234, 73–76.
[27] Tosato, G., Seamon, K.B., Goldman, N.D., Sehgal, P.B., May, L.T., Washington, G.C., Jones, K.D. and Pike, S.E. (1988) Science 239, 502–504.
[28] VanDamme, J., Opdenakker, G., Simpson, R.J., Rubira, M.R., Cayphas, S., Vink, A., Billiau, A. and VanSnick, J. (1987) J. Exp. Med. 165, 914–919.
[29] Takai, Y., Wong, G.G., Clark, S.C., Burakoff, S.J. and Herrmann, S. (1988) J. Immunol. 140, 508–512.
[30] Gauldie, J., Richards, C., Harnish, D., Lansdorp, P. and Baumann, H. (1987) Proc. Natl. Acad. Sci. USA 84, 7251–7255.
[31] Katz, Y., Revel, M. and Strunk, R.C. (1988) EMBO J., submitted.
[32] Helfgott, D.C., May, L.T., Shtoeger, Z., Tamm, I. and Sehgal, P.B. (1987) J. Exp. Med. 166, 1300–1308.
[33] Sanceau, J., Falcoff, R., Zilberstein, A., Beranger, F., Lebeau, J., Revel, M. and Vaquero, C. (1988) J. Interferon Res., in press.
[34] Ikebuchi, K., Wong, G.G., Clark, S.C., Ihle, J.N., Hirai, Y. and Ogawa, M. (1987) Proc. Natl. Acad. Sci. USA 84, 9035–9039.
[35] Revel, M., Zilberstein, A., Ruggieri, R., Chen, L., Mory, Y., Rubinstein, M. and Michalevicz, R. (1988) in: Monokines and Other Non-Lymphocytic Cytokines (Powanda, M.C. et al. eds) pp.21–27, A.R. Liss, New York.
[36] Alitalo, R., Andersson, L.C., Betscholz, C., Nilsson, K., Westermark, B., Heldin, C.-H. and Alitalo, K. (1987) EMBO J. 6, 1213–1218.
[37] May, L.T., Ghrayeb, J., Santhanam, U., Tatter, S., Shtoeger, D., Helfgott, D.C., Chiorazzi, N., Grieninger, G. and Sehgal, P.B. (1988) J. Biol. Chem. 263, 7760–7768.