Differential regulation of interleukin-6 expression in human fibroblasts by tumor necrosis factor-α and lymphotoxin

Luisa Mantovani[1], Reinhard Henschler[1], Marion A. Brach[1], Raimund Wieser[2], Michael Lübbert[1], Albrecht Lindemann[1], Roland H. Mertelsmann[1] and Friedhelm Herrmann[1]

[1]*Albert-Ludwigs-Universität Freiburg, Department of Hematology and Oncology, Freiburg, FRG* and [2]*Johannes Gutenberg Universität Mainz, Institute for Toxicology, Mainz, FRG*

Received 5 June 1990; revised version received 6 July 1990

The treatment of human diploid fibroblasts with tumor necrosis factor (TNF)-α and with lymphotoxin (LT) is associated with induction of interleukin-6 (IL-6) transcripts with TNF-α being 10-fold more potent than LT. Here we report on the TNF-α/LT-induced signaling mechanisms responsible for the regulation of IL-6 gene expression in these cells. Run-on assays demonstrated that both TNF-α and LT increase IL-6 mRNA levels by transcriptional activation of this gene. Stability studies of IL-6 transcripts in fibroblasts showed that TNF-α delayed IL-6 mRNA decay but not LT. The induction of IL-6 transcripts by TNF-α and LT was not inhibited by the isoquinoline sulfonamide derivative H7. Similarly, depletion of protein kinase C (PKC) by 12-*O*-tetradecanoyl-phorbol 13-acetate (TPA) did not change the ability of TNF-α and LT to induce IL-6 transcripts, demonstrating that stimulation by these agents may not be mediated by activation of PKC. Stimulation of IL-6 transcripts in fibroblasts did also not require new protein synthesis as exposure to the protein synthesis inhibitor cycloheximide (CHX) enhanced accumulation of IL-6 mRNA in the presence or absence of TNF-α or LT.

1. INTRODUCTION

Tumor necrosis factor (TNF)-α is a cytokine produced by inflammatory cells including activated monocytes/macrophages [1,2], polymorphonuclear leukocytes [3], T-, and B-lymphocytes [1,4]. By inducing secretion of colony stimulating factors (CSFs) in mesenchymal cells [5–7] and monocytes/macrophages [8,9], TNF-α stimulates development and functioning of hematopoietic cells. Although sharing with TNF-α the same receptor [10], lymphotoxin (LT) is less potent as a CSF-inducer [9,11,12]. To further understand the role of TNF-α and LT in hematopoietic development and activation, the ability to induce secretion of another cytokine stimulatory to hematopoiesis, interleukin-6 (IL-6), was investigated. IL-6 synergizes with CSFs to enhance growth of self-renewing blast cells [7,13] and also acts as a differentiation inducing factor [14]. Using primary human fibroblasts which constitute a major element of the bone marrow stroma, we show that TNF-alpha and LT increase the expression of IL-6. At maximum stimulatory concentrations, TNF-α was about 10-fold more potent than LT and has enhanced the expression of IL-6 by increasing its rate of transcription and mRNA stability, whereas LT had no effect on half-life of IL-6 mRNA. The action of TNF-α and LT was independent of synthesis of new proteins and did not require stimulation through the protein kinase C (PKC) pathway.

Correspondence address: F. Herrmann, Albert-Ludwigs-Universität Freiburg, Department of Hematology and Oncology, Freiburg, FRG

2. MATERIALS AND METHODS

2.1. *Cytokines and DNA probes*

Recombinant (E. coli-derived) human (rh) TNF-α (specific activity of 5×10^7 U/mg of protein) and recombinant (*E. coli*-derived) human LT (specific activity of 1×10^8 U/mg of protein) were kindly provided by Genentech, San Francisco, CA, through Dr. G.R. Adolf, Ernst Boehringer Institute for Drug Research, Vienna, Austria. Purity of TNF-α and LT was > 99% by SDS-PAGE and RP-HPLC. Endotoxin content of both preparations was < 50 pg/mg as assessed by the limulus amoebocyte assay. Mouse monoclonal antibodies (mo Abs) to rhTNF-α (lot 3314-16) and a rabbit antiserum to rhLT (lot 2970-14 B) were provided by Dr. G.R. Adolf. The neutralizing capacity of the anti-TNF-α mo Ab was 6000 U TNF-α/μg and of the antiserum to LT 2.9×10^7 U/ml. Human IL-6 cDNA (*TaqI*/*Ban*II fragment) was derived from the plasmid pBS F2.38 and was kindly provided by Drs. T. Hirano and T. Kishimoto, Institute for Molecular and Cellular Biology, Osaka University, Osaka, Japan. The 2.0 kb *Pst*I fragment of the chicken β-actin gene was derived from the pAI-plasmid (kindly provided by Dr. J. Ramadori, Dept. of Medicine, University of Mainz, Mainz, FRG). The probes were ^{32}P-labeled by random priming [15]. The specific activity was 4 to 8×10^8 cpm/μg.

2.2. *Chemical reagents*

1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H7), 12-*O*-tetradecanoylphorbol-13-acetate (TPA), cycloheximide (CHX), 2-mercaptoethanol, actinomycin-D, trypsin and EDTA were purchased from Sigma Chemicals, Munich, FRG.

2.3. *Cell Cultures*

Diploid human fibroblasts (strain FH 109) were isolated from embryonic lung tissue by proteolytic dispersion [16]. Cultures were established and cells were passaged by methods detailed elsewhere

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

[17]. In all experiments cells from passages 5–8 were used. Cultures were performed in alpha medium supplemented with low endotoxin FCS (Boehringer, Mannheim, FRG), 100 μg/ml penicillin, 100 μg/ml streptomycin, 1 mM L-glutamine, 1 mM sodium pyruvate (Gibco Laboratories, Heidelberg, FRG) in a humidified atmosphere, 7% CO_2 at 37°C. The culture period was 2–72 h. Conditioned medium (CM) from cultures of fibroblasts that were incubated with TNF-α (1 to 5 × 10^3 U/ml) or LT (10–10^4 U/ml) was collected, filtered through low protein binding millipore filters and stored at −20°C until use. In some experiments cultures received H7 (25 μM), TPA (8–24 nM), actinomycin-D (5 μg/ml), or CHX (20 μg/ml).

2.4. *IL-6 bioassay*

For measurements of IL-6 activity, the IL-6-dependent hybridoma cell line B13.29 (subline B9) was cultured at 5 × 10^4 cells/ml in Iscove's modified Dulbecco's medium (Gibco) supplemented with 50 μM 2-mercaptoethanol, 5% FCS, 100 μg/ml penicillin, 100 μg/ml streptomycin for 48 h [18]. Six hours before harvesting, all cultures were pulsed with 1 mCi/ml = 37 kBq/ml of tritiated thymidine (Amersham Buchler, Braunschweig, FRG). The cells were harvested on glass filter papers by an automated cell harvester. The radioactivity was measured by a liquid scintillation counter (Beckmann Instruments, Munich, FRG). The results are expressed as U/ml of triplicate cultures. RhIL-6 (kindly provided by Drs. T. Hirano and T. Kishimoto) was used as an internal standard. B9 cells do not proliferate in response to any of the inducing agents investigated.

2.5. *RNA extraction, Northern blot and transcriptional run-on assay*

Total cellular RNA was isolated by lysing FH 109 cells in guanidinium isothiocyanate followed by recovery of RNA by centrifugation through cesium chloride [19]. After denaturation at 60°C, RNA was electrophoresed in an agarose formaldehyde gel (1.2%) and transferred on to synthetic membranes (Schleicher and Schuell, Dassel, FRG). Filters were hybridized with labeled probe for 12–24 h at 42°C in 50% formamide, 2 × SSC, 5 × Denhardts, 0.1% SDS, 10% dextran sulfate and 100 μg/ml salmon sperm. Filters were washed to a stringency of 0.1% SSC, 65°C for 12 min and exposed to Kodak X-omat X-ray films with intensifying screens. To exclude incomplete RNA transfer in single lanes, all filters were reprobed with beta-actin cDNA. Alterations in levels of IL-6 transcripts were quantitated by laser densitometry as described [20]. The ratio of IL-6/β-actin transcripts in unstimulated cultures was compared to the ratio of experimental cultures. Changes of IL-6 mRNA from base line levels were calculated by multiplication of the ratio of density of IL-6/β-actin transcripts by the reciprocal of the ratio of base line levels. Nuclear run-on transcription assays were performed as previously described [19]. Nascent RNA chains were allowed to elongate in the presence of ^{32}P-uridine triphosphate. The ^{32}P-labeled nuclear RNA was treated with DNAse and proteinase K digestion and purified by phenol/chloroform extraction and ethanol precipitation. Equivalent amounts of TCA-precipitable ^{32}P-labeled RNAs were hybridized to vector DNA, β-actin and IL-6 probes immortalized on nitrocellulose filters. The ^{32}P-labeled RNA bound to the filters was visualized by autoradiography at −70°C by using intensifying screens.

3. RESULTS

3.1. *Induction of IL-6 release by TNF-α and LT*

IL-6 release was negligible in untreated confluent human fibroblasts (Fig. 1). However, exposure of fibroblasts to TNF-α resulted in a dose- and time-dependent increase in levels of IL-6 protein detectable in culture supernatants by biologic assay. Maximum IL-6 release was seen, when fibroblasts were exposed to 10^3 U/ml of TNF-α for 48 h. Although time-dependence of IL-6 release by LT stimulated fibroblasts was comparable to that of TNF-α stimulation, LT was

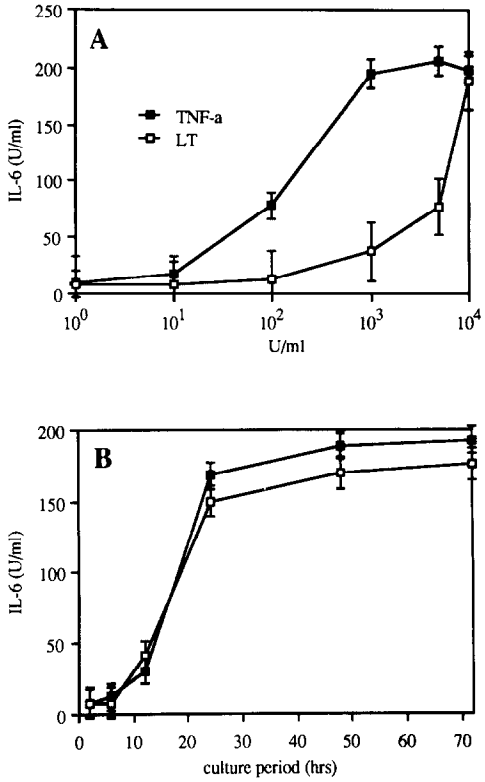

Fig. 1. Release of IL-6 from confluent fibroblasts monolayers by rhTNF-α and rhLT. Duplicate monolayers were incubated with various concentrations of TNF-α and LT for 48 h (A), or were treated with maximum stimulatory concentrations (B) of TNF-α (10^3 U/ml) or LT (10^4 U/ml). IL-6 activity in the supernatants was quantitated in the B9 proliferation assay and standardized in U/ml against serial dilutions of rhIL-6. Data represent values (±SD) of three independent experiments.

less potent (ten-fold) in inducing IL-6 release in a dose range of 10^2 to 5 × 10^3 U/ml. At maximum stimulatory concentrations of LT (10^4 U/ml), however, fibroblast-CM contained the same amount of IL-6 as compared to IL-6 present in cultures maximally stimulated with TNF-α. Endotoxin at a dose equivalent to that detectable in 10^5 U of TNF-α or LT failed to stimulate release of IL-6 protein (data not shown).

3.2. *Time- and dose-dependent effect of TNF-alpha and LT on accumulation of IL-6 mRNA*

Fibroblasts that were exposed to maximum stimulatory concentration of TNF-α or LT (10^3 U/ml and 10^4 U/ml, respectively) contained mRNA coding for IL-6, whereas unstimulated cells (passages 4–8) had undetectable IL-6 levels (Fig. 2A). Following stimulation with TNF-α, a maximum stimulation of IL-6 mRNA was observed after 8 h. Levels had decreased after 12 h. LT-stimulated fibroblasts displayed maximum levels of IL-6 mRNA after 4 h of exposure and had decreased after 8 h. Maximum levels of IL-6 mRNA were achieved after treatment with 10^3 U/ml TNF-α. Further increase in TNF-α concentrations did not result in higher con-

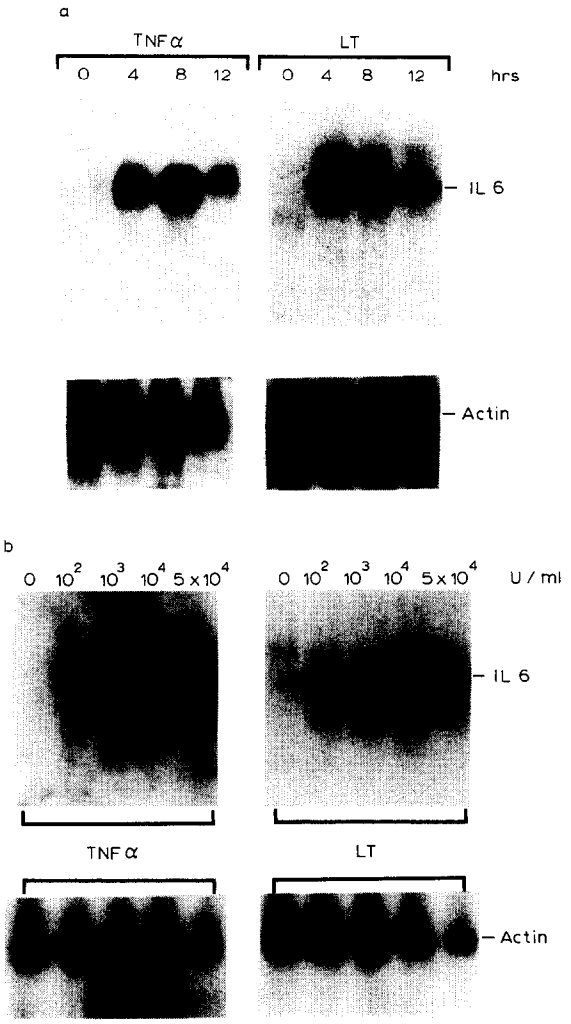

Fig. 2. (A) Time-dependent effect of TNF-α and LT on levels of IL-6 mRNA in fibroblasts. Fibroblasts (7×10^5/ml) were incubated in the presence or absence of TNF-α (10^3 U/ml) or LT (10^4 U/ml) for various periods of time. Cytoplasmic RNA (20 μg/lane) was sequentially hybridized with IL-6 and β-actin cDNA. (B) Dose-dependent effect of TNF-β and LT on levels of IL-6 mRNA in fibroblasts. Fibroblasts (7×10^5/ml) were incubated in the presence or absence of various concentrations of TNF-α for 8 h or in the presence or absence of various concentrations of LT for 4 h. Cytoplasmic RNA (20 μg/lane) was sequentially hybridized with IL-6 (1.6 kb) and β-actin cDNA (2.1 kb).

centrations of IL-6 mRNA (Fig. 2B). Similarly, LT stimulated IL-6 mRNA accumulation in fibroblasts in a dose-dependent fashion. Maximum hybridization signals were detected in fibroblasts stimulated with 10^4 U/ml of LT, being 50% less intense than with TNF-α. Again, endotoxin at dose equivalents to those present in 10^5 U of TNF-α or LT failed to induce transcripts of IL-6 (data not shown).

3.3. *Stimulation of IL-6 mRNA accumulation does not require PKC activation*

To evaluate the effects of the inhibitor of PKC (H7) on IL-6 mRNA accumulation induced by TNF-α and

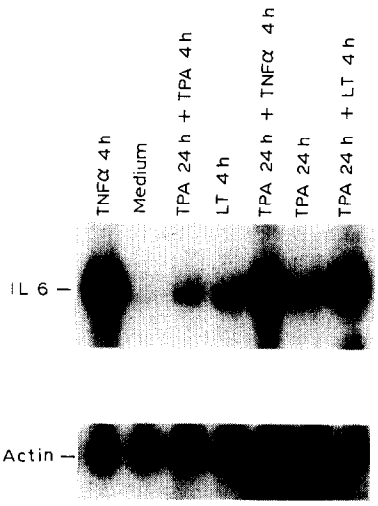

Fig. 3. Effect of PKC depletion by prolonged exposure of TPA on levels of IL-6 mRNA induced by TNF-α and LT. Fibroblasts (7×10^5/ml) were treated with TPA (24 nM) for 24 h, washed and reexposed to TPA (8 nM) without, and with TNF-α (10^3 U/ml), or LT (10^4 U/ml), for additional 4 h. Cytoplasmic RNA (20 μg/lane) was sequentially hybridized with IL-6 and β-actin-specific cDNA.

LT, fibroblasts were stimulated with TNF-α or LT in presence of H7 (25 μM). The induction of IL-6 transcript was not abrogated by H7, suggesting that the action of TNF-α and LT is independent of PKC stimulation (data not shown). In addition, depletion of PKC by prolonged exposure of fibroblasts to TPA [21], failed to prevent subsequent enhancement of IL-6 mRNA accumulation by TNF-α or LT (Fig. 3). Treatment of fibroblasts for 24 h with TPA (24 nM) and subsequent incubation of these cells for 4 h in fresh medium induced IL-6 mRNA accumulation. The IL-6 mRNA levels had decreased by 55% when culture had been treated with TPA (8 nM), for 4 h. In contrast, in cultures of fibroblasts incubated with TNF-α and LT for 4 h a reexpression of IL-6 transcripts is stimulated (Fig. 3).

3.4. *Induction of IL-6 transcripts by TNF-α or LT does not require de novo protein synthesis*

Exposure of fibroblasts to CHX has induced the accumulation of IL-6 mRNA (data not shown). Cells previously cultured in the presence of CHX, and subsequently treated with TNF-alpha for 4 h have shown about 50-fold and three-fold higher IL-6 mRNA levels compared to cultures treated with CHX and TNF-α only, respectively. Similarly, the response of LT was at least 20-fold enhanced by pretreatment with CHX. Two-fold higher levels of IL-6 transcripts were achieved by sequential CHX/LT treatment compared to cultures stimulated with LT only.

3.5. *Enhancement of IL-6 gene transcription by TNF-alpha and LT*

To evaluate the transcriptional regulation of expres-

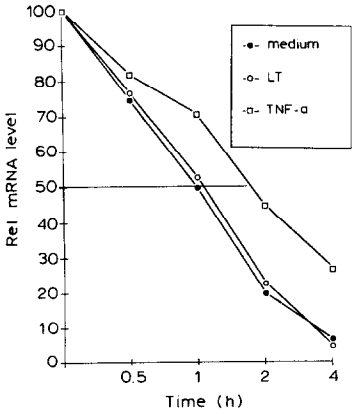

Fig. 4. Stability of base-line IL-6 mRNA levels in fibroblasts treated with TNF-alpha or LT. Fibroblasts (7×10^5/ml) were cultured in the presence or absence of TNF-α (10^3 U/ml) or LT (10^4 U/ml) for 7 h and 4 h, respectively, and then actinomycin D (5 μg/ml) was added to the cultures for 0, 1, 2, 4 h. Cytoplasmic RNA (40 μg/lane in cells cultured in medium alone, 20 μg/lane in cells exposed to TNF-α or LT) was sequentially hybridized to IL-6 and β-actin-specific cDNA.

sion of IL-6 mRNA, nuclear run-on assays were performed. Transcription of the IL-6 gene was enhanced to a comparable extent in fibroblasts after treatment with TNF-α and LT (data not shown). In order to determine if the increase of IL-6 mRNA by TNF-α and LT was also dependent on mRNA stabilization, mRNA half-life studies using actinomycin-D were performed. Aged FH 109 fibroblasts (passages 12–14), which constitutively accumulate IL-6 mRNA were treated with either without, with TNF-α, or LT at maximum stimulatory concentrations and then exposed to actinomycin-D (Fig. 4). After 0.5, 1, 2 and 4 h cells were harvested. The half-life of IL-6 mRNA in untreated cells was about 50 min and remained unchanged after stimulation with LT. However, stimulation of fibroblasts with TNF-alpha resulted in elevated IL-6 mRNA stability.

4. DISCUSSION

In the present study we confirm that not only TNF-α is a potent inducer of IL-6 in fibroblasts but also LT. In addition, we explore some mechanisms involved in the regulation of IL-6 expression by TNF-alpha and LT. We found that TNF-α was more potent than LT in inducing IL-6 mRNA accumulation and protein secretion. 10^2 U/ml of TNF-α had nearly the same stimulatory effect as 5×10^3 U/ml of LT. These findings confirm several recent reports demonstrating that certain cell types responsive to TNF-α are either partially or totally refractive to LT [9,11,12,22]. This predicts different receptors for the two proteins. Indeed, it has recently been shown that the cross-linking of the receptor of rhLT revealed two distinct bands at M_r of 100 kDa and 120 kDa, whereas affinity cross-linking with rhTNF-α provided only a single band with M_r of 100 kDa [23].

In this study we have used non-glycosylated LT, whereas the natural LT is glycosylated. Nevertheless, experimental evidence suggests that *E. coli*-derived rhLT lacking carbohydrate is not equipotent to mammalian cell-derived rhLT for cell binding [23]. In addition, we show that TNF-α augments IL-6 mRNA accumulation by increasing the rate of gene transcription and enhancing the stability of IL-6 mRNA, whereas LT had only an effect on transcription of IL-6. In this regard the recent observation of the ability of TNF-α to activate G-binding proteins [24] and to induce genes by prolonged activation of the jun/AP-1 system [24] is interesting. Moreover, the IL-6 gene possesses recognition elements for G-binding proteins and AP-1 [25]. Current experiments therefore address whether the action of LT on the induction of IL-6 is associated with c-fos/jun induction and whether inhibitors of G-binding proteins will prevent LT mediated induction of IL-6. The findings that IL-6 gene activation occurs in the absence of protein synthesis (as discussed below) and thus must involve the modification of preexisting cellular factors needs further investigation. Modification of preexisting cellular factors has been shown as a mechanism responsible for c-fos expression [26]. Similar to c-fos, IL-6 is expressed by a variety of different cells and induced by different agents. It would therefore be of interest to determine if different transcription factors regulate IL-6 expression, as already shown for c-fos [27], and if these factors are specific for cell type and inducer. Previous studies have shown that induction of IL-6 by TNF-α requires enhancement of cAMP levels and protein kinase activity [28]. Now we show that PKC activation does not play a role in induction of IL-6 expression by TNF-α and LT. Induction of IL-6 by both compounds was not prevented by PKC inhibitor H7 and fibroblasts that became refractory to TPA which activates PKC continued to be inducible to express IL-6 by TNF-α and LT.

We also demonstrate that induction of IL-6 by TNF-alpha and by LT does not require synthesis of new proteins, as the inhibitor of protein synthesis CHX failed to downregulate TNF-α- and LT-inducible accumulation of IL-6 mRNA. Stimulation of IL-6 mRNA accumulation by CHX suggests that the IL-6 gene is apparently negatively regulated by repressor proteins, a situation that may facilitate the rapid modulation of its expression. Again, the same observation applies to c-fos [29]. Interestingly the c-fos serum responsive element (SRE) was identified within the conserved region of the IL-6 promotor and it has been shown that IL-6 inducibility by IL-1 involves this c-fos SRE [30]. A number of rapidly inducible genes involved in inflammation, acute-phase response, and development of hematopoiesis have AT-rich sequences in their 3′untranslated regions [25,30,31] that are involved in the degradation of the respective mRNA by RNase. Our

experiments demonstrating increase of IL-6 mRNA levels by CHX are consistent with decrease of RNase activity.

Fibroblasts also produce granulocyte-macrophage colony-stimulating factors when exposed to TNF-alpha [32] or LT [33], and mechanisms of the regulation of this expression are similar to those shown here for IL-6 [32]. Therefore, the capacity of TNF-α/LT to regulate expression of different classes of proteins central to inflammation, acute-phase response and hematopoiesis, may represent a pivotal tool to coordinate host defense.

Acknowledgements: Supported by BMFT Grant 0319012. B.L.M. was supported by a fellowship of the Ministero Pubblica Istruzione Italia.

REFERENCES

[1] Cuturi, M.C., Murphy, M., Costa-Giomi, M.P., Weinmann, R., Perussia, B. and Trinchieri, G. (1985) J. Exp. Med. 162, 1512.
[2] Herrmann, F., Bambach, T., Bonifer, R., Lindemann, A., Riedel, D., Oster, W. and Mertelsmann, R. (1988) Int. J. Cell Clon. 6, 241.
[3] Lindemann, A., Riedel, D., Oster, W., Ziegler-Heitbrock, H.W.L., Mertelsmann, R. and Herrmann, F. (1989) J. Clin. Invest. 83, 1308.
[4] Lindemann, A., Ludwig, W.D., Oster, W., Mertelsmann, R. and Herrmann, F. (1989) Blood 73, 880.
[5] Munker, R., Gasson, J., Ogawa, M. and Koeffler, H.P. (1986) Nature 323, 79.
[6] Broudy, V.C., Kaushansky, K., Segal, G.M., Harlan, J.M. and Adamson, J.W. (1986) Proc. Natl. Acad. Sci. USA 83, 7467.
[7] Oster, W., Cicco, N.A., Klein, H., Hirano, T., Kishimoto, T., Lindemann, A., Mertelsmann, R. and Herrmann, F. (1989) J. Clin. Invest. 84, 451.
[8] Oster, W., Lindemann, A., Mertelsmann, R. and Herrmann, F. (1989) Eur. J. Immunol. 19, 543.
[9] Oster, W., Lindemann, A., Horn, S., Mertelsmann, R. and Herrmann, F. (1987) Blood 70, 1700.
[10] Aggarwal, B.B., Eessalu, T.E. and Haas, P.E. (1989) Nature 318, 665.
[11] Broudy, V.C., Harlan, J.M. and Adamson, J.W. (1987) J. Immunol. 138, 4298.
[12] Koeffler, H.P., Gasson, J., Ranyard, J., Souza, L., Shepard, M. and Munker, R. (1987) Blood 70, 55.
[13] Ikebuchi, K., Wong, G.G., Clark, S.C., Ihle, J.N., Hirai, Y. and Ogawa, M. (1987) Proc. Natl. Acad. Sci. USA 84, 9035.
[14] Brach, M., Riedel, D., Mertelsmann, R. and Herrmann, F. (1989) Virchows Archiv. Mol. Pathol. (in press).
[15] Feinberg, A.P. and Vogelstein, B. (1983) Anal. Biochem. 132, 6.
[16] Dayer, J.M., Breard, J., Chess, L. and Krane, S.M. (1979) J. Clin. Invest. 64, 1386.
[17] Dayer, J.M., Zavadil-Grob, C., Ucla, C. and Mach, B. (1984) Eur. J. Immunol. 14, 898.
[18] Aarden, L.A., de Groot, E.R., Schaap, O.L. and Lansdorp, P.M. (1987) Eur. J. Immunol. 17, 1411.
[19] Herrmann, F., Oster, W., Meuer, S.C., Lindemann, A. and Mertelsmann, R. (1989) J. Clin. Invest. 81, 1415.
[20] Brach, M., Klein, H., Platzer, E., Mertelsmann, R. and Herrmann, F. (1989) Exp. Hematol. (in press).
[21] Solanki, V., Slaga, T.J., Callaham, M.E. and Huberman, E. (1981) Proc. Natl. Acad. Sci. USA 78, 1722.
[22] Locksley, R.M., Heinzel, F.P., Shepard, H.M., Agosti, J., Eessalu, T.E., Aggarwal, B.B. and Harlan, J.M. (1987) J. Immunol. 139, 1891.
[23] Stauber, G.B. and Aggarwal, B.B. (1989) J. Biol. Chem. 264, 3573.
[24] Brenner, D.A., O'Hara, M., Angel, P., Chojkier, M. and Karin, M. (1989) Nature 337, 661.
[25] Hirano, T., Kishimoto, T., Sporn, M.B. and Roberts, A.B., (eds.), Springer, Berlin (in press).
[26] Maniatis, T., Goodbourn, S. and Fisher, J.A. (1987) Science 236, 1237.
[27] Prywes, R. and Roedder, R.G. (1986) Cell 47, 777.
[28] Zhang, Y., Lin, J.-X., Yip, Y.K. and Vilcek, J. (1988) Proc. Natl. Acad. Sci. USA 85, 6802.
[29] Sariban, E., Luebbers, R. and Kufe, D. (1988) Mol. Cell. Biol. 8, 340.
[30] Matsuda, T., Suematsu, S., Kawano, M., Yoshizaki, K., Tang, B., Tanabe, O., Nakajima, T., Akira, S., Hirano, T. and Kishimoto, T. (1989) Ann. New York Acad. Sci. 557, 466.
[31] Brawerman, G. (1987) Cell 48, 5.
[32] Koeffler, H.P., Gasson, J. and Tobler, A. (1988) Mol. Cell. Biol. 8, 3432.
[33] Akashi, M., Saito, U. and Koeffler, H.P. (1989) Blood 74, 2283.