Pergamon

European Journal of Cancer Vol. 33, No. 10, pp. 1661–1667, 1997

Printed in Great Britain
0959-8049/97 $17.00+0.00

PII: S0959-8049(97)00159-7

Original Paper

Tumour-derived, Endocrine, Exogenous and Therapeutic Factors Differentially Modulate Cytokine Secretion in Whole Blood Cell Culture

J.R. Fischer,[1] M. Schindel,[1] H. Lahm[2] and P. Drings[1]

[1]Thorax-Klinik der LVA Baden, Department of Medical Oncology, Amalienstraße 5, 69126 Heidelberg-Rohrbach, Germany; and [2]Swiss Institute for Experimental Cancer Research, Department of Cellular Biology, 1066 Epalinges, Chemin des Boveresses 155, Switzerland

Following our previous results which showed that TGF-β1 suppressed the secretion of certain cytokines, we investigated the effects of different endogenous and exogenous factors on cytokine secretion in whole blood cell culture by using an enzyme-linked immunosorbent assay (ELISA) for measurement of cytokine concentrations. Several molecules including dexamethasone, noradrenaline (NA) and ethanol differentially inhibited mitogen-induced cytokine secretion. Dexamethasone and noradrenaline suppressed secretion of IL-2, IFN α, IFN γ, TNFα, IL-1α and IL-1β. β-Endorphin and Leu-Enkephalin had no significant influence on cytokine secretion. Suppression of cytokine secretion by TGF-β1 was further intensified significantly and dose dependently by addition of noradrenaline. GM-CSF stimulated the secretion of IL-1α, IL-1β and TNF γ, but had no influence on the secretion of IL-2, IFN α and IFNγ. G-CSF, IL-3 and SCF did not significantly influence secretion of all cytokines tested. Thus, endogenous and exogenous factors differentially influence cytokine secretion by immunocompetent cells. © **1997 Elsevier Science Ltd.**

Key words: cytokine secretion, immunomodulation, immunosuppression
Eur J Cancer, Vol. 33, No. 10, pp. 1661–1667, 1997

INTRODUCTION

COMMUNICATION BETWEEN T cells, B cells, macrophages and other immunocompetent cells within the immunoregulatory network is regulated by a number of cytokines that are secreted by these cells upon activation. Thus, an effective immune defence depends on a variety of cytokines and different specific immune reactions directed against infectious agents or tumour cells appear to be influenced by a cascade of secreted cytokines. More recently, additional cytokines have been identified that suppress immune functions. The fact that immunosuppressive cytokines such as transforming growth factor β1 (TGF β1) and interleukin (IL)-10 are produced by immunocompetent cells suggests a physiological role in the downregulation of immune functions [1–3]. As evidence is accumulating that immunosuppressive factors are not only produced by immunocompetent cells but also by a variety of tumour cells [4–6], they may also mediate pathological immunosuppression in tumour patients [7–9]. Tumour cells may escape from immunosurveillance by secretion of immunosuppressive factors [10–12]. We have previously reported that small lung cancer (SCLC) cell lines secrete TGF β1 leading to inhibition of IL-2 mediated T cell proliferation [13, 14]. Further investigations have shown that cytokine suppression is dependent on tumour load. Secretion of IL-2, IFN α, IFN γ is significantly suppressed in SCLC patients with limited or extensive disease, whilst secretion of tumour necrosis factor (TNF) α is significantly suppressed in extensive but not limited disease. In contrast, secretion of IL-1α and IL-1β is not suppressed. Reduction of tumour load leads to reconstitution of cytokine secretion [15, 16]. These results suggest an interaction between tumour cells and the immune system mediated by a factor secreted by tumour cells. As SCLC cell lines secrete bioactive TGF β1, TGF β1 may be one factor mediating tumour-derived immunosuppression *in vivo*. To investigate this hypothesis further,

Correspondence to J.R. Fischer.
Received 12 Aug. 1996; revised 18 Mar. 1997; accepted 18 Mar. 1997.

we examined the effect of TGF β1 on cytokine secretion by mitogen-activated immunocompetent cells in whole blood cell culture from normal individuals [15, 16]. The results showed that TGF β1 selectively suppressed secretion of IL-2, IFN α, IFN γ and TNF α, but did not influence secretion of IL-1α and IL-1β. This cytokine pattern induced by TGF β1 is similar to the cytokine pattern found in SCLC patients [16].

Nevertheless, other factors may as well modulate cytokine secretion. In this paper, we examined the influence of additional exogenous and endogenous factors on mitogen-induced cytokine secretion in whole blood cell cultures. Concentrations of secreted IL-1α, IL-1β, IL-2, IFN α, IFN γ and TNFα were determined by using an enzyme-linked immunosorbent assay (ELISA) specific for the different cytokines.

MATERIALS AND METHODS

Reagents

PHA was purchased from Welcome (Burgwedel, Germany). Newcastle disease virus (NDV) was kindly provided by R. Zawatzki, German Cancer Research Center, Heidelberg, Germany. Highly purified human TGF β1 was purchased from British Biotechnology (Oxford, U.K.), noradrenaline from Fluka (Neu-Ulm, Germany), dexamethasone, β-Endorphin, Leu-Enkephalin from Sigma Pharmaceuticals (Deisenhofen, Germany), ethanol from Merck (Darmstadt, Germany). G-CSF(colony stimulating factor), GM-CSF and IL-3 was kindly provided from Behring (Marburg, Germany), SCF (stem cell factor) was a kind gift from Amgen (Müchen, Germany).

Blood samples

10 ml of heparinised blood were taken between 8 and 10 a.m. from healthy adults and used for further investigations within 2 h. Differential leucocyte counts were also determined.

Whole blood cell culture

Heparinised venous blood was used according to the method described [11, 12]. Briefly, blood was diluted 1:5 with RPMI 1640 medium (Gibco Laboratories, Grand Island, New York, U.S.A.) supplemented with HEPES (10 mM final concentration), L-glutamine (2 mM final concentration), penicillin (100 U/ml), streptomycin (100 μg/ml).

Stimulation of cytokine secretion

Stimulation of cytokine secretion was performed as previously described [15–18] with slight modifications. Briefly, cells were incubated in 96-well plates at a final volume of 200 μl culture medium at 37°C and 5% CO_2 in humified air and stimulated for 24 h in the presence of 10 μg/ml PHA for the secretion of TNF α and IL-1β; for 48 h in the presence of 10 μg/ml PHA for the secretion of IL-2; for 48 h in the presence of NDV at a final dilution of 1:100 for the secretion of IFN α2a; and for 72 h in the presence of 10 μg/ml PHA for the secretion of IL-1 α and IFN γ. For assessment of the influence of noradrenaline, defamethasone, β-endorphin, Leu-Enkephalin, ethanol, G-CSF, GM-CSF, IL-3 and SCF, on mitogen-induced cytokine secretion, these factors were added to the culture at the time of activation of cells at the concentrations indicated in the results.

Determination of cytokine concentrations

Supernatants were tested for the presence of cytokines by using an ELISA as previously described [15–18]. The ELISA for determination of IL-1α and IL-1β, IL-2, IFN α, IFN γ and TNF α was established by H. Gallati, Hoffman La Roche, Basel, Switzerland. Cytokines of the standard and cell supernatants were bound to a murine monoclonal antibody that previously had been absorbed to the bottom of a microtitre plate. A second murine monoclonal antibody against the cytokine conjugated with peroxidase was added. After an incubation time of 16–24 h, the peroxidase activity was determined by a redox indicator. The intensity of the colour, measured with a multi-channel photometer, is directly proportional to the cytokine concentration. The assay range is 5–100 pg/ml for IL-1α, 25-1000 pg/ml for IL-1β, 20–1000 pg/ml for IL-2, 0.5–10 U/ml for IFN α, 10–1000 pg/ml for IFN γ and 10–500 pg/ml for TNF α.

Statistical analysis

The significance of differences between the results in the test groups and controls was calculated by using Student's *t*-test.

RESULTS

Influence of different endogenous factors on cytokine secretion

We evaluated the effect of dexamethasone, noradrenaline, β-endorphin and leu-enkephalin on cytokine secretion in whole blood cell cultures from normal healthy individuals (Figure 1). Noradrenaline (Figure 1(b)) and dexamethasone (Figure 1(c)) substantially inhibited secretion of all cytokines measured. This inhibition of cytokine secretion by dexamethasone and noradrenaline was dose-dependent (data not shown). In contrast, β-endorphin and leu-enkephalin had no significant effect on cytokine secretion at all concentrations tested (Figure 1 (d) and (e)). For comparison, previously published results with TGF-β1 are shown [16], Figure 1 (a)). It suppressed the secretion of IL-2, IFN α, IFN γ and TNF α, but did not influence the secretion of IL-1α and IL-1β [16]. TGF β1-activity was dose-dependent and was most pronounced at 10 ng/ml. At concentrations below 2.5 ng/ml, no inhibitory effect of TGF β1 on cytokine secretion in whole blood cell cultures was observed any more (data not shown). This cytokine pattern induced by TGF β1 is similar to the cytokine pattern found in SCLC patients [16].

The immunosuppressive effect of TGF β1 is significantly enhanced by noradrenaline

We investigated whether the combination of TGF β1 and noradrenaline would result in an additive immunosuppressive effect. Table 1 shows that the immunosuppressive effect of the combination of TGF β1 and noradrenaline was clearly enhanced compared to the immunosuppressive effect induced by each molecule alone. This additive effect was dose-dependent and statistically significant. At a concentration of 10^{-6} M, noradrenaline significantly enhanced the immunosuppressive effect of TGF β1 (Table 1), whereas at 10^{-8} M no further enhancement of the TGF β1 effect was observed (data not shown).

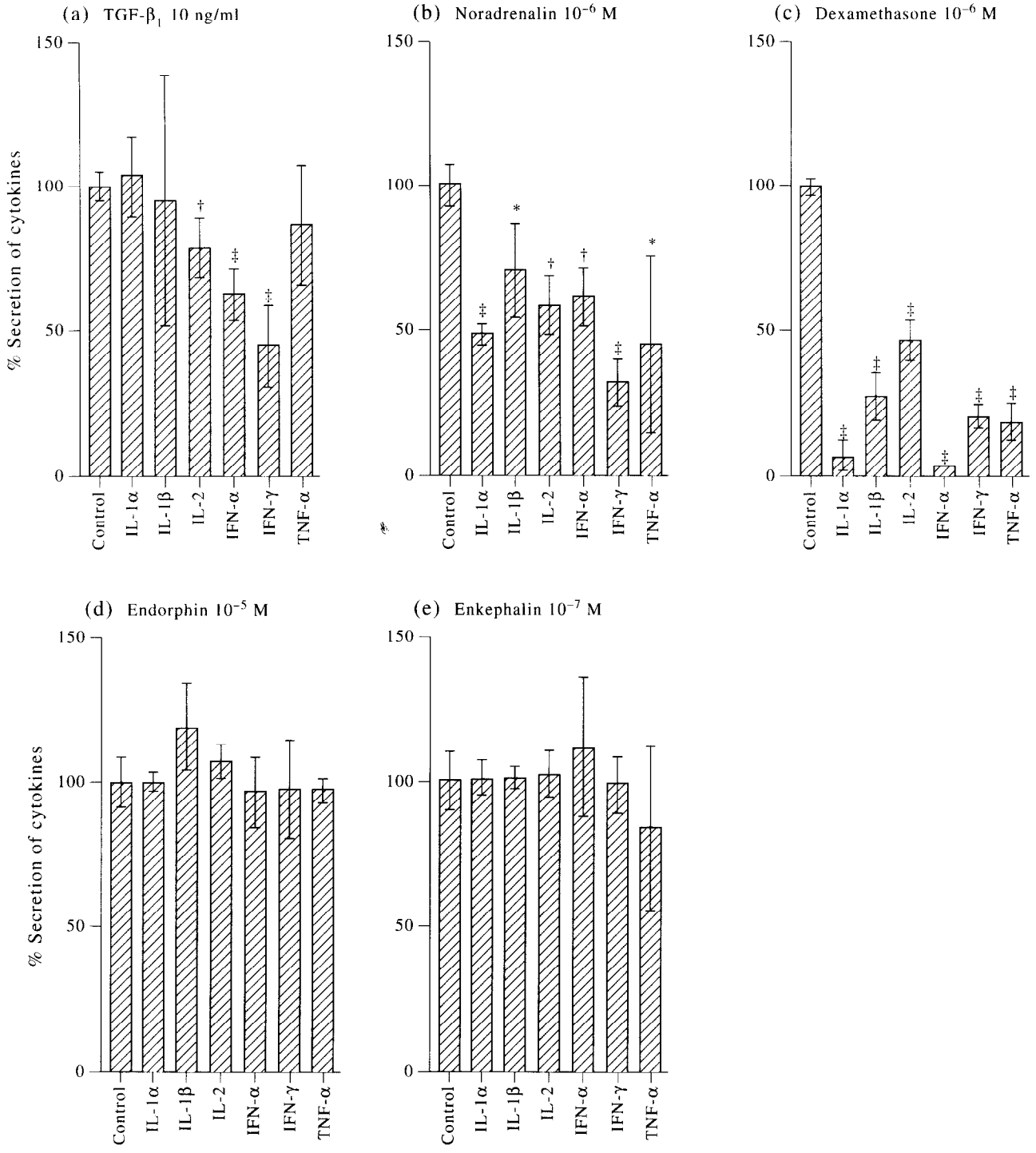

Figure 1. Modulation of mitogen-induced cytokine secretion in whole blood cell cultures by different factors. Whole blood cell cultures were activated as described in Materials and Methods. Cells were cultured in the presence or absence of the factors as indicated. Results for TGF β1 are taken from ref. 16. Cytokine concentrations were determined as described in Materials and Methods. Values represent the mean of three independent experiments. Each experiment was carried out in quadruplicate. Statistical differences between mitogen control and cytokine secretion in the presence of different factors were calculated by using Student's *t*-test. *$P < 0.05$, †$P < 0.005$, ‡$P < 0.0005$.

Influence of ethanol on cytokine secretion

We also examined the influence of ethanol (which has been shown to be immunosuppressive) on mitogen-induced cytokine secretion at concentrations from 2 mmol to 150 mmol. The results in Table 2 show that ethanol dose dependently inhibited the secretion of IL-1 α, IL-1β, IL-2 and TWF α with a significant decrease in cytokine secretion at ethanol concentration between 9 and 150 mmol. There was no significant effect on IFN α or γ.

Influence of colony stimulating factors on cytokine secretion

We investigated the effect of G-CSF, GM-CSF, IL-3 and SCF on mitogen-induced cytokine secretion in whole blood cell culture. The results are shown in Figure 2. GM-CSF

Table 1. Influence of TGF β1 and noradrenaline alone and in combination on mitogen-induced cytokine secretion in whole blood cell culture

Cytokine		Percent cytokine secretion compared to mitogen-control upon incubation with TGF β1*	NA†	TGF β1/NA‡
IL-2	Exp. 1	72	69	54
	Exp. 2	70***	71***	57***‡
IFNα	Exp. 1	63	72	47
	Exp. 2	72***	100	53**‡
IFNγ	Exp. 1	58	42	26
	Exp. 2	47***	39***	15**‡
TNFα	Exp. 1	70	47	33
	Exp. 2	100	80*	80‡

*TGF β1 10 ng/ml, †NA 10^{-6}M, ‡Difference to cytokine secretion in the presence of TGF β1 or NA alone, $*P < 0.05$, $**P < 0.01$, $***P < 0.005$. Asterisks represent the *P*-value for the mean of experiments 1 and 2. Statistical difference was calculated by using Student's *t*-test.

stimulated the secretion of IL-1α, IL-1β and TNF α, but had no influence on the secretion of IL-2, IFN α and IFN γ. G-CSF, IL-3 and SCF did not significantly influence secretion of any of the cytokines tested. Combinations of haematopoietic growth factors had no effect different from that of single CSFs (data not shown).

DISCUSSION

Whether malignant growth is connected to suppression of host immune defence is still an unanswered question. Evidence is accumulating that tumour cells secrete immunosuppressive factors that may lead to clinically relevant suppression of immune functions [4–12]. We have previously shown that SCLC cell lines secrete bioactive TGF β1 [14]. In addition, cytokine secretion was found to be selectively suppressed in SCLC patients at the time of diagnosis [15, 16]. Further investigation demonstrated that cytokine secretion is reconstituted upon tumour reduction by chemotherapy, but not upon ineffective chemotherapy. These results suggested an interaction between tumour cells and immune functions. Since SCLC cell lines secrete bioactive TGF β1 which induces the selective cytokine suppression found in SCLC patients [16], TGF β1 may be one of the factors mediating immunosuppression in SCLC. We have previously reported more than 60% inhibition of proliferation of a T cell line by TGF β1 at 1 ng/ml [14]. The results in Table 1 demonstrate a 30–50% reduction in cytokine levels in cultured whole blood upon incubation with TGF β1. This reduction is comparable to results obtained by others, who reported 50–60% inhibition of cytokines in separated PBMC with 10–15 ng/ml TGF β1 [19, 20].

However, additional endogenous factors may also influence cytokine secretion during or before the outbreak of disease. Furthermore, exogenous factors applied during treatment may modulate cytokine secretion. Recent evidence suggests a bidirectional link between the immune system and the neuroendocrine system [21, 22]. Obviously, immunocompetent cells express receptors for neuroendocrine hormones such as ACTH, cortisol, adrenaline and opioids on their surface [23–25]. Moreover, a variety of neuroendocrine molecules differentially influence various immune functions including modulation of cytokine secretion [26–31]. However, cytokines are able to modulate the secretion of neurohormones by the pituitary–hypothalamic–adrenocortical axis [21]. Interactions between the immune system and the neuroendocrine system may have clinical implications. Nevertheless, modulation of cytokine secretion by neuroendocrine factors has been only partially investigated.

We evaluated the effect of dexamthasone, noradrenaline, β-endorphin and leu-enkephalin on cytokine secretion in whole blood cell cultures from normal healthy individuals.

Table 2. Influence of ethanol on mitogen-induced cytokine secretion in whole blood cell culture

Cytokine		Per cent cytokine secretion compared to mitogen-control upon incubation with ethanol (mmol) 150	75	38	19	9	4	2
IL-1α	Exp. 1	65	78	82	115	122	148	152
	Exp. 2	55*	71*	78*	78	78	100	124
IL-1β	Exp. 1	62	62	76	100	119	145	148
	Exp. 2	49*	63†	73†	88	100	122	122
IL-2	Exp. 1	89	91	91	94	105	99	93
	Exp. 2	89†	94*	102	98	111	119	119
IFNα	Exp. 1	119	116	98	89	98	109	95
	Exp. 2	39	52	73	95	113	117	152
IFNγ	Exp. 1	76	86	105	90	86	100	105
	Exp. 2	36	51	57	72	74	77	102
TNFα	Exp. 1	87	75	87	87	100	100	100
	Exp. 2	75*	67*	83*	85*	90	94	100

$*P < 0.05$, $†P < 0.005$. Statistical difference was calculated by using Student's *t*-test. Asterisks represent the *P*-value for the mean of experiments 1 and 2.

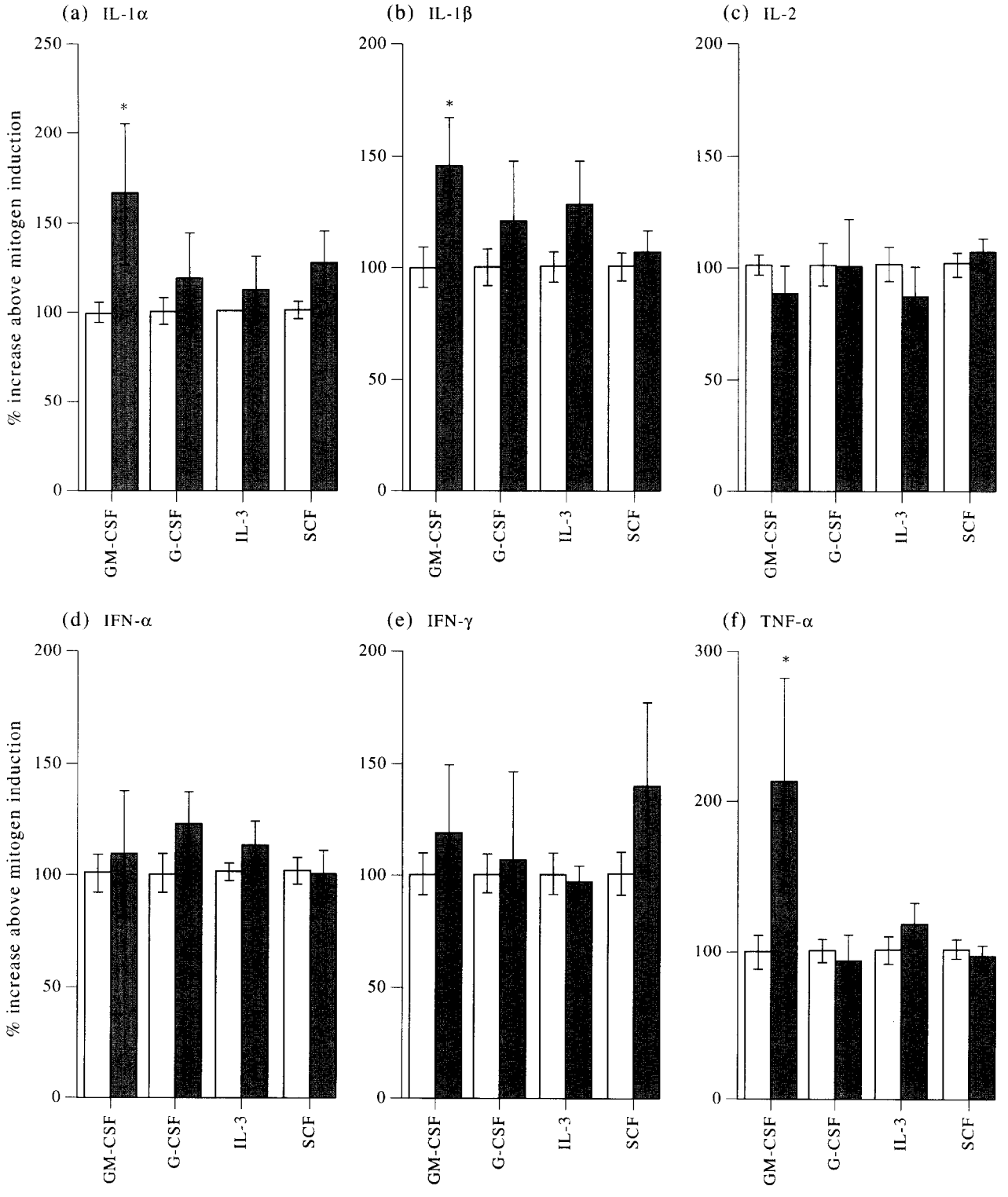

Figure 2. Modulation of mitogen-induced cytokine secretion in whole blood cell cultures by colony stimulating factors. Whole blood cell cultures were activated as described in Materials and Methods. Cells were cultured in the absence (open bars) or presence (closed bars) of CSFs as indicated (GM-CSF 100 U/ml, G-CSF 100 U/ml, IL-3 100 U/ml, SCF 100 ng/ml). Cytokine concentrations were determined as described in Materials and Methods. Values represent the mean of three independent experiments. Each experiment was carried out in quadruplicate. Statistical differences between mitogen control and cytokine secretion in the presence of different CSFs were calculated by using Student's *t*-test. *$P < 0.05$.

The immunosuppressive activity of corticosteroids is not unknown, but their influence on cytokines in whole blood has not been reported. Our results demonstrate that dexamethasone significantly inhibited secretion of all cytokines measured. The effect of dexamthasone was dose-dependent and was most pronounced at 10^{-6} M, but was still observed at lower levels in the range of 10^{-9} M. These concentrations correspond well to effective *in vivo* concentrations of cortisol

and to concentrations reached *in vivo* by the therapeutic application of dexamthasone. Noradrenaline was found to inhibit significantly secretion of all cytokines tested. Noradrenaline was used at 10^{-6} M. The effect on cytokine secretion was dose-dependent and could still be observed at 10^{-8}–10^{-9} M (data not shown). Baseline *in vivo* levels for noradrenaline are in the range of 10^{-10} M. Concentrations increase to 10^{-9} M in physiological situations and may reach 10^{-8} M, for example, under stressful conditions. Thus, concentrations used here are well in the range of concentrations that may be found *in vivo*. These results may indicate a possible immunomodulation by noradrenaline. Whether this immunosuppressive activity of noradrenaline has any clinical relevance remains to be investigated. In contrast, endorphine and enkephalin did not significantly modulate cytokine secretion.

Interestingly, the combination of TGF β1 and noradrenaline resulted in an additive effect on suppression of cytokine secretion. This additive effect was statistically significant and dose-dependent. Such a combined immunosuppressive activity of a tumour-derived factor and a neuroendocrine molecule has not been reported before. Whether this combined immunosuppression has clinical relevance remains to be shown.

Ethanol is a commonly used agent that has been suspected to exert immunosuppressive activity. It has been shown to inhibit human T-lymphocyte proliferation in concentrations equivalent to levels achieved by the ingestion of moderate amounts of alcoholic beverages [32]. Inhibition of secretion of a single cytokine has been reported [33]. Our results now extend this observation and demonstrate that ethanol inhibits the secretion of a number of cytokines. This immunosuppressive effect is dose-dependent. Interestingly, at a dosage corresponding to levels reached in the blood following ingestion of moderate amounts of alcohol (0.8 mg/ml blood) a significant inhibition of cytokine secretion was observed. These results strongly indicate that the ingestion of large amounts of alcohol may influence immune surveillance. Whether this could contribute to the development of tumours is not yet known.

Haematopoietic growth factors are now clinically used to reduce chemotherapy induced neutropenia. GM-CSF and G-CSF significantly reduce neutropenia and neutropenic fever and allow intensification of cytotoxic therapy by dose intensification or interval reduction [34, 35]. Thus, CSFs may not only decrease the frequency and severity of adverse effects related to therapy but also increase response rates to therapy and survival [36]. However, while the reduction of neutropenic fever is sufficiently supported by data, improvement of response rates and survival needs to be confirmed by further investigation [36].

Haematopoietic growth factors are secreted by tumour cells [37, 38]. As CSFs may differentially interact with cytokine secretion by immunocompetent cells [39, 40], one possible mechanism under investigation represents induction of secondary cytokines by haematopoietic growth factors that may result in improved anti-tumour immune effector reactions. We have shown here that GM-CSF stimulated the secretion of IL-1α, IL-1β and TNF α, but had no influence on the secretion of IL-2, IFN α and IFN γ. G-CSF, IL-3 and SCF did not significantly influence the secretion of all cytokines tested. Differential effects of distinct haematopoietic growth factors on the secretion of a number of secondary cytokines in whole blood cultures at the protein level may have clinical relevance. For example, that secondary cytokines are induced by GM-CSF but not by G-CSF may provide an experimental basis for the observation that side-effects, particularly inflammatory reactions, are more frequently seen with the application of GM-CSF than with G-CSF.

In conclusion, we have shown that endogenous and exogenous factors differentially influence cytokine secretion by immunocompetent cells. Insight into mechanisms of immunomodulations and possible interactions between different factors may improve our understanding of immunoregulation in the development and course of clinical disease. In addition, these findings may have clinical impact on therapeutic immunomodulation in the future.

1. Kehrl JH, Wakefield LM, Roberts AB, *et al.* Production of transforming growth factor β by human T lymphocytes and its potential role in the regulation of T cell growth. *J Exp Med* 1986, **163**, 1037–1050.
2. Kehrl JH, Roberts AB, Wakefield LM, Jakowlew S, Sporn MB, Fauci AS. Transforming growth factor β is an important immunomodulatory protein for human B lymphocytes. *J Immunol* 1986, **137**, 3855–3860.
3. Moore KW, Vieira P, Fiorentino DF, *et al.* Homology of cytokine synthesis inhibitory factor (IL-10) to the Epstein–Barr virus gene BCRFI. *Science* 1990, **248**, 1230–1234.
4. Bartlett JMS, Rabiasz GG, Scott WN, Langdon SP, Smyth JF, Miller WR. Transforming growth factor β mRNA expression and growth control of human ovarian carcinoma cells. *Br J Cancer* 1985, **65**, 655–660.
5. Bodmer S, Strommer K, Frei K, *et al.* Immunosuppression and transforming growth factor β in glioblastoma. *J Immunol* 1989, **143**, 3222–3229.
6. Coffey RJ, Shipley GD, Moses HL. Production of transforming growth factors by human colon cancer lines. *Cancer Res* 1986, **46**, 1164–1169.
7. Elsässer-Biele U, von Kleist S, Fischer R, *et al.* Impaired cytokine production in whole blood cell cultures of patients with urological carcinomas. *Cancer Res Clin Oncol* 1993, **119**, 430–433.
8. Masuno T, Ikeda T, Yokota S, Komuta K, Ogura T, Kishimoto S. Immunoregulatory T lymphocyte functions in patients with small cell lung cancer. *Cancer Res* 1986, **46**, 4195–4199.
9. Yoshino I, Yano T, Murata M, *et al.* Tumor-reactive T-cells accumulate in lung cancer tissues but fail to respond due to tumor cell-derived factor. *Cancer Res* 1992, **52**, 775–781.
10. Chen Q, Daniel V, Maher DW, Hersey P. Production of IL-10 by melanoma cells: examination of its role in immunosuppression mediated by melanoma. *Int J Cancer* 1994, **56**, 755–760.
11. Torre-Amione G, Beauchamp RD, Koeppen H, *et al.* A highly immunogenic tumor transfected with a murine transforming growth factor type β1 cDNA escapes immune surveillance. *Proc Natl Acad Sci USA* 1990, **87**, 1486–1490.
12. Arteaga CL, Hurd SD, Winier AR, Johnson MD, Fendly BM, Forbes JT. Anti-TGF β-antibodies inhibit breast cancer cell tumorigenicity and increase mouse spleen natural killer cell activity. *J Clin Invest* 1993, **92**, 2569–2576.
13. Fischer JR, Stehr S, Lahm H, Falk W, Drings P, Krammer PH. Different cytokines with autocrine and immunosuppressive activities are secreted by a small cell lung cancer cell line. In Freund M, *et al.*, eds. *Cytokines in Hemopoiesis, Oncology and AIDS II.* Berlin, Heidelberg, Springer-Verlag, 1992, 107–113.
14. Fischer JR, Darjes H, Schindel M, *et al.* Constitutive secretion of bioactive transforming growth factor β1 by small cell lung cancer cell lines. *Eur J Cancer* 1994, **30A**, 2125–2129.
15. Fischer JR, Darjes H, Schindel M *et al.*. Does TGF β1 play a role in immunosuppression in small cell lung cancer? In Zeller

WJ, *et al.*, eds. *Contributions to Oncology*. Basel, Karger-Verlag, 1995, 124–130.
16. Fischer JR, Schindel M, Darjes H, *et al.* Selective suppression of cytokine secretion in patients with small cell lung cancer. *Ann Oncol* 1995, **9**, 921–926.
17. Kirchner H, Kleinicke C, Digel W. A whole blood technique for testing production of human Interferon by leucocytes. *J Immunol Meth* 1982, **48**, 213–219.
18. Elsässer-Beile U, von Kleist S, Gallati H. Evaluation of a test system for measuring cytokine production in human whole blood cell cultures. *J Immunol Meth* 1991, **139**, 191–195.
19. Espevik T, Figari IS, Shalaby R, *et al.* Inhibition of cytokine production by cyclosporin A and TGF β. *J Exp Med* 1987, **166**, 571–576.
20. Reinhold D, Bank U, Bühling F, *et al.* TGF β1 inhibits DNA synthesis of PWM-stimulated PBMC via suppression of IL-2 and IL-6 production. *Cytokine* 1994, **6**, 382–388.
21. Besedovsky H, Del Rey A, Sorkin E, Dinarello CA. Immunoregulatory feedback between Interleukin-1 and Glucocorticoid hormones. *Science* 1986, **233**, 652–654.
22. Steinman L. Connections between the immune system and the nervous system. *Proc Natl Acad Sci USA* 1993, **90**, 7912–7914.
23. Smith EM, Brosnan P, Meyer W, Walter J, Blalock E, Edwin J. An ACTH receptor on human mononuclear leucocytes. Relation to adrenal ACTH-receptor activity. *N Engl J Med* 1987, **317**, 1266–1269.
24. Williams LT, Snydermann R, Lefkowitz RJ. Identification of β-adrenergic receptors in human lymphocytes. *J Clin Invest* 1976, **57**, 149–155.
25. Hazum E, Kwen-Jen C, Cuatrecasas O. Specific nonopiate receptors for β-endorphin. *Science* 1979, **205**, 1033–1035.
26. Heagy W, Laurance M, Cohen E, Finberg R. Neurohormones regulate T cell function. *J Exp Med* 1990, **171**, 1625–1633.
27. Alison S, Rapson NT, Hunter CA, Liew FY. Regulation of Tumor necrosis factor production by adrenaline and β-adrenergic agonists. *J Immunol* 1992, **148**, 3441–3445.
28. Spengler RN, Chensue SW, Giacherio DA, Blenk N, Kunkel SL. Endogenous norepinephrine regulates tumor necrosis factor-a production from macrophages *in vitro*. *J Immunol* 1994, **152**, 3024–3031.
29. Garcia I, Perez-Castillo A, Moreno J, Alemany S. β-Endorphin inhibits interleukin-2 release and expression of interleukin-2 receptors in concanavalin A-stimulated splenic lymphocytes. *Lymphokine Cytokine Res* 1992, **11**, 339–245.
30. Mathews PM, Froelch CJ, Sibbitt WL, Bankhurst AD. Enhancement of natural cytotoxicity by β-endorphin. *J Immunol* 1983, **130**, 1658–1662.
31. Lew W, Oppenheim JJ, Matsushima K. Analysis of the suppression of IL-1α and Il-1β production in human peripheral blood mononuclear adherent cells by a glucocorticoid hormone. *J Immunol* 1988, **140**, 1895–1902.
32. Kaplan DR. A novel mechanism of immunosuppression mediated by ethanol. *Cell Immunol* 1986, **102**, 1–9.
33. Verma BK, Fogarasi M, Gyongyi S. Down-regulation of tumor necrosis factor α activity by acute ethanol treatment in human peripheral blood monocytes. *J Clin Immunol* 1993, **13**, 8–22.
34. Drings P, Fischer JR. Biology and clinical use of GM-CSF in lung cancer. *Lung* 1990, **168**, S1059–S1068.
35. Havemann K, Klausmann M, Wolf M, Fischer JR, Drings P, Oster W. Effect of rhGM-CSF on haematopoietic reconstitution after chemotherapy in small-cell lung cancer. *J Cancer Res Clin Oncol* (suppl IV) 1991, **117**, S203–S207.
36. Fischer JR, Manegold C, Bülzebruck H, Vogt-Moykopf I, Drings P. Induction chemotherapy with and without R-methHuG-CSF support in locally advanced stage IIIA/B Non small lung cancer. *Semin Oncol* 1994, **21**, S3:20–27.
37. Lahm H, Wyniger J, Hertig S, *et al.* Secretion of bioactive GM-CSF by human colorectal carcinoma cells. *Cancer Res* 1994, **54**, 3700–3702.
38. Ramakrishnan FJX, Brandt SJ, Niedel JE, Bast RC, Brown EL. Constitutive production of macrophage colony-stimulating factor by human ovarian and breast cancer cell lines. *J Clin Invest* 1989, **83**, 921–926.
39. Jones TC. The effects of rhGM-CSF on macrophage function. *Eur J Cancer* 1993, **29**, S10–S13.
40. Sisson SD, Dinarello CA. Production of interleukin-1α, interleukin-1β and tumor-necrosis factor by human mononuclear cells stimulated with granulocyte-macrophage colony-stimulating factor. *Blood* 1988, **72**, 1368–1374.