nuclear antigen (PCNA). Structural conservation and detection of a nuclear form. *J Cell Sci* 1990, **96**, 121–129.
19. Kurki P, Ogata K, Tan EM. Monoclonal antibodies to proliferating cell nuclear antigen (PCNA/cyclin) as probe for proliferating cells by immunofluorescence microscopy and flow cytometry. *J Immunol Methods* 1988, **109**, 49–59.
20. Chiodi F, Bredberg-Raaden U, Biberfeld G, *et al.* Radioimmunoprecipitation and Western blotting with sera of human immunodeficiency virus infected patients: A comparative study. *AIDS Res Hum Retrovir* 1987, **3**, 165–176.
21. Hsu R, Raine L, Farger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparision between ABC and unlabelled antibody (PAP) procedures. *J Histochem Cytochem* 1981, **29**, 577–580.
22. Gaub J, Auer G, Zetterberg A. Quantitative cytochemical aspects of a combined Feulgen Naphtol S staining procedure for simultaneous determination of nuclear and cytoplasmic proteins and DNA in mammalian cells. *Exp Cell Res* 1985, **92**, 323–332.
23. Adams LR. A photographic cytophotometric method which avoids distributional error. *Acta Cytol* 1968, **12**, 3.
24. Wallin G, Askensten U, Backdahl M, Grimelius L, Lundell G, Auer G. Cytochemical assessment of the nuclear DNA distribution pattern by means of image and flow cytometry in thyroid neoplasms and non neoplastic thyroid lesions. *Acta Chir Scand* 1989, **155**, 251–258.
25. Shapiro HN. *Practical Flow Cytometry*. New York Alan R. Liss 1988, 2nd edition, 1–353.
26. Bloom HJG, Richardson WW. Histological grading and prognosis in breast cancer. A study of 1409 cases of which 359 have been followed for 15 years. *Br J Cancer* 1957, **11**, 359–377.
27. Sanchez MA, Ames ED, Erhadt K, Auer G. Analysis of DNA distribution in Kaposi's sarcoma in patients with and without acquired immune deficiency syndrome. *Anal Quant Cytol* 1988, **10**, 16–20.
28. Bayley AC. Aggressive Kaposi's sarcoma in Zambia. *Lancet* 1984, **i**, 1328–1320.
29. Safai B, Good RA. Kaposi's sarcoma: A review and recent developments. *CA A Cancer Journal for Clinicians* 1981, **31**, 2–12.
30. Werner SS, Hofschneider PH, Roth WK. Cells derived from sporadic and AIDS-related Kaposi's sarcoma reveal identical cytochemical and molecular properties *in vitro*. *Int J Cancer* 1989, **43**, 1137–1144.

Acknowledgements—Supported by the Swedish Medical Research Council, SAREC and ICSC World-Laboratory. The skilled assistance of Vera Nelson, Angelina De Santiago and Ingeborg May is acknowledged. Ephata E. Kaaya, Edward Mgaya, visiting scientists and Vera Nelson, visiting technican from the Department of Pathology, Muhimbili Medical Centre, Dar-es-Salaam, Tanzania, were supported by SAREC and ICSC World-Lab. project no MDC2. Carlo Parravicini, visiting scientist from the Department of Pathology, State University Hospital "L. Sacco", Milano, Italy, supported by the European Community Concerted Action on "Pathophysiology and Immunology of HIV-Related Diseases".

Eur J Cancer, Vol. 28A, No. 11, pp. 1894–1899, 1992.
Printed in Great Britain

0964–1947/92 $5.00 + 0.00

Growth Stimulation of a Human Colorectal Carcinoma Cell Line by Interleukin-1 and -6 and Antagonistic Effects of Transforming Growth Factor β_1

Harald Lahm, Danuta Petral-Malec, Aysim Yilmaz-Ceyhan, Jürgen R. Fischer, Murielle Lorenzoni, Jean-Claude Givel and Nicolas Odartchenko

We analysed the effect of interleukin-1 (IL-1), IL-6 and transforming growth factor β_1 ($TGF\beta_1$) on the growth of a panel of eight colorectal carcinoma cell lines. IL-1 stimulated growth of two lines (LS411N and LS1034) up to20-fold and IL-6 enhanced proliferation of LS1034 more than 5-fold. Both cytokines also augmented colony-formation of LS1034 in methylcellulose. Under both growth conditions IL-1 was the most potent stimulator. However, the addition of IL-6 to IL-1 synergistically enhanced proliferation of LS1034 in monolayer culture and additively augmented the number of colonies formed in methylcellulose. Furthermore, $TGF\beta_1$ strongly reduced the growth rate of LS1034. Low amounts of $TGF\beta_1$ markedly inhibited the response of LS1034 to IL-1 and totally abrogated proliferation induced by IL-6. We conclude that different cytokines can provide distinct signals for the regulation of growth of colorectal carcinoma cells.
Eur J Cancer, Vol. 28A, No. 11, pp. 1894–1899, 1992.

INTRODUCTION

THE TERM CYTOKINES covers a number of soluble mediators that deliver signals from one cell to another. Within this group, the interleukins (IL) represent an important family. The spectrum of target cells affected by a single interleukin is generally rather broad. This is particularly true for IL-1 and IL-6, both molecules exerting activities on a large variety of different cell types.

Peripheral blood monocytes were originally described as the producer cells of IL-1. Evidence has accumulated, however, that numerous other cell types are also capable of secreting IL-1[1]. The effects of IL-1 on tumour cell growth are controversial. For some tumours IL-1 is cytotoxic [2] or cytostatic [3]. In contrast, IL-1 enhances the proliferation of several tumour cell lines [4–6] including one of colorectal origin [7]. The activities of IL-6, originally described as a B cell differentiation factor are also pleiotropic. IL-6 is produced by various tumours and

tumour cell lines [8–10]. In addition, IL-6 is implicated in the growth regulation of several malignancies, either in an autocrine [11,12] or paracrine [13] fashion.

For transforming growth factor β (TFGβ) inhibitory effects on cell growth have been described for both neoplastic and non-neoplastic cells. However, TGFβ-sensitivity does not correlate with the presence of TGFβ-receptors, which are ubiquitously expressed [14]. Some tumour cells are markedly inhibited by TGFβ [15], while others are refractory though expressing functional receptors [16]. In addition, one fundamental mechanism behind the antiproliferative effect of TGFβ is its ability to antagonise the mitogenic effect of other growth factors. Such inhibitory properties of TGFβ have been reported for proliferative responses induced by several other cytokines [17, 18].

We have investigated the effects of IL-1, IL-6 and $TGF\beta_1$ on the growth of colorectal carcinoma cell lines, alone and in combination. We show, that IL-1 and IL-6 promote growth of colorectal carcinomas, but are more effective when acting in co-stimulation. In addition, we provide evidence that $TGF\beta_1$ effectively abrogates both responses.

MATERIALS AND METHODS

Culture medium

Cell lines were cultured in a 1:1 mixture of Dulbecco's modified eagle medium (DMEM) and nutrient mixture Ham's F-12 (both Gibco) supplemented with HEPES (10mmol/l final concentration), L-glutamine (1.4 mmol/l final concentration), penicillin (100 U/ml) and streptomycin (100 μg/ml). This medium is referred to as EF medium.

Cell lines and culture conditions

We have used a panel of eight human colorectal cancer cell lines derived from primary tumours: Co-115, HT-29 (ATCC: HTB38), SW480 (ATCC:CCL228), WiDr (ATCC: CCL218) and Lisp-1 (obtained from Dr D. Lopez, Ludwig-Institute for Cancer Research, Sao Paulo, Brazil). The cell lines LS411N, LS513 and LS1034 have been established in our laboratory. LS411N was established from a biopsy specimen of a poorly differentiated caecal carcinoma, LS513 from a mucin-secreting tumour located at the Bauhin valve and LS1034 from a moderately to poorly differentiated caecal carcinoma. LS411N represents a tumour of Dukes' stage B, while LS513 and LS1034 were classified as Dukes' C. All cell lines were cultured in EF medium with 5% FCS (Seromed, Berlin.) All cell lines were consistently found to be free of mycoplasma contamination using standard culture procedures (Myco Tect, Gibco).

Cytokines and reagents

Recombinant human (rhu) IL-1β was obtained from Biogen (Geneva), rhu IL-6 from Boehringer Mannheim (Bern) and $TGF\beta_1$ from porcine platelets from R & D Systems (Minneapolis). [Methyl-^{3}H]thymidine (^{3}H-TdR) was purchased from Amersham with a specific activity of 925 GBq/mmol.

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Revised 20 Feb. 1992; accepted 9 Mar. 1992.

Proliferation assay

Proliferation was assessed in EF medium. Cells were washed with PBS and cell suspensions were obtained by trypsinising monolayer cultures with 0.05% trypsin/0.02% EDTA (Seromed). They were washed with EF medium, resuspended in EF medium and distributed at 1×10^4 cells/well into 96-well flat-bottomed microtitre plates (Nunc) in a final volume of 200 μl EF medium. Cells were incubated for 5 days at 37°C and 5% CO_2 in the presence or absence of cytokines. To assess the inhibitory effect of $TGF\beta_1$ on cytokine-induced proliferation, $TGF\beta_1$ (0.5 ng/ml) was added to serial dilutions of IL-1 and IL-6 at the onset of the experiment. All samples were measured in triplicate. Proliferation was determined by measuring ^{3}H-TdR incorporation after pulsing the cells with ^{3}H-TdR (18.5 KBq/well) for the last 8 h of culture. Cells were harvested onto filter papers. The filters were dried and radioactivity was determined using a liquid scintillation counter.

Inhibition assay

Cells were distributed at 5×10^3 cells/well into 96-well flat-bottomed microtitre plates in a final volume of 200 μl of EF medium containing 5% FCS in the presence or absence of $TGF\beta_1$. All samples were measured in triplicate. Proliferation was assessed by measuring ^{3}H-TdR incorporation after pulsing the cells with ^{3}H-TdR (18.5 KBq/well) for the last 8 h of a 4-day culture period.

Methylcellulose assay

Anchorage-independent growth was examined in a methylcellulose-based clonogenic assay [19]. Briefly, cells were suspended in EF medium supplemented with 0.9% methylcellulose (Fluka, Buchs, Switzerland). 1 ml aliquots were plated into bacteriological petri dishes (35 mm diameter, Greiner, Nürtingen, Germany) at a final cell concentration of 1×10^4 cells/plate. Cytokines were added directly to the plates. Cells were incubated at 37°C in a fully humidified atmosphere of 5% CO_2, 5% O_2 and 90% N_2. Colonies of more than 50 cells were counted after 2 weeks under an inverted microscope. All samples were set up in triplicate plates.

Statistical analysis

Significance of differences between responses to growth factors and untreated control cells was calculated using the Student's *t*-test.

RESULTS

IL-1 and IL-6 stimulate proliferation of colorectal carcinomas in monolayer culture

We tested IL-1 and IL-6 for growth stimulation on eight human colorectal carcinoma cell lines. Growth of two cell lines, LS411N and LS1034, was enhanced by IL-1. In addition, LS1034 was also stimulated by IL-6. Figure 1 shows representative dose–response curves for both cell lines. IL-1 proved to be a very potent stimulator of LS1034 cells and enhanced growth up to 20-fold (Fig. 1a). IL-6 induced a lower response, but enhanced proliferation of LS1034 nearly 6-fold (Fig. 1a). The effect of IL-1 on growth of LS411N was less pronounced. IL-1 stimulated growth more than 2-fold in a dose-dependent way, while IL-6 was not effective over the whole dose range tested (Fig.1b).

A single addition of IL-1 (20 ng/ml) or IL-6 (100 U/ml) on day 0 stimulated cell division of LS1034 up to 14 days. This effect was significant from day 5 on ($P < 0.05$). By day 14, the

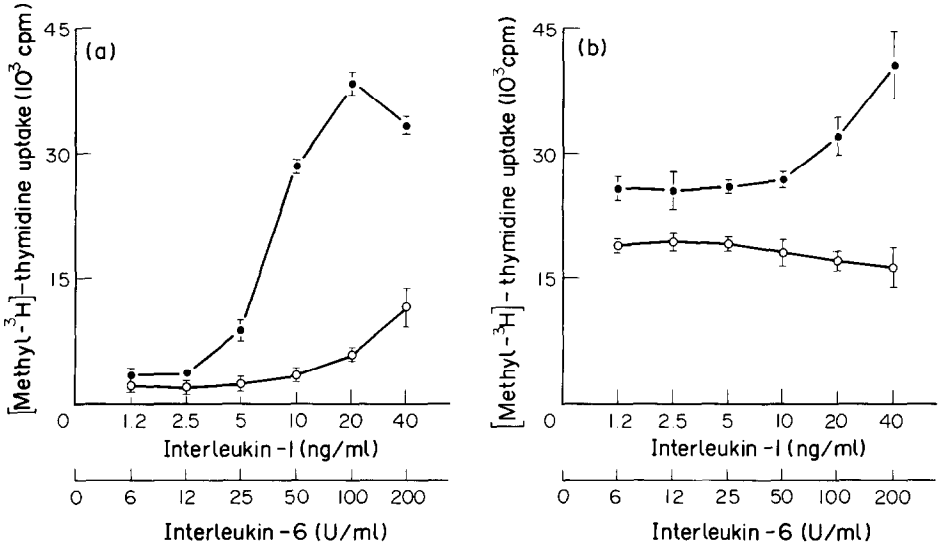

Fig. 1. Growth stimulation of human colorectal carcinoma cells by IL-1 and IL-6. (a) LS1034 and (b) LS411N cells were cultured in the presence of serial dilutions of rhu IL-1β (●) or rhu IL-6 (○). Values represent the mean of triplicate wells (S.D.). ^{3}H-TdR uptake in the presence of culture medium was 1.992 (464)cpm (LS1034) and 18.582 (1.819) cpm (LS411N), respectively. $P < 0.005$ (for IL-1 > 1.25 ng/ml), $P < 0.001$ (for IL-6 > 25 U/ml) from untreated control cells.

number of LS1034 cells cultured with IL-1 or IL-6 was enhanced by a factor of 6.2 and 3.0, respectively (data not shown).

IL-1 and IL-6 stimulate anchorage-independent colony-formation

We used the same conditions to investigate the effect of IL-1 and IL-6 on anchorage-independent colony-formation in methylcellulose. Both cytokines significantly increased the number of colonies of LS1034. In agreement with our results obtained in monolayer culture, IL-1 was more effective than IL-6 and enhanced colony-number more than 3-fold (Table 1). With IL-6 colony numbers were enhanced up to 180% with 200 U/ml (Table 1 and data not shown). LS411N cells did not form colonies under these conditions, even in the presence of IL-1 (data not shown).

IL-1 and IL-6 cooperate synergistically in stimulating cell proliferation

We next investigated the response to the concomitant addition of IL-1 and IL-6. For co-stimulation experiments we used 100 U/ml of IL-6, which *per se* induced significant proliferation ($P<0.0001$) (Fig. 2). However, in combination with IL-1 the proliferation of LS1034 was further enhanced as compared to the response obtained with IL-1 alone (Fig. 2). In fact, the dose–response curve was shifted to the left about 3-fold and maximal stimulation occurred at a higher plateau. The effect of IL-6 was most pronounced at the lowest doses of IL-1 tested. With IL-1-concentrations of 10 ng/ml or below the response upon simultaneous addition of IL-6 was synergistic ($P<0.005$) and potentiated about 5-fold. On the other hand, IL-6, which did not stimulate growth of LS411N cells, failed to augment IL-1-induced proliferation of this cell line (data not shown).

We also determined the effect of a combination of IL-1 and IL-6 on colony-formation of LS1034 in methylcellulose. IL-6 alone induced a small increase in colony number and further augmented the number of colonies obtained with IL-1 alone (Table 1). However, the effect of the combination of both factors was less pronounced than in monolayer cultures. Upon

Table 1. Induction of colony-formation by co-stimulation with IL-1 and IL-6

IL-1 (ng/ml)	Number of colonies (S.D.)* Medium	IL-6
40	351 (44)	460 (39)
20	263 (56)	308 (44)
10	185 (41)	254 (22)
5	184 (23)	196 (10)
0	97 (20)	143 (3)

*LS1034 cells were cultured in the presence of rhu IL-1β as indicated together with medium or rhu IL-6 (100 U/ml). Colonies were counted after 2 weeks. Values represent the mean of triplicate plates.

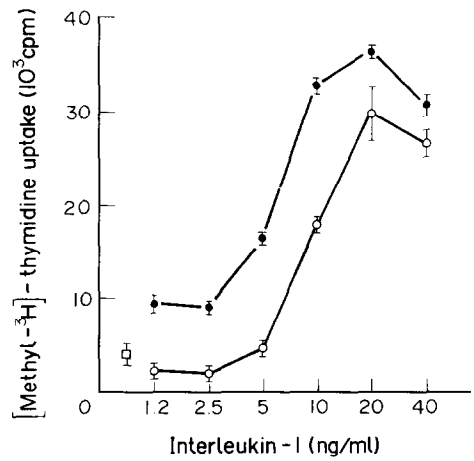

Fig. 2. IL-6 enhances IL-1 induced proliferation. LS1034 cells were cultured in the presence of serial dilutions of rhu IL-1β alone (○), rhu IL-6 alone (100 U/ml, □) or IL-1 and IL-6 (●). Values represent the mean of triplicate wells (S.D.). ^{3}H-TdR uptake in the presence of culture medium was 1.738 (433) cpm.

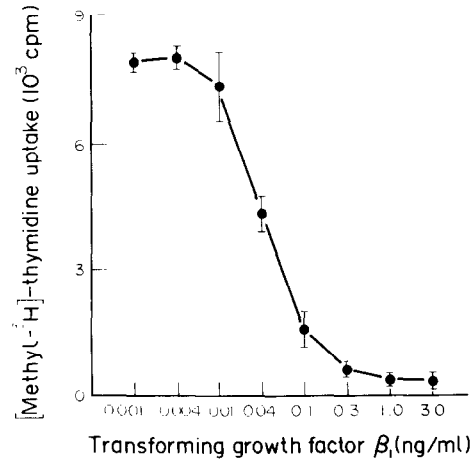

Fig. 3. TGFβ1 inhibits growth of LS1034. Cells were cultured in the presence of serial dilutions of TGFβ1. Values represent the mean of triplicate wells (S.D.)(as in Legend to Figs 1 and 2). ³H-TdR uptake in the presence of culture medium was 9.157 (645)cpm.

simultaneous addition of both cytokines the number of colonies was enhanced additively (Table 1).

TGFβ1 inhibits proliferation of LS1034

Since TGFβ inhibits growth of several tumours including colon carcinomas [15] we tested TGFβ1 for growth inhibitory effects on LS1034 and LS411N cells. Growth of LS411N was not affected (data not shown). In contrast, TGFβ1 strongly inhibited growth of LS1034 in a dose-dependent way. At 1 ng/ml the proliferation was almost completely abrogated and even concentrations below 0.1 ng/ml reduced growth significantly ($P < 0.01$) (Fig. 3). The cyto-inhibitory effect was completely neutralised by anti-TGFβ antibodies (data not shown).

TGFβ1 abrogates IL-1- and IL-6-induced cell proliferation

TGFβ effectively antagonises proliferative responses induced by several cytokines [17, 18]. Thus, we examined whether TGFβ1 would influence proliferation of LS1034 induced by IL-1 and IL-6. TGFβ1 was indeed an efficient inhibitor of both responses ($P < 0.01$). In the presence of TGFβ1, the response to IL-1 up to a concentration of 10 ng/ml was completely blocked. With higher doses the proliferation was strongly inhibited (> 78%). However, IL-1 still induced slight proliferative effects ($P < 0.0001$) at these concentrations (Fig. 4a). Likewise, TGFβ1 totally abrogated the response to IL-6 and even the highest concentration of IL-6 (200 U/ml) did not elicit any response exceeding the proliferation rate of cells incubated with culture medium alone (Fig. 4b).

DISCUSSION

We report growth stimulation of colorectal carcinoma cell lines by IL-1 and IL-6. The LS1034 cell line provides evidence that both cytokines can act together to support growth of such tumours. IL-1 has been found to be mitogenic for numerous tumour cell lines [4–6] and the same is true for IL-6 [11,12]. However, growth stimulation of colorectal carcinomas by IL-1 has only been observed with few cell lines [7,20], and growth stimulation by IL-6 has not been reported so far.

IL-1 and IL-6 display as well antiproliferative effects on tumour cell growth. IL-1 is directly cytotoxic for melanomas [2] and inhibits growth of malignant mammary cell lines [21]. Growth arrest in G_1 phase has been attributed to IL-1 and IL-6 [22]. We have shown that colorectal carcinoma cells were driven through the whole cell cycle by IL-1 and IL-6. They stimulated DNA synthesis and also increased cell number. Thus, the effects of these cytokines on the proliferation of tumour cells appear to vary with the cell type.

The biological activities of IL-1 and IL-6 partially overlap. Both factors cooperatively influence cell growth, either in stimulating proliferation [23] or in reducing cell growth [24]. The combination of IL-1 and IL-6 was an effective stimulus for the proliferation of colorectal carcinomas. We have recorded synergistic effects of LS1034 cells by measuring thymidine uptake, but only additive enhancement of colony formation. Indeed, the two systems measure different parameters. The methycellulose assay reflects the number of cells which have originally started dividing to give rise to a colony. In this context it is of note that the magnitude of colonies growing in the presence of IL-1 exceeded the size of those colonies growing in the presence of medium. In contrast, in the proliferation assay measuring thymidine uptake, every growing cell scores positive. The intermediate values of the stimulation index obtained by counting viable cells is in good agreement with this.

Solid tumours are frequently infiltrated *in vivo* by macrophages and lymphocytes. Once activated, these cells secrete several cytokines which contribute to the destruction of tumour cells, among those IL-1 and IL-6. Provided that growth of tumour cells is stimulated by these factors, as shown for LS411N

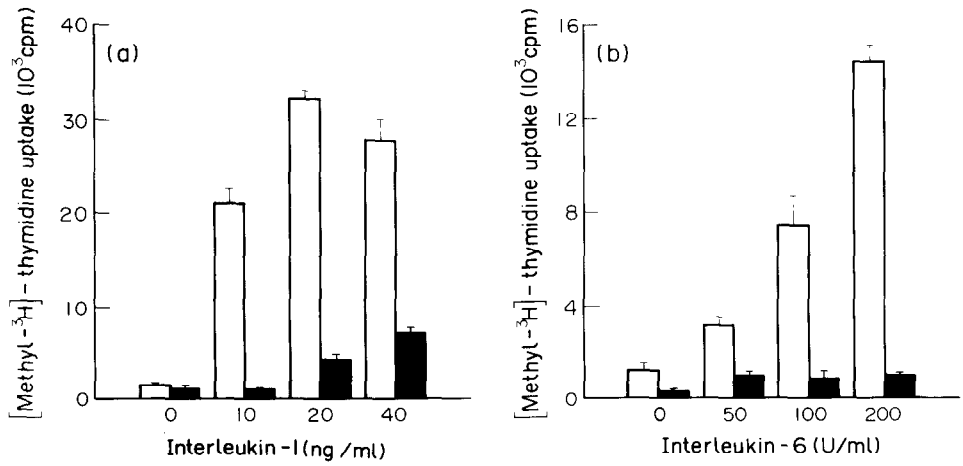

Fig. 4. TGFβ1 abrogates cytokine induced proliferation. LS1034 cells were incubated in the presence of cytokines together with medium (open bars) or TGFβ1(0.5 ng/ml) (filled bars). Values represent the mean of triplicate wells (S.D.).

and LS1034 cells, the production of IL-1 and IL-6 at the tumour site may be deleterious to the host. Certain tumour cells may gain a selective growth advantage, thereby escaping the surveillance of the host immune system. IL-1 appears to be only exceptionally expressed in host cells infiltrating primary colorectal tumours, while mRNA for tumour necrosis factor α (TNFα) has been frequently detected [25]. Since TNFα is a potent inducer of IL-1 and IL-6 may, therefore, create a favourable microenvironment to promote tumour cell growth. It is of interest that the combination of IL-1 and IL-6 was most effective at low cytokine-concentrations, which are probably closest to the *in vivo* situation.

IL-6 functions as an autocrine growth factor in several types of malignancies [11,12] and its potential role as an autocrine growth factor has been suggested for colon carcinomas [26]. In addition, IL-1 is a potent inducer of IL-6 [27]. Therefore, it is tempting to speculate that IL-6 produced by colorectal carcinoma cells themselves may contribute to growth stimulation. However, we have not obtained evidence that autocrine IL-6 contributes to proliferation of LS1034 under the culture conditions used: (a) we have performed experiments in which we delayed the addition of exogenous IL-6. The synergistic response with IL-1 was only seen if IL-6 was present throughout the experiment. A delay of 24 h caused a rapid decrease of proliferation (data not shown). (b) The addition of IL-6 shifted the dose–response curve of IL-1 to a higher plateau, indicating the participation of another receptor. If endogenous IL-6 were already present, maximal proliferation in response to IL-1/IL-6 could have been equal only. (c) Anti-IL-6 Ab did not affect proliferation of LS1034 cells (data not shown). Still, we cannot totally exclude that LS1034 cells may secrete very low levels of IL-6.

Different mechanisms account for anti-proliferative effects mediated by cytokines. IL-1 and TNFα are directly cytotoxic for tumour cells [2,22]. Though TGFβ inhibits numerous proliferative responses no direct cytotoxic effects of this molecule have been reported. We also did not observe cytotoxic effects of $TGF\beta_1$ on LS1034 cells (data not shown). However, we observed a change in morphology of LS1034 cells after exposure to $TGF\beta_1$. They exhibited a more flattened phenotype and grew in more uniform monolayers than untreated control cells. Similar effects have been observed with another human colorectal carcinoma [15] and endometrial carcinoma cell lines [28]. Such changes in cell morphology induced by TGFβ are accompanied by differentiative events [15]. In addition, TGFβ induced differentiation of colorectal tumours [29], which in turn may account for the antagonistic effect of $TGF\beta_1$ on the proliferation induced by IL-1 and IL-6.

This differentiation may indeed be reversible. In the presence of $TGF\beta_1$ colonies of LS1034 developed at a very low frequency (approximately 7×10^{-4}) in methylcellulose. However, upon retesting all were sensitive to the cyto-inhibitory effect of $TGF\beta_1$ (data not shown). Thus, upon removing $TGF\beta_1$, the cells obviously regained a state of susceptibility to this factor. The same may apply for responsiveness to IL-1 and IL-6. Of course, $TGF\beta_1$ might work via other mechanisms, such as modulation of receptors and intracellular targets for other growth factors. Multiresponsive lines such as LS1034 may, therefore, provide a useful tool to elucidate these events in further detail.

1. Oppenheim JJ, Kovacs EJ, Matsushima K, Durum SK. There is more than one interleukin 1. *Immunol Today* 1986, 7, 45–56.
2. Lachman LB, Dinarello CA, Llansa ND, Fidler IJ. Natural and recombinant human interleukin 1-β is cytotoxic for melanoma cells. *J Immunol* 1986, **136**, 3098–3102.
3. Tsai S-CJ, Gaffney EV. Modulation of cell proliferation by human recombinant interleukin-1 and immune interferon. *J Natl Cancer Inst* 1987, **79**, 77–81.
4. Hamburger AW, Lurie KA, Condon ME. Stimulation of anchorage-independent growth of human tumor cells by interleukin 1. *Cancer Res* 1987, **47**, 5612–5615.
5. Lachman LB, Brown DC, Dinarello CA. Growth-promoting effect of recombinant interleukin 1 and tumor necrosis factor for a human astrocytoma cell line. *J Immunol* 1987, **138**, 2913–2916.
6. Lahm H, Fischer JR, Reichert Y, *et al.* Autocrine growth factors secreted by the malignant human B-cell-line BJAB are distinct from other known cytokines. *Eur Cytokine Netw* 1990, **1**, 41–46.
7. Gaffney VE, Koch G, Tsai S-C, Lingenfelter SE. Correlation between human cell growth response to interleukin 1 and receptor binding. *Cancer Res* 1988, **48**, 5455–5459.
8. van Meir E, Sawamura Y, Diserens A-C, Hamou M-F, de Tribolet N. Human glioblastoma cells release interleukin 6 *in vivo* and *in vitro*. *Cancer Res* 1990, **50**, 6683–6688.
9. Watson JM, Sensintaffar JL, Berek JS, Martinez-Maza O. Constitutive production of interleukin 6 by ovarian cancer cell lines and by primary ovarian tumor cultures. *Cancer Res* 1990, **50**, 6959–6965.
10. Kirnbauer R, Köck A, Schwarz T, *et al.* IFN-β2, B cell differentiation factor 2, or hybridoma growth factor (IL-6) is expressed and released by human epidermal cells and epidermoid carcinoma cell lines. *J Immunol* 1989, **142**, 1922–1928.
11. Akashi K, Shibuya T, Harada M, *et al.* Interleukin 4 suppresses the spontaneous growth of chronic myelomonocytic leukemia cells. *J Clin Invest* 1991, **88**, 223–230.
12. Miki S. Iwano M, Miki Y, *et al.* Interleukin-6 (IL-6) functions as an *in vitro* autocrine growth factor for renal cell carcinomas. *FEBS Lett* 1989, **250**, 607–610.
13. Klein B, Zhang X-G, Jourdan M, *et al.* Paracrine rather than autocrine regulation of myeloma-cell growth and differentiation by interleukin-6. *Blood* 1989, **73**, 517–526.
14. Sporn MB, Roberts AB, Wakefield LM, Assoian RK. Transforming growth factor-β: biological function and chemical structure. *Science* 1986, **233**, 532–534.
15. Hoosein NM, Brattain DE, McKnight ML, Levine AE, Brattain MG. Characterization of the inhibitory effects of transforming growth factor-β on a human colon carcinoma cell line. *Cancer Res* 1987, **47**, 2950–2954.
16. Coffey RJ Jr, Shipley GD, Moses HL. Production of transforming growth factors by human colon cancer lines. *Cancer Res* 1986, **46**, 1164–1169.
17. 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.
18. Chantry D, Turner M, Feldman M. Interleukin 7 (murine pre-B cell growth factor/lymphopoietin 1) stimulates thymocyte growth: regulation by transforming growth factor beta. *Eur J Immunol* 1989, **19**, 783–786.
19. Eliason JF, Aapro MS, Decrey D, Brink-Petersen M. Non-linearity of colony formation by human tumor cells from biopsy samples. *Br J Cancer* 1985, **52**, 311–318.
20. Whitehead RH, Nice EC, Lloyd EC, James R, Burgess AW. Detection of colonic growth factors using a human colonic carcinoma cell line (LIM1215). *Int J Cancer* 1990, **46**, 858–863.
21. Gaffney EV, Tsai S-C. Lymphocyte-activating and growth-inhibitory activities for several sources of native and recombinant interleukin 1. *Cancer Res* 1986, **46**, 3834–3837.
22. Belizario JE, Dinarello CA. Interleukin 1, interleukin 6, tumor necrosis factor, and transforming growth factor β increase cell resistance to tumor necrosis factor cytotoxicity by growth arrest in the G_1 phase of the cell cycle. *Cancer Res* 1991, **51**, 2379–2385.
23. Houssiau FA, Coulie PG, Olive D, van Snick J. Synergistic activation of human T cells by interleukin 1 and interleukin 6. *Eur J Immunol* 1988, **18**, 653–656.
24. Onozaki K, Akiyama Y, Okano A, *et al.* Synergistic regulatory effects of interleukin 6 and interleukin 1 on the growth and differentiation of human and mouse myeloid leukemic cell lines. *Cancer Res* 1989, **49**, 3602–3607.
25. Naylor MS, Stamp GWH, Balkwill FR. Investigation of cytokine

gene expression in human colorectal cancer. *Cancer Res* 1990, **50**, 4436–4440.
26. Shirota K, LeDuy L, Yuan S, Jothy S. Interleukin-6 and its receptor are expressed in human intestinal epithelial cells. *Virch Archiv B* 1990, **58**, 303–308.
27. van Damme J, Cayphas C, Opdenakker G, Billiau A, van Snick J. Interleukin 1 and poly(rI) * poly(rC) induce production of a hybridoma growth factor by human fibroblasts. *Eur J Immunol* 1987, **17**, 1–7.
28. Boyd JA, Kaufmann DG. Expression of transforming growth factor β1 by human endometrial carcinoma cell lines: inverse correlation with effects on growth rate. *Cancer Res* 1990, **50**, 3394–3399.
29. Schroy P, Rifkin J, Coffey RJ, Winawer S, Friedman E. Role of transforming growth factor β1 in induction of colon carcinoma differentiation by hexamethylene bisacetamide. *Cancer Res* 1990, **50**, 261–265.

Acknowledgements—This work was supported by a grant from the European Community (B/MR4*-900924) to H. Lahm and a grant from the Swiss National Foundation.

Eur J Cancer, Vol. 28A, No. 11, pp. 1899–1904, 1992.
Printed in Great Britain

0964–1947/92 $5.00 + 0.00

Differential Expression of Insulin-like Growth Factor Binding Proteins in Human Non-small Cell Lung Cancer Cell Lines

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The possible expression and secretion of insulin-like growth factor binding proteins (IGFBPs) by non-small cell lung cancer (NSCLC) cell lines was investigated and compared with possible IGFBP expression by primary NSCLC tumours. Cells growing under serum-free conditions released binding proteins with apparent molecular masses of 26–43 kD when analysed by a ligand blotting method under non-reducing conditions. Additionally, northern blot analysis of total RNA from NSCLC cell lines and tumours was performed using cDNAs coding for each of IGFBP-1, IGFBP-2, and IGFBP-3. This analysis revealed expression of all three mRNAs to varying degrees by all cell lines. In contrast all primary tumours analysed expressed predominantly IGFBP-2 and IGFBP-3 and none showed any evident expression of IGFBP-1. Both NSCLC cell lines and tumours synthesise IGFBPs but the pattern of expression differs significantly between cell lines and primary tumours.
Eur J Cancer, Vol. 28A, No. 11, pp. 1899–1904, 1992.

INTRODUCTION

INSULIN-LIKE GROWTH FACTORS (IGFs) are peptides which modulate growth in a variety of tissues and cell types [1]. IGFs are present in the circulation, in tissues, and in cell culture media and in all these locations are found to be bound to specific binding proteins [2]. These IGF-binding proteins (IGFBPs) bind both IGF-I and IGF-II with high affinity and specificity, and do not bind insulin [3]. There are at least four distinct forms of human IGFBPs which differ in terms of molecular mass, binding specificities and distribution in biological fluids and which have been cloned: IGFBP-1, IGFBP-2, IGFBP-3 and IGFBP-4.

IGFBP-1 is the predominant IGFBP in amniotic fluid and IGFBP-1 mRNA is detected in decidua and secretory endometrium [4]. Elsewhere IGFBP-1 mRNA is found only in the liver (and in the hepatic HEP G2 cells) with concentrations higher in fetal compared to adult liver [5]. Analysis of mRNA in various tissues and cell lines reveals a different pattern of expression for IGFBP-1 and IGFBP-2. IGFBP-2 mRNA is detected in adult liver, brain, in Jurkat and kidney 293 cells, but not in HeLa, Namalwa and HEPG2 cells [6]. IGFBP-1 and IGFBP-2 are proteins with 40% sequence identity [6]. Both proteins contain regions of clustered Pro, Glu, Ser and Thr residues (PEST regions), a Arg–Gly–Asp sequence (RGD motif) and a cystein-rich amino-terminus [5, 6]. The major IGFBP in human adult serum is a 125–150 kD complex which dissociates under acidic conditions, releasing free IGFs and an acid stable IGFBP subunit. This binding subunit is referred to as IGFBP-3 (formerly IGFBP-53) with an apparent molecular mass of 53 kD under non-reduced and 43 kD under reduced conditions. Sequencing of cloned IGFBP-3 cDNA reveals a cysteine-rich primary structure of 264 residues and a predicted molecular mass of 28.7 kD [7]. The sequence has a 33% aminoacid identity including conservation of all 18 cysteine residues with IGFBP-1. Recently two additional IGFBPs have been described. One in conditioned medium from human bone cells and has limited aminoterminal sequence identity with IGFBP-1, IGFBP-2 and IGFBP-3 and appears to inhibit the action of IGF [8]. The other is a binding protein found in cerebrospinal fluid which specifically binds IGF-2 [9].

The biological functions of these binding proteins remain

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Revised 22 Apr. 1992; accepted 28 Apr. 1992.