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Modulation of in vitro cell growth of human and murine urothelial tumor cell lines under the influence of interleukin-3, granulocyte-, macrophage- and granulocyte-colony-stimulating factor*

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Summary. To elucidate possible growth-modulating effects of interleukin 3 (IL-3), granulocyte-macrophage-colony-stimulating factor (GM-CSF) and granulocyte-colony-stimulating factor (G-CSF), human transitional cell carcinoma (TCC) cell lines T24, RT112, EJ and 647V were solitarily and continuously exposed to these hematopoietic growth factors at concentrations of 1–100 ng/ml. The murine line MBT-2 was used as a negative and the colon carcinoma cell line HTB38 as a positive control, because of species specificity and known proliferation in response to growth factors, respectively. In the T24 TCC-line solitary and continuous exposure to IL-3, GM-CSF and G-CSF at the highest concentration of 100 ng/ml led to a significant proliferation of cell growth in vitro. Significant proliferation in the RT112 line was only achieved with continuous exposure to IL-3 and GM-CSF (100 ng/ml); G-CSF failed to induce growth modulation in the RT112 line. No significant proliferative effect of any of cytokines administered was observed in the 647V line. Exposure of the EJ line to cytokines at the highest activity levels had a proliferative effect only in suboptimal growth conditions.

Key words: Interleukin 3 – Granulocyte-macrophage-colony-stimulating factor – Granulocyte-colony-stimulating factor – Human transitional cell carcinoma lines – In vitro growth modulation

Hematopoietic growth factors have been implicated in the regulation and differentiation of hematopoietic precursor cells. One of them, the granulocyte-macrophage-colony stimulating factor (GM-CSF), stimulates the production and function of granulocytes and macrophages in vivo. Therefore, in phase I and II trials GM-CSF is administered during chemotherapy for various non-hematopoietic tumor diseases to minimize therapy-related toxicity. In the treatment of urothelial cell carcinoma with methotrexate, vinblastine, doxorubicin and cisplatin (MVAC); these beneficial effects of G-CSF were confirmed by Gabrilove [6].

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In 32 patients with urothelial tumors that had formerly proved refractory to other antineoplastic agents, Logothetis [9] reported excellent results, with complete and partial remission rates of 23% and 17%, respectively, when they received combined treatment with rh-GM-CSF and MVAC chemotherapy with escalated dosages. Logothetis interpreted these results as the consequence of an enhanced efficacy of antineoplastic agents due to the escalated dosages of the cytostatic agents or of increased sensitivity of urothelial tumor cells to antineoplastic agents due to GM-CSF [9].

The idea of a growth-modulating effect of hematopoietic growth factors on various non-hematopoietic human tumor cell lines remains controversial [2, 4 12, 13]. Therefore, in this study the modulation of cell growth under the influence of IL-3, GM-CSF, and G-CSF in vitro was studied in four different human transitional cell carcinoma (TCC) lines, in one adenocarcinoma cell line and in one murine TCC line (as controls) and correlations with cell differentiation and T stages were sought.

Materials and methods

Hematopoietic growth factors

Recombinant human IL-3, GM-CSF and G-CSF at a specific activity of 5×10^7 units per mg protein were kindly provided by Behringwerke (Marburg, FRG). Aliquots were stored at −20°C. For experimental use these factors were resuspended in adequate media with an optimal pH of 7.4 at concentrations of 1, 10 and 100 ng/ml. Cytokines were administered both solitarily and continuously (see below). Polygeline, in which GM-CSF is stabilized, was also applied to cell lines solitarily and continuously to exclude a possible growth-modulating effect of this compound.

Cell lines and culture conditions

TCC lines were derived from different tumor types and were kindly supplied by the Tumor Bank, German Cancer Research Center (Heidelberg, FRG). MBT-2 and HTB-38 lines were kindly provided by Dr. Ratliff (St. Louis/Mio.) and Dr. Berdel (Berlin, FRG), respectively. Characteristics and culture conditions of the cell lines

Table 1. Characteristics and culture conditions of cell lines

Cell line	Histology	Source	T-Stage	Grading	Medium	FCS
EJ	TCC	Human	2–4	4	MEM-Earle	10%
RT112	TCC	Human	*	1–2	RPMI 1640	10%
T24	TCC	Human	*	3	MEM-Dulbecco	15%
647V	TCC	Human	*	2	RPMI 1640	10%
MBT2	TCC	Murine	*	3	RPMI 1640	10%
HTB38	AdenoCa	Human	*	3	McCoy's	10%

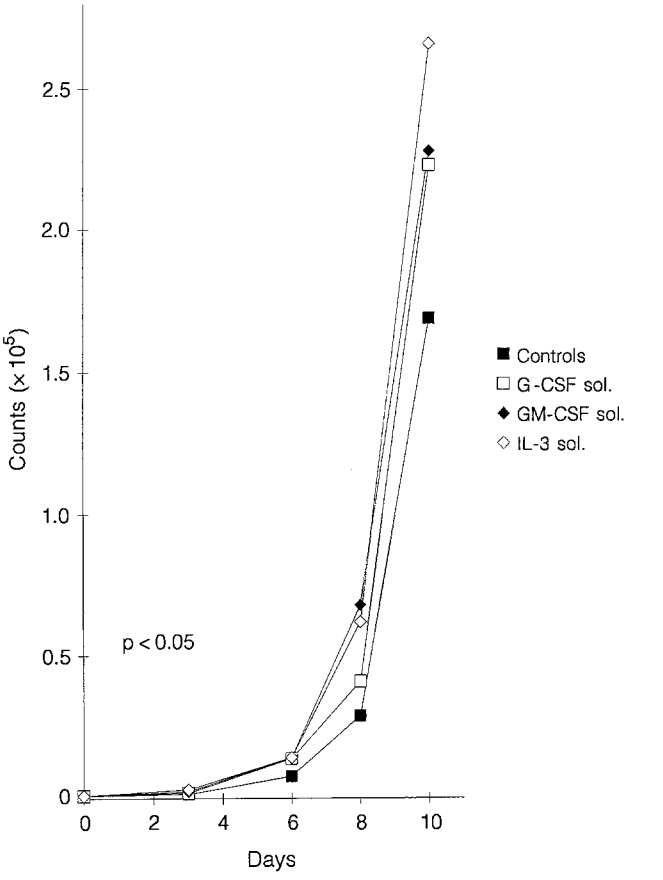

Fig. 1. Proliferative effects of IL-3, GM-CSF and G-CSF (100 ng/ml) on solitary exposure to the human T24 TCC line. Abbreviations for all figures: IL-3, interleukin 3; GM-CSF, granulocyte-macrophage-colony-stimulating factor; G-CSF, granulocyte-colony-stimulating factor; TCC, transitional cell carcinoma

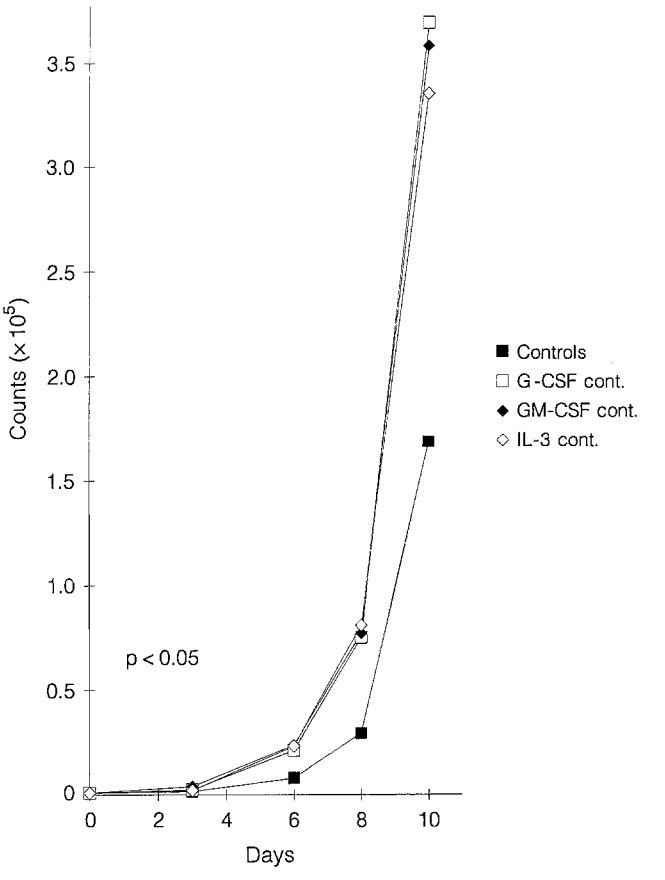

Fig. 2. Proliferative effects of IL-3, GM-CSF and G-CSF (100 ng/ml) on continuous exposure to the human T24 TCC line

used are displayed in Table 1. Optimal growth conditons were determined in each TCC line. Cell lines MBT-2 and HTB-38 were used as controls because of the known species specificity of human recombinant cytokines and the reported proliferative effect due to continuous exposure to IL-3, GM-CSF [2], respectively.

Culture media contained fetal calf serum (FCS), 1% non-essential aminoacids, 2 mM glutamine and gentamicin (50 µg/ml). All cell lines were maintained at 37°C in 5% CO_2 in a humidified atmosphere.

To reveal any modulation of cell proliferation by growth factors under suboptimal growth conditions, IL-3, GM-CSF and G-CSF were added to cells of the 647 V and EJ lines in medium in which FCS was substituted by 1% fetal calf albumin.

Cells were screened for mycoplasma, and the results were negative throughout these studies.

Monolayer proliferation assay

Cells of each cell line were incubated for 10 days in 24-well plates (Costar, Cambridge, UK) in 1 ml specific medium with and without cytokines and polygeline, respectively. Different concentrations of IL-3, GM-CSF, and G-CSF were diluted as already described and added to the medium at the initiation of experiments ("solitary exposure"). For "continuous exposure" cells were incubated with IL-3, GM-CSF and G-CSF at days 3, 6, and 8 additionally. Control groups exposed to polygeline and untreated cells were always incubated at the same time. To evaluate the proliferation rates in each experimental group, cell counts were performed with a hemocytometer (MD Kova, Madaus-Diagnostik, Köln, FRG) on days 3, 6, 8 and 10. Cell viability, as assessed by the trypan blue exclusion technique, was observed to be at least 98% for each experiment. All experiments were carried out at least in duplicate.

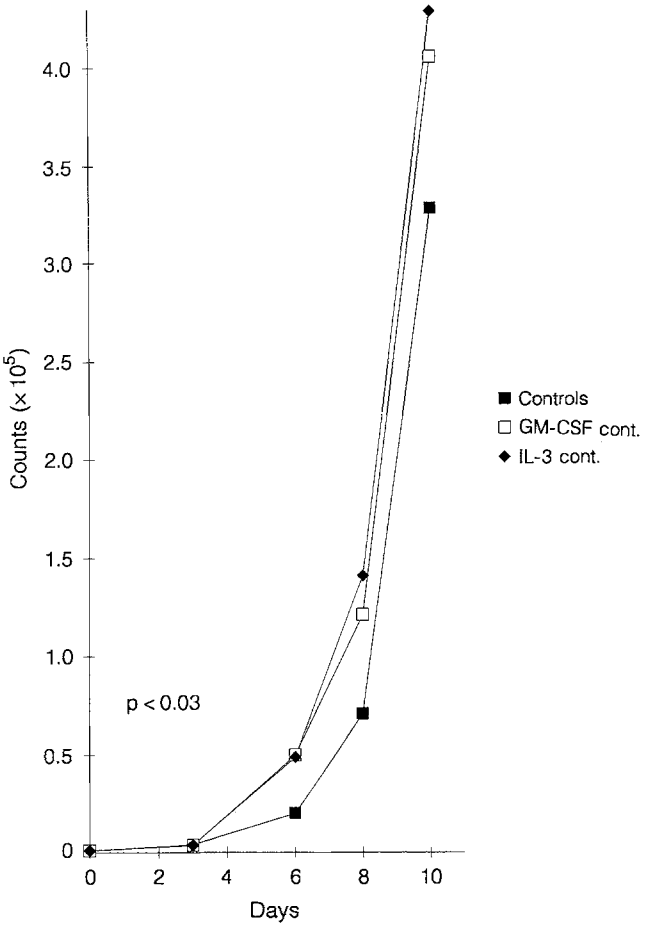

Fig. 3. Growth modulation of cells of the human RT112 TCC line on continuous exposure to IL-3 and GM-CSF (100 ng/ml)

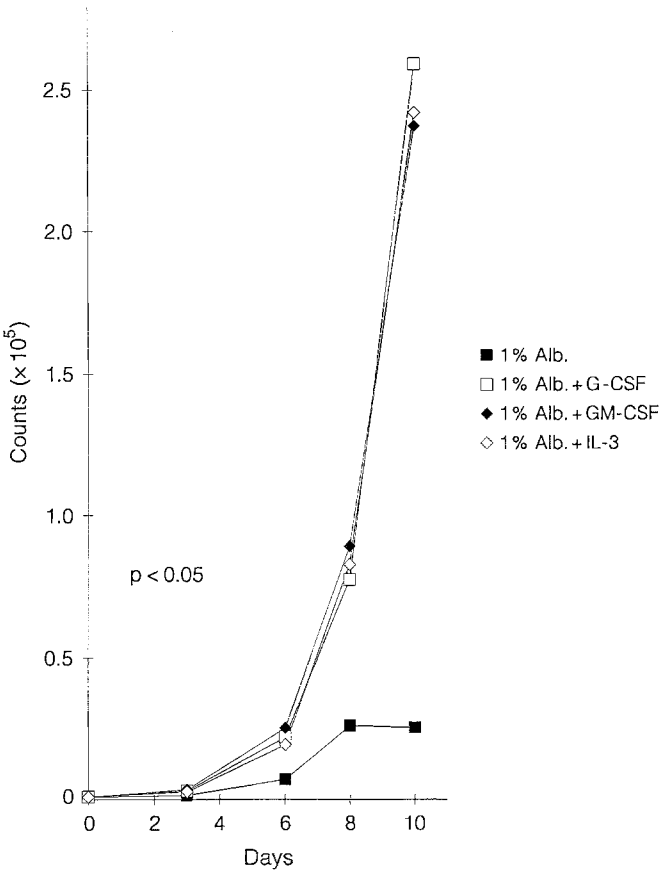

Fig. 4. Growth modulation of cells of the human EJ TCC line following addition of IL-3, GM-CSF and G-CSF in FCS-free medium

Statistical methods

Differences of cell growth between described experimental groups were analyzed by means of the Friedman and Wilcoxon-Wilcox tests by R. Busch.

Results

As expected, the MBT2 line showed no growth modulating effect due to human growth factors because of their known species specificity; IL-3 and GM-CSF stimulated proliferation of the HTB-38 colon cancer cell line in continuous exposure in dosages of 100 ng/ml. Polygeline was unable to enhance in vitro cell growth in any of the lines tested.

The best proliferative effect (Figs. 1, 2) was achieved in the T24 TCC line by solitary and continuous exposure to IL-3, GM-CSF and G-CSF at the highest activities of 100 ng/ml. Significant differences from controls occurred from days 6 to 10 ($P<0.05$).

Cells of the RT112 line were only significantly stimulated ($P<0.03$) by continuous exposure to IL-3 and GM-CSF at 100 ng/ml from days 6 to 10 (Fig. 3). Solitary administration of IL-3 and GM-CSF, and both solitary and continuous exposure to G-CSF failed to induce cell growth in the RT112 line.

No significant differences in the modulation of growth response were determined between IL-3 and GM-CSF in T24 and RT112 cell lines. In optimal culture conditions cells of the lines 647V and EJ showed no proliferative response to any of the cytokines administered. In FCS-free medium, however, continuous administration of IL-3, GM-CSF and G-CSF lead to enhancement of cell growth ($P<0.05$) in the EJ TCC line compared with growth in medium containing 1% albumin only (Fig. 4).

In the 647V line, FCS-free medium induced immediate cytolysis, so that in the 647V TCC line the effects of cytokines in suboptimal culture conditions could not be assessed.

Discussion

Effects of growth factors on cell growth in various non-hematopoietic human tumor cell lines remain controversial. Some in vitro studies have suggested a proliferative effect on tumor cells [2, 4], while another demonstrated no modulation of tumor cell growth [13] and Ruff et al. [12] observed an inhibiton of cell proliferation.

Therefore, the aim of this study was to determine possible growth-modulating effects in human TCC lines, because hematopoietic growth factors are administered

during chemotherapy for advanced TCC to treat or to circumvent chemotherapy-induced myelosuppression [6, 9]. Significant dose-dependent and proliferation in the T24 and RT112 human TCC lines may indicate an effect of certain cytokines on cell growth in vitro. Response to growth factors was correlated to cell density. At high cell concentrations a decrease of growth response to cytokines was noted. This effect might be explained by the density-induced down-regulation of growth factor receptors due to cell-cell interaction [11].

No correlation was found between T stage and differentiation of the TCC lines tested and response to cytokines.

Compared with controls, the EJ and 647V lines showed no differences in cell growth on exposure to any of the factors. However, in the EJ line a growth-modulating effect was determined in suboptimal growth conditions.

A stimulatory capacity of GM-CSF and IL-3 in human TCC lines in vitro was also observed with the clonogenic assay [2] and ^{3}H-thymidine uptake [10]. The results shown, achieved with the monolayer proliferation assay, confirm the general possibility of a growth-modulating effect on TCC lines in vitro. However, the EJ line showed no significant response in optimal growth conditions. A significant proliferative effect on cell growth was only achieved in suboptimal growth conditions. This effect has also been reported by other authors [3, 5]. Cytokine-related cell proliferation in the EJ TCC line in FCS-free medium suggests a regulation of urothelial carcinoma-cell-specific metabolism [1, 7, 8].

It also seems possibled that there is a heterogeneous response to proliferative effects due to these cytokines within the TCC tumor type. Similar data were obtained in the colon carcinoma cell line HTB38 – tested in this study as a positive control – and the WIDR cell line, with significant and insignificant growth modulation in vitro, respectively [2].

It is of clinical interest that Logothetis, in a phase II study, reported complete and partial remission rates of 23% and 17%, respectively, in urothelial tumors primarily refractory to MVAC, CISCA and CMV chemotherapy when escalated MVAC treatment was given (methotrexate 30 mg/m^2, vinblastine 4 mg/m^2, doxorubicin 60 mg/m^2, cisplatin 100 mg/m^2) with concomitant administration of rh-GM-CSF (120 μg/m^2 for 10 consecutive days) in 32 patients [9]. These remissions – described by the authors as unexpectedly positive – might be interpreted as evidence of better chemotherapeutic efficacy because of the higher dosages used or of an increased sensitivity of tumor cells to antineoplastic agents because of synchronized cells in the cell cycle due to GM-CSF [8, 9].

Because of the growth modulation shown in TCC lines under the influence of hematopoietic growth factors in vitro and the clinical results reported by Logothetis, possible cell-cycle-specific effects of IL-3, GM-CSF and G-CSF are now being investigated by flow-cytometric analysis. To assess the possibility of a higher sensitivity to chemotherapeutic agents, the cell lines EJ, T24, RT112 and 647V are now being exposed to methotrexate, vinblastine, doxorubicin and cisplatin with and without IL-3, GM-CSF and G-CSF. Possible interactions between antineoplastic agents and hematopietic growth factors in TCC would mean modifying time schedules for cytokines and chemotherapy, which might have implications for experimental and for clinical treatment.

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