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ORIGINAL ARTICLE

Stefan Berger · Antje Siegert · Carsten Denkert
Martin Köbel · Steffen Hauptmann

Interleukin-10 in serous ovarian carcinoma cell lines

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Abstract Interleukin-10, one of the most potent anti-inflammatory cytokines, is expressed in ovarian carcinomas in vivo. In contrast to the high levels of IL-10 in ascites and tumour tissue, the expression of this cytokine appears to be a rare event in ovarian carcinoma cell lines in vitro. Virtually nothing is known about the regulation of IL-10 expression in ovarian carcinoma cell lines. We investigated the expression of IL-10 in four cell lines originally derived from ovarian serous adenocarcinoma: OVCAR-3, SKOV-3, CAOV-3 and OAW-42. IL-10-specific mRNA was detected in OVCAR-3 and only this cell line produced IL-10 constitutively under serum-free conditions as well as in serum-containing medium. Our studies on the regulation of IL-10 secretion in OVCAR-3 revealed that (1) proinflammatory stimuli IL-1β and TNF-α, but not LPS, enhance IL-10 secretion, (2) IL-6 has no influence on the release of IL-10, (3) prostaglandin E2 influences neither the spontaneous nor the TNF-α- or IL-1β-stimulated IL-10 production and (4) interferon-γ inhibits IL-10 secretion. We conclude that only a minority of serous ovarian carcinoma cells maintain the ability to produce IL-10 in vitro. Our data on the regulation of IL-10 production in OVCAR-3 indicate that ovarian carcinoma cells share some, but not all, of the regulatory features typical for the monocytic IL-10 secretion.

Key words Interleukin-10 · Ovarian cancer

Introduction

Expression of a wide range of cytokines has been found in ovarian carcinomas, further substantiating the morphologist's experience of an ongoing inflammatory process within solid tumours and malignant effusions, in ovarian carcinoma as well as in many other types of malignant tumours. While at first the presence of inflammatory cells within tumour tissue was regarded as sufficient explanation for the expression of inflammatory mediators, it is now clear that malignant epithelial cells of ovarian carcinoma themselves also produce cytokines with immunoregulatory capacities [8, 11, 15, 16, 20–22].

Over the past decade, evidence has accumulated that the expression of cytokines plays an important role in development and progression of ovarian carcinoma. Among many cytokines described in ovarian carcinoma, interleukin (IL)-6 [17, 23], macrophage colony stimulating factor (M-CSF) [6] and vascular endothelial growth factor (VEGF) [13] appear to be of particular importance for autocrine growth regulation, invasion and neovascularization.

Another important question is how malignant tumours manage to escape immunosurveillance despite the inflammatory response to the tumour, consistently observed. In this context, the finding of abundant levels of IL-10 in peritoneal effusions, in the serum and in solid tumour tissue of patients with ovarian carcinoma is particularly interesting [9, 12, 19]. IL-10, originally described as cytokine synthesis inhibitory factor (CSIF), is a highly potent anti-inflammatory cytokine, which primarily acts on cell-mediated (Th1-like) immune responses. Indeed, an immunosuppressive soluble factor in the malignant effluent of ovarian carcinoma patients has been described long before today's knowledge on the complexity of cytokine networks had emerged [2].

To date, it is not entirely clear whether epithelial cells from ovarian carcinoma produce IL-10, or whether inflammatory cells fully account for the abundant cytokine production. Moreover, no data exist on the regulation of IL-10 production in ovarian carcinoma. Here we report the constitutive and partially regulated expression of IL-10 by an ovarian carcinoma cell line, OVCAR-3, in vitro, indicating that epithelial cells contribute to the

S. Berger (✉) · A. Siegert · C. Denkert · M. Köbel
S. Hauptmann
Institute of Pathology, Charité Hospital Berlin,
Schumannstrasse 20/21, 10117 Berlin, Germany
e-mail: stefan.berger@charite.de
Tel.: +49-30-28023420; Fax: +49-30-28021296

production of IL-10 observed in ovarian carcinomas in vivo.

Materials and methods

Antibodies, recombinant cytokines and reagents

Neutralizing mouse-anti-human IL-1β mAb (clone IL1B-H67) and neutralizing polyclonal rabbit-anti-human TNF-α Ab were purchased from Endogen (Woburn, Mass., USA).

Neutralizing polyclonal goat-anti-human IL-6 Ab and recombinant human (rh) IL1-β, rhTNF-α, rhIL-6 and rhIFN-γ were from R&D Systems (Minneapolis, Minn., USA).

Prostaglandin E2 and LPS from *Escherichia coli* 026:B6 were obtained from Sigma Chemical (St. Louis, Mo., USA). Dulbecco's minimum essential medium (DMEM) was from BioWhittaker Europe (Verviers, Belgium), foetal calf serum (FCS) and L-glutamine from PAA-Laboratories (Linz, Austria).

Ovarian carcinoma cell lines and cell culture

CAOV-3 and NIH-OVCAR-3 were obtained from the American Type Culture Collection; OAW-42 and SKOV-3 were from Cell Lines Service (CLS; Heidelberg, Germany). Cells were maintained in a humidified atmosphere in DMEM supplemented with 10% FCS and 1% L-glutamine at 37 °C/ 5% CO_2 in air. All culture media contained <0.1 EU/ml endotoxin, as tested by the limulus amebocyte lysate assay.

Cells were plated in 96-well flat-bottomed plates at 2×10^5 cells/well in a final volume of 250 µl. After 2 days confluent monolayers were obtained. Medium was exchanged and cells were further incubated in medium alone or medium supplemented with 0.01–1 ng/ml rhIL-1β, or 0.1–10 ng/ml rhTNF-α, or 1–100 ng/ml rhIL-6, or 0.01–100 ng/ml rhIFN-γ, or 50–5000 ng/ml PGE2, or 0.01–100 ng LPS. In some experiments, 10 µg/ml of neutralizing anti-IL-1β, or anti-IL-6, or anti-TNF-α antibodies were present throughout the culture. In other experiments, the medium was replaced by serum-free DMEM or DMEM containing 2%, 5% or 10% FCS.

Determination of cytokine levels by ELISA

A mouse anti-human IL-10 capture mAb (clone 9D7), a biotinylated mouse anti-human IL-10 detection mAb (clone 12G8), a mouse anti-human IL-6 capture mAb (clone 5IL6) and a biotinylated mouse anti-human IL-6 detection mAb (clone 7IL6) (all from Endogen) were used to develop specific ELISAs for the detection of IL-10 and IL-6, respectively, as previously described [3]. Standard curves were obtained using serial dilution of rhIL-10 and rhIL-6 (R&D Systems). The detection limit was 2 pg/ml for IL-10 and 8 pg/ml for IL-6.

Detection of mRNA transcripts

Total RNA, from confluent cultures grown in 10 cm Ø dishes in the absence or presence of 10 ng/ml TNF-α or 1 ng/ml IL-1β, was extracted by the phenol/isothiocyanate method using Trizol reagent (Gibco). After cDNA synthesis, a control PCR for glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) was performed using the following primers: 5′-ACCACAGTCCATGCCATCAC-3′ and 5′-TCCACCACCCTGTTGCTGTA-3′. Primers used for amplification of IL-10 specific mRNA fragments were: 5′-CCAGGCAACCTGCCTAACAT-3′ and 5′-TGCTCCACGGCCTTGCTCTT-3′.

PCR was performed in a reaction mixture containing 3.5 mM $MgCl_2$ and 5% DMSO with 28 cycles of 94 °C for 1 min, 60 °C for 1 min and 72 °C for 15 s.

Amplification products were visualized on a 2% agarose gel stained with ethidium bromide.

Results

Constitutive and cytokine-induced secretion of IL-6 and IL-10 in ovarian carcinoma cell lines

The expression of IL-10 was studied in four permanent epithelial cell lines originally derived from serous ovarian adenocarcinomas. Figure 1 shows the expression of IL-10-specific mRNA as detected by RT-PCR. Since IL-10 production in monocytes is triggered by proinflammatory cytokines, we studied the constitutive expression of IL-10-specific mRNA as well as the expression stimulated by IL-1β or TNF-α. Among the four cell lines tested only OVCAR-3 showed the expression of IL-10-specific mRNA. Stimulation of OVCAR-3 clearly resulted in an upregulation of IL-10 mRNA expression.

Table 1 shows IL-10 secretion by ovarian carcinoma cell lines, unstimulated or stimulated with TNF-α or IL-1β, compared with the secretion of IL-6. All cell lines tested constitutively secreted abundant levels of IL-6, which could be further enhanced by stimulation with TNF-α, while a stimulatory effect of IL-1β was confined to OVCAR-3 and SKOV-3.

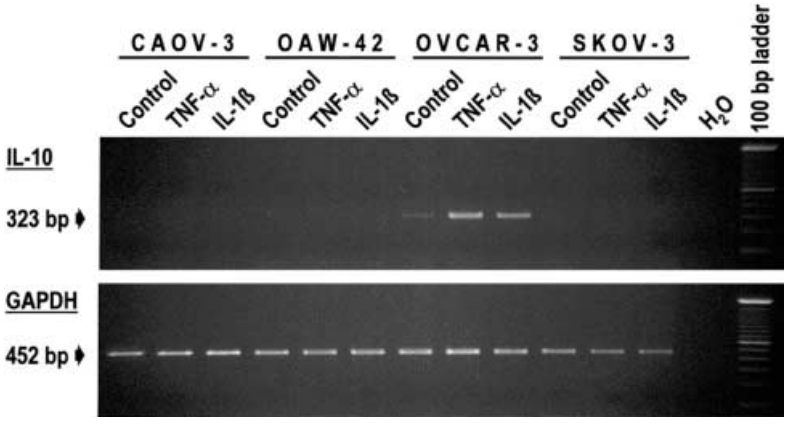

Fig. 1 Expression of IL-10-specific mRNA in ovarian carcinoma cell lines. Total RNA was isolated from confluent ovarian carcinoma cells that were grown in medium alone or medium supplemented with 1 ng/ml IL-1β or with 10 ng/ml TNF-α and analysed for IL-10-specific transcripts by RT-PCR. Detection of GAPDH-specific mRNA transcripts served as internal control. A commercially available 100 bp DNA ladder was use as molecular weight marker

Table 1 Production of IL-6 and IL-10 by ovarian carcinoma cell lines. Ovarian carcinoma cell lines were incubated in medium alone or medium supplemented with 1 ng/ml rhIL-1β or 10 ng/ml rhTNF-α. Supernatants were assayed for the concentration of IL-6 and IL-10 by specific ELISAs. Data represents mean ± SEM from five independent experiments, where each condition was at least tested in duplicate (< indicates concentration below assay threshold)

Cell line	Stimulated with	IL-6 (ng/ml)	IL-10 (pg/ml)
CAOV-3	Medium	10.8 ± 4.3	<
	IL-1β	9.7 ± 4.1	<
	TNF-α	34.5 ± 10.5	<
OAW-42	Medium	0.7 ± 0.1	<
	IL-1β	0.8 ± 0.0	<
	TNF-α	6.2 ± 2.2	<
OVCAR-3	Medium	0.1 ± 0.0	14.7 ± 3.7
	IL-1β	32.0 ± 7.7	2098.0 ± 196.9
	TNF-α	1.6 ± 0.2	473.4 ± 76.6
SKOV-3	Medium	0.4 ± 0.2	<
	IL-1β	3.7 ± 1.0	<
	TNF-α	1.7 ± 0.8	<

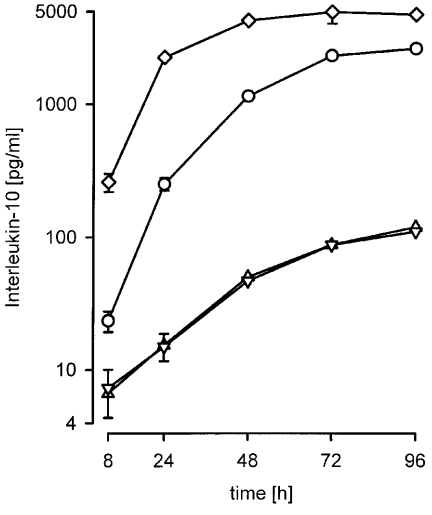

Fig. 2 Time-dependent expression of IL-10 in OVCAR-3. Confluent cultures of OVCAR-3 cells were incubated in medium alone ($\triangledown$), or in medium supplemented with 1 ng/ml IL-1β ($\diamond$) or 10 ng/ml TNF-α ($\circ$) or 10 ng/ml IL-6 ($\triangle$). Cell-free supernatants were collected after 8, 24, 48, 72 or 96 h and analysed for the presence of IL-10 by specific ELISA. Results represent mean ± SEM from three independent experiments, where each condition was tested in duplicate

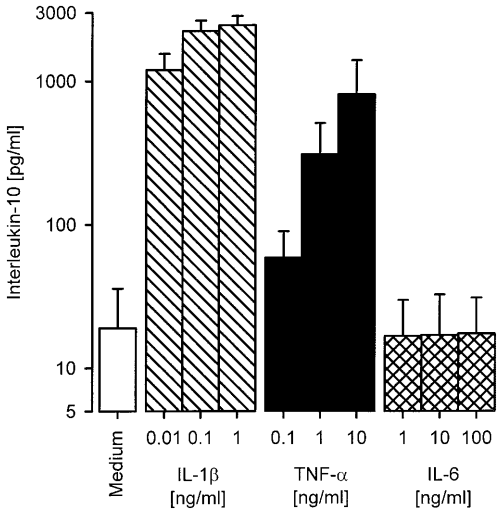

Fig. 3 Dose-dependent stimulatory effect of proinflammatory cytokines on IL-10 secretion by OVCAR-3. Confluent cultures of OVCAR-3 cells were incubated in medium alone (*hollow bar*), or in medium supplemented with 0.01–1 ng/ml IL-1β (*striped bars*) or 0.1–10 ng/ml TNF-α (*filled bars*) or 1–100 ng/ml IL-6 (*cross-hatched bars*). Cell-free supernatants were collected after 48 h and analysed for the presence of IL-10 by specific ELISA. Results represent mean ± SEM from three independent experiments, where each condition was tested in duplicate

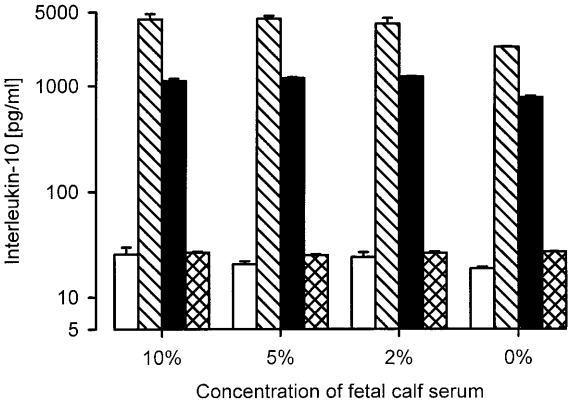

Fig. 4 Constitutive secretion of IL-10 by OVCAR-3. OVCAR-3 cells were grown to confluency in DMEM/10% FCS. The medium was then changed to DMEM alone or supplemented with various concentrations of FCS. Cells were further incubated in medium alone (*hollow bars*) or with 1 ng/ml IL-1β (*striped bars*) or with 10 ng/ml TNF-α (*solid bars*) or with 10 ng/ml IL-6 (*cross-hatched bars*). After 48 h, cell-free supernatants were analysed for the presence of IL-10 by specific ELISA. Results represent mean ± SEM from three independent experiments, where each condition was tested in duplicate

In contrast, only OVCAR-3 secreted detectable levels of IL-10, which were greatly elevated after stimulation with TNF-α and IL-1β in a dose- and time-dependent manner (Figs. 2 and 3). The secretion of IL-10 was essentially independent of the serum concentration in the medium (Fig. 4), indicating that IL-10 was constitutively expressed and not stimulated by an exogenous serum factor.

Ovarian carcinoma cell lines are known to produce a wide range of proinflammatory cytokines. In order to investigate the influence of endogenously produced proinflammatory cytokines on the secretion of IL-10, we studied IL-10 expression in the presence of neutralizing antibodies (Fig. 5). While 10 µg/ml anti-IL-1β was sufficient to completely suppress the stimulatory effect of exogenously added IL-1β, it influenced neither the constitutive nor the TNF-α-induced IL-10 secretion in OVCAR-3. Correspondingly, 10 µg/ml anti-TNF-α completely antagonized the effect of exogenous TNF-α,

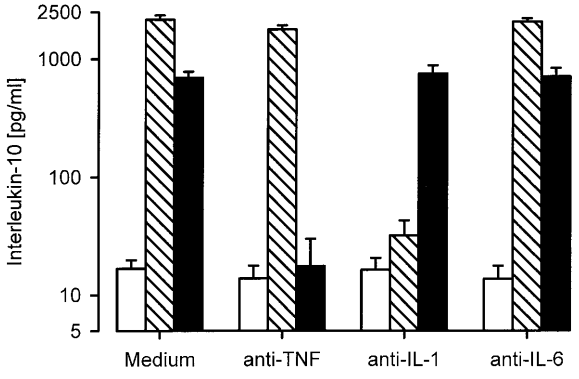

Fig. 5 Role of endogenously produced proinflammatory cytokines on IL-10 secretion. Confluent cultures of OVCAR-3 cells were incubated in medium alone (*hollow bar*), or in medium supplemented with 1 ng/ml IL-1β (*striped bars*) or 1 ng/ml TNF-α (*filled bars*). In some cultures a neutralizing anti-TNF-α antibody, or a neutralizing anti-IL-1β antibody, or a neutralizing anti-IL-6 antibody, was present throughout the culture. Cell-free supernatants were collected after 48 h and analysed for the presence of IL-10 by specific ELISA. Results represent mean ± SEM from four independent experiments, where each condition was tested in triplicate

but did not influence the constitutive or the IL-1β stimulated IL-10 secretion. Neutralizing anti-IL6 antibodies had no effect on IL-10 expression, either constitutive or IL-1β- or TNF-α-stimulated.

Regulation of IL-10 secretion in OVCAR-3 by IFN-γ, LPS and PGE-2

In monocytes the secretion of IL-10 is greatly enhanced by bacterial endotoxins and prostaglandins, while IFN-γ potently suppresses monocytic IL-10 production. We have tested the influence of IFN-γ, LPS and PGE-2 on the constitutive and the IL-1β- and TNF-α- stimulated IL-10 secretion in OVCAR-3 (Fig. 6).

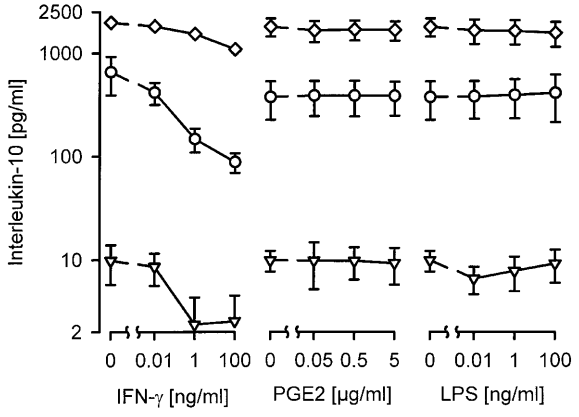

Fig. 6 Effect of IFN-γ, PGE-2 and LPS on IL-10 secretion by OVCAR-3. Confluent cultures of OVCAR-3 cells were incubated in medium alone ($\triangledown$), or in medium supplemented with 1 ng/ml IL-1β ($\diamond$) or 10 ng/ml TNF-α ($\circ$). Various concentrations of IFN-γ or PGE-2 or LPS were added. Cell-free supernatants were collected after 48 h and assayed for IL-10 concentration by specific ELISA. Results represent mean ± SEM from three independent experiments, where each condition was at least tested in duplicate

IFN-γ effectively suppressed the constitutive and cytokine-stimulated IL-10 secretion in a dose-dependent manner. However, the capacity of IFN-γ to suppress IL-10 secretion was dependent on type and strength of the stimulatory signal. While IFN-γ in concentrations of 1 ng/ml and above completely suppressed the constitutive IL-10 secretion, the TNF-α- and IL-1β-triggered IL-10 secretion was maximally reduced to 13% and 55%, respectively, even when concentrations of up to 10 ng/ml IFN-γ were used.

In contrast, neither LPS nor PGE2 exerted any influence on IL-10 secretion by OVCAR-3.

Discussion

We have investigated the expression of IL-10 in four ovarian carcinoma cell lines derived from serous carcinoma. IL-10-specific mRNA was detected in OVCAR-3, but not in CAOV-3, OAW-42 or SKOV-3.

Only OVCAR-3 secreted detectable amounts of IL-10; this appears to be a factual constitutive secretion, since it occurred in the absence of serum proteins. LPS did not enhance the secretion of IL-10 in OVCAR-3, making it very unlikely that the constitutive IL-10 secretion was the result of a possible low-level endotoxin contamination.

The secretion of IL-10 by OVCAR-3 was greatly enhanced by the proinflammatory cytokines IL-1β and TNF-α, but not by IL-6, in a dose- and time-dependent manner. This upregulation appears to be controlled at the transcriptional level, since we detected a considerable increase of IL-10-specific mRNA accumulation after stimulation with IL-1β or TNF-α, using a semiquantitative PCR approach.

Although IL-10 secretion was greatly enhanced by proinflammatory cytokines, the constitutive IL-10 expression occurred independently from endogenously secreted TNF-α and IL-1β, as it could not be suppressed by the presence of neutralizing anti-IL1β or anti-TNF-α antibodies. Furthermore, studies with neutralizing antibodies showed: (a) that the IL-1β- and TNF-α-induced enhancement of IL-10 secretion was specific for the respective cytokine and not due to a possible contamination of our cytokine preparation; (b) that the enhancement caused by IL-1β was mainly independent of the concomitantly IL-1β-induced secretion of TNF-α and vice versa; (c) that the constitutive, as well as the cytokine-induced, secretion of IL-10 was not connected to endogenously secreted IL-6.

Despite the potency of IL-1β and TNF-α to enhance IL-10 secretion in OVCAR-3, these cytokines had no effect on the IL-10 concentration measured in the supernatants of the other three cell lines tested, nor did they induce expression of IL-10-specific mRNA. However, different cell lines derived from ovarian carcinoma show distinct reaction patterns towards the stimulatory effects

of cytokines, as can be seen by the failure of IL-1β to enhance IL-6 secretion in CAOV-3 and OAW-42. It is possible that CAOV-3, SKOV-3 and OAW-42 need other stimuli in order to induce IL-10 expression in vitro.

IL-10 and IFN-γ act as antagonists in the balance between cell-mediated and antibody-dependent immune responses. IFN-γ has been used in therapeutic trials as well as in xenograft models of ovarian carcinoma in order to treat residual disease [1, 7]. It proved to have some beneficial effect with regard to survival time and formation of peritoneal metastases. In analogy to the effect of IFN-γ on the monocytic IL-10 production, we found that IFN-γ strongly suppresses the IL-10 secretion in OVCAR-3, suggesting that the suppression of IL-10 secretion by ovarian carcinoma cells in vivo might contribute to the beneficial effect of IFN-γ.

In contrast to regulatory features observed in the monocytic IL-10 expression, PGE-2 or LPS had no stimulatory effect in OVCAR-3.

IL-10 is a potent anti-inflammatory cytokine that preferentially acts on the cell-mediated (TH1-like) immune response, which is generally regarded as the adequate immune reaction against malignant tumours. IL-10 thus interferes with tumoricidal mechanisms, it suppresses secretion of TNF-α and of reactive oxygen intermediates in macrophages [4], downregulates MHC I expression on tumour cells and interferes with accessory cell function of antigen-presenting cells. On the other hand, a tumour-suppressing effect of IL-10 has been reported in a xenograft model of IL-10-transfected malignant melanoma, where IL-10 impedes neovascularization and tumour growth [10].

An immunosuppressive soluble factor has been described earlier in the ascites of patients with ovarian carcinoma that closely resembles known features of IL-10, in that it affects the spontaneous cytotoxicity against tumour cells, but not the antibody-mediated cytotoxicity [2].

In vivo, abundant expression of IL-10-specific mRNA and IL-10 protein has been detected in malignant ascites, serum and within tumour tissue [9, 12, 18]. The question of which cell population is responsible for the production of IL-10 in ovarian carcinoma has so far been addressed solely by the investigation of ovarian carcinoma cell lines in vitro. To date, the general view is that ovarian cancer cell lines are not capable of producing IL-10. Three different groups have reported that ovarian carcinoma cell lines do not produce IL-10 [9, 14, 18]. However, although OVCAR-3 is a well-defined cell line, derived from a serous ovarian adenocarcinoma that is known to produce proinflammatory cytokines, it has not been included in these studies on IL-10 expression. Only one group that reported on their cytokine profile screening in ovarian carcinoma cell lines hinted at an expression of IL-10-specific mRNA in one out of six cell lines, without any further specification or comment on this issue within the text [5]. Our results indicate that epithelial cells from ovarian carcinoma are in principle capable of producing IL-10 and that OVCAR-3 may serve as a model cell line to investigate the regulation of IL-10 expression.

In contrast to the abundant IL-10 expression in vivo, the expression of IL-10 in ovarian carcinoma cell lines appears to be a rather rare event. Still, contrasting in vivo and in vitro expression patterns have been reported for other cytokines in ovarian carcinoma. For example, a constitutive expression of high TNFα levels has been reported in freshly isolated ovarian carcinoma cells, while established cell lines secrete TNFα only upon stimulation with proinflammatory cytokines [24]. In parallel, freshly isolated ovarian carcinoma cells were reported to secrete IL-1β, while established cell lines do not, and, moreover, IL-1β secretion could be augmented by LPS in the former, but not in the latter [11].

Our finding that epithelial cells from ovarian carcinoma are in principle capable of expressing IL-10 in vitro makes it worthwhile to investigate the expression pattern of IL-10 in suitable in vivo models.

References

1. Allavena P, Peccatori F, Maggioni D, Erroi A, Sironi M, Colombo N, Lissoni A, Galazka A, Meiers W, Mangioni C (1990) Intraperitoneal recombinant gamma-interferon in patients with recurrent ascitic ovarian carcinoma: modulation of cytotoxicity and cytokine production in tumor-associated effectors and of major histocompatibility antigen expression on tumor cells. Cancer Res 50: 7318
2. Badger AM, Oh SK, Moolten FR (1981) Differential effects of an immunosuppressive fraction from ascites fluid of patients with ovarian cancer on spontaneous and antibody- dependent cytotoxicity. Cancer Res 41: 1133
3. Berger S, Chandra R, Ballo H, Hildenbrand R, Stutte HJ (1997) Immune complexes are potent inhibitors of interleukin-12 secretion by human monocytes. Eur J Immunol 27: 2994
4. Bogdan C, Vodovotz Y, Nathan C (1991) Macrophage deactivation by interleukin 10. J Exp Med 174: 1549
5. Burke F, Relf M, Negus R, Balkwill F (1996) A cytokine profile of normal and malignant ovary. Cytokine 8: 578
6. Chambers SK, Wang Y, Gertz RE, Kacinski BM (1995) Macrophage colony-stimulating factor mediates invasion of ovarian cancer cells through urokinase. Cancer Res 55: 1578
7. Colombo N, Peccatori F, Paganin C, Bini S, Brandely M, Mangioni C, Mantovani A, Allavena P (1992) Anti-tumor and immunomodulatory activity of intraperitoneal IFN-gamma in ovarian carcinoma patients with minimal residual tumor after chemotherapy. Int J Cancer 51: 42
8. Erroi A, Sironi M, Chiaffarino F, Chen ZG, Mengozzi M, Mantovani A (1989) IL-1 and IL-6 release by tumor-associated macrophages from human ovarian carcinoma. Int J Cancer 44: 795
9. Gotlieb WH, Abrams JS, Watson JM, Velu TJ, Berek JS, Martinez-Maza O (1992) Presence of interleukin 10 (IL-10) in the ascites of patients with ovarian and other intra-abdominal cancers. Cytokine 4: 385
10. Huang S, Ullrich SE, Bar-Eli M (1999) Regulation of tumor growth and metastasis by interleukin-10: the melanoma experience. J Interferon Cytokine Res 19: 697
11. Huleihel M, Maymon E, Piura B, Prinsloo I, Benharroch D, Yanai-Inbar I, Glezerman M (1997) Distinct patterns of expression of interleukin-1 alpha and beta by normal and cancerous human ovarian tissues. Eur Cytokine Netw 8: 179
12. Merogi AJ, Marrogi AJ, Ramesh R, Robinson WR, Fermin CD, Freeman SM (1997) Tumor-host interaction: analysis of

cytokines, growth factors, and tumor-infiltrating lymphocytes in ovarian carcinomas. Hum Pathol 28: 321
13. Mesiano S, Ferrara N, Jaffe RB (1998) Role of vascular endothelial growth factor in ovarian cancer: inhibition of ascites formation by immunoneutralization. Am J Pathol 153: 1249
14. Nash MA, Lenzi R, Edwards CL, Kavanagh JJ, Kudelka AP, Verschraegen CF, Platsoucas CD, Freedman RS (1998) Differential expression of cytokine transcripts in human epithelial ovarian carcinoma by solid tumour specimens, peritoneal exudate cells containing tumour, tumour-infiltrating lymphocyte (TIL)-derived T cell lines and established tumour cell lines. Clin Exp Immunol 112: 172
15. Naylor MS, Malik ST, Stamp GW, Jobling T, Balkwill FR (1990) In situ detection of tumour necrosis factor in human ovarian cancer specimens. Eur J Cancer 26: 1027
16. Naylor MS, Stamp GW, Foulkes WD, Eccles D, Balkwill FR (1993) Tumor necrosis factor and its receptors in human ovarian cancer. Potential role in disease progression. J Clin Invest 91: 2194
17. Obata NH, Tamakoshi K, Shibata K, Kikkawa F, Tomoda Y (1997) Effects of interleukin-6 on in vitro cell attachment, migration and invasion of human ovarian carcinoma. Anticancer Res 17: 337
18. Pisa P, Halapi E, Pisa EK, Gerdin E, Hising C, Bucht A, Gerdin B, Kiessling R (1992) Selective expression of interleukin 10, interferon gamma, and granulocyte-macrophage colony-stimulating factor in ovarian cancer biopsies. Proc Natl Acad Sci USA 89: 7708
19. Punnonen R, Teisala K, Kuoppala T, Bennett B, Punnonen J (1998) Cytokine production profiles in the peritoneal fluids of patients with malignant or benign gynecologic tumors. Cancer 83: 788
20. Ramakrishnan S, Xu FJ, Brandt SJ, Niedel JE, Bast RC Jr, Brown EL (1989) Constitutive production of macrophage colony-stimulating factor by human ovarian and breast cancer cell lines. J Clin Invest 83: 921
21. Sowter HM, Corps AN, Evans AL, Clark DE, Charnock-Jones DS, Smith SK (1997) Expression and localization of the vascular endothelial growth factor family in ovarian epithelial tumors. Lab Invest 77: 607
22. Watson JM, Sensintaffar JL, Berek JS, Martinez-Maza O (1990) Constitutive production of interleukin 6 by ovarian cancer cell lines and by primary ovarian tumor cultures. Cancer Res 50: 6959
23. Watson JM, Berek JS, Martinez-Maza O (1993) Growth inhibition of ovarian cancer cells induced by antisense IL-6 oligonucleotides. Gynecol Oncol 49: 8
24. Wu S, Boyer CM, Whitaker RS, Berchuck A, Wiener JR, Weinberg JB, Bast RC Jr (1993) Tumor necrosis factor alpha as an autocrine and paracrine growth factor for ovarian cancer: monokine induction of tumor cell proliferation and tumor necrosis factor alpha expression. Cancer Res 53: 1939