EUROPEAN JOURNAL OF CANCER 47 (2011) 1883–1889

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Cytokine analysis as a tool to understand tumour–host interaction in ovarian cancer

Refika Yigit [a,*], *Carl G. Figdor* [b], *Petra L.M. Zusterzeel* [a], *Jeanette M. Pots* [b], *Ruurd Torensma* [b], *Leon F.A.G. Massuger* [a]

[a] *Department of Obstetrics and Gynaecology (791), Radboud University Nijmegen Medical Centre, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands*

[b] *Department of Tumour Immunology, Nijmegen Centre for Molecular Life Sciences (278), Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands*

ARTICLE INFO

Article history:
Received 12 February 2011
Received in revised form 20 March 2011
Accepted 22 March 2011
Available online 20 April 2011

Keywords:
Ovarian cancer
Immune suppression
Cytokines
Ascites
IL-6

ABSTRACT

Epithelial ovarian cancer (EOC) is an immunogenic tumour and exploits many suppressive ways to escape immune eradication. EOC is known to spread primarily by tumour cell implantations in peritoneal cavity. Therefore, ascites may be an ideal fluid compartment to unravel the immune status of the peritoneal cavity.

We analysed the expression of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IFN-γ, TNF-α, TNF-β, TGF-β and CCL22 in ovarian cancer ascites, representing immune activating and suppressing cytokines.

We observed high expression of pro-inflammatory cytokines IL-6, IL-8 and immune suppressive cytokines IL-10, CCL22 and TGF-β in most samples whereas Th1 (IL-12p70, IFN-γ) and Th2 (IL-4, IL-5) cytokines were only detectable in 13% of the samples. TGF-β was only detected in latent form, questioning its immune suppressive role. CCL22 was in similar levels present in early stage compared to advanced stage tumours. At advanced stage, we observed a negative correlation with CCL22 levels and Th1/2 cytokine expression. We found a positive correlation between IL-6 concentration in ascites and residual disease after debulking. Additionally, IL-6 levels were remarkably higher at recurrence compared to primary advanced disease, which opens an opportunity for inhibition of IL-6 expression in the prevention of recurrence. Despite the heterogeneity of EOC and the complexity of cytokine functions, our results show that cytokine analysis in ascites may aid in understanding tumour–host interaction in EOC.

1. Introduction

Epithelial ovarian cancer (EOC) is the leading cause of death amongst gynaecological malignancies. EOC is an immunogenic tumour, indicated by the presence of tumour infiltrating lymphocytes (TILs) which correlate positively and strongly with patient survival.[10,13,31,39] The influx and activities of TILs are mediated by cytokines. The cytokine profile is, therefore, a reflection of the host immune status and may serve as a way to visualise the immune response. EOC is known to create an immune suppressive microenvironment in order to escape from immune elimination.[11,37] Diverting the immune response from Th1(IL-12p70, IFN-γ) towards Th2 (IL-4, IL-5) response is considered as one of the escape mechanisms of

* *Corresponding author:* Tel.: +31 24 3668760; fax: +31 24 3668597.
E-mail address: R.yigit@obgyn.umcn.nl (R. Yigit).
0959-8049/$ - see front matter
doi:10.1016/j.ejca.2011.03.026

EOC.[7] Th1-type immune response stimulates cellular immunity by activating macrophages and dendritic cells and recruiting CD8+ cytotoxic T-cells and NK-cells. Th2-type immune response inhibits cell-mediated immunity and favours the humoral immune response. The generation of either Th1-type or a Th2-type response depends on the balance between the cytokines.[2,35,40] Another mechanism reported to be implemented by EOC to escape from immune elimination is up regulation of immune inhibitory cytokines IL10, CCL22 and TGF-β. CCL22 attracts the immune suppressive regulatory T cells (Tregs) to the tumour microenvironment.[7,30] Both TGF-β and IL-10 have a role in the conversion of functional antigen presenting cells into dysfunctional ones, which in their turn stimulate expansion and differentiation of Tregs. Furthermore, IL-10 and IL-6 have been shown to induce B7-H4 expression on tumour macrophages which in their turn can lead to T-cell cycle arrest.[16–18]

IL-6 and IL-8 are pro-inflammatory cytokines that can cause uncontrolled proliferation in epithelial cancers.[8,21,23,27,34] Both IL-6 and IL-8 are produced by tumour cells as well. Over the past years, increasing evidence shows that both IL-6 and IL-8 are important factors in promoting the progression of EOC.[4,25,26]

EOC is known to spread primarily by tumour cell implantations in the peritoneal cavity. Therefore, ascites may be an ideal fluid compartment in which the interaction between the host's immune system and the tumour cells may be adequately reflected. The aim of our study was to map the cytokine expression profiles in ascites from EOC patients in order to characterise the type of immune responses and correlate the findings with clinical prognostic parameters.

2. Patients and methods

2.1. Sample collection

2.1.1. Ascites fluid

Patients suspected for EOC, undergoing either ascites puncture for diagnosis or primary surgery, patients with remaining ascites after neo-adjuvant chemotherapy undergoing intervention surgery and patients with recurrent EOC with ascites were enroled in the study. From these patients ascites was collected prospectively between January 2009 and September 2010 at the Radboud University Nijmegen Medical Centre. The collected samples were filtered and centrifuged at 1400 rpm for 10 minutes. The supernatant was then stored at −20 °C until analysis.

2.2. Cytokine measurements

Cytokine concentrations in ascites samples were determined using the Human Th1/Th2 11plex Flow Cytomix Multiplex (Bender Med Systems) according to the manufacturer's instructions. The assay evaluates the levels of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IFN-γ, TNF-α, and TNF-β. TGF-β concentration was measured using the Flow Cytomix Human TGF-β1 Simplex Kit and CCL22 was measured using Human MDC Enzyme-linked immunosorbent assay (ELISA) kit (R&D systems and Bender Med Systems) according to the manufacturer's instructions.

All values above 14 pg/ml were considered positive for TGF-β. All results on TGF-β concern values after acidification. Samples without acidification were assayed in parallel. The detection limit for CCL22 was 270 pg/ml, for IL-8 14 pg/ml, and for all the other cytokines 27 pg/ml, based on the standard curve run for each cytokine.

2.3. Statistical analysis

Results were expressed as medians and ranges. The Mann–Whitney test was used to test statistical significance. A p-value <0.05 was considered to be statistically significant.

3. Results

3.1. Characterisation of the study groups

We collected ascites prospectively from 63 patients. Nine patients were excluded from analysis because the final diagnosis showed no EOC. The remaining 54 patients were divided into five groups. The first group consisted of patients with borderline ovarian malignancy ($n = 4$); 3 of them had a mucinous tumour and 1 had a serous tumour. The second group concerned early stage EOC (FIGO stage I) ($n = 3$), 1 serous and 2 mucinous tumours. In the third group 35 patients with FIGO stage III–IV disease were included from whom ascites was obtained before any treatment. Twenty-eight out of the 35 patients completed their primary treatment (debulking surgery and chemotherapy), 2 patients only had surgery, 1 patient only chemotherapy and 4 patients had no treatment due to poor general condition. Fifteen out of the 28 patients who completed their treatment had a complete debulking (no macroscopic tumour), 13 had an incomplete debulking (macroscopic residual tumour). The characteristics of those patients are shown in Table 1. The fourth group ($n = 5$) included patients with primary advanced disease (FIGO stage IIIc–IV) from whom ascites was obtained after 3 courses of neo-adjuvant chemotherapy. In this group, 4 patients had serous adenocarcinoma and 1 patient had adenocarcinoma not otherwise specified. The last group consisted of 7 patients with recurrent disease; all of them had stage IIIc disease at primary treatment including 5 serous, 1 mixed epithelial and 1 borderline serous tumour. All cytokines were measured in all 54 samples except for CCL22 which was measured in 35 out of 54 samples (in 23 primary advanced disease, 3 early stage disease, 4 recurrence, 4 after neo-adjuvant chemotherapy and 1 borderline tumour) and TGF-β which was measured in 52 out of 54 samples.

3.2. Expression of pro-inflammatory cytokines in EOC ascites ($n = 54$)

High concentrations of IL-6 in ascites were observed in all but 2 patients with a median of 1762 pg/ml (range 0–107849). All median concentration values with range for IL-6 are shown in Table 2. The highest median concentration was observed in the group with recurrent disease (4090 pg/ml). The lowest median concentration was found in the group with early stage disease (719 pg/ml). In the group of neo-adjuvant chemotherapy the median IL-6 level (1082 pg/ml) was lower com-

Table 1 – Patient and disease characteristics of patients with primary advanced ovarian cancer.

Characteristics	No. of patients ($n = 35$)
Age median (range)	67 (33–95)
FIGO stage	
III	30
IV	5
Histological subtype	
Serous	27
Mucinous	2
Adenocarcinoma NOS	5
Sarcoma	1
Cytoreductive surgery	
Primary	6
Intervention	23
No surgery	6
Operability	
No residual tumour (complete debulking)	15
Residual tumour (incomplete debulking)	13
No surgery	7
CA125 after 3 courses <35 iU/L	
Yes	12
No	15
No chemo or not measured	8
CA125 after 6 courses <35 iU/L	
Yes	17
No	6
No chemo or not measured	12

Abbreviations: FIGO, Fédération Internationale de Gynécologie Obstétrique; NOS, not otherwise specified.

pared to the median level in untreated primary advanced disease (2226 pg/ml). The differences for IL-6 were statistically not significant.

IL-8 was detectable in 80% of the ascites samples (43/54). The highest median IL-8 concentration (2996 pg/ml) was observed in patients with borderline disease ($n = 4$). In patients with early stage EOC ($n = 3$), the median concentration was 276 pg/ml. The difference in IL-8 levels between both borderline and early stage EOC compared to IL-8 levels in advanced stage was statistically significant ($P < 0.05$). All median concentration values with range for IL-8 are shown in Table 2. Fig. 1 displays the different expression levels for IL-6 and IL-8 in the various groups.

The pro-inflammatory cytokines IL-1β, TNF-α and TNF-β were only expressed in a few samples. Detectable levels of IL-1β were observed in just 5 out of all 54 patients (9%); all had untreated primary advanced stage tumours. TNF-α was detectable in 13% of the patients, 5 in primary untreated advanced stage tumours and 2 in early stage tumours. TNF-β was not detectable in any of the samples.

3.3. *Expression of immune suppressive cytokines in EOC ascites (n = 54)*

IL-10 was expressed in 25 of all patients (46%) and in 22 out of 35 cases with untreated primary advanced cancer (63%). Median concentration levels with range are shown in Table 2 for the various groups. In advanced stage tumour ascites, IL-10 concentrations were significantly higher in untreated primary disease than in ascites after neo-adjuvant treatment ($p < 0.02$). In the latter group ($n = 5$), IL-10 was not detectable in any of the samples. IL-10 concentrations were significantly higher in untreated primary tumour ascites than in ascites obtained at recurrence ($p < 0.05$). In early stage borderline ($n = 4$) and malignant ($n = 3$) tumours, only in 2 patients IL-10 was detectable and at very low levels (<35 pg/ml). Taking all early stage tumours (borderline and malignant) together, the difference in IL-10 levels between early and advanced stage was significant ($p < 0.03$).

High TGF-β expression was observed in 98% (51/52) of the ascites. All samples were tested after acidification and the detected values concerned total TGF-β (active + latent form). The median concentration of latent TGF-β in all samples was 2330 pg/ml (range 0–9196). We found no significant difference in TGF-β levels between ascites from untreated primary disease versus ascites after neo-adjuvant treatment or recurrent disease. In early stage and borderline ovarian tumours, the median values are similar. Median concentration levels with range are shown in Table 2 for the various groups. In order to detect only active TGF-β, we evaluated the expression without acidification. No TGF-β in active form was detectable in any of the samples.

CCL22 was detected in 35 out of 54 patients with a median concentration of 501/ pg/ml (range 307–1336). In advanced stage tumours, the median CCL22 concentration was significantly higher at recurrence compared to primary disease ($p < 0.02$) (Table 2).

3.4. *Expression of T-helper Type 1 and Type 2 cytokines in EOC ascites (n = 54)*

IL-12p70 and IFN-γ (Th1 cytokines) were observed in 13% (7/54) of the patients. IL-2 also considered to be a Th1 cytokine was detectable in 7% (4/54) of the patients. Th2 cytokines IL-4 and IL-5 were detected in 13% (7/54) and 4% (2/54), respectively. Th1 and Th2 cytokines were only observed in ascites from untreated primary advanced tumours, and mostly co-expressed. The expression of Th1 (IL-12p70, IFN-γ) and Th2 (IL-4) cytokines was significantly associated with low CCL22 concentrations within untreated primary advanced stage tumours ($p < 0.05$) (median 372 pg/ml (317–457) versus 501 pg/ml (307–1336)]. Th1/2 cytokines were totally absent in ascites after chemotherapy and in ascites at recurrence.

3.5. *Prognostic value of cytokine profiles in EOC ascites*

We analysed the prognostic value of cytokines in the homogenous group of 35 patients with primary advanced EOC. We found no correlation between cytokine expression and age or histology in this group. We observed significantly lower IL-6 concentrations in patients with complete debulking ($n = 16$) (primary and intervention) [median 1169 pg/ml (0–4142)] compared to patients with incomplete debulking ($n = 14$) [median 5125 pg/ml (512–107849)] ($p < 0.05$). The median IL-6 level in the group of 5 patients who had no surgery was 3245 (296–11670). IL-8 values were considerably lower in

Table 2 – Median [range] cytokine concentrations in ascites from patients with advanced versus early stage, patients after neo-adjuvant chemotherapy and patients with recurrent disease.

	Borderline malignancy	Stage I	Stage III-IV	After neo-adjuvant Treatment	Recurrence
	Median [range] $N = 4$	Median [range] $N = 3$	Median [range] $N = 35$	Median [range] $N = 5$	Median [range] $N = 7$
IL-10	0 [0–33]	0 [0–28]	54 [0–4266]	ND	0 [0–100]
CCL22[a]	1275	568 [533–694]	461 [307–1336]	455 [334–743]	990 [555–1236]
TGF-β	1355 [1259–3237]	1201 [906–2041]	2543 [0–9196]	2561 [2019–6152]	2712 [706–7017]
IL-6	1575 [187–2892]	719 [189–9115]	2226 [0–107849]	1082 [0–1518]	4090 [74–9291]
IL-8	2997 [0–14697]	276 [199–1303]	101 [0–5995]	41 [0–318]	127 [19–323]
TNF-α	ND	34 [0–50]	0 [0–6498]	ND	ND
				ND	
IL-1β	ND	ND	0 [0–2863]		ND
	ND	ND		ND	
TNF-β			ND		
IL-12	ND	ND	0 [0–8664]	ND	ND
INF-γ	ND	ND	0 [0–7466]	ND	ND
IL-2	ND	ND	0 [0–5330]	ND	ND
IL-4	ND	ND	0 [0–4036]	ND	ND
IL-5	ND	ND	0 [0–1410]	ND	ND

Abbreviations: ND, Not Detectable; TGF-β, Transforming Growth Factor-β; IL, interleukin; IFN-γ, interferon-γ; TNF, tumour Necrosis Factor.
[a] Analysed in 35 ascites: untreated primary advanced disease ($n = 23$), early stage ($n = 3$), recurrence ($n = 4$), after neo-adjuvant chemo ($n = 4$), and borderline tumour ($n = 1$).

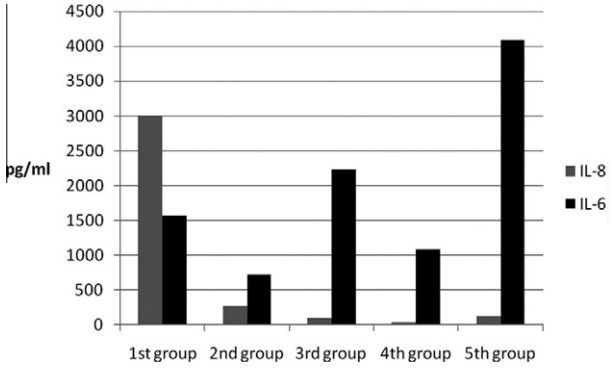

Fig. 1 – The median expression levels of IL-6 and IL-8 for the different groups. (1st group: Borderline tumour related ascites, 2nd group: Early stage ovarian cancer, 3rd group: Untreated advanced stage ovarian cancer, 4th group: Advanced stage ovarian cancer after 3 courses of chemotherapy, 5th group: Recurrent ovarian cancer.)

patients with complete debulking [(median 44 pg/ml (0–5995)] compared to patients with residual tumour [median 101 pg/ml (0–2016)], however, the difference was not significant. Looking at the group with intervention surgery ($n = 18/35$), we found both IL-6 and IL-8 significantly lower in patients with complete debulking compared to incomplete debulking. The 3 highest IL-6 concentrations (>10000 pg/ml) were found in patients with primary advanced disease with bulky lymph node metastasis on CT-scan. No significant correlation was found between IL-6 and IL-8 profiles and CA 125 level at diagnosis, CA125 response after 3 or 6 courses of chemotherapy.

In 7 out of the 35 patients with untreated primary advanced EOC IL-12p70 and IFN-γ were detected and in 6 of them IL-4 was also expressed. Out of those 7 patients only 2 had a normalised CA-125 (<35 iU/L) after 3 cycles of chemotherapy whereas 3 of them still had high CA 125 levels and 2 of them had had no treatment due to old age and general condition. Four out of the 7 patients had a normalised CA 125 after 6 courses of chemotherapy and 3 out of them are

still in remission, however, two patients just finished their treatment and for the 3rd one the follow-up is 9 months.

4. Discussion

In the present study, the cytokine production profiles in EOC ascites were measured. We found both IL-6 and IL-8 expressed in most ascites samples. Hypoxia and necrosis are common features of EOC and other solid tumours and may be the trigger for the release of those cytokines.[14,36]

Our data show remarkably higher IL-6 levels at advanced stage compared to early stage tumours. Furthermore, we found high IL-6 levels at primary advanced disease and considerably lower levels after neo-adjuvant chemotherapy. At recurrence, we found IL-6 levels remarkably higher than at primary disease and within the group with primary disease, we found the highest IL-6 levels in patients with bulky lymph node metastases on CT-scan. These results show a different IL-6 expression depending on the state and progression of the disease, suggesting a role for IL-6 in the progression of EOC, as has been reported for various other cancers.[1,8,21,23,27,33] Moreover, IL-6 strengthens the immune suppressive status by inducing B7-H4 on tumour associated macrophages. High expression of B7-H4 in macrophages is indicative of decreased overall survival.[18]

We found a significant correlation between low IL-6 concentrations and the achievement of complete debulking (no macroscopic residual tumour), which is considered as a predictor of survival. There are no earlier reports on correlation between IL-6 levels in ascites and operability. Penson et al. showed that in serum, increased concentrations of both IL-6 and IL-8 correlated with a poor initial response to chemotherapy and a poor final outcome in EOC patients.[28] The pattern of IL-6 expression as presented here opens new therapeutic approaches by blocking the effects of IL-6. This was described for other diseases like rheumatoid arthritis by using monoclonal antibodies towards IL-6 receptors.[12] Via an alternative signalling cascade where IL-6 binds to soluble IL-6 receptors, endothelial cell apoptosis by chemotherapeutics can be prevented.[20] Interference with this pathway may offer opportunities for anti IL-6/IL-6 receptor therapy in ovarian cancer.

IL-8 levels were significantly lower in patients with complete debulking compared to incomplete debulking, only when surgery was performed after neo-adjuvant chemotherapy. This might suggest a role for IL-8 in chemotherapy responsiveness.[28] We found no significant relationship between IL-8 levels and CA 125 response after 3 or 6 courses of chemotherapy.

IL-10 was expressed in the ascites of most untreated primary advanced cancer patients, but nearly absent in ascites at recurrence. In the latter group no Th1 (IL-12p70, IFN-γ) or Th2 (IL-4, IL-5) cytokines were detectable either. Additionally, CCL22 concentrations in recurrent ascites were significantly higher than in ascites at primary disease. This suggests a higher influx of Tregs at recurrence contributing to the creation of an immune suppressive environment, where Th1 and Th2 cytokines are totally absent. Low expression of CCL22 in the presence of Th1 and Th2 cytokines of untreated primary advanced tumour ascites supports this finding. The CCL22 concentrations after neo-adjuvant chemotherapy are comparable with the levels in ascites of primary untreated patients. The absence of Th1 and Th2 cytokines after chemotherapy treatment may be a transient suppressive effect of cytotoxic drugs on both humoral and cell mediated immunity. Additionally, we found no IL-10 and low IL-6 and IL-8 concentrations in ascites after chemotherapy as compared to ascites before treatment. Although the role of IL-10 in cancer has been studied extensively, the exact relationship with the tumour remains to be elucidated. Especially, the role of this cytokine in the immune response against cancer is still controversial. IL-10 is commonly regarded as cytokine that allows malignant cells to escape from immune surveillance. By contrast, other reports suggest that IL-10 might favour immune-mediated rejection of cancer.[24] Our findings question the immune suppressive role of IL-10 in ascites. We detected expression of Th1 (IFN-γ and IL-12) and Th2 (IL-4) cytokines only in the presence of high IL-10 concentrations. We believe that under certain circumstances this cytokine may not act as an immune suppressive molecule or even may support an effective immune attack against malignant cells.

Interestingly, CCL22 was already detectable in early stage tumours and there was no remarkable difference in its expression levels between early and advanced stage disease. This suggests that the creation of an immune suppressive environment with influx of Tregs already starts in early stage tumours. Curiel et al. also detected high levels of CCL22 in ascites and demonstrated that *in vivo* treatment with monoclonal antibody to CCL22 significantly decreased Treg cell migration into tumours.[5]

Our findings question the immune suppressive role of TGF-β in EOC ascites. We found no active TGF-β in any of the samples. Active TGF-β might be the lacking trigger leading to the epigenetic suppression of the TGF-β pathway in EOC as reported recently by Matsumura et al.[22] TGF-β has been described to have an inhibiting effect on the immune system in various studies.[15,19] Only a few studies have investigated the presence of TGF-β in ascites.[3,32] In those reports, no distinction was made between latent and active forms. In colon cancer, it has been shown that active TGF-β is more indicative of malignant progression, stage and survival than total TGF-β.[9]

Similar to Giuntoli et al.[7], we observed high IL-6 and IL-10 levels and reduced IL-2 levels in EOC ascites. Giuntoli et al. characterised both IL-6 and IL-10 as Th2 type cytokines, and reported skewing towards Th2 phenotype in ovarian cancer ascites. On the other hand, Punnonen et al. did not define IL-6 as a Th2 cytokine and reported no skewing towards Th2 type cytokines in the peritoneal fluids of patients with benign and malignant gynaecologic tumours.[29] This reflects the fact that the nomenclature of the type1/type2 cytokine model and the experimental methodology testing this model still continue to evolve. In this manuscript, we did not define IL-6 and IL-10 as Th2 type cytokines. We found the Th1 (IL-12, IFN-γ) and Th2 (IL-5 and IL-4) response cytokines only detectable in a limited number of patients and usually co-expressed. Based on these results, we found no evidence of skewing from Th1 towards Th2 phenotype in ascites as underlying mechanisms of EOC to escape the immune system. Furthermore, it has also been shown that IL-6 is able to shift the differentiation of CD4

cells towards Th2 in the presence of IL-4.[6] The limited presence of IL-4 in ovarian cancer related ascites suggests that Th1 differentiation is hampered.

Interestingly, we observed that patients with expression of Th1 cytokines IL-12 and IFN-γ had a poor CA 125 response after 3 courses of chemotherapy which is a predictor of survival. This is the opposite of what one would expect and may be because more aggressive malignancies elicit a better immune response but finally manage to escape from it. Another explanation could be the larger areas of necrosis in those aggressive tumours with a subsequent activation of the scavenging function of the macrophages.[38] Zeimet et al. reported earlier that high ascitic IL-12 levels are associated with disease progression in EOC.[38]

In conclusion, despite the heterogeneity of EOC and the complexity of cytokine functions, our results show that cytokine analysis in ascites may aid in understanding tumour–host interaction in EOC patients.

Conflict of interest statement

None declared.

REFERENCES

1. Barille S, Bataille R, Amiot M. The role of interleukin-6 and interleukin-6/interleukin-6 receptor-alpha complex in the pathogenesis of multiple myeloma. *Eur Cytokine Netw* 2000;**11**:546–51.
2. Belardelli F. Role of interferons and other cytokines in the regulation of the immune response. *APMIS* 1995;**103**:161–79.
3. Chen LL, Ye F, Lu WG, et al. Evaluation of immune inhibitory cytokine profiles in epithelial ovarian carcinoma. *J Obstet Gynaecol Res* 2009;**35**:212–8.
4. Curiel TJ, Cheng P, Mottram P, et al. Dendritic cell subsets differentially regulate angiogenesis in human ovarian cancer. *Cancer Res* 2004;**64**:5535–8.
5. Curiel TJ, Coukos G, Zou L, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med* 2004;**10**:942–9.
6. Diehl S, Rincon M. The two faces of IL-6 on Th1/Th2 differentiation. *Mol Immunol* 2002;**39**:531–6.
7. Giuntoli RL, Webb TJ, Zoso A, et al. Ovarian cancer-associated ascites demonstrates altered immune environment: implications for antitumor immunity. *Anticancer Res* 2009;**29**:2875–84.
8. Goldberg JE, Schwertfeger KL. Proinflammatory cytokines in breast cancer: mechanisms of action and potential targets for therapeutics. *Curr Drug Targets* 2010.
9. Hawinkels LJ, Verspaget HW, van der Reijden JJ, et al. Active TGF-beta1 correlates with myofibroblasts and malignancy in the colorectal adenoma–carcinoma sequence. *Cancer Sci* 2009;**100**:663–70.
10. Hayashi K, Yonamine K, Masuko-Hongo K, et al. Clonal expansion of T cells that are specific for autologous ovarian tumor among tumor-infiltrating T cells in humans. *Gynecol Oncol* 1999;**74**:86–92.
11. Kandalaft LE, Powell DJ, Jr., Singh N, et al. Immunotherapy for ovarian cancer: what's next? *J Clin Oncol* 2010.
12. Kishimoto T. IL-6: from its discovery to clinical applications. *Int Immunol* 2010;**22**:347–52.
13. Kooi S, Freedman RS, Rodriguez-Villanueva J, et al. Cytokine production by T-cell lines derived from tumor-infiltrating lymphocytes from patients with ovarian carcinoma: tumor-specific immune responses and inhibition of antigen-independent cytokine production by ovarian tumor cells. *Lymphokine Cytokine Res* 1993;**12**:429–37.
14. Koong AC, Denko NC, Hudson KM, et al. Candidate genes for the hypoxic tumor phenotype. *Cancer Res* 2000;**60**:883–7.
15. Kriegel MA, Li MO, Sanjabi S, et al. Transforming growth factor-beta: recent advances on its role in immune tolerance. *Curr Rheumatol Rep* 2006;**8**:138–44.
16. Kryczek I, Wei S, Zhu G, et al. Relationship between B7–H4, regulatory T cells, and patient outcome in human ovarian carcinoma. *Cancer Res* 2007;**67**:8900–5.
17. Kryczek I, Wei S, Zou L, et al. Cutting edge: induction of B7–H4 on APCs through IL-10: novel suppressive mode for regulatory T cells. *J Immunol* 2006;**177**:40–4.
18. Kryczek I, Zou L, Rodriguez P, et al. B7–H4 expression identifies a novel suppressive macrophage population in human ovarian carcinoma. *J Exp Med* 2006;**203**:871–81.
19. Li MO, Wan YY, Sanjabi S, et al. Transforming growth factor-beta regulation of immune responses. *Annu Rev Immunol* 2006;**24**:99–146.
20. Lo CW, Chen MW, Hsiao M, et al. IL-6 trans-signaling in formation and progression of malignant ascites in ovarian cancer. *Cancer Res* 2011;**71**:424–34.
21. Lou W, Ni Z, Dyer K, et al. Interleukin-6 induces prostate cancer cell growth accompanied by activation of stat3 signaling pathway. *Prostate* 2000;**42**:239–42.
22. Matsumura N, Huang Z, Mori S, et al. Epigenetic suppression of the TGF-beta pathway revealed by transcriptome profiling in ovarian cancer. *Genome Res* 2010.
23. Miki S, Iwano M, Miki Y, et al. Interleukin-6 (IL-6) functions as an *in vitro* autocrine growth factor in renal cell carcinomas. *FEBS Lett* 1989;**250**:607–10.
24. Mocellin S, Marincola FM, Young HA. Interleukin-10 and the immune response against cancer: a counterpoint. *J Leukoc Biol* 2005;**78**:1043–51.
25. Nilsson MB, Langley RR, Fidler IJ. Interleukin-6, secreted by human ovarian carcinoma cells, is a potent proangiogenic cytokine. *Cancer Res* 2005;**65**:10794–800.
26. Ning Y, Manegold PC, Hong YK, et al. Interleukin-8 is associated with proliferation, migration, angiogenesis and chemosensitivity *in vitro* and *in vivo* in colon cancer cell line models. *Int J Cancer* 2010.
27. Okamoto M, Lee C, Oyasu R. Interleukin-6 as a paracrine and autocrine growth factor in human prostatic carcinoma cells *in vitro*. *Cancer Res* 1997;**57**:141–6.
28. Penson RT, Kronish K, Duan Z, et al. Cytokines IL-1beta, IL-2, IL-6, IL-8, MCP-1, GM-CSF and TNF-alpha in patients with epithelial ovarian cancer and their relationship to treatment with paclitaxel. *Int J Gynecol Cancer* 2000;**10**: 33–41.
29. Punnonen R, Teisala K, Kuoppala T, et al. Cytokine production profiles in the peritoneal fluids of patients with malignant or benign gynecologic tumors. *Cancer* 1998;**83**:788–96.
30. Qin XJ, Shi HZ, Deng JM, et al. CCL22 recruits CD4-positive CD25-positive regulatory T cells into malignant pleural effusion. *Clin Cancer Res* 2009;**15**:2231–7.
31. Raspollini MR, Castiglione F, Rossi DD, et al. Tumour-infiltrating gamma/delta T-lymphocytes are correlated with a brief disease-free interval in advanced ovarian serous carcinoma. *Ann Oncol* 2005;**16**:590–6.
32. Santin AD, Bellone S, Ravaggi A, et al. Increased levels of interleukin-10 and transforming growth factor-beta in the plasma and ascitic fluid of patients with advanced ovarian cancer. *BJOG* 2001;**108**:804–8.

33. Smith PC, Hobisch A, Lin DL, et al. Interleukin-6 and prostate cancer progression. *Cytokine Growth Factor Rev* 2001;**12**:33–40.
34. Torres MP, Ponnusamy MP, Lakshmanan I, et al. Immunopathogenesis of ovarian cancer. *Minerva Med* 2009;**100**:385–400.
35. Trinchieri G. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Annu Rev Immunol* 1995;**13**:251–76.
36. Vaupel P, Kallinowski F, Okunieff P. Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: a review. *Cancer Res* 1989;**49**:6449–65.
37. Yigit R, Massuger LF, Figdor CG, et al. Ovarian cancer creates a suppressive microenvironment to escape immune elimination. *Gynecol Oncol* 2010;**117**:366–72.
38. Zeimet AG, Widschwendter M, Knabbe C, et al. Ascitic interleukin-12 is an independent prognostic factor in ovarian cancer. *J Clin Oncol* 1998;**16**:1861–8.
39. Zhang L, Conejo-Garcia JR, Katsaros D, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *N Engl J Med* 2003;**348**:203–13.
40. Zhu J, Paul WE. Peripheral CD4+ T-cell differentiation regulated by networks of cytokines and transcription factors. *Immunol Rev* 2010;**238**:247–62.