

RESEARCH ARTICLE

Expression of IL-10 in human normal and cancerous ovarian tissues and cells

Alex Rabinovich^{1,3*}, Liat Medina^{2,3*}, Benjamin Piura^{1,3}, Mahmoud Huleihel^{2,3}

¹ Unit of Gynecologic Oncology, Division of Obstetrics and Gynecology, Soroka University Medical Center, Beer-Sheva, Israel

² The Shraga Segal Department of Microbiology and Immunology

³ Faculty of Health Sciences, Ben-Gurion University, Beer-Sheva, Israel

Correspondence: Prof. M. Huleihel, Ph.D, The Shraga Segal Department of Microbiology and Immunology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel
<huleihel@bgu.ac.il>

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ABSTRACT. IL-10 is an 18-kd polypeptide that has been shown to be secreted by multiple cell types, including T and B cells, monocytes and some human tumors. However, which cell population is responsible for the elevated IL-10 levels in the serum and ascites of ovarian cancer patients, whether ovarian carcinoma cells produce IL-10, and how IL-10 influences the development and progression of ovarian carcinoma are issues that remain unclear. The aim of our study was to examine IL-10 production and secretion by ovarian carcinoma tissues and cells, and to determine its possible role in the cell and tumor micro-environment. The mean IL-10 protein levels expressed in normal ovarian tissue homogenates were significantly higher compared to cancerous ovarian tissue ($p = 0.002$). Yet, the IL-10 mRNA expression was significantly higher in cancerous ovarian tissues as compared to normal tissues ($p = 0.021$). The IL-10 receptor mRNA expression levels of the cancerous ovarian tissue homogenates were slightly, but not significantly, higher than the normal tissues. IL-10 immunostaining revealed that in both normal and cancerous ovarian tissues, IL-10 expression could be detected mainly in epithelial cells. In normal ovarian tissues, similar levels of IL-10R were demonstrated in epithelial and stromal cells. However, in cancerous ovarian tissues, epithelial cells expressed higher levels of IL-10R than the stroma. Primary normal and cancerous ovarian cell cultures and SKOV-3 cells secreted similar amounts of IL-10 after 24 hours of incubation. Our results suggest that epithelial cells are the main source of IL-10 in the ovary. Nevertheless, the target cells for IL-10 are different in normal and cancerous ovarian cells. Thus, IL-10 and its receptor could be involved in the pathogenesis of ovarian carcinoma.

Keywords: IL-10, IL-10 receptor, IL-10 mRNA, SKOV-3, ovarian carcinoma, primary cell line

Ovarian cancer is the most frequent cause of death among gynecological malignancies, and the fifth leading cause of death from all malignancies in women. Despite standard treatment, cytoreductive surgery followed by platinum/paclitaxel-based chemotherapy, the overall survival for ovarian cancer is only 35% [1]. Ovarian carcinoma disseminates by exfoliation of cells from the primary tumor site into the peritoneal cavity. Even in an advanced stage disease, the tumor cells usually remain confined to the abdominal cavity, either as free-floating cellular aggregates or as peritoneal implants [2].

It has been suggested that the growth and progression of ovarian carcinoma might be related to a local phenomenon of immunosuppression [3] induced by immunoinhibitory cytokines such as IL-10, which can provide a status of immune privilege at the tumor site, allowing the developing tumor to escape immune surveillance [4].

IL-10, originally called cytokine synthesis inhibitory factor, is an 18-kd polypeptide that lacks detectable glycosylation

and is expressed as a noncovalent homodimer of two, interpenetrating polypeptide chains [5]. The IL-10 receptor is composed of at least two subunits that are members of the interferon receptor family. Signaling events mediated through the IL-10 receptor, use a Jak/STAT pathway. IL-10 binding to its receptor activates the Jak1 and Tyk2 kinases and leads to the activation of STAT1, STAT3, and in nonmacrophage cells, STAT 5.

Since its original description, IL-10 has been shown to be secreted by multiple cell types, including T cells, monocytes, activated B cells, and some human tumors [3]. However, which cell population is responsible for the elevated IL-10 levels in the serum and ascites of ovarian cancer patients, whether ovarian carcinoma cells produce IL-10, and how IL-10 influences the development and progression of ovarian carcinoma [6] remain unclear.

In our previous studies, we have shown that both normal and cancerous ovarian tissues produce IL-1 α , IL-1 β , IL-6 and TNF- α . However, cancerous tissues expressed higher levels of these cytokines compared to normal ovarian tissues. IL-6 produced by ovarian cancer cells was

* These authors contributed equally to this work.

shown to increase the secretion of MMP-9, but did not affect MMP-2 secretion [7-9].

The aim of our study was to examine the levels and cellular origin of IL-10 in normal and cancerous ovarian tissues and cells.

DONORS AND METHODS

Reagents

Phosphate-buffered saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM), Essential Medium- α (MEM- α), FCS (Fetal Calf Serum), L-glutamine, antibiotics (a combination of streptomycin and penicillin), and trypsin-EDTA were all purchased from Biological Industries (Beit-Haemek, Israel). Collagenase and hyaluronidase were obtained from Sigma (St. Louis, MO, USA). BSA (bovine serum albumin) and tween-20 were purchased from ICN Biomedicals, Inc. (Aurora, OH, USA).

Antibodies

The mouse anti-human IL-10 antibodies set for ELISA (primary antibodies, cat # M-010-E; secondary antibodies, cat # M-011-B) were purchased from ENDOGEN (Woburn, MA, USA), and the recombinant human IL-10 (cat # 2226-01) was purchased from Genzyme Diagnostics (Cambridge, MA, USA). The polyclonal goat anti-human IL-10 (cat # AF-217-NA, R&D Systems, Inc., Minneapolis, MN, USA) and the polyclonal rabbit anti-mouse IL-10R (cat # sc-25478, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) were used for immunohistochemical stainings.

Origin and handling of ovarian tissues

Fresh ovarian tissues were collected under sterile conditions from the operating room of the Department of Obstetrics and Gynecology, Soroka Medical Center, Beer-Sheva, Israel. The Institutional Review Board approved this study before its initiation. Epithelial ovarian tissue samples were obtained from 25 women with ovarian carcinoma at various stages of the disease (18 serous papillary adenocarcinoma, 3 endometrioid, 2 undifferentiated and 2 clear cell), and normal ovarian tissue from 31 women who underwent surgery for benign or malignant gynecological diseases other than ovarian carcinoma. The histopathological diagnosis was confirmed in formalin-fixed, paraffin-embedded tissues. Fresh tissue samples were immediately washed with cold PBS in order to eliminate residual blood cells. Approximately one-two grams of each fresh ovarian tissue was used for the establishment of primary cell line. The remaining tissue was stored at -70°C and was later used for the evaluation of protein and mRNA levels.

Establishment of primary normal and cancerous ovarian cell lines

All procedures and cell manipulations were carried out under sterile conditions. Primary ovarian cancerous cells were established as described by Huleihel *et al.* [7].

Briefly, ovarian tissue was minced with a scalpel into small pieces and dissociated by stirring with collagenase (0.05% w/v) and hyaluronidase (0.01% w/v) for two-three hours at 37°C , with stirring until complete dissociation. The cell suspension was filtered through sterile gauze and centrifuged at 1,200 RPM for 10 min. The cells were suspended in growth medium [DMEM containing 10% FCS, L-glutamine (2 mM) and an antibiotic combination of streptomycin (0.1 mg/mL) and penicillin (100 U/mL)], and cultured in 25 cm² flasks at 37°C in a humidified air atmosphere containing 5% CO₂. After 7-10 days, monolayers were formed and the cultures were trypsinized and passed to fresh flasks (first passage). At each passage, cells were cultured in fresh bottles to remove contaminating macrophages. Assays were performed following up to six passages.

SKOV-3 cells (ovarian adenocarcinoma cell line) were cultured in MEM- α containing 5% FCS, L-glutamine (2 mM) and an antibiotic combination of streptomycin 0.1 mg/mL and penicillin 100 U/mL.

Immunohistochemical staining

Immunoperoxidase assay was carried out on paraffin-embedded, normal or cancerous ovarian sections from archival material of the Department of Pathology, Soroka University Medical Center, using the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA, USA). Briefly, four micron-thick sections from formalin-fixed, paraffin-embedded tissue blocks were mounted on SuperFrost Plus slides, dried at 37°C for 48 hours and stored at room temperature. Before the primary antibodies were applied, the slides were deparaffinized in xylene, rehydrated in graded alcohol and warmed twice in 6M UREA for five minutes. Blocking of the nonspecific background staining was achieved with PBS containing 2.5% of either goat or rabbit serum. This solution was also used to dilute the primary antibodies. The biotinylated antibody and the streptavidin-peroxidase conjugate (Avidin-Biotin Complex [ABC]) were applied according to the supplier's instructions (Vector Laboratories). Blocking of the endogenous peroxidase was done with 0.01% H₂O₂ in 80% methanol for 25 min before the ABC was applied. Development was performed with 3,3' diaminobenzidine (DAB) and Mayer's haematoxylin was used for counterstaining. For the negative control, we used the blocking solution instead of primary antibodies.

Preparation of conditioned medium from primary ovarian cell cultures and SKOV-3 cells

SKOV-3 cells (4×10^5 cells/mL/well) were seeded in 24-well plates in MEM- α . Primary cells obtained from normal or cancerous ovarian tissues (5×10^4 cells/mL/well) were seeded in 24-well plates in DMEM and FCS 2.5%. After 24-96 hours of incubation, conditioned medium samples (supernatants) were collected and stored at -20°C until examination of IL-10 levels using a specific ELISA kit. SKOV-3 cells were trypsinized and collected into a fresh tube for mRNA extraction.

Preparation of normal and cancerous ovarian homogenates

Normal or cancerous ovarian tissues were homogenized in one ml of cold saline in ice. At the end of the homogenization process, the mixture was centrifuged at 13,000 RPM for 15 min, the supernatant was collected and stored at -70°C . Total protein was examined using Bio-Rad reagent (BIO-RAD, Hercules, CA, USA). IL-10 levels were examined using a specific ELISA kit.

Extraction of RNA and semi-quantitative RT-PCR analysis

Total RNA was extracted from ovarian tissues using the EZ-RNA Reagent protocol (Biological Industries) according to the manufacturer's instructions. mRNA was extracted from SKOV-3 cells using the Dynabeads mRNA Direct Kit (Dynal Biotech, Oslo, Norway), according to the manufacturer's instructions.

First-strand complementary DNAs (cDNAs) were synthesized from 2.5 μg total RNA (ovarian tissues) or 50 ng mRNA (SKOV-3) with 5 x RT buffer, 2 μM oligo (dT) primers (Sigma), 0.5 mM dNTP mix (ORNAT, Rehovot, Israel), 10 U Rnase Out (Invitrogen, Carlsbad, CA, USA) and 200 U M-MLV (Invitrogen) in a final volume of 20 μL . The reverse transcriptase (RT) reaction was performed for one h at 37°C and stopped for 10 min at 65°C . The volume of 20 μL was subsequently made up to 60 μL with DEPC-(Sigma) treated water. Negative controls for the reverse transcriptase reaction (RT-) contained DEPC-treated water instead of RNA.

The semi-quantitative RT-PCR was performed by calculating the ratio between the intensity of each band (obtained by densitometry, using TINA 2.0 software) and the intensity of the β -actin band of the same cDNA sample. In brief, 2.5 μL of cDNA were amplified by PCR in a final volume of 25 μL containing 10 x PCR buffer, 0.2 mM dNTP mix, 2 mM Mg^{++} , 0.25 U DNA polymerase (BIOLINE- London, UK) and 0.5 μM of the following primers: forward - 5' gacgaggccagacgaagag 3', reverse- 5' gggccggactcgtcatactc 3' for the human β -actin (935 bp); forward - 5' cctctgatacctcaacccccca 3', reverse- 5' aatccctccgagacactgga 3' for hIL-10 (393 bp); and forward - 5' acctttaggggaccctccat 3', reverse- 5' ccagaggaggttcagtacca 3' for hIL-10R (416 bp). Negative controls for the polymerase chain reaction contained DEPC-treated water instead of cDNA (cDNA-). The PCR reactions were carried out on a T personal Thermal Cycler (Biometra, Goettingen, Germany). The β -actin cDNA was amplified at 63°C for 30 cycles (homogenates of primary ovarian tissues) or 25 cycles (SKOV-3), the IL-10 was amplified at 62°C for 30 cycles (homogenates of primary ovarian tissues) or 35 cycles (SKOV-3), and the IL-10R was amplified at 59°C for 30 cycles. Twenty microliters of each PCR product were run on a 2% agarose gel, containing ethidium bromide, and then photographed under UV light.

Evaluation of IL-10 levels

Levels of IL-10 were recorded by reference to a standard curve obtained with human recombinant IL-10. The

range of the standard curve was 5-2,500 pg/mL, sensitivity was < 10 pg/mL. ELISA was performed by an overnight incubation of the first antibody (0.2 $\mu\text{g}/\text{ml}$) in 96-well ELISA plates, followed by washing (PBS with 0.05% tween-20) and addition of blocking buffer (PBS with 10% FCS) for two hours at 37°C . Thereafter, recombinant cytokine and samples were added. After one hour of incubation at 37°C , plates were washed and the second antibody (0.6 $\mu\text{g}/\text{mL}$) was added for one hour of incubation at 37°C . After washing, Streptavidin HRP (Peroxidase-conjugated Streptavidin, Jackson Immuno-Research, West Grove, PA, USA) was added for 15 minutes at 37°C . After another washing, TMB (Tetramethylbenzidine, Dako, Carpinteria, CA, USA) was added for 10 minutes and the reaction was stopped by adding 2N H_2SO_4 (sulphuric acid, Gadot, Natania, Israel). Absorbance was read using an ELISA reader at 450 nm.

Statistical analysis

Samples were examined in triplicate in each experiment. Each experiment was repeated at least three times. Results are expressed as the mean \pm SEM.

To evaluate the statistical significance of the results, Student's t-test was performed. A p-value < 0.05 was considered as significant.

RESULTS

IL-10 protein levels in normal and cancerous ovarian tissue homogenates

ELISA examination revealed that IL-10 protein was expressed in all 31 normal and 25 cancerous ovarian tissue homogenates (figure 1). The mean value of IL-10 expressed by the normal ovaries was significantly higher compared to cancerous ovarian samples; 0.019 ± 0.0023 pg/ μg protein and 0.0098 ± 0.001 pg/ μg protein, respectively ($p = 0.002$).

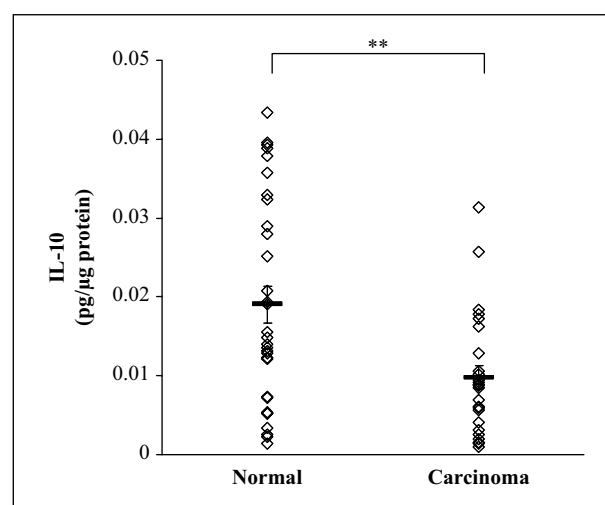


Figure 1

IL-10 levels in normal and cancerous ovarian tissue homogenates. Normal ($n = 31$) and cancerous ($n = 25$) ovarian samples were homogenized. IL-10 protein levels were evaluated using a specific ELISA kit. IL-10 levels are expressed as pg/ μg protein; each point represents one sample. Horizontal and vertical lines indicate mean \pm SEM. Statistical significance according to t-test: ** $p = 0.002$.

IL-10 mRNA and IL-10R mRNA levels in normal and cancerous ovarian homogenates

Cancerous ovarian tissues expressed significantly higher IL-10 mRNA levels than normal ovarian tissues, with a mean value of $79.7 \pm 15.1\%$ compared to $37.6 \pm 7.17\%$, respectively ($p = 0.021$) (figure 2A).

The IL-10R mRNA expression levels of the cancerous ovarian tissues were slightly, but not significantly, higher than the normal samples; $92.3 \pm 13.3\%$ compared to $68.4 \pm 8.8\%$, respectively (figure 2B). Four representative samples from normal (figure 2C, 1-4) and carcinoma (figure 2C, 5-8) ovarian tissues that express IL-10 and IL-10R differently are presented.

The percentage of the IL-10 or IL-10R mRNA-positive samples in the cancerous ovarian tissues was higher than in normal tissues; 93.3% compared to 81.8%, respectively, for the IL-10 mRNA and 100% compared to 92.3%, respectively, for IL-10R mRNA (table 1).

IL-10 and IL-10R immunostaining in normal and cancerous ovarian tissues

Both normal and cancerous (serous papillary and mucinous) ovarian tissues were positively stained when anti-human IL-10 antibodies were applied. IL-10 expression could be detected mainly in epithelial cells, while stromal cells expressed basal levels of IL-10 (figure 3A-C). The pattern of IL-10 expression in endometrioid type cancerous tissue was different; similar levels of IL-10 expression could be seen in both epithelial and stromal cells (figure 3D).

Normal ovarian tissues expressed similar levels of IL-10R in both epithelial and stromal cells (figure 3F). Cancerous ovarian tissues showed differences in the expression pattern of IL-10R. In the serous papillary and mucinous tissues, the cancerous epithelial cells expressed higher levels of IL-10R than the stroma (figure 3G and H, respectively). In contrast,

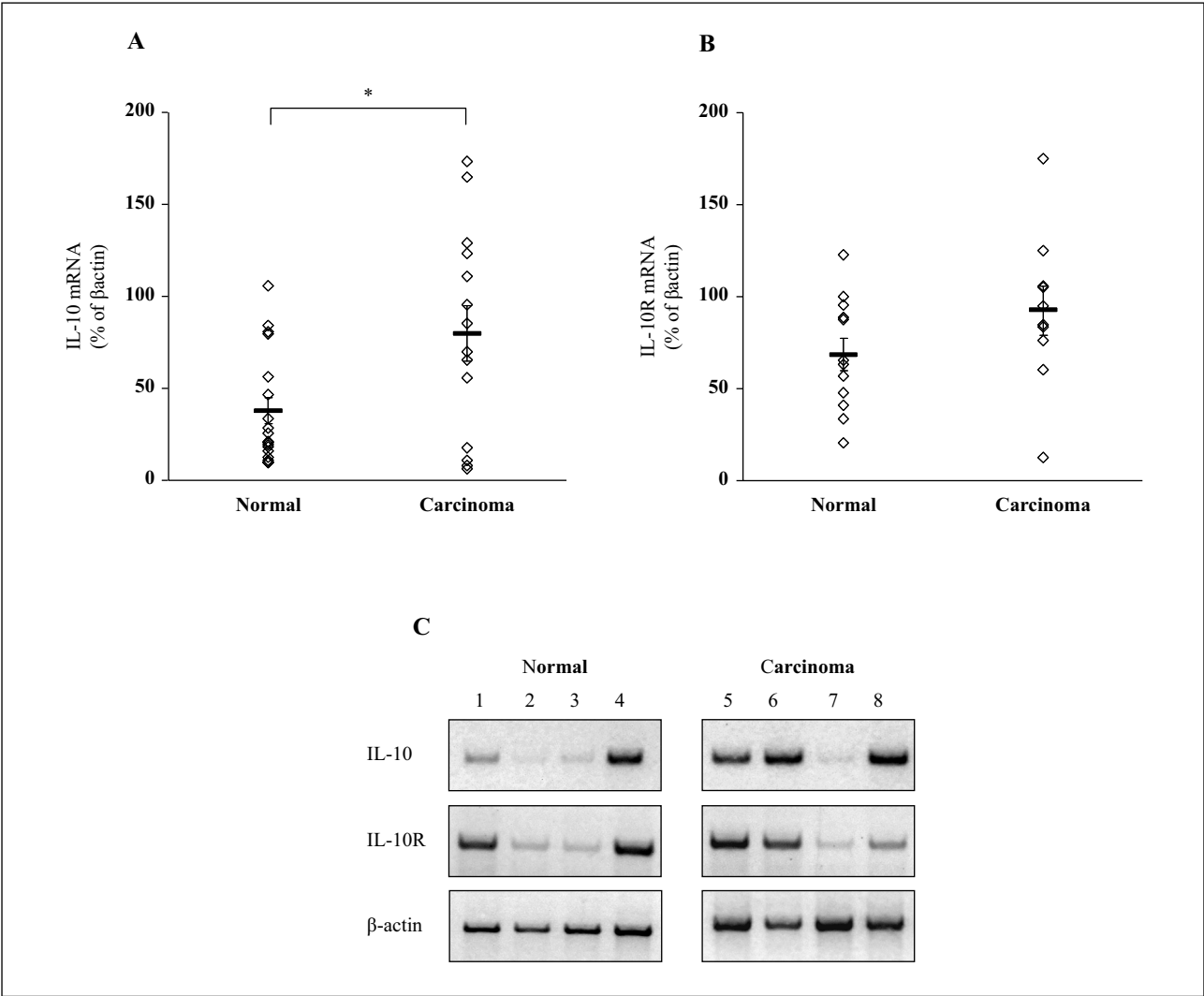


Figure 2

IL-10 mRNA and IL-10R mRNA expression levels in normal and cancerous ovarian homogenates. Normal and cancerous ovarian samples were used. The expression levels of IL-10 mRNA (A $n = 22$ and $n = 15$, respectively) and IL-10R mRNA (B $n = 13$ and $n = 10$, respectively) were evaluated by semi-quantitative RT-PCR using specific primers, and the PCR products were separated on 2% agarose gel containing ethidium bromide. Quantitative evaluation of the positive PCR products was performed by densitometry. Each point represents the ratio between IL-10 mRNA and β-actin bands or IL-10R mRNA and β-actin bands of the same sample. Horizontal and vertical lines indicate MEAN \pm SEM. C) representative samples of normal (1-4) and cancerous (5-8) ovarian tissues are presented.

* Indicates statistical significance according to t-test: $p = 0.021$.

Table 1
IL-10 and IL-10-receptor (IL-10-R) mRNA expression in normal and cancerous ovarian tissues

mRNA expression	Normal			Carcinoma		
	N (total)	n (total)	Positive samples (%)	N (total)	n (total)	Positive samples (%)
IL-10	22	18	81.8	15	14	93.3
IL-10R	13	12	92.3	10	10	100

Normal and cancerous ovarian samples were examined for IL-10 mRNA (n = 22 and n = 15, respectively) and IL-10-R mRNA (n = 13 and n = 10, respectively) levels by semi-quantitative RT-PCR. The sum of the total samples tested (N), the sum of the positive expression samples (n), and the percentage of the positive samples of the total samples examined (%) are presented.

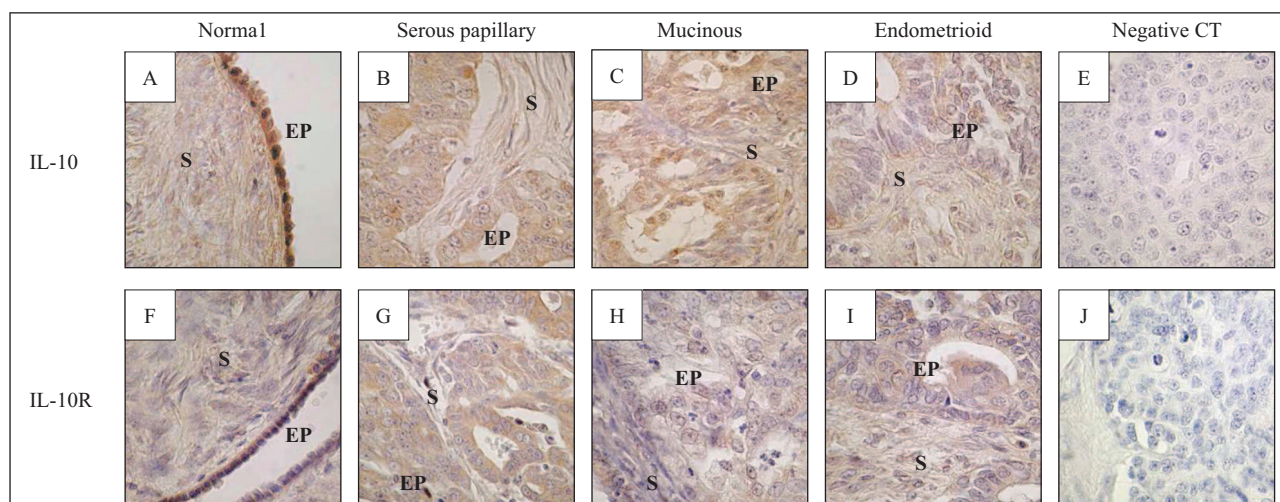


Figure 3

Immunohistochemical staining for IL-10 and IL-10-R in normal and cancerous ovarian tissues. Immunohistochemical staining of normal (A and F, respectively), and different types of cancerous ovarian tissues: serous papillary (B, G), endometrioid (C, H) and mucinous (D, I) are presented. Ovarian cancerous tissues were used as negative control for IL-10 (E) and IL-10R (J). EP: epithelial cells; S: stroma. Magnification- x 600.

the endometrioid type expressed similar levels of IL-10R in both epithelial and stromal cancerous cells (figure 3I).

IL-10 and IL-10R expression patterns were similar in cancerous ovarian tissues. Both were strongly expressed in the epithelial cells and weakly expressed in the stroma of papillary serous and mucinous carcinomas. Both were expressed to the same level in the epithelial cells and the stroma of endometrioid carcinoma. In contrast, differences could be seen in the expression patterns of IL-10 and IL-10R in normal ovarian tissues. While the IL-10 expression patterns were similar to papillary serous and mucinous carcinomas, *i.e.* a dominant expression in the epithelial cells, IL-10R expression was similar in both stromal and epithelial cells.

IL-10 protein levels in supernatants of primary cell cultures from normal or cancerous ovarian tissues and SKOV-3 cells

Ovarian primary cell cultures (obtained from fresh normal and cancerous ovarian tissues), and SKOV-3 cell cultures constitutively secreted IL-10 as demonstrated by ELISA (figure 4). Similar amounts of IL-10 were secreted after 24 hours of incubation of normal ovarian primary cell cultures (40.5 ± 10.8 pg/mL), ovarian carcinoma primary cell culture (60.9 ± 11.5 pg/mL), and SKOV-3 cell cultures (37.1 ± 17.7 pg/mL).

DISCUSSION

More than a decade after its original description, the source of IL-10 in ovarian carcinoma, and its role in the cell and tumor micro-environment remain unresolved. Our results show that both normal and cancerous ovarian tissues produce and secrete IL-10. In our study, IL-10 protein levels were significantly higher in normal ovarian tissues compared to cancerous tissues. In normal ovarian cells, IL-10 probably maintains its original autocrine role as a “cytokine synthesis inhibitory factor” by inhibiting the secretion of numerous other cytokines such as IL-1, IL-6, TNF α , TNF γ , MMPs, and other growth factors essential for tumor progression and development [10-14]. In cancer cells, we expect to find mechanisms that regulate IL-10 levels; on one hand they prevent the inhibition of cytokines and MMPs secretion (thus increase their secretion), and on the other hand they provide protection to cancer cells from the immune system (mainly cell-mediated, T helper-like immune responses).

IL-10 concentrations in peritoneal fluid and serum of ovarian cancer patients were found to be higher than in patients with benign ovarian diseases [15, 16]. In ovarian cancer patients, the IL-10 concentration in ascites is generally higher than in the corresponding serum [16, 17]. This observation may indicate that IL-10 synthesis

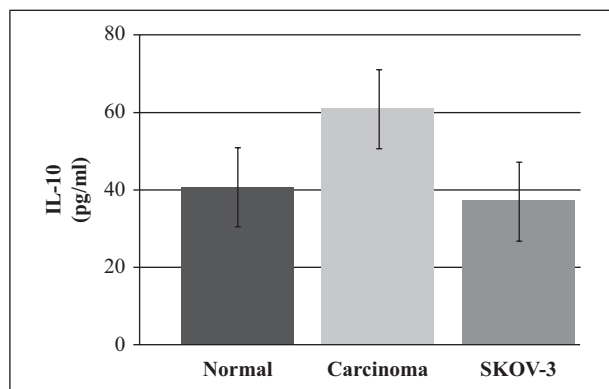


Figure 4

IL-10 protein levels in supernatants of primary cell cultures from normal or cancerous ovarian tissues and SKOV-3 cells. Ovarian primary cell cultures, obtained from normal ($n = 6$, 5×10^4 cells/mL), cancerous ($n = 3$, 5×10^4 cells/mL) tissues, and SKOV-3 cells (4×10^5 cells/mL), were incubated for 24 hours. Levels of IL-10 were evaluated in supernatants of these cultures using a specific ELISA kit. Levels are expressed as pg/ml and presented as mean \pm SEM.

occurs mostly in the tumor micro-environment. Tumor cells may produce IL-10 by themselves [18] or induce IL-10 secretion by other cell populations, such as macrophages or tumor infiltrating lymphocytes [3]. Our results corroborate other studies [6, 19] by proving that ovarian cancer cells secrete IL-10. However, our results contradict those studies by showing lower levels of IL-10 in ovarian cancerous tissues compared to normal ovarian tissues, or similar levels of IL-10 secreted from normal and cancerous ovarian cell cultures.

Contrary to others [20-22], we have shown that IL-10 mRNA can be demonstrated in both normal and cancerous ovarian tissues. In contrast to the results for IL-10 levels, the expression of IL-10 mRNA was significantly higher in ovarian cancer cells. The dissimilarity in the final IL-10 levels in normal and cancerous cells is probably the result of a different type of control by IL-10 expression in normal and cancerous ovarian tissues/cells of either protein translation or mRNA transcription and/or stability. For instance, Brewer *et al.* [23] have shown an extended half-life of IL-10 mRNA in melanoma cells due to reduced levels of AUF1 binding protein at the 3'-untranslated region of IL-10 mRNA.

To the best of our knowledge, this is the first report of either qualitative or quantitative results involving the IL-10 receptor in ovarian carcinoma. Our results indicate that both normal and cancerous tissues express IL-10R, but IL-10R mRNA expression levels in cancerous ovarian tissues were slightly, although not significantly, higher than in the normal samples. This may indicate a possibly greater effect of IL-10 on ovarian cancerous tissues compared to normal tissues.

Immunohistochemical staining revealed the cell origin of IL-10 and IL-10R. In normal ovarian cells, dominant IL-10 expression was demonstrated in epithelial cells. However, similar levels of IL-10R expression were found in epithelial and stromal cells. In mucinous and serous papillary ovarian carcinoma cells, dominant IL-10 and IL-10R expression in epithelial cells was shown. In endometrioid-type ovarian carcinoma cells,

similar levels of IL-10 and IL-10R expression were shown in both epithelial and stromal cells.

These results suggest that epithelial cells are the main source of IL-10 in the ovary. However, the target cells for IL-10 are different in normal and cancerous ovarian cells. It is reasonable to assume that in mucinous and serous papillary ovarian carcinoma cells, IL-10 affects epithelial cells in an autocrine/paracrine manner. In normal ovarian cells and endometrioid-type ovarian carcinoma cells, IL-10 binds to receptors in both stromal and epithelial cells. Thus, different regulatory mechanisms control the effect of IL-10 in the cell micro-environment of normal and cancerous ovarian cells.

Contrary to others [3, 12, 17], we detected IL-10 in supernatants of primary cell cultures of normal and cancerous ovarian cells, as well as SKOV-3 cells. IL-10 concentrations were almost identical in these cells. These results show that epithelial cells of ovarian origin could be, at least, part of the source of IL-10 in the tissue and serum.

Ovarian carcinoma progresses by exfoliation, and remains confined to the abdominal cavity. The control of peritoneal dissemination is crucial to improve the prognosis of ovarian cancer patients [24]. IL-10 has been shown to suppress the secretion of TNF- α , IL-2 and IFN- γ , down-regulate MHC class I expression on tumor cells, interfere with the accessory cell function of antigen-presenting cells [25-27], and inhibit antigen presentation by macrophages and Langerhans cells [28-30], thus allowing tumor cells to escape immune surveillance [31]. IL-10 produced by ovarian carcinoma tissues and cells could be involved in the pathogenesis of this tumor.

IL-10, IL-4 and IL-6 are examples of cytokines that induce a state of immune-privilege that prevents effective anti-tumor reactivity. Ovarian epithelial carcinoma has been proposed to be a cytokine-propelled disease [32]. Therefore, single anti-cytokine therapy does not appear to be effective. Continued analysis of the relationship between cytokines and ovarian carcinoma might help us to design a novel, immune therapy for this tenacious disease.

Disclosure. None of the authors has any conflict of interest to disclose.

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