High Serum and Ascitic Soluble Interleukin-2 Receptor α Levels in Advanced Epithelial Ovarian Cancer

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This study was undertaken to determine if advanced epithelial ovarian cancer was associated with increased serum and ascitic levels of soluble interleukin-2 receptor α (sIL-2RA). Serum and ascitic fluid samples from 23 ovarian cancer patients were analyzed for sIL-2Ra using an enzyme-linked immunosorbent assay and compared with the serum and peritoneal levels in 18 normal females. The samples were analyzed for CA-125 levels using a radioimmunossay and the total protein was also measured. Normal individuals had low serum levels of sIL-2Ra (367.5 ± 44.6 U/mL) with similar levels of sIL-2Ra in the normal peritoneal fluid (438.6 ± 48.8 U/mL). In contrast, the serum and ascitic fluid levels in ovarian cancer patients were significantly higher (746.7 ± 82.9 U/mL, P = .0006; 2,656.7 ± 373.7 U/mL, P = .00002, respectively). The results for sIL-2Ra were also significant when the levels were expressed per milligram of total protein. More importantly, in almost every ovarian cancer patient the ascitic sIL-2Ra level far exceeded the serum level, a pattern also observed for CA-125. There was no correlation between the serum and ascitic sIL-2Ra levels, or between the serum and ascitic CA-125 levels. Although the serum levels of sIL-2Ra and CA-125 were elevated in the same patient, overall there was no correlation between the serum sIL-2Ra and serum CA-125 levels, either when the levels were expressed in absolute units or per milligram of total protein. Similarly, there was no correlation between sIL-2Ra and CA-125 levels in individual ascitic samples. While CA-125 levels may reflect an independent index of tumor burden, these results suggest that selective accumulation of sIL-2Ra in the ascites may be one of the factors associated with the known nonresponsiveness of the infiltrating lymphocytes against ovarian carcinoma cells.

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INTERLEUKIN-2 (IL-2) is a multipotential cytokine involved in the mediation of a wide range of immunologic functions, particularly in T cells and natural killer (NK) cells. Three receptor types for IL-2 have been identified: the low-affinity receptor (p55 or IL-2Ra), the intermediate-affinity receptor (p75 or IL-2Rβ), and the high-affinity receptor (a noncovalent complex of p55 and p75). The IL-2Ra is expressed only when the cell has been activated and then only in association with IL-2Rβ. The p55 receptor, which was initially identified on activated T cells, has now been identified on activated NK cells, activated B cells, monocytes, and eosinophils. More recently, a third subunit of the IL-2 receptor, designated IL-2Rγ, has been identified and the gene cloned. Activated T cells secrete IL-2 and express cell-surface IL-2Ra, but also shed IL-2Ra (soluble IL-2Ra or sIL-2Ra). This soluble receptor is a truncated form of the p55 receptor with a molecular weight of 45 to 50 kD and an IL-2 binding affinity similar to that of the membrane-bound p55. It has been proposed that sIL-2Ra is a marker for T-cell activation and possibly could negatively modulate the local immune response. Increased levels of sIL-2Ra are found in many leukemic malignancies and in autoimmune diseases characterized by excessive activation of the immune system. However, although increased serum levels of sIL-2Ra are found in different solid malignancies, including lung, breast, colon, and stomach cancer, no study has reported specifically on levels in ovarian cancer. In light of our previous findings, and those of others, that the lymphocytes infiltrating the peritoneal cavity of ovarian cancer patients have no initial reactivity against autologous tumor cells and that IL-2 could activate tumoricidal function in these lymphocytes in vitro, we undertook this study to determine the serum and ascitic levels of sIL-2Ra in patients with advanced (FIGO stage III and IV) epithelial ovarian cancer.

MATERIALS AND METHODS

Blood samples. Eighteen normal women and 23 patients with advanced (International Federation of Gynecology and Obstetrics [FIGO] stage III or IV) epithelial ovarian cancer had preoperative peripheral venous blood samples (clotted) drawn. The serum was removed and centrifuged at 400g for 10 minutes to remove contaminating cells. The supernatant was then aspirated, passed through a 0.22-μm filter (Costar, Cambridge, MA), and frozen in 1-mL aliquots at −70°C.

Normal peritoneal fluid. In 18 normal women who were undergoing laparoscopic sterilization, a sample of the normal peritoneal fluid (the volume of which is less than 30 mL) was aspirated from the pelvis under laparoscopic control without installation of any fluid into the peritoneal cavity, and collected in a sterile container without heparin. The samples were centrifuged at 400g for 10 minutes. The supernatant was withdrawn, passed through a 0.22-μm filter, and frozen in 1-mL aliquots at −70°C. These samples will be referred to as normal peritoneal fluid.

Malignant ascites. In 23 patients with advanced ovarian cancer undergoing exploratory laparotomy, ascites was collected into a sterile vacuum bottle without heparin. A 10-mL sample was withdrawn and centrifuged at 400g for 10 minutes. The supernatant was removed, passed through a 0.22-μm filter, and frozen in 1-mL aliquots at −70°C. These cell-free ascitic samples will be referred to as ascitic fluid.

This study was approved by the institutional review board of the University of South Florida Health Sciences Center, in accordance with the precepts of the Helsinki Declaration.

sIL-2Ra assay. The levels of sIL-2Ra were determined by the sandwich enzyme immunoassay (Cellfree Interleukin-2 Receptor Test Kit, T Cell Sciences, Inc, Cambridge, MA) according to the manufacturer's instructions and the absorbance was read at 490 nm. The ascitic fluid samples from the cancer patients were assayed at 1:10 dilution.
dilution. The lower standard for the enzyme-linked immunosorbent assay (ELISA) was 200 U/mL and the upper standard was 3,200 U/mL. Within this range there is a linear relationship between the corrected mean absorbance at 490 nm and the concentration of sIL-2Ra ($r = .99$). The mean intra-assay coefficient of variation was 2.7% and the mean interassay coefficient of variation was 5.0%.

CA-125 assay. The serum and normal peritoneal fluid samples from normal females and the preoperative serum and ascitic fluid samples from ovarian cancer cases were analyzed for CA-125, as previously described, using a monoclonal antibody immunoradiometric assay with the Centocor CA-125 RIA kit (Centocor, Inc, Malvern, PA) according to the manufacturer's instructions. The lower standard for the assay was 6.5 U/mL and the upper standard 500 U/mL. Within this range there is a linear relationship between the cpm and the corresponding CA-125 concentration ($r = .99$). The normal serum and normal peritoneal samples were analyzed undiluted. The serum and ascitic samples from the ovarian cancer patients were analyzed at 1:10 to 1:20 dilution and 1:10 to 1:40 dilution, respectively.

Total protein assay. Serum, normal peritoneal fluid, and ascitic fluid samples were analyzed for total protein (milligrams per milliliter) using the BioRad Protein Assay (BioRad, Richmond, CA).

Statistical methods. Results are reported as mean and standard error of the mean. The method of statistical analysis was the Student's t-test, with the level of significance $P < .05$. A paired t-test was used to analyze paired data (serum and ascitic levels of sIL-2Ra and of CA-125). Linear regression analysis was used to compute the correlation between the sIL-2Ra and CA-125 levels in the serum and ascitic fluid of individual cancer patients.

RESULTS

Serum sIL-2Ra levels. In the initial experiments, the serum levels of sIL-2Ra in 18 normal females and in 23 ovarian cancer patients were measured before surgery (Fig 1A). The absolute levels in the cancer patients (746.7 ± 82.9 U/mL) were found to be significantly greater ($P = .0006$) than those in normal individuals (367.5 ± 44.6 U/mL). Only one normal individual had an sIL-2Ra level greater than the mean serum level in the cancer patients. When the sIL-2Ra levels were expressed in units per milligram of total protein (Fig 1B), this difference between the serum levels was more significant ($P = .00001$).

sIL-2Ra levels in normal peritoneal fluid and in ascitic fluid. We next determined whether sIL-2Ra could be detected in the peritoneal cavity, ie, in the ascites, where the tumor cells have infiltrated. For comparison, peritoneal fluid collected at laparoscopic sterilization of normal females was tested for sIL-2Ra. The peritoneal fluid levels of sIL-2Ra in 18 normal women (438.6 ± 48.8 U/mL) were similar to the normal serum levels. In contrast (Fig 2A), the ascitic fluid levels (2,656.7 ± 373.7 U/mL) were markedly higher than the serum levels in cancer patients ($P = .007$) and greater than the normal peritoneal fluid levels ($P = .00002$). When the normal peritoneal fluid and ascitic fluid levels of sIL-2Ra were expressed in units per milligram of total protein (Fig 2B), the difference between the normal and malignant peritoneal sIL-2Ra levels was also significant ($P = .0001$). More importantly, in those 18 cancer patients with paired preoperative serum levels and ascitic fluid levels, the level of sIL-2Ra was significantly ($P = .00019$) increased locally (ie, in the peritoneal cavity) compared with that in the systemic circulation (Fig 3). Of the 18 cases shown, 15 had a serous papillary adenocarcinoma of the ovary, 2 had a mucinous carcinoma (cases 1 and 3), and 1 had an endometrioid carcinoma (case 2). When these peritoneal levels were expressed...
in units per milligram of total protein, the difference was also significant \((P = .0096; \text{results not shown})\). The mean ratio of the absolute ascitic levels of sIL-2Ra to the serum levels was \(4.0 \pm 0.9\). There was no correlation \((r^2 = .145)\) between the serum and ascitic levels of sIL-2Ra. These results suggest that there is preferential margination of sIL-2Ra in the ascites of patients with ovarian cancer localized to the sites of malignant disease.

**Serum CA-125 levels.** In the normal controls \((n = 17)\), the mean serum level was \(15.5 \pm 1.9\) U/mL. In the 23 cancer cases, the mean preoperative serum levels were significantly higher \((P = .003)\) than the normal levels \((\text{mean}, \ 994.8 \pm 279.1; \text{Fig 4A})\). When the CA-125 levels were expressed in units per milligram of total protein, this difference was also significant \((P = .0005)\) \((\text{Fig 4B})\).

**CA-125 levels in normal peritoneal fluid and in ascitic fluid.** In the samples of normal peritoneal fluid \((n = 18)\), the mean level of CA-125 was \(70.1 \pm 12.5\) U/mL. The mean CA-125 level in the ascitic samples \((n = 23)\) was significantly higher \((P = .0007)\) than the normal level \((\text{mean}, \ 3,679.5 \pm 756.3; \text{Fig 5A})\). When the levels were expressed in units per milligram of total protein, the difference was also significant \((P = .002)\) \((\text{Fig 5B})\). As with the sIL-2Ra levels, when paired \((n = 17)\) preoperative serum and ascitic levels of CA-125 were compared in cancer patients, the ascitic level was higher than the serum level \((P = .01)\) \((\text{Fig 6})\). When the paired serum and ascitic CA-125 levels were expressed in units per milligram of total protein, the difference was also significant \((P = .45)\). The ascitic level of CA-125 exceeded the serum level in almost every cancer patient. However, there was no significant correlation between individual serum and ascitic levels of CA-125 \((r^2 = .00035)\).

**Correlation between sIL-2Ra levels and CA-125 levels.** There was no correlation in individual samples between the absolute serum sIL-2Ra and CA-125 levels \((r^2 = .204)\) \((\text{Fig 7})\), or between the ascitic sIL-2Ra and CA-125 levels \((r^2 = .159)\) \((\text{Fig 8})\). When the serum and ascitic levels were expressed as units per milliliter of total protein, there was also no correlation \((r^2 = .044\) and \(r^2 = .009\) respectively).

**Discussion**

This is the first study to report specifically on serum and ascitic levels of sIL-2Ra in ovarian cancer patients. The results clearly demonstrate elevated levels of sIL-2Ra in advanced epithelial ovarian cancer. Despite the large intraperitoneal tumor burden in many patients with advanced ovarian cancer, disease outside the abdomen is uncommon and patients usually die from persistent intra-abdominal (intraperitoneal) disease. In the majority of patients, the ascitic level of sIL-2Ra far exceeded the serum level of sIL-2Ra. More significantly, the paired data showed that sIL-2Ra localizes mainly in the tumor site (intraperitoneal), a finding consistent with the clinical observations. Similar patterns were observed for the serum and ascitic levels of CA-125. These high ascitic levels of sIL-2Ra may contribute to the known pathologic behavior of these cancers and may have important clinical and immunologic implications.

Quantitatively, there is a large pool of sIL-2Ra in the peritoneal cavity, where the volume of ascites commonly exceeds the blood volume. Clinically, it was our impression that the highest ascitic levels of sIL-2Ra were in those patients whose tumor burden was greatest. However, there is no reliable method to quantify volume of disease (tumor burden) in these patients. The FIGO staging system estimates the extent of, or spread of, disease and not tumor burden. For practical purposes, the volume of ascites in many cases would be an approximation. We therefore did not express the sIL-2Ra and CA-125 levels in terms of the tumor burden or volume of ascites.

Cell-free ascitic fluid from ovarian cancer patients has immunosuppressive activity, and contains growth-enhancing factors. The lymphocytes and NK cells infiltrating malignant ovarian ascites have impaired cytotoxicity that can
be reversed by in vitro culture with IL-2. It is known that sIL-2Rα can bind IL-2 with an affinity similar to that of the membrane-bound sIL-2Rα. Furthermore, the sIL-2R–IL-2 complex is of sufficient stability in vitro to permit purification on an affinity chromatographic column. While the affinity of membrane-bound p55 is relatively low compared with that of the IL-2Rβ and of the high-affinity IL-2Rα/β complex, the quantity of sIL-2Rα in the ascites may abrogate IL-2–mediated effects, including those from intraperitoneally administered IL-2. This is one mechanism by which lymphocytes infiltrating the peritoneal cavity may remain unreactive to the tumor cells in vivo. Just as one or more soluble factors present in the ascites may be responsible for the lack of immunoreactivity in the infiltrating lymphocytes, they may, on the other hand, also contribute to tumor growth. Transforming growth factor (TGF)-like activity, TGF-β, and tumor necrosis factor have been identified in ascitic fluid, and of these, TGFβ has known immunosuppressive properties. IL-6 is produced by ovarian cell lines and by primary ovarian tumor cultures and high levels of IL-6 have been found in the ascites and serum of ovarian cancer patients. Our data therefore add to the diversity of soluble immunologic factors in malignant ovarian ascites.

The most common cells of the ascitic cellular infiltrate, besides tumor cells, are T lymphocytes and macrophages. Few cells in the cellular infiltrate of malignant ascites from serous ovarian adenocarcinoma express IL-2Rα. This may be because of receptor internalization and/or shedding, the latter of which may account for the high sIL-2Rα levels in the ascitic fluid observed in this study. We are unaware of any report indicating that ovarian cancer cells express IL-2Rα or shed sIL-2Rα. In a recent report, in situ hybridization was used to determine messenger RNA (mRNA) for IL-2Rα (p55) in the tumor-infiltrating mononuclear cells in ovarian and breast adenocarcinoma. Only a few mononuclear cells at the tumor site expressed mRNA for IL-2Rα.

It is possible that different cell types are a source of sIL-2Rα. In the peripheral blood, as lymphocytes are the predominant cell type and as activated T cells express IL-2Rα and shed sIL-2Rα, they most likely are the major source of circulating sIL-2Rα. It is unknown if the pool of sIL-2Rα in the ascites contributes to this circulating soluble receptor level, or vice versa. In the ascites, the phenotypic composition of the cellular infiltrate peripheral is variable. As IL2Ra expression has been identified on different activated cell types and as T cells and macrophages are more commonly found in the ascitic cell infiltrate, we would speculate that they are the major source of the sIL-2Rα in ascites. Malignant ovarian ascites is commonly blood stained secondary to hemorrhage from a friable necrotic tumor mass, and the peripheral blood cells sequestered in the ascites may contribute to the sIL-2Rα ascitic level. However, we believe that this is not a major source of sIL-2Rα in malignant ovarian ascites.
The tumor-associated antigen CA-125 is the most useful marker for epithelial ovarian cancer, especially for the serous type of tumor, in which the serum CA-125 level increases as the FIGO stage of disease advances. In addition, serum CA-125 is a useful indicator of residual and recurrent disease and has an important place in the clinical management of patients undergoing chemotherapy and those being considered for a second look laparotomy. Although we found no correlation between the serum or ascitic levels of CA-125 and sIL-2Ra, it is of interest that the pattern of elevated serum and ascitic CA-125 levels, i.e., significantly higher ascitic levels, was similar to that of sIL-2Ra. As was observed with the sIL-2Ra levels, there was no correlation between serum and ascitic levels of CA-125.

The cells responsible for the shedding of sIL-2Ra in the ascites and peripheral blood of ovarian cancer patients need to be identified. Elucidation of the signalling mechanisms for IL-2Rα–mRNA production and IL-2Rα expression is central to understanding the host-tumor interaction. This knowledge hopefully will lead to improved treatments of a cancer whose survival statistics for advanced disease have remained dismal.

REFERENCES


High serum and ascitic soluble interleukin-2 receptor alpha levels in advanced epithelial ovarian cancer [see comments]

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