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Radical Oncologic Surgery Affects the Circulatory Levels of Interleukin 10

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Background and Objective: Interleukin 10 (IL-10) has been shown to be elevated in the plasma of cancer-bearing patients. The source of systemic IL-10 may be the tumor microenvironment. We therefore tried to evaluate if ablative surgery for gastrointestinal cancer could affect the levels of circulating IL-10.

Methods: Plasma IL-10 concentration was measured in 45 patients with adenocarcinoma of the gastrointestinal tract. Forty healthy subjects, 15 women undergoing hysterectomy for uterine fibroma, and 15 patients undergoing palliative operation for pancreatic cancer were used as control groups. Plasma IL-10 was assessed 1 day before surgery (baseline) and 1, 4, and 8 days after operation.

Results: The baseline concentration of IL-10 was significantly higher in cancer patients than in healthy subjects and in women with fibroma (8.6 ng/mL, 2.1 and 1.8 respectively; $P = 0.015$). After radical surgery, the IL-10 levels significantly dropped in cancer patients (from 8.6 ng/mL to 3.8; $P = 0.024$), whereas in subjects undergoing palliative operation, the concentration remained elevated (8.5 ng/mL baseline versus 7.9 on day +1).

Conclusions: The origin of circulating IL-10 may be the tumor microenvironment. *J. Surg. Oncol. 1997;66:244–247.*

KEY WORDS: **Interleukin 10; neoplasm; adenocarcinoma; oncologic surgery**

INTRODUCTION

Interleukin 10 (IL-10) is a pleiotropic cytokine produced by macrophages, T-helper 2 cells, and B lymphocyte (CD5 subset) and capable of both stimulating and suppressing the immune response [1,2]. Moreover, IL-10 has potent anti-inflammatory properties by inhibiting the production and release of pro-inflammatory cytokines, particularly TNF-alpha, IL-1, and IL-6 [3]. Recently, IL-10 has been hypothesized to play a key role in the oncogenetic and metastatic ability of neoplasms [4,5], and it has been demonstrated to be increased in the plasma of patients with different histotype of solid and hematopoietic tumors [6–9]. The elevated levels of IL-10 in cancer-bearing patients may be due to a direct release of the cytokine by neoplastic cells or to an indirect production by tumor-infiltrating lymphocytes (TIL) and/or macrophages [10,11].

Radical surgery may represent a valid *in vivo* model to confirm that the tumor microenvironment is a possible source of circulating IL-10 in cancer patients. Thus we designed a clinical prospective study to evaluate if ablative operation for gastrointestinal cancer could affect the levels of plasma IL-10.

MATERIALS AND METHODS

Forty-five patients with histologically documented adenocarcinoma of the stomach (n = 15), colorectum (n =

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TABLE I. Interleukin 10 Study: Patient Characteristics and Surgical Parameters (Values Mean ± SD)

	Stomach (n = 15)	Pancreas (n = 15)	Colorectum (n = 15)	Uterine fibroma (n = 15)	Palliative surgery (n = 15)
Age (years)	63.5 ± 11.3	66.4 ± 12.8	62.8 ± 7.1	58.3 ± 11.1	65.2 ± 9.4
Male/female	9/6	9/6	7/8	0/15	8/7
Albumin (g/L)	38.2 ± 5.1	37.5 ± 6.2	40.1 ± 5.6	41.2 ± 3.5	38.0 ± 6.4
Weight loss (%; Pts.)	46.6% (7/15)	40.0% (6/15)	13.3% (2/15)	0% (0/15)	46.6% (7/15)
Hematocrit (%)	38.7 ± 2.8	40.2 ± 4.5	40.3 ± 3.1	36.7 ± 2.5	38.5 ± 3.3
Surgical procedure	Total gastrectomy: 7 Subtotal gastrectomy: 8	Pancreatoduodenectomy: 11 Distal pancreatectomy: 4	Left hemicolectomy: 7 Right hemicolectomy: 4 Low anterior resection: 4	Laparotomic hysterectomy: 15	Gastric by-pass: 5 Biliary by-pass: 10
Duration of surgery (min)	254 ± 39	305 ± 67	208 ± 56	128 ± 45	142 ± 37
Intraoperative blood loss (mL)	461 ± 271	625 ± 295	480 ± 98	265 ± 95	315 ± 75
Type of transfusion Autologous/ homologous	7/2	9/3	5/2	2/1	3/1

15), or pancreas (n = 15) were considered eligible for the study. To qualify for radical surgery, all patients were subjected to chest X-ray, abdominal ultrasound, and scintigraphic bone scan to exclude the presence of distant metastases. Patients with pancreatic cancer also underwent a color-Doppler ultrasound to evaluate the possible tumor infiltration of the superior mesenteric artery and vein. During operation, frozen section was performed to exclude the presence of residual tumor on both proximal and distal margins of the resected organ.

The following parameters were recorded in all patients: age, sex, malnutrition (weight loss >10%) [12], albumin, hematocrit, duration of surgery, intraoperative blood loss, rate and type (homologous or autologous) of blood transfused, and cancer stage. Patients with pancreatic and gastric cancer were stratified according to the UICC recommendation [13]. Astler and Coller classification was used for patients with colorectal cancer.

Healthy subjects (n = 40; mean age 57.3 ± 12.5; 23 male, 17 female), women (n = 15) with benign tumor (uterine fibroma), and patients (n = 15) undergoing palliative surgery (gastric or biliary by-pass) for nonresectable cancer of the pancreas were considered as control groups. The clinical characteristics and the surgical parameters of the patients are reported in Table I.

To measure IL-10 circulating levels, 5 mL of blood was collected 1 day before surgery (day − 1) in vials containing citrate phosphate dextrose adenine anticoagulant and centrifuged at 3,000 rpm for 10 minutes. The plasma was collected and stored at −80°C until measurement. Plasma IL-10 (ng/mL) concentration was assessed by a commercially available ELISA kit (Cytokit™, Genzyme, Cambridge, MA) according to the recommendation of the manufacturer. The detection limit of the assay was 0.1 ng/mL. Optical density was determined at 405 nm using a microtiter plate reader (Titerket Multiskan MCC, ICN Biomedicals, Opera, Italy). Every sample was tested in duplicate. Blood collection and IL-10 plasma measurement was repeated 24 hours after operation (day + 1) and 4 and 8 days after surgery.

The nonparametric, two-tailed test (Mann-Whitney rank sum test) was used to compare continuous variables among groups. No differences were found in the *P* values before and after Bonferroni correction. Thus the *P* values are shown after Bonferroni correction as it was considered to be more appropriate for analysis of the data. To study the correlation between plasma levels of IL-10 and age, sex, albumin, hematocrit, weight loss, duration of surgery, operative blood loss, amount of type of transfusion, and cancer stage, stepwise multiple regression analysis was applied. Confidence intervals (95%) for the regression coefficients were also calculated. The data are shown as mean ± standard deviation.

RESULTS

The cancer stages of the patients studied are summarized in Table II. Figure 1 depicts the kinetics of plasma IL-10 before and after operation in the different groups. At day − 1 (baseline), IL-10 level was significantly lower in healthy subjects than in patients bearing gastric ($P = 0.015$), pancreatic ($P = 0.018$), or colorectal cancer ($P = 0.014$) (Table III). Also, women with benign tumors of the uterus had IL-10 levels significantly lower than patients with gastric ($P = 0.013$), pancreatic ($P = 0.016$), or colorectal cancer ($P = 0.013$). The site of

TABLE II. Interleukin 10 Study: Cancer Stage of Patients

	Stomach (n = 15)	Pancreas (n = 15)	Colorectum (n = 15)
Stage			
I	2	5	—
II	8	2	—
III	5	8	—
B1	—	—	3
B2	—	—	4
C1	—	—	5
C2	—	—	3

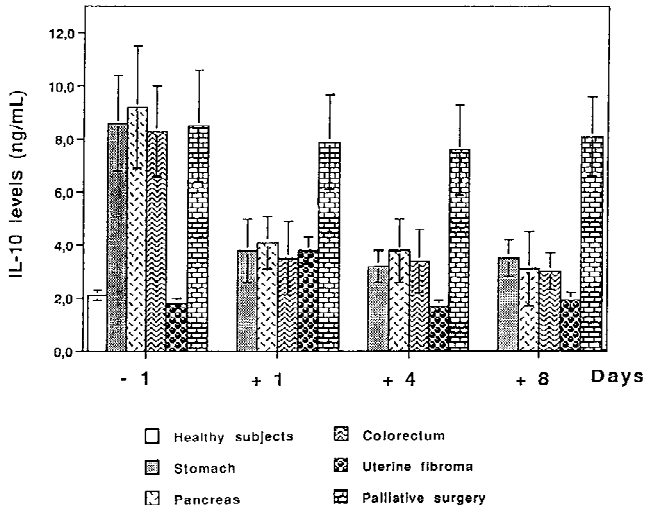

Fig. 1. Interleukin 10 kinetics in the different groups studied. Values are mean ± standard deviation.

TABLE III. Comparison of Interleukin 10 Values at Day −1 and Day +1 in Various Study Groups (Values ± Standard Deviation)

Day −1	*p* value
Healthy subjects (2.1 ± 0.2) compared to stomach (8.6 ± 1.8)	0.015
Healthy subjects compared to pancreas (9.2 ± 2.3)	0.018
Healthy subjects compared to colorectum (8.3 ± 1.7)	0.014
Healthy subjects compared to palliative (8.5 ± 2.1)	0.016
Uterine fibroma (1.8 ± 0.2) compared to stomach	0.013
Uterine fibroma compared to pancreas	0.016
Uterine fibroma compared to colorectum	0.013
Uterine fibroma compared to palliative	0.015
Day +1	
Palliative (7.9 ± 1.8) compared to stomach (3.8 ± 1.2)	0.024
Palliative compared to pancreas (4.1 ± 1.0)	0.023
Palliative compared to colorectum (3.5 ± 1.4)	0.024
Palliative compared to uterine fibroma (3.8 ± 0.5)	0.020

	Day −1	Day +1	
Stomach	8.6 ± 1.8	3.8 ± 1.2	0.022
Pancreas	9.2 ± 2.3	4.1 ± 1.0	0.023
Colorectum	8.3 ± 1.7	3.5 ± 1.4	0.022
Uterine fibroma	1.8 ± 0.2	3.8 ± 0.5	0.043
Palliative	8.5 ± 1.7	7.9 ± 1.8	0.72

cancer did not affect the baseline levels of IL-10. In fact no significant difference was observed among patients with adenocarcinoma of the stomach, pancreas, or colorectum. In these groups, who underwent radical operation, the plasma concentration of IL-10 significantly dropped 1 day after surgery (baseline stomach vs. +1 stomach, $P = 0.022$). Similar statistical results were obtained by comparing baseline IL-10 levels with +4 and +8 values for matching tumor sites. The patients who underwent palliative surgery had IL-10 concentration comparable to baseline levels of tumor-bearing patients ($P = 0.73$). In these subjects, IL-10 values remained similar to the preoperative levels throughout the study period. Women subjected to laparotomic hysterectomy showed a temporary increase of IL-10 at day +1 ($P = 0.043$ vs. baseline) and a decrease of concentration (similar to baseline) at days +4 ($P = 0.041$ vs. +1) and +8 ($P = 0.043$ vs. +1).

By regression analysis, no significant relationship between IL-10 and age, sex, albumin, hematocrit, weight loss, duration of surgery, intraoperative blood loss, type and amount of transfused blood, and cancer stage was observed, except for a weak correlation between IL-10 and cancer grade ($r = 0.435$; $P = 0.07$; 95% confidence intervals = 0.247–0.655).

DISCUSSION

Neoplastic cells might escape the immune surveillance by creating an alteration of host response at the tumor microenvironment. In particular, IL-10 seems to be involved in this mechanism by inhibiting T-helper 1 cell proliferation and effector functions. This is mediated by the ability of IL-10 to reduce IL-2 production [1,2,4] and by the diminished release of other cytokines essential for the antitumor response, such as TNF-alpha and interferon-gamma [3,5,14].

Transcription of genes encoding IL-10 was demonstrated by Filigueira et al. [15] in renal cell carcinoma, and selective expression and production of this cytokine was reported in ovarian cancer biopies [16] and in human melanoma [10,17]. Similar findings were shown by Smith et al. [18], who studied tissue homogenates of bronchogenic carcinomas, and by Gastl and co-workers in nonhematopoietic cancer lines [11]. In tumor-bearing patients, IL-10 synthesis and production do not seem to be limited at the tumor microenvironment, but the production appears to be in sufficient amount to be released systemically. In fact, elevated serum concentration of IL-10 was found in subjects with prostate cancer [7], multiple myeloma [8], and non-Hodgkin's lymphoma

[9]. We have recently reported that patients with advanced solid cancer (stage IV) had high levels of plasma IL-10 compared to normal volunteers. Moreover, the cancer histotype appeared to affect the IL-10 plasma concentration, whereas the site of tumor origin seemed to be irrelevant [6].

Based on the above observations, the present study was designed with the original hypothesis that if IL-10 is produced at the tumor microenvironment and released in the blood stream, radical exeresis of neoplasm might reduce the circulating levels of IL-10. The results obtained confirm our hypothesis. Radical surgery for adenocarcinoma of the gastrointestinal tract markedly reduced plasma concentration of IL-10, whereas in patients undergoing palliative operations the levels remained similar to baseline values. The hypothesis that circulating IL-10 is linked to the presence of cancer cells, tumor-infiltrating lymphocytes, or macrophages is also sustained by the observation that the kinetics of IL-10 following major surgery for benign disease is profoundly different. Patients who underwent coronary artery bypass grafting showed undetectable IL-10 the day before operation with a significant increase shortly after surgery. Moreover, IL-10 levels returned to preoperative values 2 days after surgery [19]. A similar IL-10 kinetics was reported in victims of trauma with a sharp increase of IL-10 levels immediately after injury, and it was undetectable within a few days posttrauma in the absence of inflammatory complications [20]. This event may be interpreted as a counteracting mechanism to control the overwhelming proinflammatory response mediated by TNF and IL-1. The temporary augmentation of circulating IL-10 after major nononcologic surgery is also shown by our results in women undergoing hysterectomy.

The present results confirm that cancer bearing patients have higher levels of IL-10 than healthy subjects or patients with benign tumor such as uterine fibroma and that the site of origin of cancer does not influence the levels of IL-10, since no difference among gastric, colonic, or pancreatic adenocarcinoma was found. In contrast, our data do not help to discriminate if IL-10 is produced and released by neoplastic cells, tumor-infiltrating lymphocytes, or macrophages since the surgical ablation of the tumour involves all these components.

In conclusion, these findings taken together strongly suggest that neoplastic cells and/or tumor-infiltrating lymphocytes or macrophages are capable of producing IL-10. The data also support the hypothesis that IL-10 is somehow involved in the immune alterations observed in cancer patients. It is appealing to speculate that the synthesis and release of IL-10 directly from the tumor microenvironment represent one of the mechanisms carried out by neoplastic cells to escape the control of host defences, but the precise biologic consequences of high plasma levels of this cytokine remain to be clarified.

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