Pre-operative Immunoprophylaxis with Interleukin-2 may Improve Prognosis in Radical Surgery for Colorectal Cancer Stage B-C

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Abstract. Cancer-associated immunodeficiency is seriously worsened by surgical trauma. Short-term pre-operative interleukin-2 (IL-2) administration abolished post-operative immunodeficiency. The effects of a pre-operative IL-2 immunotherapy on the prognosis of colorectal cancer patients (Dukes’ stages B and C), undergoing radical surgery, are reported. The study included, after post-operative stratification, 86 consecutive patients with colorectal cancer Dukes’ stage B (57) and C (29), undergoing radical laparotomic surgery, randomised to be treated pre-operatively, with or without a short-term course of subcutaneous (s.c.) IL-2 immunotherapy. Human recombinant IL-2 was given s.c. at 6x10⁶ I.U. twice daily pre-operatively for 3 consecutive days. Surgery was performed 36 hours after the last IL-2 injection. Dukes’ C patients of both groups received standard adjuvant chemotherapy consisting of 5-FU plus folates and radiotherapy for rectal cancer patients. After a median follow-up of 54 months (range 18-86), the progression rate was significantly lower in patients pre-treated with IL-2 than in controls: 9/42 (21.4%) IL-2 group vs. 19/44 (43.1%) controls, (p<0.03). The positive effect of immunotherapy was detected both in the Dukes’ B group, with 5/29 (17%) progression in the IL-2 group vs. 9/28 (32%) in controls, and Dukes’ C patients with 4/13 (30%) vs. 10/16 (62%). This study shows that a 3-day pre-operative course of IL-2 immunotherapy may improve prognosis in patients with colorectal cancer at Dukes’ stages B and C, as previously demonstrated in patients with more advanced disease. Therefore, the early activation of the antineoplastic immune system in the first post-operative days following a presurgical activation with IL-2 may counteract the growth of minimal residual disease and prevent late disease progression.

In order to understand the significance of the immunological approach to oncological surgery, a major question needs to be resolved: among two patients with the same tumor site, the same histological staging and grading, operated on by the same surgical team, why will one be cured, while the other will die due to the malignancy? Looking only at tumour characteristics is not sufficient. An explanation might be found by examining the host immune status prior to surgery and the host’s immune response to surgical trauma. It is well known that cancer patients suffer from deficiency of cell-mediated immune function (1-7). Among patients with the same stage or tumour grading, different degrees of immune response status exist. Thus, the immunodeficiency pattern explains the known relevance to the overall prognosis of quality and quantity of tumour-infiltrating lymphocytes and other immune cells in tumour tissue or around it (8-12). This factor is demonstrated to be an independent prognostic factor in Jass’ classification of rectal cancer (9); notably, it is the only host-related and not cancer-related prognostic factor. A second factor consists of the peripheral immune response consequent to the surgical trauma. Surgical trauma induces a severe post-operative decrease of total circulating lymphocytes and mainly of lymphocytes subsets, such as T helper (CD 4) lymphocytes and T (CD3) lymphocytes (2). The post-operative lymphocytopenia associated with the high serum levels of Interleukin-6 (IL-6) (13-15) and the decrease of IL-2 availability (16-19), makes the post-operative course a vulnerable period. During this period, the immune control of cancer cell growth is severely impaired, since the implant...
of eventual micrometastases is not counteracted by the host immune surveillance.

Accordingly, the disease relapse that can be documented by imaging after 12 to 18 months finds its biological beginning in the post-operative period, when minimal residual disease spread (20) is not controlled by the immune system, which has been severely impaired by surgical trauma. This theoretical model was clinically validated by the results of a retrospective study in which total circulating lymphocytes, CD3 lymphocytes and CD4 lymphocytes, assessed before surgery and on the 7th post-operative day, were correlated with long-term prognosis (21). Patients who post-operatively recovered a circulating CD3 and CD4 lymphocyte number close to that of the pre-operative period had a significantly better prognosis, in comparison with patients whose post-operative drop in CD3 and CD4 lymphocytes did not recover after 7 days.

Other reports suggested that the peripheral lymphocyte count is related to prognosis (1-3, 5). These observations, together with the demonstration of the prognostic relevance of infiltrating tumour lymphocytes by Jass’ colorectal tumour classification, clearly indicate that the host immune function is measurable and has a significant impact on the prognosis of malignancy after radical surgery.

It is known that the antitumour immune system can be selectively activated with the specific growth factor IL-2, in addition to generation of LAK cells (22). Such a treatment is necessary to counteract post-operative lymphocytopenia, with the goal of promoting an active antitumour immune response in the post-operative period in order to counteract the eventual growth of minimal subclinical residual disease. In our previous report, we demonstrated that a pre-operative short-term immunotherapy with subcutaneous IL-2 was active in preventing post-operative lymphocytopenia, mainly on CD3 and CD4 lymphocytes (23, 24). This biological activity was also confirmed by other authors (25-28). On the other hand, experimental models in animals had confirmed the ability of IL-2 immunotherapy to prevent tumour spreading after standard surgical stress (29).

In a phase II study, the long-term disease progression rate after radical surgery in colorectal cancer patients was effectively reduced by IL-2 immunotherapy (30).

The aim of the present study was to evaluate the effects of IL-2 pre-surgical immunotherapy on the peri-operative lymphocyte profile and its impact on the survival time in colorectal cancer patients undergoing surgery at Dukes’ stages B and C.

**Materials and Methods**

From February 1998 to December 2003, 88 patients with locally limited colorectal cancer entered the study at the Department of Surgery of San Gerardo Hospital, Monza, Italy. Patients were randomised to be treated with (43) or without (45) pre-operative immunotherapy with IL-2. The protocol was explained to each patient and informed consent was obtained. The inclusion/exclusion criteria were as follows: histologically documented adenocarcinoma of the colon or rectum without metastases at pre-operative staging scan, only elective surgery, only laparotomic surgery, the laparoscopic approach was excluded, no second tumour, no inflammatory or perforated tumour, no familial polyposis, age between 20-80 years, no cardiovascular or major symptomatic disease, no hepatic or renal failure, no chronic inflammatory or autoimmune disease, no chronic treatment influencing the immune system (e.g., corticosteroids or immunosuppressive agents) and the ability to give informed consent. The antibiotic prophylaxis with cefoxitine was the same in all patients and was interrupted after 24 hours post-operatively.

Pre-operative orograde bowel lavage was performed in all patients undergoing surgery for left colon or rectal cancer with PEG 4.000. IL-2 immunotherapy was administered subcutaneously at a dose of 6x10^6 I.U. twice a day for 3 consecutive days starting the fourth day before programmed surgery. Surgery was performed at least 36 hours after the last IL-2 injection, in order to permit the occurrence of lymphocyte rebound in the peripheral blood. Patients treated with anterior resection of the rectum underwent a total mesorectal excision. In this study, patients treated pre-operatively with radiotherapy or radiochemotherapy for tumour down-staging were excluded. After post-operative stratification, one patient in the IL-2-treated group was excluded because of inflammatory tumour with bladder invasion. Another patient in the control group died in the post-operative period due to myocardial acute infarction after a Miles procedure. In the remaining 86 patients (42 treated with IL-2 and 44 controls), tumour and lymph node histological examination documented a Dukes’ stage B in 57 (28 IL-2 group and 29 controls) and Dukes’ stage C in the remaining 29 patients (13 IL-2 and 16 controls) (Asler and Cooler modification).

The characteristics of the patients and the type of surgical operation are listed in Table I. Surgical complications in the post-operative course were recorded in both groups. Dukes’ stage B patients were subject to standard follow-up, while patients in Dukes’ stage C received adjuvant chemotherapy for 6 months with 5-FU and folic acid, plus radiotherapy in patients with rectal cancer.

The data were statistically analysed by the Fischer’s exact test, the Student’s t-test and analysis of variance, as appropriate.

**Results**

No significant difference was observed in the mean basal values of lymphocytes between the two groups of patients. In contrast, the mean lymphocyte numbers occurring in the post-operative period were significantly higher in patients pre-surgically treated with IL-2 than in control patients, as illustrated in Figure 1.

IL-2 pre-operative immunotherapy was substantially well tolerated: fever occurred in all 42 patients treated, but only 12 patients had fever over 39°C. One patient showed cutaneous rash with prurigo, controlled by an antihistaminic drug, but in particular, no cardiovascular complications occurred in the peri-operative period. Major post-operative complications, consisting of clinical
anastomotic leakage, were documented in 4 patients in the IL-2 group and 5 control patients.

After a median follow-up of 54 months (range 18-86 months), the percent of disease progression observed in patients pre-operatively treated with IL-2 was significantly lower with respect to that observed in the control group 9/42 (21.4%) vs. 19/44 (43.1%), (p<0.03). As regards the recurrence rate in relation to the stage of disease, Dukes’ stage B patients treated with IL-2 showed a lower percent of relapse than in controls at the same stage of disease 5/29 (17%) vs. 9/28 (32%) (N.S.). The percent of progression of disease observed in Dukes’ stage C patients receiving IL-2 was also lower than in the controls: 4/13 (30%) vs. 10/16 (62%) (Table II).

The actuarial survival rate for the IL-2 group was 81% (34/42). Six patients died in relation to cancer progression, one due to cerebrovascular stroke 1 year after surgery and one due to a secondary gastric cancer. The survival rate was 68% (30/44) in the control group and only one patient died from a non-related cancer disease.

The survival curve according to the Kaplan Meier method is reported in Figure 2.

### Discussion

Currently there are several clinical experiences that confirm the feasibility of IL-2 pre-operative immunotherapy without any clinical side-effects on the post-operative course (24-28). The s.c. administration route reduces the side-effects of therapy and is able to promote lymphocyte replication (31, 32). After 15 years of clinical experience, we believe that the optimal schedule of IL-2 pre-operative immunotherapy effective in inducing post-operative lymphocytosis with limited side-effects is a short-term course of 3 days with 6 million I.U., twice a day with a day of rest before the programmed surgery (33).

According to results obtained previously for colorectal cancer patients with the Dukes’ stage D (34), as well as to the report of a phase II study, this experience shows that
IL-2 pre-operative immunotherapy may also completely abolish surgery-induced lymphocytopenia in colorectal cancer patients with less extended disease. In addition to previous results showing the positive influence of IL-2 pre-operative immunotherapy on the survival time of surgically-treated colorectal cancer patients (30), this study demonstrated the efficacy of IL-2 pre-surgical therapy in reducing the progression rate of disease in colorectal cancer patients undergoing surgery for locally limited disease, by confirming the fundamental role of the immune reaction in the clinical history of colorectal carcinoma. The positive influence of IL-2 pre-surgical therapy can be summarised by the following documented biological effects: i) The increase of IL-6 (cytokine with suppressive effects on lymphocyte function and replication) (15) serum concentration during the early post-operative period (13, 14) is statistically reduced in patients treated with IL-2 (36). ii) The reduction of circulating dendritic cells, especially of mature dendritic cells (CD 11c), generally detected in the post-operative period (37) is reduced by IL-2 pre-operative administration (38). iii) The post-operative decline of IL-12 serum levels is reduced by immunotherapy, and the increase of angiogenic factor VEGF is counteracted (39). iv) IL-2 immunotherapy is able to induce a lymphocytes and eosinophil infiltration in tumour stroma (28-40). v) Clonal expansion of T lymphocytes and T helper lymphocytes is the natural effect of IL-2 and may be also proven in the post-operative period in patients treated preoperatively with IL-2 immunotherapy, where an increase of these lymphocyte subsets is observed, compared with pre-operative levels (24-27).

All these biological effects are able to induce new immune conditions in the post-operative period with consequent control of the growth of eventual micrometastases.

The ability of pre-operative IL-2 immunotherapy appeared to be evident in both the Dukes’ stage B and C groups, even if the statistical significance, for the limited number, is documented only for the total number of patients considered.

The results of this study suggest that the association of IL-2 pre-surgical immunotherapy in Dukes’ stage C patients may further enhance the efficacy of adjuvant chemotherapy in preventing distant organ recurrence. Further multicentric studies, with a larger number of patients, are required to confirm the ability of IL-2 pre-surgical immunotherapy to prolong the disease-free survival of colorectal cancer patients with locally limited disease, as well as to establish its influence on the overall survival time.

References


