

Review

Modulation of Systemic and Intestinal Immune Response by Interleukin-2 Therapy in Gastrointestinal Surgical Oncology. Personal Experience in the Context of Current Knowledge and Future Perspectives

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Abstract. Interactions between host and malignant tumor is currently under intensive investigation. The immune system seems to have a key role in cancer development and spread. Novel strategies to actively modulate the immune system have been proposed to improve the outcome of disease in patients with neoplasms. Our experience with systemic immunomodulation by interleukin-2 (IL-2) focused on both systemic and local immunity in surgical gastrointestinal cancer. Preoperative IL-2 subcutaneous injection was effective in counteracting postoperative immunosuppression, with a reduction of serum levels of IL-6 and the maintenance of preoperative levels of IL-12, a higher number of circulating total lymphocytes, and CD3⁺ and CD4⁺ T-cells, and a smaller decrease in circulating mature and immature dendritic cells (DCs), as well as a reduction in postoperative serum levels of vascular endothelial growth factor. At the intestinal level, in patients with colorectal cancer, preoperative administration of IL-2 affected both phenotype and function of resident dendritic cells and T-cells, skewing local immunity toward a more immunogenic one. Our data showed that immunomodulation by IL-2 was effective in counteracting the systemic postoperative immune suppression related to

surgical stress. IL-2 was also active at a local level on intestinal immunity, affecting both phenotype and function of resident T-cells and DCs. Future studies will encompass the possibility of reaching more adequate intratumoral IL-2 concentrations by direct intralesional injection to maximize immunostimulatory effects and minimize adverse effects.

Immunosurveillance, Immunoediting and Immunoescape

The ability of the immune system to function as a primary defence against cancer, identifying and eliminating nascent tumor cells, is referred to as tumor immune surveillance. The existence of immunosurveillance is nowadays supported by strong evidence, in both animal models of cancer and in humans (1). This might provide new opportunities for developing strategies for cancer therapy. Yet, cancer may develop even in the presence of a functioning immune system. This has been explained by further updating the immunosurveillance theory into the immunoediting theory (2).

In particular, it has been hypothesized by Swann *et al.* (1) that if the initial elimination of tumor cells is not complete, cancer cells are further developed by accumulating different genomic mutations and/or gene expressions. Under a selective pressure, by eliminating susceptible neoplastic clones, this process may eventually lead to the selection of tumoral variants. These variants are able to avoid or suppress the immunological response, with the final result of tumor growth and spread.

Multiple mechanisms can exert an inhibitory effect on immune effector cells, impairing their ability to promote efficient elimination of target cells, leading to tumor escape

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(2, 3). Both the adaptive and innate immune systems can be affected by tumor escape (3-5).

Several direct and indirect mechanisms of immune evasion are currently known: loss or down-regulation of HLA class I antigens; loss of tumor antigens; defective death receptor signaling; lack of co-stimulatory molecules; production of immunosuppressive cytokines, such as vascular endothelial growth factor (VEGF), prostaglandin (PG)-E₂, IL-10 and transforming growth factor (TGF)-beta (3).

Moreover, in tumor-bearing patients, other factors may negatively affect the immune system, such as malnutrition (6), postoperative infections (7, 8) and surgical trauma itself (9). In fact, after major surgical procedures, a complex pathophysiological response results in transient immunosuppression. In particular, the postoperative period is characterized by increased circulating levels of anti-inflammatory mediators, such as IL-6, IL-10 and PGE₂, and a decrease of proinflammatory cytokines, including IL-2 and IL-12 (10). Moreover, cell-mediated immunity is also hampered, with a significant decrease in circulating lymphocytes and a functional shift of T-cells toward a tolerogenic, T-helper 2 response (11, 12).

Postoperative immunosuppression has been also correlated to septic complications (13). In cancer patients, another negative factor is impaired immunological control of minimal residual disease (microneoplastic foci) that might eventually lead to tumor growth and formation of metastases.

The developing relationship between host and cancer has in recent years resulted in progressive changes in approaching neoplastic diseases, focusing more on host immune-related aspects, particularly at a local and intratumoral level.

In humans, a correlation between tumor-infiltration by T-cells, natural killer (NK) cells or NK T-cells, and better oncological outcome has been reported for a number of different tumor types. In patients with cutaneous melanoma, a high density of tumor-infiltrating lymphocytes (TIL), in particular CD8⁺ T-cells, has been related to a better survival compared to patients with low TIL density (14, 15). Similar results have been described for other types of tumor, such as ovarian carcinoma (16), endometrial cancer (17), and esophageal cancer (18).

In colorectal carcinoma (CRC), several studies have focused on the correlation between TIL density and patient prognosis (19-22), finding an association between higher density of TILs and a survival advantage. The density of TIL is now considered as an independent predictor of oncological outcome, at least for patients with stage II CRC (23).

IL-2 Immunotherapy

Recent knowledge on the potential role of the immune system in patients with neoplastic disease, stimulated several attempts to intervene in the mechanism of tumor immune

tolerance. One of the conceivable ways to actively modulate the immune system consists in its complete stimulation using proinflammatory cytokines, such as IL-2.

IL-2 is a cytokine produced by several immune effectors, such as T-helper lymphocytes and dendritic cells, and can act both in autocrine and in paracrine fashion. IL-2 is fundamental for T-cell growth (24) and for generation of memory T-cells (25). Additionally, IL-2 is able to enhance NK cell functions and induce their cytolytic activity, and is effective in stimulating B-cell proliferation and antigen production (26).

These characteristics make IL-2 a potential agent for passive immunotherapy in patients with neoplasms. Immunotherapeutic properties of IL-2 have been described in animal models since 1983 (27). In the early 1990's IL-2 was approved by the Food and Drug Agency (FDA) as an anticancer agent, mainly for advanced kidney cancer and advanced melanoma (28).

Subsequent phase I and phase II studies reported a role for IL-2 administration for therapy of a number of different tumor types: head and neck cancer, lung cancer with malignant pleural effusions, ovarian cancer, bladder carcinoma, colorectal carcinoma, and others (29). Nevertheless, several side-effects, in some case life-threatening, have limited IL-2 administration. In particular, hypotension and vascular leakage syndrome were reported in patients treated with high doses or intravenous route of administration. Lesser toxicities, such as fever, chills, flu-like malaise and skin rash, were more commonly observed using low-doses of cytokine or subcutaneous administration (29, 30). Such a route may have several advantages when compared to intravenous injection, if given as a bolus or as progressive infusion. Subcutaneous IL-2 injection, by forming depots in subcutaneous tissue, results in a prolonged release, with lower plasma levels and more extended effects, and less pronounced toxicity (29).

Biologic Effects of Systemic Subcutaneous IL-2 Immunotherapy

Our first experiences in immunomodulation were focused on counteracting surgery-induced immunosuppression by preoperative immunoprophylaxis. This was hypothesized as a key factor in improving cancer patient outcome.

We studied 12 consecutive patient candidates for elective radical surgery for CRC. They were preoperatively treated by subcutaneous IL-2 injections (12 million IU/day for 3 consecutive days before surgery). We did not observe the classic increase in serum IL-6 levels in the postoperative period compared to an age- and disease-matched group (30).

In a further preliminary study (31) carried out in 14 gastrointestinal tract cancer patients, we observed a significant reduction of circulating immature (CD123⁺) and mature (CD11⁺) dendritic cells on postoperative day 7 compared to the preoperative period. Subsequently, in a

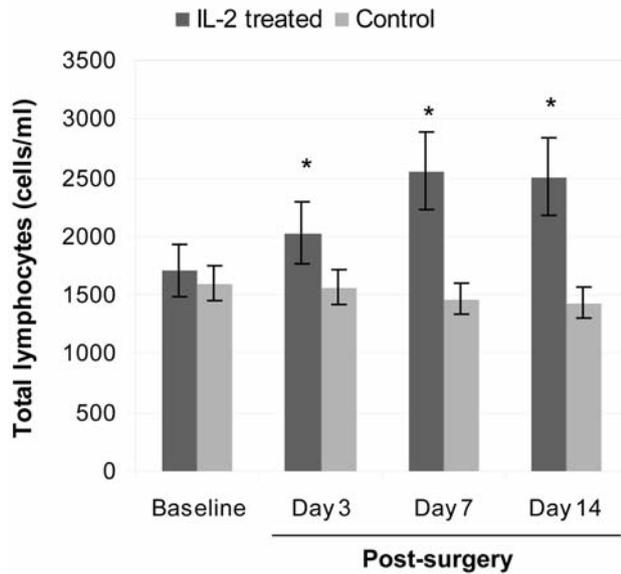


Figure 1. Total lymphocyte count in peripheral blood of patients with colorectal cancer undergoing resection and treated with IL-2 (12 million IU/day), and of controls. * $p < 0.01$ vs. Controls (non-parametric Mann-Whitney U-test). Baseline: Preoperative value. Data are presented as the means \pm SEM.

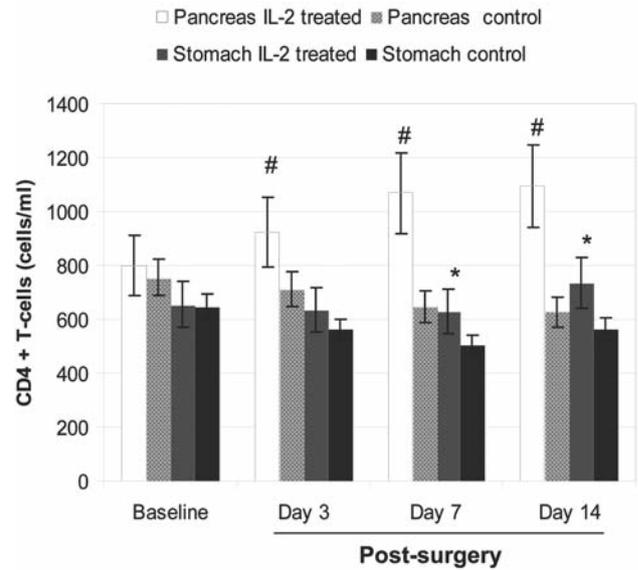


Figure 2. Total lymphocyte count in peripheral blood of patients undergoing pancreatic resection for cancer and treated with IL-2 (12 million IU/day), and of controls. * $p < 0.01$ vs. Controls (non-parametric Mann-Whitney U-test). Baseline: Preoperative value. Data are presented as the means \pm SEM.

randomized fashion, we evaluated the impact of preoperative subcutaneous IL-2 (12 million IU/day for 3 consecutive days before surgery) on such immunological parameters in patients with CRC (32). The trial showed the efficacy of IL-2 treatment in counteracting the decrease of circulating immature DCs on postoperative days 3 and 7 when compared to baseline, and a restoration of the number of mature DCs on day 7.

In a group of 68 patients with gastric carcinoma (33) randomized to preoperative subcutaneous IL-2 treatment (9 million IU/day twice a day, for 3 consecutive days) or surgery alone, we observed a significantly smaller decrease in the number of circulating CD3⁺ T-cells after surgery (difference in cell numbers preoperatively and 7 days after surgery was -195 cells/mm³ in controls and -71 cells/mm³ in treated patients, $p < 0.05$). A similar trend was observed for circulating CD8⁺ T-cells and NK cells.

The role of preoperative subcutaneous treatment with IL-2 on total circulating lymphocytes was also investigated in patients with pancreatic cancer (Figure 1). The classic lymphocytopenia observed in control patients was effectively counteracted by preoperative IL-2 therapy. When a low dose of subcutaneous IL-2 (9 million IU/day) was given, we did not observe any distinct advantages compared with controls (34).

The ability of IL-2 therapy to modulate the immune response was also reflected by evaluating the circulating levels of CD4⁺ T-cells in patients operated on for pancreatic or gastric cancer (Figure 2).

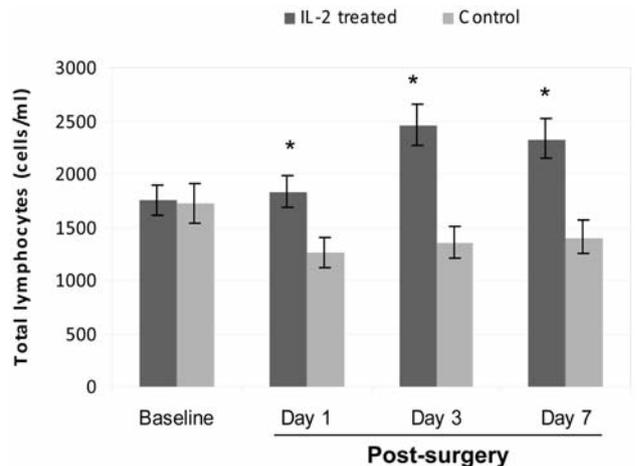


Figure 3. CD4⁺ T-cell count in peripheral blood of patients undergoing pancreatic surgery and gastric surgery for cancer and treated with IL-2 (12 million IU/day), and of matched controls. $P < 0.05$ vs. matching controls (*stomach; #pancreas) (non-parametric Mann-Whitney U-test). Baseline: Preoperative value. Data are shown as the means \pm SEM.

Interestingly, an observation was made that the less pronounced postoperative immunosuppression by IL-2 therapy, as measured by the total blood lymphocyte count (Figure 3), correlated well with the patient's long-term oncological outcome. In fact, in patients with Dukes' stage B and C CRC, the progression rate was significantly lower in these pre-treated

Table I. Percentage of different T lymphocyte populations isolated from the lamina propria (LP) and neoplasm of treated and control patients.

	CD4 ⁺	CD8 ⁺	CD45RA (naive)	CD45RO (memory)
LP control	52.5±45.0	75.8±33.6	28.6±7.9	57.3±41.1
IL-2 treated	35.6±38.1	67.2±30.4	36.6±25.2	43.3±37.2
Tumor control	12.8±5.1*	46.2±22.8	13.5±11.7*	27.1±19.3
IL-2 treated	9.4±5.3*	43.2±19.8	10.5±26.8	22.5±18.7

Numbers are shown as the percentage of FACS gate (mean±standard deviation). **p*<0.05 vs. LP of matching group (non-parametric Mann-Whitney *U*-test).

with IL-2 than in controls: 9/42 (21.4%) IL-2 group vs. 19/44 (43.1%) controls (*p*<0.03), median follow-up time: 54 months. The positive effect of immunotherapy was detected both in the Dukes' B group, with 5/29 (17%) progressions in the IL-2 group vs. 9/28 (32%) in controls, and Dukes' C patients, with 4/13 (30%) vs. 10/16 (62%) (35).

In another trial (36), 30 patients with CRC were randomized to receive preoperative subcutaneous IL-2 injection (12 million IU/day for 3 consecutive days) or surgery alone. IL-2 treatment significantly reduced the postoperative circulating VEGF levels. Moreover, postoperative plasma levels of IL-12 were higher in treated patients than in controls.

In all our experience, toxicity related to subcutaneous injection of IL-2 was always mild, with fever (grades I and II according to WHO criteria) associated with flu-like symptoms and skin erythema.

Local Effects of Systemic Immunotherapy

The effect of systemic IL-2 was also analyzed at a local level, evaluating peritumoral immune infiltrate by histopathological and immunohistochemical analysis of surgical specimens.

In pancreatic cancer, preoperative IL-2 (6 million IU/twice a day, for 3 days before surgery) did not induce a significant intratumoral infiltration of eosinophil, white cells, or NK cells (37). In contrast, in patients with gastric and CRC treated with preoperative IL-2, we observed a significant increase in peritumoral eosinophil infiltration (33, 38). Moreover, in immunotreated patients with gastric cancer, a significant increase in lymphocytic peritumoral infiltration was reported (33).

The different results in local response observed in patients with pancreatic cancer are consistent, in our opinion, with the peculiarity of this neoplasia, which is not typically associated with a rapid inflammatory infiltrate.

We further analyzed the local effects of systemic IL-2 administration on intestinal immunity more in detail, in

Table II. Percentage of T lymphocytes isolated from lamina propria (LP) and neoplasm producing interleukin-4 (IL-4) and interferon-gamma (INF-γ).

	IL-4	IFN-γ
LP Control	62.2±17.1	6.6±9.0
IL-2 treated	9.7±11.2*	58.4±30.3*
Tumor Control	67.0±23.5	25.4±14.3
IL-2 treated	10.2±9.6*	50.7±27.2*

Numbers are shown as the percentage of positive cells (mean ±standard deviation). **p*<0.05 vs. LP or neoplasm of control group (non-parametric Mann-Whitney *U*-test).

patients with adenocarcinoma of the colorectum and candidates for curative surgery. They were randomized to receive preoperative subcutaneous injection of IL-2 at a dosage of 6 million IU twice a day for 3 days or no treatment (control group).

We analyzed tissue specimens isolated from both the tumor itself and from healthy mucosa (at least 10 cm from the tumor) from the same patient. This allowed us to evaluate the effect of IL-2 on the tumor and on the lamina propria (LP) of healthy adjacent tissue.

Colon DCs and lymphocytes were isolated from specimens of both healthy mucosa and from the tumor itself of patients receiving IL-2 or not as previously described (39). Cell phenotype was analysed by fluorescence-activated cell sorter (FACS) analysis and quantified as the percentage of viable cells expressing the marker in the gate.

DCs isolated from both LP and tumor were incubated or not with *Salmonella typhimurium* and then incubated with allogeneic CD45RA⁺ purified T-cells to detect intracellular IL-4 and interferon-gamma (IFN-γ) expression. The same procedure was applied for lymphocytes.

We not only found that there were significantly fewer CD4⁺ T-cells in the tumor than in the LP, but also that their number did not increase after IL-2 treatment. The same trend was observed for total naïve and memory T-cells, as well as for CD8⁺ T-cells (Table I). This might suggest that IL-2 administration is not capable of affecting the proliferation of intestinal lymphocytes as occurs for peripheral cells (40, 41). Yet it cannot be excluded that the lymphocytopenia that characterizes peripheral blood 36 to 48 h after IL-2 administration (40), and that is followed by an increase of total blood lymphocytes, is delayed in the intestine. However, we found that both in the neoplasm and in the LP treatment with IL-2, the phenotype of resident T-cells was changed. T-Cells isolated from IL-2-treated patients are skewed toward a Th1 response, while those in untreated patients toward a Th2 response (Table II). This suggests that preoperative IL-2 treatment may favor the recovery of the

Table III. Analysis of the phenotypes of dendritic cells (DCs) isolated from the lamina propria (LP) and neoplasm of treated and control patients.

	CD11c	CD1a	HLA-DR ⁺	CD83 ⁺	CD123
LP control	19.2±15.0	17.1±20.7	14.8±5.8	49.8±21.9	33.9±16.7
IL-2 treated	19.1±12.5	31.6±25.1	41.3±19.9	81.6±7.8*	74.6±11.8*
Tumor control	20.1±14.2	14.2±16.9	25.1±12.1	40.2±15.3	27.7±20.4
IL-2 treated	34.9±26.3	29.8±11.9	48.8±26.8*	71.9±17.8*	55.3±17.4*

Numbers are shown as the percentage of the FACS gate (mean±standard deviation). Activated mature DCs (CD83⁺ and HLA-DR⁺); myeloid DCs (CD11c and CD1a); and plasmacytoid DCs (CD123). **p*<0.05 vs. LP control or tumor control (non-parametric Mann-Whitney *U*-test).

antineoplastic response. Indeed, IFN- γ release by Th1 cells is a hallmark of a good anticancer immune response due to its ability to activate several immune effector cells with anticancer properties (42). The possible role of local intestinal Th2 cells in the pathogenesis of tumor escape mechanisms is also reinforced by the observation of high levels of circulating IL-10 in cancer patients and by its disappearance after radical surgery (43).

DCs are recognized as the most powerful antigen-presenting cells, unique in their ability to activate T-cells (44) and, according to their phenotype, to drive T-cell polarization towards Th1 or Th2 phenotypes (45). Therefore, we investigated the *in vivo* effect of IL-2 administration on DC recruitment in normal colon LP and carcinoma. Our results suggest that IL-2 plays a central role in recruiting different subtypes of DCs. In particular, a significant increase of activated mature DCs, myeloid, and plasmacytoid subsets was observed in both LP and cancer tissue. In this setting, except for HLA-DR, IL-2 did not appear to increase the expression of other surface antigens (Table III).

We confirmed results by others (46), showing that T-cells isolated from colon LP and cancer tissue drive a default Th2 type of response, suggesting that T-cell polarization is dictated by the activation status of local DCs. By contrast, we observed that T-cells isolated from IL-2-treated patients produced IFN- γ , suggesting that the phenotype of local DCs had changed. When we analyzed the ability of DCs to polarize allogeneic naïve T-cells, we found that DCs isolated from IL-2-treated patients acquired the ability to drive IFN- γ -producing T-cells in response to bacteria, whereas those isolated from control patients did not (Figure 4). Therefore, the phenotype of T-cells isolated directly from mucosal and neoplastic tissue is paralleled by a similar potential for T-cell polarization acquired by DCs. However, T-cells isolated directly from the excised tissues of IL-2-treated patients showed a polarization towards a Th1 phenotype, and T-cells activated by DCs isolated from either tumor or LP of IL-2-treated patients displayed a phenotype that is more reminiscent of a Th0 type. It is possible that IL-2 may activate NK cells that in turn could drive Th1-promoting DCs as recently suggested by Martin-Fontecha *et al.* (47).

Intratumoral Immunotherapy: Toward a More Efficient Administration

Different routes of administration have been also investigated in the attempt to achieve an optimal concentration of IL-2 at the tumor site. It is conceivable that optimal oncological results depend on the cytokine concentration within the tumor mass (49). The intratumoral administration may better shift the local immune microenvironment toward a more immunogenic one and allow doses to be optimized, reducing systemic toxicity.

Systemic administration of IL-2 results in a rapid clearance from the body. Moreover, plasma levels of high-affinity IL-2 soluble receptors, that are three to eightfold higher in patients with neoplasm than in the normal population, may have a negative impact on intratumoral cytokine concentration following systemic administration (29, 49).

To reach target tissues more directly and predictably, alternative approaches have been investigated.

Local or locoregional treatment includes intra- or peritumoral injection, intracavitary or intrahepatic infusion, and inhalation. In experimental studies, the antineoplastic effects of intratumoral IL-2 administration have been investigated. Local or locoregional treatment have been shown to be superior when compared to systemic administration (50, 51). More interestingly, local immunotherapy has been described to exert an antineoplastic action not only directly at the site of the injection but also systemically, having an impact on distant metastases (50). It is hypothesized that locally stimulated effector cells may represent a prerequisite for consequent systemic activity by their migration to the lymphoid organs.

In humans, the effect of locoregional or local IL-2 treatment have been described in different advanced malignancies, such as head and neck squamous cell carcinoma, lung cancer and lung metastases, liver metastases, hepatoma, ovarian cancer and bladder cancer (50). In 2003, Radny *et al.* reported the results of a phase II trial evaluating 24 patients with advanced melanoma and skin or soft tissue metastases (52). IL-2 was administered intralesionally into the metastases (doses ranging from 0.6 to 6 million IU, 2-3 times weekly over 1-57 weeks),

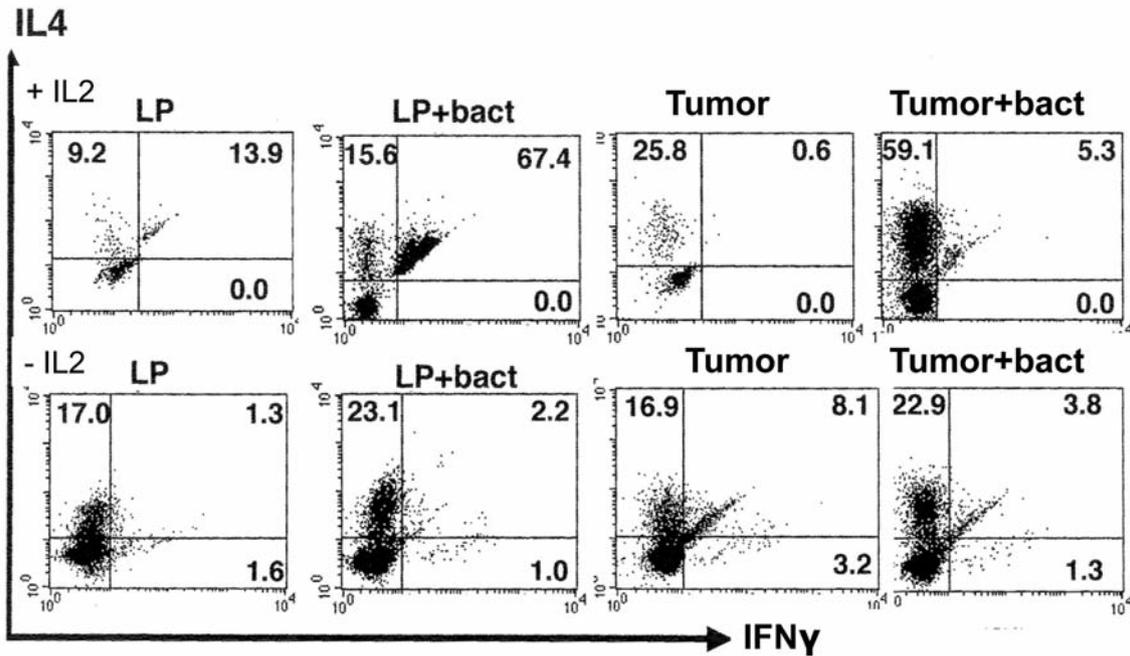


Figure 4. Polarization of naïve allogenic T-lymphocytes producing interleukin-4 (IL-4) and interferon-gamma (IFN- γ) in a mixed leukocyte reaction. LP: lamina propria; +IL2: treated patients; -IL2: control patients; Bact: dendritic cells pretreated with *Salmonella typhi*.

achieving a 63% rate of complete response and 83.5% rate of overall response. With the exception of one patient, no grade 3 or 4 adverse events were documented, suggesting a good tolerability to the local treatment.

In 2006, a phase I/II study published by Green *et al.* (53), evaluated the effects of topical Imiquimod (a synthetic molecule binding and activating toll-like receptor 7), and intralesional IL-2 in patients with cutaneous or subcutaneous melanoma. A total of 182 lesions were treated. A clinical response was observed in 50.5% of the treated lesions, with a complete response in 40.7% of the cases. Again, local treatment was well tolerated. The most frequently adverse events were flu-like symptoms and fever. Only one case of grade III toxicity was reported. Moreover, different immunological parameters have been evaluated by collecting peripheral blood mononuclear cells. An increase of the CD4/CD8 ratio during the treatment course was documented, mainly due to an expansion of activated T-cells and a polarization toward a Th1 response (54).

Based on these observations, we are currently planning a prospective trial in patients with rectal cancer that will be randomized to preoperative, endoscopically-guided, intratumoral IL-2 injection, or surgery alone. In our previous clinical experience, rectal cancer seems to be more aggressive and less susceptible to systemic subcutaneous IL-2 administration compared to colon cancer.

Our hypothesis is that rectal antineoplastic immunity might be better modulated by intratumoral rather than

systemic immunotherapy. Local administration might also offer the advantage of using lower doses of IL-2 with less pronounced adverse effects.

Conclusion

Increasing evidence supports a key role of the immune system in cancer development and spread. This provides the rationale for novel approaches to improve the outcome of cancer patients.

In our experience, preoperative subcutaneous IL-2 immunotherapy was safe and well tolerated, with low-grade toxicity. Such immunomodulation was shown to be effective in counteracting postoperative immune suppression related to surgical stress in cancer patients. Furthermore, IL-2 was also active at a local level on intestinal immunity, affecting both phenotype and function of resident T-cells and DCs.

Future studies will encompass the possibility of reaching more adequate intratumoral IL-2 concentrations by direct intralesional injection, to maximize immuno-stimulatory effects at the relevant sites and minimize adverse effects.

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