Local Activation of Immune Response in Bladder Cancer Patients Treated with Intracaval Infusion of Recombinant Interleukin-2

Francesca Velotti,1 Antonella Stoppacciaro, Luigi Ruco, Andrea Tubaro, Antonio Pettinato, Stefania Dormone, Tiziano Napolitano, Pia C. Bossola, Christopher R. Franks, Peter Palmer, Caterine N. Pourreau, Mario Piccoli, Carlo D. Baroni, Luigi Frati, Lucio Miano, and Angela Santoni


ABSTRACT

Tumor regression induced in cancer patients by i.v. infusion of interleukin-2 (IL-2) is often accompanied by severe side effects. To investigate whether local administration would affect immune response without the side effects, two 5-day cycles of continuous intraarterial intracaval IL-2 (rIL-2) were performed in 12 patients with transitional cell carcinoma (tumor stage 1, node stage 0, metastasis stage 0, and grade 1-2) of the bladder. Four groups of 3 patients were treated at each of 4 escalating doses of rIL-2 (18 x 10^4, 18 x 10^5, and 18 x 10^6 IU/m^2/day) throughout the course of the two IL-2 cycles. This treatment was effective in inducing a marked intratumol inflammatory response, consisting mainly of T-lymphocytes and macrophages. A remarkable dose-dependent increase in the levels of soluble CD25 was observed in the urine of all patients, which was associated constantly with an enhanced number of intratumor CD25+ cells. Intratumor macrophages were often immunoactive for interleukin-1 and/or tumor necrosis factor, suggesting an activated status. Increased levels of soluble CD25 and CD25+ lymphocytes were observed in peripheral blood only at the two highest doses of rIL-2, while increased percentages of circulating HLA-DR+ and CD71+ lymphoid cells and enhancement of CD3+/CD16+ T-lymphocytes were found at lower doses. Peripheral blood eosinophils were augmented in almost all patients but were rarely increased in situ. We provide evidence that continuous intraarterial infusion of rIL-2 activates host immune response, acting preferentially at the tissue level.

INTRODUCTION

rIL-21 infused i.v. at high doses in cancer patients affects host immune responses and may induce tumor regression (1, 2); however, the high doses of IL-2 that are effective for therapy cause severe toxicity (1-3). Alternative treatments with IL-2 have been evaluated that would increase host immunostimulation and decrease dose-dependent systemic toxicity. Encouraging experimental and clinical results have been obtained for some tumors with more localized administration of IL-2, capable of activating local and systemic immune responses (4-9).

One anatomic region suitable for local therapy is the urinary bladder. Pizza et al. (10) reported preliminary results of intrareglional injections of partially purified natural IL-2 in invasive bladder cancer. More recently, intravesical perfusion of natural IL-2 in patients with invasive inoperable bladder cancer has been reported by Huland et al. (11). The urinary bladder derives its blood supply from the internal iliac artery, a system that can be catheterized, with a relatively low flow rate, making it appropriate for drug administration (12).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact. This work was supported partially by grants from the Istituto Superiore di Sanita' (Italy-USA Program on Therapy of Neoplasias) and the Italian Association for Cancer Research.

1 To whom requests for reprints should be addressed.
2 The abbreviations used are: IL-2, interleukin-2; rIL-2, recombinant IL-2; i.a., intracaval; TUR-BT, transurethral resection of bladder tumor; PBMC, peripheral blood mononuclear cell; sCD25, soluble CD25; MAb, monoclonal antibody; ADCC, antibody-dependent cellular cytotoxicity; NK, natural killer; LAK, lymphokine-activated killer; FACS, fluorescence-activated cell sorter.

Received 8/23/90; accepted 2/19/91.
LOCAL IMMUNE RESPONSE INDUCED IN BLADDER CANCER BY IL-2

Lysis was assessed in a 51Cr-release assay, as previously described (18). Effector cells were incubated with 5 x 10^5 51Cr-labeled targets, at effector to target ratios of 100, 50, 25, and 12.5, in a total volume of 200 µL. After a 4-h incubation at 37°C in a humidified 5% CO2 atmosphere, plates were centrifuged at 500 x g for 10 min, and 100 µL of supernatant was collected and counted in a gamma counter (Pharmacia LKB, Uppsala, Sweden). All tests were performed in quadruplicate with an SD always ±5% of the mean. The results were expressed as percentages of specific 51Cr release.

\[
\text{cpm sample - cpm spontaneous release} \times 100 \div \text{cpm total release - cpm spontaneous release}
\]

Spontaneous release was ≤10% of total release in all experiments presented. Cryopreserved cells were run in parallel in each assay, thus providing a measure of the assay variability. Serial testing of frozen cells and controls enable us to compute a mean ± 2 SD for NK and ADCC activities.

RESULTS

Clinical Findings. Toxic effects were observed in only two patients (F. T. treated with 18 x 10^6 and T. M. with 18 x 10^6 IU/m²/day), who developed grade III (World Health Organization classification) hypotension/mental confusion and fever, respectively. All side effects disappeared rapidly when treatment was stopped.

Objective responses were present in 5 of 12 patients. Two histologically proven complete responses were observed in two patients, P. E. and S. E., treated with 18 x 10^4 and 18 x 10^5 IU/m²/day of IL-2, respectively. Three partial responses (>50% decrease in the sum of products of cross-sectional diameter of bladder tumor) occurred in two patients (R. A. and L. A.) treated with 18 x 10^5 IU/m²/day and in one (C. T.) receiving 18 x 10^6 IU/m²/day of IL-2. After a mean follow-up of 19 months (range, 9–29 months), recurrence was observed at 3 months in only two patients (P. L. and B. R.), in whom no objective clinical response was observed at the time of TUR-BT.

A more detailed description of the clinical findings will be published elsewhere.4

Peripheral Blood Leukocytes. No significant change in the number of peripheral blood lymphocytes was seen during and after treatment. Increased eosinophil counts were seen in 11 of 12 patients (data not shown), sometimes 24–48 h after the beginning of IL-2 administration. This enhancement was more pronounced after the second cycle of IL-2.

The percentages of CD3+, CD4+, and CD8+ T-lymphocytes did not change significantly, while an increased percentage of CD16+ cells was observed in 9 of 11 patients (the two remaining had high baseline levels) (Fig. 2). This enhancement was observed at the end of the first or the second cycle of treatment, with individual variability, and independently of the dose of IL-2 administered. The cell population was better characterized by analyzing lymphocytes for coexpression of CD3/CD16 antigens in dual color analysis. Fig. 2 shows that the increased percentage of CD16+ cells was due mostly to enhancement of CD3+/CD16+ T-lymphocytes, except in those patients treated with the highest dose of IL-2, in whom NK cells (CD3+/CD16+) were increased. After the first 5-day cycle of therapy, the percentage of monocytes (CD14+) tended to increase in most patients.


2457
Patients; a slight increase in the percentage of B-lymphocytes (CD19+) was found in 3 of 11 patients.

All modifications of PBMC distribution tended to return to baseline levels at the end of IL-2 treatment.

Analysis of cell activation markers showed no change in the percentage of CD25+ cells (baseline levels, ≤5%; data not shown) in patients treated with the first two doses of IL-2, but all patients treated with the two highest doses exhibited 2-8-fold increases in the percentages of CD3+/CD25+ lymphocytes (Fig. 3). A smaller increase of CD16+/CD25+ cells was observed in patients treated with 18 x 10^4 IU/m^2/day of IL-2 (Fig. 3). The increase in CD25+ cells paralleled a marked increase in plasma-soluble IL-2 receptor (sCD25) (Fig. 3). Peak levels were reached after the first IL-2 cycle, and a slow decline was observed after treatment.

In contrast, an increase in the percentage of lymphocytes with CD71+ (8 of 11 patients) and HLA-DR+ (6 of 11 patients) was also observed at the lower doses (Figs. 4 and 5). Interestingly, the percentage of CD71+ or HLA-DR+ cells was not increased in patients treated with the highest dose of IL-2. Similarly to CD25 antigen, enhancement of CD71 and HLA-DR was due mostly to an increase in the percentage of CD3+/CD71+ and CD3+/HLA-DR+ T-lymphocytes (Figs. 4 and 5).

NK and ADCC activities were affected variably, independently of the dose of IL-2 administered. LAK activity was seen in only 4 of 12 patients. Interestingly, modification of ADCC activity did not correlate strictly with changes in the percentage of CD16+ cells, which are the major mediators of this cytotoxic activity (19), or with variations in NK cytotoxicity. However, increased NK activity was always associated with the development of LAK activity.

Tumor-infiltrating Leukocytes. Prior to treatment, focal inflammatory infiltrates (CD45+ cells) were detected in tumor connective stroma and in submucosa (Table 1; Fig. 6A) in all patients. They were mainly composed of CD4+ T-lymphocytes and macrophages (DakoMac+, Pami+), with variable numbers of B-lymphocytes (CD19+), plasma cells (CD38+), and eosinophils. The neoplastic urothelium was infiltrated by scattered CD8+ T-lymphocytes and by some CD1+ Langerhans cells.

Treatment with IL-2 was followed by a detectable increase in the number of CD45+ leukocytes, both in tumor connective stroma and in neoplastic urothelium (Table 1; Fig. 6B). Macrophages and T-lymphocytes were still prevalent in submucosa and in tumor stroma, but a relative increase in the number of CD8+ lymphocytes was noted. In neoplastic urothelium, CD4+ lymphoid cells and macrophages became as numerous as CD8+ lymphocytes. Rare intraurothelial CD16+ cells were observed in 5 of 9 cases. A consistent increase in the number of eosinophils was detected in the connective stroma in only 3 of 9 cases (2 patients treated with 18 x 10^4 and one treated with 18 x 10^5 IU/m^2/day of IL-2).

A dose-dependent increase in the number of CD25+ (Fig. 6, C and D) and/or HLA-DR+ lymphoid cells was observed after IL-2 treatment. Activated cells were demonstrated in the stroma and in the neoplastic urothelium. Some intratumor CD25+
LOCAL IMMUNE RESPONSE INDUCED IN BLADDER CANCER BY IL-2

Table 1  Tumor CD45+ infiltrating cells in four groups of three bladder cancer patients before and after treatment with rIL-2

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dose of rIL-2 (IU/m²/day)</th>
<th>Pretreatment biopsy</th>
<th>Posttreatment biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Urothelium⁹</td>
<td>Stroma</td>
</tr>
<tr>
<td>P. L.</td>
<td>18 × 10⁵</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>F. C.</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>F. S.</td>
<td>ND</td>
<td>ND</td>
<td>42 + 25</td>
</tr>
<tr>
<td>P. E.</td>
<td>18 × 10⁵</td>
<td>22 + 5</td>
<td>±</td>
</tr>
<tr>
<td>S. E.</td>
<td>16 + 6</td>
<td>±</td>
<td>176 + 39</td>
</tr>
<tr>
<td>B. R.</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>F. T.</td>
<td>18 × 10⁴</td>
<td>125 + 37</td>
<td>±</td>
</tr>
<tr>
<td>R. A.</td>
<td>ND</td>
<td>ND</td>
<td>409 + 117</td>
</tr>
<tr>
<td>L. A.</td>
<td>29 + 13</td>
<td>±</td>
<td>317 + 89</td>
</tr>
<tr>
<td>S. G.</td>
<td>18 × 10⁶</td>
<td>71 + 24</td>
<td>±</td>
</tr>
<tr>
<td>C. T.</td>
<td>18 + 11</td>
<td>±</td>
<td>453 + 101</td>
</tr>
<tr>
<td>T. M.</td>
<td>87 + 32</td>
<td>±</td>
<td>468 + 67</td>
</tr>
</tbody>
</table>

⁹ Number of CD45+ cells in 10 microscope fields at ×400.
* ND, not done; ±, scattered CD45+ cells; +, scattered CD45+ cells and aggregates of CD45+ cells; ++, confluent aggregates of CD45+ cells.

Several CD45+ cells were located beneath the tumor basement membrane.

lymphoid cells were detected, however, even in patients with the two lowest doses of IL-2, who had no evidence of circulating CD25+ cells. After treatment, stromal and intraurothelial macrophages were often immunoreactive for IL-1 and/or tumor necrosis factor (Fig. 6E), suggesting an activated status. At the time of tumor biopsy, local proliferation of infiltrating leukocytes was marginal, as estimated by the low number of Ki67+ cells. In the two cases (C. T., T. M.) in which fragments of normal urothelium were examined before and after treatment, IL-2 infusion induced changes in normal urothelium similar to those in the tumor, suggesting that the inflammatory reaction was not directed selectively against tumor cells. In all cases, normal and neoplastic urothelium was HLA-DR- before and after treatment.

Soluble IL-2 Receptor in Urine. A marked, dose-dependent increase in the levels of soluble IL-2 receptors (sCD25) was observed in urine of all patients (Fig. 7). The urinary levels of sCD25 were already increased 48–72 h after IL-2 infusion, reaching peak levels after the first IL-2 cycle and returning to baseline values after a few days. At the two lowest doses, a clear dissociation was noted between high levels of sCD25 in urine and baseline levels of sCD25 in the plasma.

DISCUSSION

Continuous i.a. infusion of rIL-2 is effective in inducing systemic and local activation of the host immune response. Dose-dependent increased levels of sCD25 were observed in the urine of patients. sCD25 in urine might thus represent an important marker for evaluating the local effectiveness of IL-2 treatment in diseases of the urogenital tract. We also observed the appearance of numerous CD25+ cells at the tumor site and in normal urothelium. These findings are in agreement with a previous report by Huland et al. (11), describing the appearance of CD25+ cells in the urine of bladder cancer patients after intravesical perfusions of IL-2. Similarly, Cohen et al. (20) described the augmentation of intratumoral CD25+ cells after IL-2 therapy in melanoma patients.

Circulating CD25+ lymphocytes and sCD25 in the plasma were increased only at the two highest doses of IL-2. Similarly, Lotze et al. (21) and Voss et al. (22) reported marked increases in the number of CD25+ cells and in sCD25 plasma levels in patients treated with high doses of i.v. rIL-2.
Fig. 6. Biopsy specimens from a patient before (A and C) and after (B-E) treatment with $18 \times 10^5$ IU/m²/day of recombinant interleukin-2. Cryostat sections were immunostained with monoclonal antibodies to CD45 (A and B), CD25 (C and D), and α-tumor necrosis factor (E), using the peroxidase-antiperoxidase method and counterstaining with hematoxylin. Before treatment, CD45+ leukocytes were few and were located mainly in the tumor stroma (A). After treatment, a marked increase in the number of CD45+ cells was seen in tumor stroma and in urothelium (B). A very small number of CD25+ tumor-infiltrating lymphoid cells was observed before treatment (C), which augmented after interleukin-2 administration (D). Some stromal macrophages were immunoreactive for α-tumor necrosis factor after treatment (E). Original magnification: A and B, × 100; D and E, × 400; C, × 1000.

The increased number of tissue CD25+ cells, enhanced sCD25 antigens in urine, and the simultaneous lack of detectable CD25 in peripheral blood suggest that urinary sCD25 is released by locally activated cells. Our results indicate a more pronounced activity of IL-2 after i.a. infusion at the tissue level, probably because of preferential delivery of IL-2 at this site.

The immunochemical study revealed activation of tissue macrophages, as indicated by the appearance of intracellular cytokines such as IL-1 and tumor necrosis factor. It is thus conceivable that these cytokines are directly active against the tumor and may act synergistically with IL-2 in the activation of T-lymphocytes (23, 24). We showed previously (25) that lymphoid and nonlymphoid tissue macrophages contain virtually no IL-1 or tumor necrosis factor, suggesting that the expression of these cytokines is related to a particular functional status of these effector cells.
A potential antitumor role of eosinophils was emphasized recently (26). Also, a remarkable increase in the number of eosinophils in urine was reported in bladder cancer patients after intravesical perfusion of IL-2 (11). We did not observe any increase in tumor-infiltrating eosinophils (although eosinophilia was found in peripheral blood); moreover, we were not able to establish a correlation between eosinophil infiltration and tumor regression.

The finding of only a few proliferating Ki67+ cells after IL-2 treatment may indicate that the increase in the number of infiltrating leukocytes depends on recruitment from the systemic circulation rather than local proliferation. The validity of this hypothesis can be shown only through the use of sequential biopsies.

Interestingly, the inflammatory infiltration pattern was similar in normal and neoplastic urothelium, before and after treatment. This finding further supports the idea that the local efficacy of i.a. treatment is the result of IL-2 activity at the level of the bladder.

The percentage of peripheral blood HLA-DR+, CD71+, and CD16+ T-lymphocytes was increased even with lower doses of IL-2. CD16 antigen, commonly expressed by NK cells (19), was found mostly on CD3+ cells, suggesting that continuous i.a. IL-2 treatment can modulate its expression on T-lymphocytes. Previous studies (27) showed that CD16 antigen is expressed on only a small proportion of T-cells (≤2-5%) from healthy donors and does not change significantly following short-term culture with IL-2 in vitro. Voltarelli et al. (28), however, reported a 3.5-fold increase in the percentage of CD3+/CD16+ T-cells when lymphocytes were cultured for 20 days with rIL-2. Furthermore, several CD3+/CD16+ clones have been described (27), and functional studies have shown that this T-cell subset displays ADCC, while NK and LAK activities are absent. In our study, ADCC activity increased in only a small number of patients and did not always correlate with the number of CD3+/CD16+ cells, suggesting that they are not the main effectors of ADCC activity.

Finally, our data indicate that i.a. infusion of IL-2 does not exert major effects on the level of circulating and tissue NK cells. In fact, the percentage of NK (CD3−/CD16+) cells did not increase consistently, except in those patients treated with the highest dose of IL-2. This finding, together with the evidence that the number of NK cells is enhanced by high doses of i.v. IL-2 (29), indicates that only high concentrations of IL-2 in the peripheral blood can increase the number of NK cells. Moreover, no consistent change in NK activity was observed, and no correlation between NK cell numbers and cytotoxicity against K562 was noted. This finding could be explained by the presence, among the PBMC, of either accessory cells capable of modulating NK activity negatively (30) or T-cytotoxic cells with "promiscuous" activity (31). The augmentation of NK activity always correlated with the appearance of LAK activity, corroborating the finding that most LAK cytotoxicity is mediated by NK cells (32). At the tissue level, rare CD16+ cells were sometimes present, independently of the dose of IL-2. This observation is in accord with previous results showing that serial biopsies of regressing melanoma lesions did not contain infiltrating CD16+ NK cells (20) and supports the finding that T-lymphocytes, rather than NK cells, display a preferential trafficking to the tumor site (33).

No clear correlation between the modulation of the immune response and tumor regression was observed. Whether immunological data, however, correlate with the local relapse rate of tumor will be evaluated at the end of a 5-year follow-up.

ACKNOWLEDGMENTS

The skillful technical assistance of S. Ferraro is gratefully acknowledged.

REFERENCES


Local Activation of Immune Response in Bladder Cancer Patients Treated with Intraarterial Infusion of Recombinant Interleukin-2

Francesca Velotti, Antonella Stoppacciaro, Luigi Ruco, et al.