In Vivo Interleukin 2-induced Activation of Lymphokine-activated Killer Cells and Tumor Cytotoxic T-Cells in Cervical Lymph Nodes of Patients with Head and Neck Tumors

Licia Rivotini, Carlo Gambacorti-Passerini, Massimo Squadrelli-Saraceno, Maria Ilaria Grosso, Giulio Cantù, Roberto Molinari, Attilio Orazi, and Giorgio Parmiani

Division of Experimental Oncology D [L. R., C. G.-P., G. P.], Division of Head and Neck Surgical Oncology [M. S.-S., M. I. G., G. C., R. M.], and Division of Pathology [A. O.], Istituto Nazionale dei Tumori, Milan, Italy

ABSTRACT

To study whether regional injection of recombinant interleukin 2 (rIL-2) can induce an in vivo lymphocyte activation in cervical lymph nodes (LNs) of patients with head and neck carcinoma, 12 patients, candidates for prophyactic dissection, were treated for 7-10 days prior to surgery with rIL-2, 10^8 units/day, injected in the perimastoid region. A marked induction of cytotoxic activity against allogeneric (K562 and Daadli lines) and autologous target cells (fresh spindle cell carcinomas of the tongue) was observed in lymphocytes obtained from jugular, spinal, and, to a lesser extent, submandibular LNs of all treated patients. An increase of cytotoxicity was also present in LNs contralateral to the rIL-2 injection site. On the other hand, only a border line increase in spontaneous proliferation was detected. Moreover, in the two cases tested, a marked and apparently autologous tumor (Auto-Tu)-specific lysis was found in CDS^+ lymphocytes obtained from LNs, whereas lymphokine-activated killer activity was mainly exerted by CD16^+ natural killer cells. T-lymphocytes, when cultured with irradiated Auto-Tu cells and low doses of rIL-2, showed an increased Auto-Tu lysis, while cytotoxicity against allogeneic tumor cells (including K562) was not observed. These data indicate that regional injection of rIL-2 can activate lymphokine-activated killer cells from LN lymphocytes but also induce and/or expand a T-cell population expressing a restricted Auto-Tu cytotoxicity.

INTRODUCTION

High doses of rIL-2^2 alone, or in combination with LAK cells given to patients with advanced cancer, resulted in partial and even complete durable clinical responses (1-3). A series of immunological modifications appeared in these patients, the most significant being the cytotoxic and proliferative activation of PBL (1, 2, 4). The cost, the logistic complexity, the significant toxicity, and the limited frequency of responses are the major limitations of such a therapeutic approach.

As indicated by several animal models, rIL-2 toxicity can be avoided and a therapeutic effect achieved when locally growing tumors are treated with multiple low doses of rIL-2 or rIL-2 plus lymphocytes (5, 6). An ideal model for locoregional immunotherapy in man is represented by squamous cell carcinoma of the H&N. In fact, this disease is characterized by a relatively rare occurrence of distant metastases, the spread of the tumor being limited to the regional lymph nodes. However, the majority of advanced or relapsing H&N carcinomas are difficult to control and may result in the death of the patients.

Despite the fact that most H&N carcinomas are probably due to chemical carcinogens contained in tobacco or alcohol (7, 8) and, like chemically induced mouse tumors, should induce a lymphocyte-mediated response (9), systemic and local immune mechanisms are impaired early in these patients and are further exacerbated by therapy (10-12). The presence of phenotypically T-suppressor cells was described along with an impaired LAK activity in invaded and noninvaded cervical lymph nodes (13, 14). More recently, metastatic lymph nodes of patients with H&N cancers were shown to contain a T-cell population that proliferates to autologous tumor cells when OKM1^+ Leu^+ suppressor lymphocytes were removed and appropriate stimuli, such as IL-1 or IL-2, were added (15). Thus, NK and/or T-cells may be involved in the immune control of these tumors, but this anti-tumor activity appears to be hampered by local suppressive factors.

In two pilot studies (16, 17), rIL-2 was injected into the perimastoid region of patients with advanced H&N cancers, where lymphographic analyses have shown that injection of radiopaque substances gives visualization of the whole lymphatic network of the neck (18). Preliminary results suggest that locoregional immunotherapy can produce significant responses in such patients (16).

However, it is unknown whether the antitumor effect induced by the local treatment with low dose rIL-2 is due to activation of lymph node lymphocytes and whether this activation may involve, in addition to LAKs, tumor-specific T-cells. To evaluate these possibilities we have examined the phenotype and immunological functions of lymphocytes derived from lymph nodes of H&N cancer patients undergoing local treatment with multiple daily injections of rIL-2.

MATERIALS AND METHODS

Patients and Treatment Schedule. Twelve patients (10 males and 2 females, between 55 and 73 years of age) with carcinoma of the N&H (T1-T4) without clinically detectable lymph node metastases (N0) were candidates for prophylactic cervical dissection. They were treated for 7-10 days before surgery with 10^9 units/day of rIL-2 (Glaxo IMB, Geneva, Switzerland); rIL-2 was resuspended in saline with 12.5% human albumin to induce a preferential lymphatic draining (19). rIL-2 injections were performed in the perimastoid region with a 26-gauge needle and 15 mm penetration as described by Cortesina et al. (16). All patients submitted to rIL-2 treatment after informed consent. Seven patients were used as negative controls; three of them were given injections of 1 ml/day of 12.5% albumin in saline for 10 days and the remaining four were untreated.

Lymphocyte Separation. LNs from the following lymphatic regions were isolated from surgical specimens: upper, middle, and lower JUG; upper, middle, and lower SP and SM nodes. LNs were mechanically disaggregated and the resulting lymphocytes purified by Ficoll centrifugation. LNLs were then resuspended in complete medium, consisting of RPMI 1640 (MA Bioproducts, Walkersville, MD), 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (MA Bioproducts), 100
1. **Cr Release Assay.** Cytotoxic activity against allogeneic (K562 and Daudi lines) and autologous (fresh spindle cell carcinomas of the tongue) targets was assessed by 1. Cr release assay performed as previously described (20). Assays were considered evaluable when spontaneous 1. Cr release of target cells, including fresh tumor cells, was <25% of the total release. Cytotoxic activity (in LU) was calculated according to the equation of Pross et al. (21). One LU defined as the number of effector cells required to lyse 30% of 1000 targets (at E/T ratios of 100/1, 50/1, 25/1, and 12/1), and cytotoxicity in LU/10^6 effector cells was computed. The total lytic activity generated/culture was calculated as LU/10^6 cells multiplied by number of cells/culture × 10^6.

**Spontaneous Proliferation.** Lymphocytes (10^5/well) were seeded in 96-well plates in a final volume of 0.2 ml of complete medium. Cells were then pulsed with [3H]TdR (1 μCi/well) and incubated for 18 h at 37°C. Lymphocytes were subsequently harvested and radioactivity was counted in a beta-counter.

**Immunohistochemistry.** Labeling by MAbs was carried out on air-dried acetone-fixed (10 min; BDH) slides obtained from frozen LNs; slides were then incubated overnight with the first MAb and a nonimmune mouse serum for negative control. After two 10-min washes in PBS, a biotin-conjugated horse anti-mouse antibody (dilution 1:200; Vector, Burlingame, CA) was applied for 45 min, washed twice, and labeled with modified avidin-biotin-peroxidase complexes (Vector) (22). Slides were then developed with diaminobenzidine (Sigma Chemical Co., St. Louis, MO). MAbs against the following markers or molecules were used: CD5 (Leu-1; Becton Dickinson, Mountain View, CA), CD4 (Leu-4; Becton Dickinson), B mix (B1 and T015 mixture; Coulter Immunology, Hialeah, FL, and Dako, Glostrup, Denmark, respectively), CD45R (8.11.13; Third International Workshop on Human Differentiation Antigens, Oxford, England, 1986), TCR (β-F1; Technogenetics, Trezzano SN, Italy), HLA-DR (AA3.84, Technogenetics), IL-2R (CD25; Dako), and Leu19 (CD56; Becton Dickinson).

**Isolation of CD5+ and CD16+ Lymphocytes.** Positive selection was performed by the “panning” technique (23). Briefly, after 2 h of plastic adherence, two suspensions of 30 and 100 × 10^6 nonadherent LNLs, respectively, were treated with anti-CD5 (Leu1) and anti-CD16 (Leu11, Becton Dickinson) MAbs. After 45 min incubation at 4°C, cells were washed in PBS plus 5% human serum, resuspended in the same medium, and seeded in 100-mm diameter tissue culture dishes (Corning) precoated with affinity-purified goat anti-mouse IgG antibody (Kierkegaard and Perry Laboratories, Gaithersburg, MA). After 2 h incubation at 4°C, each dish was gently washed with PBS plus 5% human serum; adherent CD5+ and CD16+ lymphocytes were then collected by vigorous pipetting or scraping. Precoated dishes were prepared by incubation for 60 min at room temperature with 10 ml of the purified anti-mouse IgG antibody (10 μg/ml in 0.05 m Tris buffer, pH 9.5), followed by washing with PBS plus 5% human serum.

**Lymphocyte Phenotype.** Cell surface phenotypes of lymphocyte subpopulations obtained by panning were assessed by indirect immunofluorescence. Lymphocytes (10^5/well) were seeded in 96-well flat-bottomed plates and incubated for 30 min at 4°C with anti-CD5 or CD16 MAbs. Since we have previously seen that soon after panning lymphocytes remain coated with the primary MAb (mouse anti-human CD5 or CD16), a polyclonal donkey anti-rabbit immunoglobulin fluorescein-linked MAb (Amersham, Buckinghamshire, United Kingdom) was used at a 1:30 dilution as a negative control, since in the panning technique a mouse anti-human MAb was used and it coated the lymphocytes. After 3 washings, lymphocytes were treated with goat anti-mouse fluorescein-conjugated MAb for 30 min at 4°C and all samples were analyzed by a fluorescence-activated cell sorter. T4/T8 and anti-Leu11 fluorescein-conjugated (Becton Dickinson) MAbs were also used as controls.

**MLTC.** MLTC was performed as described by Fossati et al. (24). CD5+ lymphocytes (2 × 10^6/well) were cultured for 7 days in 24-well plates (Costar), in 2 ml final volume, in the presence of 30% autologous irradiated PBL (4,000 rads) and 20% fresh irradiated (10,000 rads) Auto-Tu cells with or without rIL-2 (3 units/ml). After 7 days of coculture, lymphocytes restimulated with Auto-Tu and rIL-2 were split and maintained with either rIL-2 or with rIL-2 and rIL-4 (50 units/ml; Immunex, Seattle, WA) in the presence or absence of Auto-Tu cells. After one week, lymphocytes were restimulated or not with 20% of the irradiated Auto-Tu cells and rIL-2 or rIL-2 plus rIL-4. At the end of each culture week, CD5+ cells were tested for cytotoxic activity against autologous and allogeneic target cells and for spontaneous proliferation. On day 21 of culture, the lymphocyte phenotype was determined by indirect immunofluorescence with MAbs as described before.

**Statistical Analysis.** Correlation between the variables evaluated in this study was assessed by linear regression. Significance of the association was studied by considering the correlation coefficient and the 2-tailed related P value; P ≤ 0.05 was considered significant.

**RESULTS**

**Characteristics of Patients and Treatment.** Primary tumors (T1–T4) from patients entered into this protocol were localized on the tongue (7 patients), floor of the mouth (4 patients), or larynx (1 patient). Patients were treated for 7–10 days with regional injections of 10^6 units of rIL-2 before surgery; therapy was well tolerated, causing no significant toxic effects. Edema of injected sites and of the nearest lymph nodes was observed in most cases (details of this study will be presented elsewhere). In 4 patients the primary tumors were present at the time of rIL-2 treatment and of lymph node dissection; 2 of these patients had LN metastases detected by histological analysis, which were not observed during clinical evaluation (N0 → N1). Control patients (given either 12.5% of albumin in saline or not treated with injections) were matched with rIL-2-treated patients for age and tumor site.

**Cytotoxic Activity and Spontaneous Proliferation of LNLs after In Vivo rIL-2 Treatment.** To determine whether regional treatment with rIL-2 can induce an in vivo immunostimulation, LNLs from each of the 12 patients treated with rIL-2 were first tested for their ability to lyse tumor target cells. All data refer to LNs obtained from monolateral dissection, i.e., homolateral
to the rIL-2 injection site. Scattergrams in Fig. 1 show the cytotoxic activity exerted against K562 (Fig. 1, top) and Daudi (Fig. 1, bottom) cell lines by lymphocytes from the different cervical LNs in comparison to PBL. A statistically significant (P ≤ 0.05) increase of cytotoxicity was seen in LNs from each JUG and SP station, while SM LNs showed a lower activity, probably due to distal localization of SM LNs from the rIL-2 injection site. NK activity of PBL from these patients was much lower than that of a group of 10 healthy donors [5 ± 2 versus 49 ± 12 (SE) LU, on K562 after 18 h incubation]. A similar finding could be explained by the presence of immunosuppressive molecules released by tumor cells or/and by the effect of anesthesia and/or stress, since PBL were obtained during surgery (25, 26).

Table 1 reports a representative case (patient 10) in which it was also possible to test LNLs against Auto-Tu cells. In vivo exposure to rIL-2 generated a marked lysis of K562 and Daudi and a lower but significant lysis of Auto-Tu cells by JUG and SP LNLs, while cytotoxic activity of SM stations was weaker on all 3 targets and PBL were practically ineffective (Table 1). An increase in cytotoxic activity against K562 and Daudi was not seen in any LN station of the 3 control patients given albumin only, nor in any of the 4 untreated patients. In fact, in the latter the LNL cytotoxicity never exceeded 4 LU (mean ± SE, 2.5 ± 1.2 LU) against K562 and 1.9 LU (1.1 ± 0.3 LU) against Daudi cells.

Relevant changes in spontaneous proliferation of rIL-2 treated LNLs were never observed, although a clear trend to increase [3H]TdR incorporation was visible in JUG and SP LNLs in comparison to PBL (Fig. 2); the increased proliferation of these latter LNLs, although relatively weak, was statistically significant (P ≤ 0.05). Spontaneous proliferation of untreated cervical LNLs, although slightly higher than that of PBL, was also not modified by albumin injection (Fig. 3). In conclusion, anti-tumor cytotoxic activity appeared in LNLs after regional rIL-2 injection in all treated patients, whereas only a borderline increase of spontaneous proliferation was detectable.

Table 1 Lysis of allogeneic and autologous target cells by LNLs and PBL of patient 10 after regional treatment with rIL-2

<table>
<thead>
<tr>
<th>Patient</th>
<th>Lymphocytes from:</th>
<th>Targets</th>
<th>K562</th>
<th>Daudi</th>
<th>Auto-Tu</th>
</tr>
</thead>
<tbody>
<tr>
<td>03</td>
<td>PBL</td>
<td>2.7</td>
<td>0.4</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>05</td>
<td>PBL</td>
<td>2.1</td>
<td>1.5</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>09</td>
<td>PBL</td>
<td>1.9</td>
<td>1.05</td>
<td>1.9</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3. Spontaneous proliferation of LNLs from albumin-treated patients. Given that rIL-2 was reseeded before injection in saline with 12.5% human albumin to induce a preferentially lymphatic draining, three patients were treated with albumin alone as negative controls. Data refer to 18-h incubation assay. Columns, mean values of 3 different cases; bars, ±SE, upp. upper; mid. middle; low, lower.

Table 2 Cytotoxic activity of rIL-2-treated LNLs obtained from patients who submitted to bilateral dissection

<table>
<thead>
<tr>
<th>Patient</th>
<th>Lymphocytes from:</th>
<th>Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>03</td>
<td>PBL</td>
<td>K562</td>
</tr>
<tr>
<td>05</td>
<td>PBL</td>
<td>Daudi</td>
</tr>
<tr>
<td>09</td>
<td>PBL</td>
<td>Auto-Tu</td>
</tr>
</tbody>
</table>

Values are expressed in LU/10⁶ effector cells.

*a ND, not done.

Fig. 2. Spontaneous proliferation of LNLs from rIL-2-treated patients. LNLs were seeded at 10⁶ cells/well and incubated for 18 h in complete medium pulsed with 1 µCi/well [3H]TdR. In 7 of 12 treated patients LNs of every cervical station were obtained. Points, means; bars, ±SE. Each mean was statistically significant (P ≤ 0.05) in comparison with PBL. Each symbol represents lymphocytes obtained from the same patient.

Fig. 3. Spontaneous proliferation of LNLs from albumin-treated patients. Given that rIL-2 was reseeded before injection in saline with 12.5% human albumin to induce a preferentially lymphatic draining, three patients were treated with albumin alone as negative controls. Data refer to 18-h incubation assay. Columns, mean values of 3 different cases; bars, ±SE, upp. upper; mid. middle; low, lower.
showing follicular hyperplasia with increases of paracortex dimensions. Phenotypic analysis, however, showed a significant increase of IL-2 receptors (Fig. 4b) and Leu19+ cells (Fig. 4d) in treated as compared to untreated (Fig. 4, a and c) LNs. No obvious changes of T- and B-lymphocyte and macrophage numbers or distributions were seen (data not shown). In patient 8, LNs from the bilateral dissection were available for immunoperoxidase staining. Augmentation of CD25* and Leu19* lymphocytes was more clear in homolateral than in contralateral LNs, suggesting that expression of these activation markers was directly related to rIL-2 injection (data not shown).

Analysis of Lymphocyte Subpopulations Responsible for Cytotoxic Activity. Further experiments were performed to investigate the role of subpopulations of LNLs (NK or T) in the in vitro tumor cytotoxic activity. Owing to the frequent absence of the primary tumor lesion at the time of dissection (most of the 12 patients had previously submitted to tumor excision or radiotherapy) and the high spontaneous release of fresh tumor cells in an 18-h cytotoxic assay, in only two of the four available tumors was it possible to evaluate cytotoxicity of LNLs against Auto-Tu cells. To this end, lymphocytes from JUG and SP nodes were pooled, and CD5* and CD16* cells were separated according to phenotype and immediately tested for cytotoxic activity against K562, Daudi, and Auto-Tu cells. The phenotypes of the fractionated subpopulations is shown in Table 3. While the CD5* subpopulation was relatively pure, composed of 96–98% T-cells, the CD16* subpopulation contained about 15–20% T4/T8* cells in the first case and probably B-cells in the second one. Phenotype analysis with anti-pan B MAb could not be performed on CD16* lymphocytes owing to the reduced number of available cells.

In both cases studied, bulk population efficiently lysed the three target cells (Table 4), although Auto-Tu were the least lysed ones. CD5* lymphocytes significantly lysed Auto-Tu but not K562 and Daudi, whereas CD16* cells, which markedly killed K562 and Daudi, displayed a borderline Auto-Tu cytotoxicity. These findings indicate that rIL-2-treated LNs contain a majority of T-lymphocytes, as detected by phenotype analysis, which appear to be capable of a restricted recognition of autologous neoplastic cells. As expected, lysis of NK and LAK targets was mainly due to CD16* lymphocyte fraction.

Reactivity of CD5* cells to Auto-Tu Cells, as Assessed by MLTC. To further investigate the features of tumor-restricted T-cells, CD5* lymphocytes obtained from LNs of patient 12 were kept in an MLTC for 3 weeks and at the end of the first, second, and third weeks of culture, lymphocytes were assessed for proliferation (Table 5) and cytotoxic activity (Tables 6 and 7). After 1 week of culture in medium alone, CD5* lymphocytes were unable to proliferate (Table 5) or lyse (Table 6) any target; the presence of irradiated Auto-Tu cells alone induced a significant proliferation but a borderline cytotoxicity against Auto-Tu cells. When rIL-2 (3 units/ml) was added to the coculture, a strong increase of CD5* lymphocyte activation occurred, as indicated by proliferation (Table 5) and lysis (Table 6) of Auto-Tu. For the second week of culture, lymphocytes previously cultured with Auto-Tu and rIL-2 were maintained in the same wells of the primary culture (i.e., in the presence of residual Auto-Tu cells) or were split in new wells and cultured for

Fig. 4. Expression of CD25 and Leu19 antigens in control (a and c, respectively) and rIL-2 treated (b and d, respectively) LNs, as assessed by immunohistochemistry (avidin-biotin-peroxidase). ×160; insets, ×400.
Table 3 Phenotypic analysis of CD5+ and CD16+ cells obtained after separation of LNLs

Lymphocytes of each subpopulation (10^5/well) were incubated with different monoclonal antibodies for 30 min at 4°C and then with goat anti-mouse fluorescein conjugated for 30 min. Samples were analyzed by cell sorting. Reported values represent % of positive cells.

<table>
<thead>
<tr>
<th>Case</th>
<th>Effector lymphocytes</th>
<th>Monoclonal antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD3</td>
<td>CD5</td>
</tr>
<tr>
<td>1</td>
<td>Bulk</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>CD5*</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>CD16*</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>Bulk</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>CD5*</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>CD16*</td>
<td>ND</td>
</tr>
</tbody>
</table>

* ND, not done.

Table 4 Cytotoxicity of rIL-2-treated LNLs tested as bulk populations, CD5+ and CD16+ subpopulations from two cases

T-lymphocytes (CD5+) and NK cells were obtained by MAb separation (panning technique) and were incubated for 18 h with tumor cells at E/T ratio of 100/1, 50/1, 25/1, and 12/1.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Target</th>
<th>Effector lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K562</td>
<td>82*</td>
</tr>
<tr>
<td></td>
<td>Daudi</td>
<td>86*</td>
</tr>
<tr>
<td>07</td>
<td>Auto-Tu</td>
<td>42*</td>
</tr>
<tr>
<td></td>
<td>K562</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>Daudi</td>
<td>224</td>
</tr>
<tr>
<td>12</td>
<td>Auto-Tu</td>
<td>47</td>
</tr>
</tbody>
</table>

*LU/10⁶ effector cells.

another week with rIL-2 or rIL-2 plus rIL-4; rIL-4 was added in an attempt to selectively inhibit the development of LAK activity (27). CD5+ lymphocytes cultured with rIL-2 or rIL-2 + rIL-4 showed an impairment of both proliferation (Table 5) and Auto-Tu lysis (Table 7), in comparison with CD5+ lymphocytes maintained in the presence of residual Auto-Tu cells (Tables 5 and 7). At the onset of the third week, aliquots of lymphocytes were restimulated with rIL-2 or rIL-2 plus rIL-4 in the presence, or absence, of newly added fresh irradiated Auto-Tu. CD5+ lymphocytes markedly increased their proliferation when cultured with Auto-Tu plus rIL-2 (Table 5); no significant lysis of allogeneic tumor cells was observed (data not shown), while cytotoxicity against Auto-Tu could not be assessed. At this latter point (21 days), CD5+ lymphocytes were composed of 99% CD3+ cells (49% CD4+ and 51% CD8+) as evaluated by phenotype analysis (data not shown). These data suggest that noninfiltrated lymph nodes contain T-cells reacting in an autologous MLTC and that the presence of both Auto-Tu and rIL-2 is needed for their maximal proliferation and lysis. CD5+ lymphocytes maintained the Auto-Tu-restricted cytotoxicity even after 3 weeks of in vitro culture. rIL-4 did not improve CD5+ Auto-Tu lysis but abrogated the weak cytotoxicity (18% lysis at E/T ratio of 50/1) against allogeneic target cells that the evaluation in LU, however, fails to show.

DISCUSSION

The present results show that in vivo regional rIL-2 administration induces a significant immune reaction in cervical LNLs obtained from patients affected by H&N carcinoma. This response consists of induction of cytotoxic activity against allogeneic and autologous neoplastic target cells. IL-2-induced immunostimulation was not limited to homolateral LNs. In fact, monolateral rIL-2 injection in patients undergoing bilateral dissection induced a lower but significant anti-tumor activity of contralateral LNLs as well. This activation may be mediated by the presence of lymphatic vessels connecting right and left neck regions or by recirculation of activated lymphocytes and preferential migration in H&N LNs. Indeed, a nonrandom distribution of lymphocytes from lymph to LNs was described (28). Nevertheless, a weak increase in LNL spontaneous proliferation was detected. This may be due to the presence of inhibitory factors bound to anesthetics used during surgery, which could exert a differential immunosuppressive role on lymphocytes (25, 26). In fact, data from our laboratory have shown that adding local anesthetics (e.g., lidocaine) in different doses to the culture medium inhibits lymphocyte proliferation but not cytotoxic activity (data not shown). The failure to detect a significant increase in proliferation may also be due to the presence of suppressor T-cells in the invaded LNs from patients with H&N carcinomas (15). A further possibility is that the low proliferation reflects a more general systemic immunodeficiency which is supported by the low or absent NK activity in the PBL of such patients.

Regional injection of rIL-2 induced a marked enhancement of cytotoxicity against K562 and Daudi tumor lines in lymphocytes from JUG and SP nodes, while a lower effect was detectable in SM, suggesting that an additional submental rIL-2 injection could be required to stimulate this lymphatic region. Injection of rIL-2 with 12.5% of human albumin has been chosen to induce preferentially lymphatic, rather than systemic, draining (19), with the aim of obtaining selective activation of cervical LNs. LNL stimulation is probably a local phenomenon, given the fact that systemic LAK activity or toxic effects were not observed. Induction of lymphocyte activation in LNs is also supported by the increase of Leu19+ and CD25+ lymphocytes, i.e., of cells bearing molecules expressed in rIL-2-activated T-cells (4, 23, 24).

Although T-cells with LAK activity have been described (29–31), our data suggest that lysis of K562 and Daudi lines was mainly due to CD16+ (NK-LAK) cells, since CD5+ lymphocytes obtained from IL-2-treated LNLs never showed a significant
cytotoxicity against these tumor targets. The local injection of rIL-2 could stimulate different cellular subsets. Cervical LNs from patients affected by H6N spindle cell carcinoma were reported to contain an increased number of CD8+ cells and a parallel lower number of LAK progenitors (14). The presence of CD3+ and Leu19+ cells in tumor infiltrate (32) or of specific antitumor T-lymphocytes in tumor-draining LNs (15) has been described. It is, therefore, conceivable that regional injection of rIL-2 may have a differential effect on these different subsets of effector cells. To evaluate this possibility we have separated CD3+ CD5+ and CD3 CD16+ cells and tested them against autologous and allogeneic tumor targets in a 4-h 51Cr release assay at E/T ratios of 100/1, 50/1, 25/1, and 12/1.

Clinical implications of these data are evident. Local or regional immunotherapy with IL-2 in animal models showed that injections of IL-2 alone at tumor sites inhibit tumor growth indirectly by triggering nonreactive lymphocytes given together with IL-2 to exert anti-tumor activity (5). Further data showed that immunogenic (but not nonimmunogenic) murine tumors were cured by local injection of IL-2, owing to their ability to attract leukocytes with anti-tumor activity (6). Locoregional immunotherapy with IL-2 or IL-2 plus activated lymphocytes in patients with H&N carcinomas, bladder carcinomas, or cerebral gliomas was also performed with promising results (16, 17, 33, 34). It should be noted that most of these neoplasms are known potentially to bear tumor-specific antigens, as defined by a specific cellular immune response (15, 35).

In the present report, we have explored the possibility of directly stimulating LNLs by regional lymphatic IL-2 treatment. Our results suggest that IL-2 can induce Auto-Tu-restricted T-cells. The activation of such effectors can be instrumental for achieving the eradication of LN micrometastasis and, given the tumor-targeting ability of tumor-specific T-lymphocytes (36), even for attacking the primary lesion. These data can, therefore, contribute to designing novel local immunotherapy studies in cancer patients.

ACKNOWLEDGMENTS

The skillful technical assistance of Adua Marinoni and Edoardo Marchesi is gratefully acknowledged. We thank the Glaxo Institute for Molecular Biology SA for the generous supply of rIL-2 and Dr. Steven Gillis (Immunex Co., Seattle, WA) for providing us with rIL-4.

REFERENCES


<table>
<thead>
<tr>
<th>Target</th>
<th>Medium</th>
<th>Auto-Tu</th>
<th>Auto-Tu + rIL-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>K562</td>
<td>&lt;1#</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Daudi</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>23</td>
</tr>
<tr>
<td>Auto-Tu</td>
<td>1</td>
<td>33.5</td>
<td>326</td>
</tr>
</tbody>
</table>

* LU/10^6 effector cells.

<table>
<thead>
<tr>
<th>Target</th>
<th>rIL-2</th>
<th>rIL-2 + rIL-4</th>
<th>Auto-Tu</th>
<th>Auto-Tu + rIL-2</th>
<th>Auto-Tu + rIL-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>K562</td>
<td>&lt;1*</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Daudi</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Auto-Tu</td>
<td>13</td>
<td>16</td>
<td>144</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>LoVo/Dx</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

* LU/10^6 effector cells.
LOCALLY ACTIVATED LYMPHOCYTES IN HEAD-NECK TUMORS


In Vivo Interleukin 2-induced Activation of Lymphokine-activated Killer Cells and Tumor Cytotoxic T-Cells in Cervical Lymph Nodes of Patients with Head and Neck Tumors

Licia Rivoltini, Carlo Gambacorti-Passerini, Massimo Squadrelli-Saraceno, et al.


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/50/17/5551