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Intratumoral interleukin-2 immunotherapy: activation of tumor-infiltrating and splenic lymphocytes in vivo*

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Abstract. Direct intratumoral injection of interleukin-2 (IL-2) was evaluated in a murine model. Balb/c mice received 5×10^4 Line 1 alveolar carcinoma cells (L1C2) by subcutaneous injection. On the third day following tumor implantation, mice received injections of IL-2 (5×10^3–5×10^4 units) or diluent twice daily, either by i.p. or intratumoral injection, 5 days/week for 3 weeks. Intratumoral injection of 5×10^4 units IL-2 significantly reduced tumor volume ($P < 0.05$ versus control), increased median survival time ($P = 0.0001$), and resulted in a 23.5% cure rate ($P = 0.008$). There were no long-term survivors in the other treatment groups. Both tumor-infiltrating lymphocytes (TIL) and splenic lymphocytes isolated directly from IL-2-treated mice demonstrated enhanced cytolytic activity compared to diluent-treated controls. To determine whether non-T-cell-mediated antitumor responses were active in our model, intratumoral immunotherapy was evaluated in athymic Balb/c *nu/nu* mice. In order to decrease the recruitment of lymphocyte precursors, nude mice were splenectomized and received cyclophosphamide prior to tumor injection and IL-2 therapy. Intratumoral IL-2 immunotherapy also significantly decreased tumor volume in these immunodeficient mice ($P < 0.02$), but did not lead to long-term survival. We conclude that both TIL and splenic lymphocytes are activated in vivo in response to intratumoral IL-2 immunotherapy, suggesting that intratumoral therapy with IL-2 activates both local and systemic antitumor responses.

Key words: Interleukin-2 – Immunotherapy – Tumor-infiltrating lymphocytes

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Introduction

Adoptive transfer of activated lymphocytes and interleukin-2 (IL-2), a 15-kD a lymphocyte activator and T cell growth factor, can reduce tumor burden in mice [6, 24, 26] and in humans [19, 20, 28, 29, 40]. Although adoptive immunotherapy with activated lymphocytes and IL-2 has been demonstrated to have significant antitumor effects against several malignancies, this therapy has been accompanied by severe toxicities that are associated with high doses of IL-2 [12, 15, 17, 25, 28]. In an effort to augment the efficacy of therapy and decrease toxicity, a number of modifications are being evaluated. Important among these alterations is the injection of IL-2 directly into the tumor site.

Direct injection of tumors with IL-2 may have several advantages over systemic infusions. First, lower doses can be used when injecting into the tumor site, thereby decreasing toxicity. Second, following activation in vivo, tumor-infiltrating lymphocytes (TIL) may have antitumor reactivity against metastatic foci as well as against the primary lesion. Local injection of IL-2 at the site of one subcutaneous tumor has been shown to recruit host lymphocytes into the lymph node draining the tumor area [11] and to decrease tumor volume at a distant subcutaneous site [35]. Also, in clinical trials TIL were reactive against metastatic foci following systemic adoptive transfer [18–20, 29]. Third, direct administration of IL-2 may lead to increased local production of IL-2 and other cytokines crucial to the maintenance of an ongoing antitumor immune response [10, 30]. Finally, local injection of IL-2 may directly activate endogenous TIL, leading to an augmentation of cytolytic capacity and lymphokine production [3, 5, 7]. This has been suggested in experimental models that demonstrate that very low doses of IL-2 injected at the tumor site can activate lymphocytes in tumor-bearing mice [11].

The current study evaluates the in vivo activation of TIL and splenic lymphocytes in response to intratumoral IL-2 therapy in a murine model of localized alveolar cell carcinoma. We report that intratumoral immunotherapy mediates antitumor responses with less apparent toxicity and

enhances survival compared to systemic immunotherapy. These responses are accompanied by augmented cytolytic activity by host tumor-infiltrating and splenic lymphocytes.

Materials and methods

Animals. Pathogen-free female Balb/c mice (H-2^d) (8–12 weeks of age) were obtained from NIH and Simenson Laboratories (Gilroy, Calif.). Mice were housed in the Wadsworth Animal Research Facility and permitted access to food and water ad libitum. Nude mice (Balb/c-*nu/nu*) were obtained from the NIH and maintained in cages housed in laminar-flow hoods under pathogen-free conditions. Nude mice were permitted access to sterile water and food ad libitum. In order to decrease the recruitment of T lymphocytes, nude mice were splenectomized and received cyclophosphamide (200 mg/kg) during the week prior to tumor injection and IL-2 therapy [31, 38].

Tumors. Line 1 alveolar cell carcinoma (L1C2), a weakly immunogenic, natural-killer(NK)-resistant, and highly malignant tumor of moderate metastatic potential, which arose spontaneously in a female Balb/c mouse [41], was used to establish the in vivo model. The following tumor lines were obtained from American Type Culture Collection (Rockville, Md.) and utilized for targets in cytotoxicity assays: YAC-1, an NK-sensitive, Moloney-virus-induced lymphoma derived from A/SN (H-2^a) mice; Lewis lung carcinoma (3LL), a highly immunogenic tumor syngeneic to C57BL/6 (H-2^b) mice; and WEHI 164, a 3-methylcholanthrene-induced fibrosarcoma syngeneic to Balb/c (H-2^d) mice.

Schedule of IL-2 immunotherapy. On day 0 mice received 5×10^4 L1C2 cells via subcutaneous injection. On the third day following tumor implantation, mice were randomized to receive injections of either IL-2 ($5 \times 10^3 - 5 \times 10^4$ units) or diluent (D5W), either locally or i.p., twice daily, 5 days/week for 3 weeks. Recombinant human IL-2 (3×10^6 U/mg) was provided by Cetus (Emeryville, Calif.).

Evaluation of tumor growth. Tumor growth was assessed and recorded every other day during therapy. Two bisecting diameters of each tumor were measured with calipers. The volume was calculated using the formula $(0.4)(ab^2)$, with a = larger diameter and b = smaller diameter.

Evaluation of TIL activation. The effects of local IL-2 injections on the in vivo generation of TIL were evaluated using L1C2. Balb/c mice received 10^6 L1C2 cells by subcutaneous injection in the suprascapular area. On the third day following tumor implantation, mice were randomized to receive injections either at the tumor site or i.p. with IL-2 (5×10^4 units) twice daily for 7–9 consecutive days. Control mice were diluent-treated tumor bearers. Following therapy, TIL were isolated from subcutaneous tumors using a modification of a previously described method [4]. Subcutaneous tumors were harvested and minced into 1- to 2-mm pieces. Fragments of tumor were digested at 30-min intervals over a 2-h period in a triple-enzyme mixture containing 0.033% collagenase (type IV), 0.01% hyaluronidase (type V), and 0.002% DNase, type I (Sigma Chemical Co., St. Louis, Mo.). A single-cell suspension was prepared by filtering digested cells through tissue-culture sieves of 200 mesh and 41-μm Nitex. Thy1.2$^+$ lymphocytes were isolated by incubating cells with previously prepared anti-Thy1.2 antibody(PharMingen, San Diego, Calif.)-coated M-450 Dynabeads (Dynal Inc., Great Neck, N. Y.) at a cell : bead ratio of 2 : 1 for 75 min at 4° C. The lymphocyte/bead complexes were extracted with a magnet and cultured at $(5-10) \times 10^6$ cells/ml in complete medium containing RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (Hyclone Laboratories, Logan, Utah), 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 100 μg streptomycin, 100 units penicillin (J. R. H. Scientific, Woodland, Calif.), and 50 μM 2-mercaptoethanol (Sigma Chemical Co., St. Louis, Mo.). Following an overnight incubation in complete medium containing 20 units/ml IL-2, the cytolytic activity of TIL was determined by short-term ^{51}Cr-release assays as described below.

Splenocyte isolation. Splenocytes were obtained by aseptically removing spleens from mice, perfusing with complete medium, and teasing to separate cells from tissue. The suspension was treated briefly with sterile distilled water to deplete erythrocytes.

Lymphocyte cytotoxicity assays. Lysis of tumor cells was measured in 4-h ^{51}Cr-release assays [6]. Target cells were labeled with 100 μCi $Na^{51}CrO_4$ (Amersham Corp., Arlington Heights, Ind.) for 45 min at 37° C, and washed three times with phosphate-buffered saline (PBS). Next, 5×10^3 cells were added to wells that contained various numbers of effector cells in complete medium in U-bottomed 96-well microtiter plates. The plates were incubated for 4 h at 37° C in a humidified atmosphere containing 5% CO_2. The supernatants were harvested and counted in a gamma spectrometer (Packard Instrument Co., Sterling, Va.). Spontaneous isotope release was measured by incubation of the target cells in complete medium alone. Maximum isotope release was produced by incubating target cells in 5% Triton X (Sigma Chemical Co., St. Louis, Mo.). The percentage specific lysis was calculated as:

$$\text{lysis (\%)} = \frac{\text{experimental release (cpm)} - \text{spontaneous release (cpm)}}{\text{maximum release (cpm)} - \text{spontaneous release (cpm)}} \times 100\%$$

All determinations were made in triplicate and calculated as the mean ± SD. One lytic unit of cytotoxic activity was defined as the number of effector cells required to produce 10% specific lysis of 5×10^3 target cells [26].

Analysis of lymphocyte phenotype. TIL were stained for 30 min at 4° C with fluorochrome-conjugated anti-(mouse Ig) mAb, washed twice in PBS, and fixed in 1% paraformaldehyde/PBS. The mAb used included the following: fluorescein-isothiocyanate(FITC)-conjugated anti-Thy1.2, which reacts with murine peripheral T lymphocytes; anti-L3T4-PE (phycoerythrin), which reacts with class-II-MHC-restricted T lymphocytes and macrophages; and anti-Lyt-2-FITC, which reacts with class-I-MHC-restricted T lymphocytes (Caltag Laboratories, S. San Francisco, Calif.). Analysis of the lymphocyte cell-surface membrane phenotype was performed on a FACS Scan cytofluorometer (Becton-Dickinson, Mountain View, Calif.) after gating on the lymphocyte population as judged by its forward-angle (0°) and side-angle (90°C) light-scattering characteristics.

Histology and immunohistology. At least four mice from each treatment group were sacrificed on the 8th day of therapy to assess the accompanying lymphoid response. The tumors were harvested and fixed in 10% buffered formalin, embedded in paraffin, and sectioned at 4–5 μm. A portion of each tumor was harvested for immunohistology. Tissue was frozen rapidly at –25° C in OCT medium (Miles Scientific, Naperville, Ill.), stored at –70° C, sectioned at 4 μm in a cryostat, and stained with an avidin-biotin-immunoperoxidase kit (Vector Laboratories Inc., Burlingame, Calif.). The following primary monoclonal antibodies were used: rat anti-(mouse Thy-1.2) and mouse anti-(mouse H-$2K^d$), which reacts with class I MHC antigens (PharMingen, San Diego, Calif.). Secondary biotin-conjugated antibodies included goat anti-(mouse IgG) and goat anti-(rat IgG) (Jackson ImmunoResearch Laboratories, Inc., West Grove, Pa.).

Statistical analysis. Differences between experimental groups were analyzed using a one-way analysis of variance with repeated measures. Post-hoc analysis was performed using the Bonferroni *t*-test. The Kaplan-Meier method and log rank test were employed for survival analyses. Analysis of complete remission rate was performed using the Fisher's exact test.

Results

The antitumor effect of intratumoral IL-2 therapy

Intratumoral IL-2 immunotherapy with 5×10^4 units IL-2 twice daily significantly reduced tumor volume compared

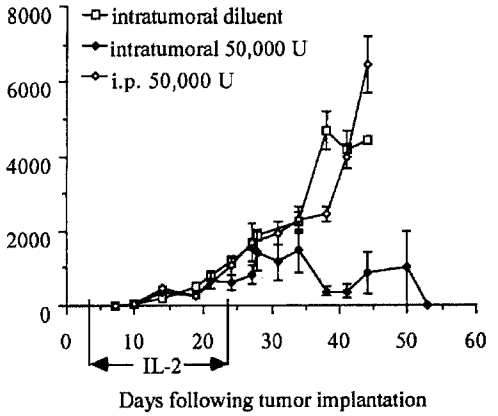

Fig. 1. Immunotherapy of L1C2 by intratumoral or i.p. injection of interleukin-2 (IL-2; 5×10^4 units) or diluent twice daily, 5 days/week for 3 weeks. Mean tumor volumes are shown for each group of 16–18 mice. Intratumoral immunotherapy with 5×10^4 units IL-2 significantly reduced tumor volume compared to both systemic therapy and control ($P < 0.05$)

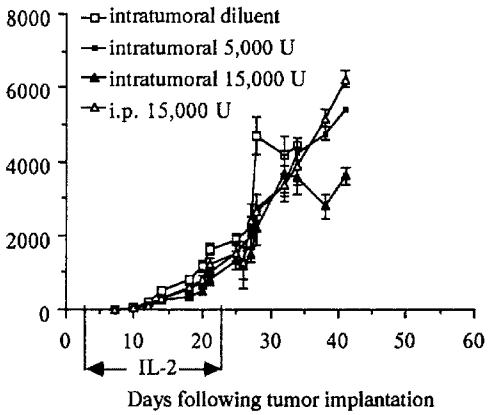

Fig. 3. Immunotherapy of L1C2 by intratumoral or i.p. injection of IL-2 ($5 \times 10^3 - 1.5 \times 10^4$ units) or diluent twice daily, 5 days/week for 3 weeks. Mean tumor volumes are shown for each group of 16–23 mice. Immunotherapy with IL-2 administered intratumorally ($0 - 1.5 \times 10^4$ units) or systemically (1.5×10^4 units) did not significantly alter tumor growth or enhance survival

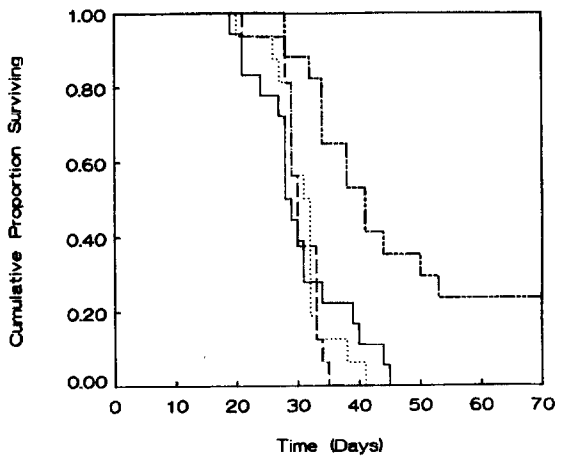

Fig. 2. Survival curves demonstrate a survival benefit for mice treated with intratumoral IL-2 (5×10^4 units) compared to systemic IL-2 and diluent controls. Intratumoral IL-2 enhanced median survival time ($P = 0.0001$) and resulted in a 23.5% cure rate ($P = 0.008$). – – –, intratumoral IL-2; ———, systemic IL-2; – – –, intratumoral diluent; ..., systemic diluent

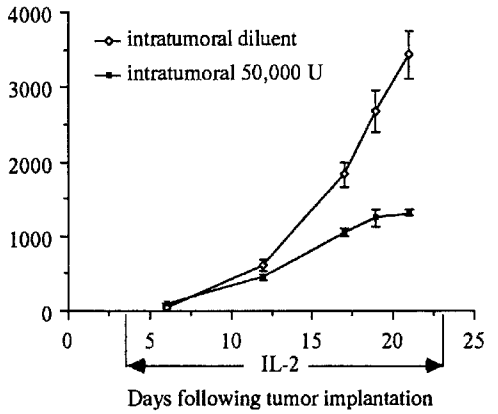

Fig. 4. Immunotherapy of L1C2 in nude mice by intratumoral injection of IL-2 (5×10^4 units) or diluent twice daily, 5 days/week for 3 weeks. Mean tumor volumes are shown for each group of 7 or 8 mice. Intratumoral IL-2 therapy significantly decreased tumor volume compared to the control ($P < 0.02$), but did not enhance survival ($P = 0.21$)

to both systemic therapy and control ($P < 0.05$) (Fig. 1). In addition, mice treated with intratumoral injections of 5×10^4 units IL-2 twice daily had a cure rate of 23.5%, as indicated by survival beyond 150 days without evidence of tumor; there were no long-term survivors in any of the other treatment groups, nor did any of these mice survive past 45 days ($P = 0.008$). The median survival time for mice treated with intratumoral IL-2 (5×10^4 units) also was significantly different from the median survival times for mice treated with systemic IL-2 and for diluent-treated controls ($P = 0.0001$) (Fig. 2). In contrast, IL-2 administered i.p. (5×10^4 or 1.5×10^4 units twice daily) and lower doses of intratumoral IL-2 (1.5×10^4 or 5×10^3 units twice daily) did not significantly reduce tumor growth (Fig. 3) or enhance survival. Both intratumoral IL-2 and diluent therapies appeared to be less toxic than were equal doses of systemic IL-2 therapy. Because tumor volumes in each group were comparable up to day 28, we attributed the increased number of deaths in the systemic IL-2 (5×10^4 units) group prior to this point to IL-2-induced toxicity (mortality at day 28: intratumoral IL-2 = 12%; diluent = 19%; systemic IL-2 = 50%). To determine whether the overall survival advantage for mice treated locally with IL-2 was due exclusively to a decrease in toxicity-associated early deaths, we calculated median survival times for mice surviving past 28 days. We found that there was a significant survival advantage for mice treated locally with IL-2 (5×10^4 units), even when the deaths prior to day 28 were excluded ($P < 0.0001$).

Table 1. Tumor-infiltrating lymphocytes (TIL) and splenocyte cytolytic activity following interleukin-2 (IL-2) immunotherapy[a]

Route of injection	Effector	Cytotoxicity (LU/10^7 cells)[b]			
		L1C2	WEHI164	YAC-1	3LL
Control	Splenocytes	0	22	85	20
Intratumoral IL-2	Splenocytes	213	200	1709	320
i.p. IL-2	Splenocytes	160	ND	2000	ND
Control	TIL	0	0	0	0
Intratumoral IL-2	TIL	40	667	87	80
i.p. IL-2	TIL	20	ND	ND	ND

[a] Following intratumoral or i.p. injection of IL-2 (5×10^4 units) or intratumoral injection of diluent twice daily for 7–9 days, TIL and splenic lymphocytes were isolated and incubated overnight in complete medium containing 20 units/ml IL-2. Cytolytic activity was determined in 4-h ^{51}Cr-release assays. Data presented are representative of three assays

[b] LU, the number of effector cells required for 10% target lysis, expressed as the number of lytic units/10^7 cells. ND, not determined

Table 2. Phenotype of TIL following intratumoral IL-2 immunotherapy[a]

Antibody	Positive cells (%)	
	Diluent	Intratumoral IL-2
Anti-Thy1	5.8	17.0
Anti-L3T4	5.3	5.2
Anti-Lyt2	6.5	18.3

[a] Following intratumoral injection of IL-2 (5×10^4 units) or diluent twice daily for 9 days, TIL were isolated and evaluated by flow cytometry for cell-surface phenotype. Data presented are representative of two assays

The antitumor effect of intratumoral IL-2 therapy in athymic Balb/c-nu/nu mice

In order to evaluate the contribution of non-T-cell-mediated activity to the antitumor response, intratumoral IL-2 therapy was evaluated in athymic Balb/c-*nu/nu* mice. Because previous studies have documented that T cells are present in the nude mouse spleen [33], and that IL-2 can lead to T cell induction in nude mice [37], the mice were splenectomized and received cyclophosphamide (200 mg/kg) during the week prior to tumor injection and IL-2 therapy. This method previously has been demonstrated to abrogate lymphocyte responses in nude mice treated with exogenous IL-2 [31, 38]. Intratumoral IL-2 therapy significantly decreased tumor volume compared to diluent-treated controls ($P < 0.02$) (Fig. 4), but did not enhance survival ($P = 0.21$).

Activation of local and systemic antitumor immune responses in vivo during intratumoral IL-2 immunotherapy

To determine the role of IL-2 immunotherapy in regulating local and systemic antitumor immune responses, the cytolytic capacity of TIL and splenic lymphocytes from treated and control mice was assessed. Compared to diluent-treated controls, intratumoral IL-2 therapy augmented nonspecific cytolytic activity in both spleen and TIL populations, as demonstrated by increased activity against autologous (L1C2), syngeneic (WEHI), allogeneic (3LL), and NK-sensitive (YAC-1) targets (Table 1). Compared to diluent-treated controls, intratumoral IL-2 immunotherapy augmented the percentage of both Thy1$^+$ and Lyt2$^+$ TIL (Table 2).

Histology and immunohistology

Compared to both systemic therapy and controls, intratumoral IL-2 treatment led to increases in the frequency of Thy1$^+$ lymphocytes in the stroma immediately around the tumor. In addition, we saw increased penetration of lymphoid cells into the tumor tissues solely in the group treated with intratumoral IL-2 (Figs. 5, 6). MHC class I expression was not altered in tumors from mice receiving either local or systemic therapy (data not shown).

Discussion

In this study, intratumoral immunotherapy with 5×10^4 units IL-2 significantly reduced tumor volume ($P < 0.05$) (Fig. 1) and enhanced survival ($P = 0.0001$) (Fig. 2) compared to both systemic immunotherapy and controls. In addition to greater tumor reduction, local IL-2 therapy appeared to be less toxic than were equal doses of systemic IL-2 therapy. This was suggested by the increased number of early deaths and decreased median survival time in the group treated systemically with 5×10^4 units of IL-2. The increased number of deaths in the high-dose systemic IL-2 group during the period when tumor volumes in all groups were comparable (up to day 28) suggests that differences in survival up to this point were secondary to treatment-associated toxicities. In addition, we evaluated median survival times for mice surviving past 28 days to investigate the possibility that the survival benefit for intratumoral IL-2 therapy was due solely to a reduction in toxicity-associated early deaths. When these early deaths were excluded, the survival advantage for local IL-2 therapy still was clearly evident ($P < 0.0001$).

It has been shown that, when TIL are isolated, expanded in vitro with IL-2, and administered intravenously, they can mediate significant antitumor activity both in mice [26] and in humans [20, 29]. This form of therapy, however, can

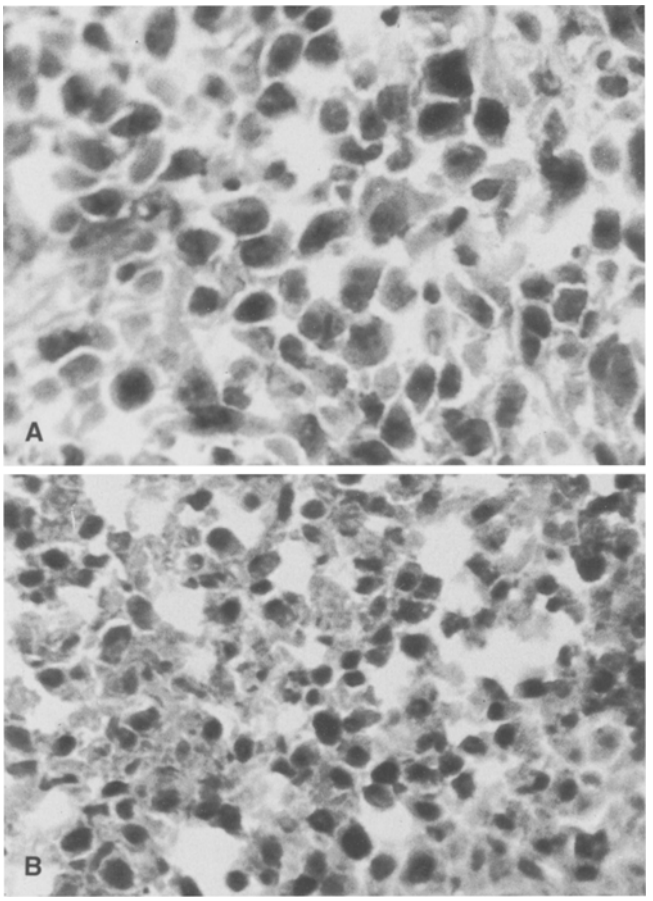

Fig. 5. **A** Tumor histology from a mouse that received intratumoral diluent injections. Lymphocytes are infrequent. H&E stain (×450). **B** Tumor from a mouse that received intratumoral IL-2 injections (5×10^4 units twice daily). Lymphocytes are present at increased frequency. H&E stain (×450)

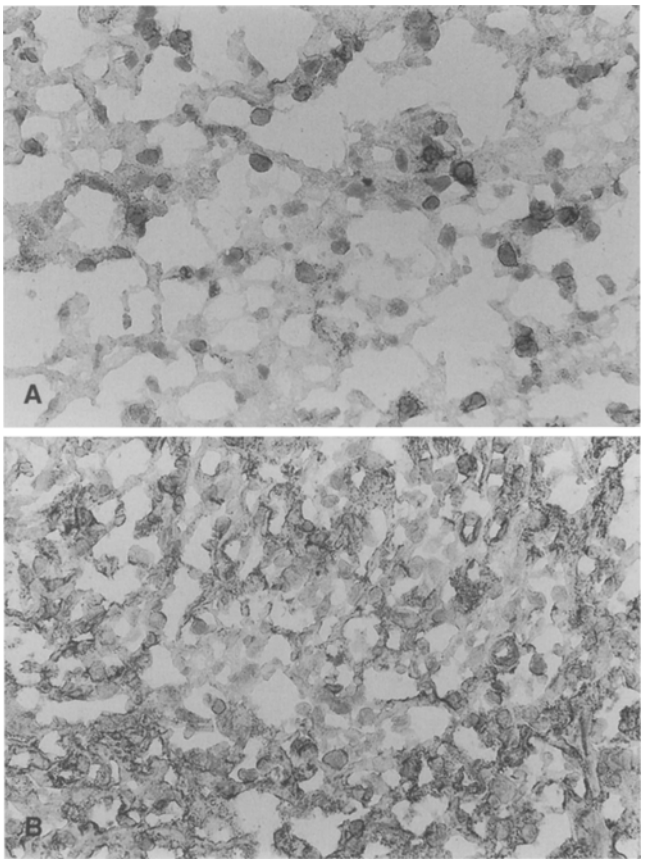

Fig. 6. **A** Scattered Thy1.2+ lymphocytes in the tumor of a mouse that received intratumoral diluent injections. Auidin-biotin-peroxidase complex (ABC) technique (×340). **B** Numerous Thy1.2+ lymphocytes in the tumor of a mouse that received intratumoral IL-2 (5×10^4 units twice daily). ABC technique (×300)

be accompanied by severe toxicities, is expensive, and is often ineffective. Anderson et al. demonstrated that augmentation of TIL NK activity can be achieved by intravenous IL-2 administered to patients with lung cancer prior to tumor resection [1]. The current study is the first to demonstrate that TIL can be activated in vivo by intratumoral injection of IL-2. In situ immunohistological analysis revealed that local IL-2 therapy led to an increased density of $Thy1^+$ lymphocytes in the intratumoral stroma (Fig. 6). In addition, lymphocytes were seen to infiltrate into the tumor proper, maximally within the periphery of the tumor, but also at its center, suggesting that the cells may move from the peritumoral area inwards. Intratumoral immunotherapy was accompanied by augmentation of TIL cytolytic activity (Table 1) and an increase in the percentage of $Thy1^+$ and $Lyt2^+$ TIL isolated directly from the tumor (Table 2). The enhanced activity of TIL following intratumoral IL-2 therapy is consistent with the known paracrine properties of cytokines [32]. In the context of previous studies that have demonstrated the efficacy of local therapy [10, 11, 30, 35], our findings add strong support for the use of intratumoral immunotherapy.

In addition to TIL activation, splenocyte populations also were activated following both local and systemic IL-2 therapy (Table 1). The activation of splenic cytolytic activity indicates the presence of a systemic antitumor immune response. At least three explanations for a systemic response to intratumoral therapy exist: (a) TIL activated at the tumor site in response to therapy may have migrated to the spleen; (b) TIL or macrophages activated at the tumor site in response to therapy may have produced cytokines that subsequently generated a systemic response; (c) IL-2 injected in the intratumoral area may have gained access to the systemic circulation in sufficient quantity to generate a systemic antitumor immune response. Further studies will be necessary to determine which of these mechanisms are operative. The elevation of both local and systemic antitumor immune responses with intratumoral therapy suggests that this form of treatment may play a role in metastatic disease. This is supported by the studies of Vaage [35] and Forni et al. [11], in which local injection of IL-2 at the site of a subcutaneous tumor was shown to decrease tumor volume at a distant subcutaneous site.

Also relevant to the current findings with intratumoral immunotherapy are recent studies that utilize cytokine-gene-transfected murine tumors [2, 8, 9, 13, 14, 16, 22, 34, 39]. Fearon et al. [9] reported that a murine tumor transfected with IL-2 established a cytotoxic T lymphocyte re-

sponse in vivo. This response required CD8+, but not CD4+, populations to maintain an antitumor effect. The authors hypothesized that the host's failure to respond to tumor antigens might be due at least partially to a lack of T cell help. The study was designed to bypass T cell help in vivo by providing a constant local source of IL-2 via the transfected tumor. After subcutaneous injection of the IL-2-producing tumor, specific antitumor activity was found in the spleen following secondary in vitro stimulation. The majority of the in vitro activity was due to activation of MHC-class-I-restricted CD8+ cytotoxic T lymphocytes. After rejection of the IL-2-producing tumor, a specific antitumor immune response was generated in vivo; the parental tumor was rejected on rechallenge. However, a delay in tumor growth was seen when both CD4+ and CD8+ T lymphocytes were depleted. This indicates that a CD4-8-effector cell population also contributed to the antitumor response in the cytokine-gene-transfected model; the authors hypothesized that NK cells, lymphokine-activated killer (LAK) cells or macrophages might be among the cells responsible. Recent studies by Gansbacher et al. [13, 14] confirm the findings of Fearon et al.

To determine whether non-T-cell-mediated antitumor responses were active in our model, we studied local IL-2 therapy in splenectomized, cyclophosphamide-treated nude mice. Intratumoral IL-2 immunotherapy effectively decreased tumor volume in these immunodeficient mice ($P < 0.02$) (Fig. 4) but did not enhance survival ($P = 0.21$). Although intratumoral therapy in immunocompetent mice increased the number and cytolytic activity of lymphocytes infiltrating the tumor, host lymphocytes may not have been entirely responsible for the antitumor response. The inhibition of tumor growth in cyclophosphamide-treated, splenectomized nude mice suggests that non-lymphocyte-mediated mechanisms of tumor inhibition may be operative. These findings are in accord with those of Sacchi et al. [31], who found that intratumoral IL-2 therapy was effective in the treatment of human tumor xenografts in a nude mouse model. It has been suggested that in the absence of host T cells, the efficacy of intratumoral IL-2 therapy may be due to macrophage activation and elaboration of cytokines, such as tumor necrosis factor α (TNFα) [7, 18, 23, 36]. Previously, we have found that the administration of IL-2 to mice gives rise to peritoneal macrophages and blood monocytes that are primed to produce TNFα. Macrophages from IL-2-treated athymic nude mice also responded but produced less TNFα than did those from euthymic mice [21]. However, the fact that IL-2 induces an antitumor response but no increased survival in nude mice suggests that NK cells or other non-specific inflammatory cells may have been operative early, during IL-2 administration, but the long-term curative effect of immune T cells, presumably present in immunocompetent mice, was not operative in nude mice. Further studies are necessary to assess non-T-lymphocyte-mediated mechanisms of tumor reduction with local therapy in immunodeficient models.

In addition to non-T-cell-mediated antitumor responses in nude mice, we found that TIL and splenocytes from the IL-2-treated immunocompetent mice demonstrated cytolytic activity against a broad array of tumor targets. These findings imply that one of the important mechanisms of tumor reduction in our model may be via nonspecific effector populations. This differs from the findings of Gansbacher et al. [13, 14] and Fearon et al. [9], who found a predominance of MHC-restricted killing in cytokine-gene-transfected models. There are at least three possible explanations for this apparent difference between the models. (a) In order to assess in vivo activities directly, we measured cytotoxicity in cells derived directly from tumor and spleen, without in vitro expansion or tumor exposure. Because our studies did not employ secondary in vitro stimulation, it is unlikely that in vitro specificity would be demonstrated. (b) The IL-2 dose administered (5×10^4 units twice daily) in our studies exceeded the amount of IL-2 (<50–400 units) that was estimated by Gansbacher to have been produced by the IL-2-transfected tumor. The higher intermittent dose may have preferentially increased non-MHC-restricted killing. (c) The L1C2 tumor may be less immunogenic than were those used by Gansbacher et al. and Fearon et al. In more recent preliminary studies using L1C2, we have found that the intratumoral administration of interferonγ in addition to IL-2 leads to increased complete antitumor responses, up-regulation of tumor MHC class I expression, and long-term specific antitumor immunity.

We conclude that both TIL and splenic lymphocytes are activated in vivo in response to intratumoral IL-2 immunotherapy, suggesting that intratumoral therapy with IL-2 activates both local and systemic antitumor responses. Intratumoral therapy was significantly more effective than equivalent-dose systemic therapy in promoting tumor reduction and enhancing survival. In addition, there appeared to be less toxicity associated with intratumoral therapy. The ability to activate TIL effectively in vivo without in vitro expansion could greatly reduce the morbidity and expense of cellular adoptive immunotherapy. Future clinical trials may utilize the intratumoral administration of cytokines to augment the generation and antitumor efficacy of TIL in vivo.

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