Activation of Human Tumor-infiltrating Lymphocytes by Monoclonal Antibodies Directed to the CD3 Complex

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ABSTRACT

We have used high concentrations of recombinant-methionyl human interleukin 2 (rIL-2) for the initial growth and expansion of human tumor-infiltrating lymphocytes (TIL). Early in the life of the TIL bulk culture, cytotoxicity was non-major histocompatibility complex restricted. Under these culture conditions antitumor cytotoxicity was observed to decline with increasing age of the bulk culture. In addition, TIL became refractory to rIL-2-induced expansion. We have used solid-phase anti-CD3 antibodies for TIL activation followed by culture in reduced concentrations of rIL-2 to reactivate TIL previously grown in high concentrations of rIL-2. TIL refractory to rIL-2 in terms of growth and antitumor cytotoxicity proved sensitive to anti-CD3 activation. The use of solid-phase anti-CD3 was also more effective than high concentrations of rIL-2 in the expansion of TIL when used at the start of culture. Finally, TIL could be induced to secrete IL-2 following solid-phase activation with anti-CD3. These data suggest that human TIL are susceptible to activation by signals directed at the CD3 complex of the TIL cell surface.

INTRODUCTION

The initial accounts of the cellular immunotherapy for metastatic cancer in humans, which used rIL-2-activated PBMC as effectors (LAK cells), have been reported (1-3) and are now yielding to second-generation immunotherapy protocols in which alternative lymphocyte populations with antitumor cytotoxicity are being evaluated (4-6). Lymphocytes present in solid tumor at the time of surgery can be isolated from both murine (7) and human (8) tumors and activated in vitro with high concentrations of rIL-2. Furthermore, TIL cultured in high concentrations of rIL-2 for several weeks to the point where rIL-2-induced proliferation and cytotoxicity abated, could be activated by alternative signals elicited by solid-phase anti-CD3.

MATERIALS AND METHODS

Human Tumor. Discarded human tumor was obtained from the Department of Pathology, Brigham and Women’s Hospital. Tumor was divided with scissors into 2- x 2-mm pieces and further dissociated with an enzyme solution composed of hyaluronidase (0.1%; Sigma Chemical Co., St. Louis, MO), collagenase (2.5 units/ml; Sigma), DNase (0.01%; Sigma), and 1 mM (4-2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (Grand Island Biologicals, Grand Island, NY) dissolved in HBSS (Whittaker, M.A. Bioproducts, MD). The tumor fragments were magnetically stirred for 3 to 4 h at room temperature. Undigested material was allowed to settle and the cell suspension was aspirated into a 50-ml tube (Becton Dickinson Labware, Lincoln Park, NJ). The cells were washed several times with HBSS and counted by Trypan blue (Gibco) exclusion. Tumor cells were resuspended in RPMI-1640 (M.A. Bioproducts) supplemented with 10% type AB serum, 50 units/ml penicillin/50 μg/ml streptomycin (Gibco), and 2 mM L-glutamine (Gibco).

Isolation of TIL. Single cell suspensions of tumor were cultured at a density of 1-5 x 10^6 tumor cells/ml in T-25 culture flasks (Corning Glass Works, Corning, NY) with 1000 units/ml rIL-2 (Ala 125; Ortho Pharmaceutical Corporation; Raritan, NJ). Lymphocyte growth was monitored biweekly and cultures were split as needed by using rIL-2-supplemented RPMI-1640. When required, cellular debris was removed from bulk cultures by centrifugation over a Ficoll (lymphocyte separation medium; Litton Bionetics, Charleston, SC) density gradient.

Solid-Phase Activation. Monoclonal anti-CD3 (generously provided by Ortho) was diluted to a concentration of 1 μg/ml with sterile 0.05 M borate buffer, pH 8.6. Diluted antibody was washed over the surface of a T-25 flask or the well of a microtiter plate and stored in a refrigerator. The wells of microtiter plates were coated with monoclonal antibodies directed to the human CD3 molecule and the cells were plated into either 6-well plates (Costar, Cambridge, MA) or T-25 flasks in RPMI-1640. IL-2 was added to a concentration of 50 units/ml.

IL-2 Assay. Cell culture supernatants of 3- to 5-week-old TIL bulk cultures activated on CD3-coated microtiter plates (Costar) (1 x 10^6 cells/well) were obtained after 48 h. These were assessed for IL-2 by using the IL-2-dependent cell line CTLL-2 in a 24-h assay (14). Standard curves were prepared with the Biologic Response Modifiers Program reference reagent human IL-2 derived from the Jurkat cell line (Biological Resources Branch, NCI-Frederick Cancer Research Facility, Frederick, MD). Results were calculated by logit analysis (15).
Flow Cytometry. Lymphocyte surface antigens were assessed with monoclonal antibodies to CD3, CD4, CD8 (fluorescein conjugates: Ortho), Leu-11b, WT31, and CD25 (Recton Dickinson Immunocytometry Systems, Mountain View, CA). One million cells suspended in HBSS (without phenol red) plus 5% fetal calf serum and 0.1% sodium azide (Sigma) were incubated with each monoclonal antibody and placed in ice for 30 min. Cells were washed and fixed in 1% paraformaldehyde (Sigma) and stored at 4°C. Flow cytometric analysis of stained lymphocytes was performed on a Coulter Epics C cytometer (Coulter Electronics, Hialeah, FL).

Proliferative Assays. TIL growth was monitored by incubating cells (1 × 10^5/well) in a 96-well plate (Costar) with different concentrations of rIL-2 for 5 days followed by an 18-h pulse with [³H]thymidine (0.5 μCi; Du Pont NEN Research, Boston, MA). Cells were harvested onto glass fiber strips (PHD Cell Harvester; Cambridge Technology, Inc., Watertown, MA). Filters were dried and counted in liquid scintillation fluid (Du Pont NEN) with a scintillation counter (LKB RackBeta 1212, Finland).

Cytotoxicity. Cytotoxicity against tumor targets was estimated in 4-h ^51Cr-release assays. Autologous and heterologous tumor, and the cell lines Daudi (American Type Culture Collection, Rockville, MD) and K562 (ATCC) were labeled for 60 min at 37°C with chromium (200 μCi; NEN), washed, and reincubated for an additional 30 min. Target cells (5 × 10^4) were incubated with various numbers of effector cells at 37°C. The supernatant was collected (Supernatant Collection System; Skatron, Inc., Sterling, VA) and the samples were counted in a gamma counter (GammaTrac 1191, TM Analytic; Elk Grove Village, IL). Results were converted to percentage of specific release:

\[
\text{% of specific release} = \frac{\text{cpm(test)} - \text{cpm(background)}}{\text{cpm(total)} - \text{cpm(background)}} \times 100
\]

Spontaneous release of chromium was always less than 30% for tumor cell lines and fresh tumor. Positive effector cell controls for each cytotoxicity assay consisted of human lymphokine-activated killer cells. LAK cells were generated from normal donors by incubating 2 × 10^6 cells/ml peripheral blood mononuclear cells, suspended in RPMI-1640 for 3 days in 1000 units/ml rIL-2 (3).

RESULTS

Long-Term Culture of TIL in rIL-2. Human TIL isolated and expanded under standard conditions which include activation with 1000 units/ml rIL-2 displayed a characteristic pattern of cytotoxicity (Fig. 1). In these examples of long-term bulk culture growth, cytotoxicity early in the life of the bulk culture was always observed against a variety of tumor cell lines and fresh tumor targets. The vigorous antitumor responses cannot be distinguished from those which characterize the rIL-2-induced cytotoxicity of peripheral blood mononuclear cells. As the age of the TIL bulk culture increased a second feature emerged. The capacity of the bulk culture to kill autologous fresh tumor as well as nonspecifically kill the LAK cell targets Daudi and K562 diminished considerably and eventually became extinguished. As bulk cultures became refractory to rIL-2-induced cytotoxicity they also lost the ability to proliferate and became much more difficult to expand (see below).

TIL Proliferation. The growth of TIL is linked to the extracellular concentration of rIL-2. TIL cultured in various concentrations of rIL-2 were observed to increase thymidine incorporation in a dose-dependent manner (Fig. 2). Maximum TIL proliferation required between 200 and 1000 units/ml rIL-2, with only modest proliferation observed at concentrations below 100 units/ml. Individual TIL bulk cultures also displayed differences in responsiveness to rIL-2-induced proliferation over the entire concentration range of exogenous rIL-2 with one (renal) TIL population relatively insensitive to low concentrations of rIL-2 and the other (renal) sensitive to rIL-2-induced growth. Continued maintenance of TIL in 1000 units/ml rIL-2 eventually resulted in a refractory state in which cells failed to thrive and continued expansion stops. To determine whether TIL growth could be sustained beyond this barrier, TIL which failed to proliferate to rIL-2 were subjected to activation with solid-phase anti-CD3. In these experiments TIL isolated and grown in 1000 units/ml rIL-2 were monitored for cell growth
against an aliquot of TIL from the same bulk culture which were stimulated for 48 h in the absence of exogenous rIL-2 on solid-phase anti-CD3, followed by 96 h in 50 units/ml rIL-2 (Table 1). In each case TIL maintained in 1000 units/ml rIL-2 failed to significantly increase in cell number, consistent with IL-2 unresponsiveness. TIL cultured on solid-phase anti-CD3 consistently demonstrated significantly higher cell yields (P < 0.05). The increased growth advantage observed with anti-CD3 was not simply a manifestation of different culture conditions unrelated to the use of anti-CD3, since TIL cultured in exactly the same way without solid-phase anti-CD3 followed by culture in 50 units/ml rIL-2 failed to grow (not shown).

The TIL used in these experiments were derived from the same bulk culture following prolonged growth in rIL-2 but differences in cell growth were apparent. To determine whether the discrepancies in growth were related to differential expression of IL-2 receptor, TIL were stained with an antibody (anti-CD25) to the p55 chain of the IL-2 receptor. Cells maintained in 1000 units/ml rIL-2 expressed little or no p55 (Table 2). In contrast, cells from the same bulk population subjected to anti-CD3 activation and cultured in low-dose rIL-2 revealed that significantly more TIL displayed the p55 chain (P < 0.05). Thus, the expression of p55 was found to be greatly reduced in poorly proliferating TIL populations cocultured with high concentrations of rIL-2. In control experiments, TIL were cultured on identical but noncoated surfaces (data not shown), suggesting that anti-CD3 activation induces the expression of the p75 receptor for high-affinity IL-2 receptor.

Anti-CD3-aided TIL Isolation. To determine the usefulness of solid-phase anti-CD3 for the isolation of human TIL, the growth of TIL using high-dose rIL-2 or solid-phase anti-CD3 was compared. We assessed 8 consecutive tumor samples submitted for the culture of TIL (Table 3). Tumor cell suspensions were incubated in flask precoated with anti-CD3 for 48 h then expanded in 50 units/ml rIL-2. Companion cultures were set up in noncoated flasks using 1000 units/ml rIL-2. Cells from both groups were fed after 1 week and TIL growth was measured.

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Standard culture</th>
<th>Anti-CD3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal</td>
<td>67</td>
<td>167</td>
</tr>
<tr>
<td>Renal</td>
<td>84</td>
<td>360</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>62</td>
<td>115</td>
</tr>
<tr>
<td>Melanoma</td>
<td>24</td>
<td>339</td>
</tr>
<tr>
<td>Ovarian</td>
<td>100</td>
<td>237</td>
</tr>
<tr>
<td>Colon</td>
<td>40</td>
<td>115</td>
</tr>
</tbody>
</table>

Table 2 Comparison of CD25 (p55) expression by TIL grown in rIL-2 or activated by anti-CD3

<table>
<thead>
<tr>
<th>Tumor histology</th>
<th>rIL-2</th>
<th>Anti-CD3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>&lt;5</td>
<td>65</td>
</tr>
<tr>
<td>Renal cell</td>
<td>&lt;5</td>
<td>80</td>
</tr>
<tr>
<td>Ovarian</td>
<td>13</td>
<td>72</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>&lt;5</td>
<td>95</td>
</tr>
</tbody>
</table>

* Tumor histology from which TIL bulk cultures were derived.

Table 3 Comparison of anti-CD3-induced TIL growth with standard conditions 2 weeks following culture initiation

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Standard</th>
<th>Anti-CD3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal</td>
<td>1.2 x 10^6 (1)</td>
<td>7.0 x 10^4 (12)</td>
</tr>
<tr>
<td>Renal</td>
<td>3.0 x 10^6 (1)</td>
<td>9.6 x 10^4 (4)</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>8.0 x 10^6 (3)</td>
<td>1.0 x 10^4 (4)</td>
</tr>
<tr>
<td>Renal</td>
<td>2.9 x 10^6 (10)</td>
<td>1.6 x 10^4 (57)</td>
</tr>
<tr>
<td>Renal</td>
<td>1.3 x 10^6 (17)</td>
<td>1.3 x 10^3 (17)</td>
</tr>
<tr>
<td>Uterine</td>
<td>8.8 x 10^6 (ND)</td>
<td>2.7 x 10^4 (ND)</td>
</tr>
<tr>
<td>Breast</td>
<td>4.4 x 10^6 (4)</td>
<td>3.5 x 10^4 (3)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>7.2 x 10^6 (15)</td>
<td>1.3 x 10^4 (26)</td>
</tr>
</tbody>
</table>

* Four million tumor cells were plated onto T-25 flasks. Cells plated on flasks coated with anti-CD3 were transferred to uncoated T-25 flasks after 48 h and incubated with 50 units/ml rIL-2. All cells were harvested after 7 days and incubated with fresh medium. Cell were counted after 14 days and the total number of TIL harvested are reported.

This number represents the fold increase between the number of lymphocytes plated at the start of culture and those harvested after 2 weeks.

Table 4 Production of IL-2 by TIL following activation by solid-phase anti-CD3

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Standard culture</th>
<th>Anti-CD3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal cell</td>
<td>&lt;0.1</td>
<td>28</td>
</tr>
<tr>
<td>Melanoma</td>
<td>&lt;0.1</td>
<td>9</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>&lt;0.1</td>
<td>62</td>
</tr>
<tr>
<td>Ovarian</td>
<td>&lt;0.1</td>
<td>31</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>&lt;0.1</td>
<td>64</td>
</tr>
</tbody>
</table>

* IL-2 half-maximal units/ml. Supernatants were collected from TIL cultures 48 h following anti-CD3 activation. Standard culture refers to control cultures incubated in the absence of exogenous rIL-2.

by trypan blue exclusion 2 weeks following the start of culture. TIL proliferation was enhanced in 6 of the 8 samples where solid-phase anti-CD3 was used to initiate bulk cultures (P < 0.05). In 2 cases where TIL growth was not enhanced, bulk culture growth was at least as good as standard conditions despite the use of much less rIL-2 in the cell cultures. Additional interest is focused on the first 2 samples studied in this series. Both cultures failed to respond to high-dose rIL-2 in terms of cell growth, yet both cultures responded to anti-CD3 activation.

Cytokine Secretion. TIL bulk cultures maintained in 1000 units/ml rIL-2 could be further distinguished from TIL activated by solid-phase anti-CD3. Short-term TIL cultured exclusively in 1000 units/ml rIL-2 were counted and resuspended in rIL-2-free RPMI-1640 and dispensed into microtiter wells either untreated or coated with anti-CD3 and assessed for the release of rIL-2 into the culture supernatant after 48 h. We were unable to detect IL-2 in the supernatants of TIL cultured exclusively in rIL-2 (Table 4). In contrast, cells activated with anti-CD3 produced IL-2 measurable in the culture supernatants 48 h after anti-CD3 (P < 0.05). No differences in bulk culture phenotype were observed following anti-CD3 activation (not shown).

Anti-CD3-induced Activation. As improvements are made in the ability to sustain bulk culture growth for 2-3 months or more one can observe the emergence of phenotypically stable TIL bulk populations dominated by CD8+ cells. A typical example of a TIL bulk population cultured for more than 8 weeks with anti-CD3 activation reveals a culture composed of CD3+, CD8+, and T-cell antigen receptor-positive (as defined by reactivity with WT31) lymphocytes, but lacking CD4 and CD16 (Fig. 3). We have assessed TIL bulk cultures possessing this phenotypic profile for cytotoxicity against K562, Daudi, autologous fresh tumor, and histologically matched heterologous fresh tumor, at a time when tumor-specific cytotoxicity and cell proliferation were low (age of bulk cultures ranged from 7 to 20 weeks; Table 5). Several patterns of tumoricidal activity were observed. The first cultures activated with solid-
phase anti-CD3 followed by culture in a reduced concentration of rIL-2 demonstrated cytotoxicity that was not accompanied by large increases in killing of Daudi, K562, and heterologous tumor, although autologous cell killing could be demonstrated. Long-term CD4+ TIL bulk cultures failed to demonstrate enhanced antitumor cytotoxicity following anti-CD3 activation, suggesting a difference in susceptibility between CD4 and CD8 populations to anti-CD3-induced cytotoxicity.

**DISCUSSION**

Human tumor-infiltrating lymphocytes have been isolated and expanded with techniques which depend on the addition of exogenous rIL-2 and studied as potential lymphocyte populations for use in the adoptive cellular immunotherapy of metastatic cancer (5, 6, 12). Our experience with the isolation, early growth, and expansion of human TIL with high concentrations of rIL-2 revealed inconsistencies with the early growth and expansion of TIL to therapeutic numbers. Previous work has demonstrated the capability of CD3 antibodies to activate normal peripheral blood lymphocytes or T-cell clones to proliferate, secrete cytokines, become cytotoxic, and express IL-2 receptors (16-21). These studies indicated that feeder cells were required for optimal cell activation (16, 22-23). As a result, we evaluated an alternative method of activating human TIL by using solid-phase anti-CD3 without the addition of feeder cells. Specifically, we found that in 2 of 8 consecutive clinical samples, rIL-2-induced growth of TIL failed after 2 weeks in culture. In contrast, identical samples activated on solid-phase anti-CD3 for 48 h and transferred to a low concentration of rIL-2 demonstrated a vigorous capacity to proliferate. These observations reveal that not all clinical samples respond to 1000 units/ml rIL-2 despite the presence of lymphocytes in the sample, and are similar to observations of others which suggest the potential of tumor-derived suppression of T-cell activation (24). These studies substantiate the fact that alternative activation signals in the form of CD3-specific antibodies are efficient in rendering lymphocytes, present in freshly resected tumor, responsive to growth in an environment of reduced exogenous rIL-2.

A second difficulty inherent in the development of TIL is that rIL-2-induced expansion of TIL must sustain growth up to $10^{10}$-10$^9$ lymphocytes. We have extended previous observations that the growth of TIL is dependent on high extracellular concentrations of rIL-2 for maximum cell proliferation by describing how, in the course of growing these cells, they become unresponsive to rIL-2 in terms of cell proliferation and tumoricidal activity. Our analysis shows that the majority of TIL maintained in an environment of high rIL-2 concentrations fail to express the p55 chain of the high-affinity IL-2 receptor and do not proliferate. This seriously jeopardizes the number of cells available for adoptive transfer. Periodic stimulation with autologous tumor cells can be shown in some cases to overcome both of these difficulties. However, serial stimulation of TIL bulk cultures with autologous tumor requires manipulation of TIL bulk cultures as well as frozen tumor, both of which are subject to the introduction of microbial agents. In addition, the availability of sufficient quantities of tumor for serial stimulation is highly variable from patient to patient. This constraint makes it impossible to routinely use serial tumor stimulation and is one issue which prompted the present study.

Tac antigen expression has been described in populations of normal purified T-cells following stimulation with phorbol esters, plant mitogens, and anti-CD3 (20). In the present study, increased TIL expression of p55 was demonstrated following withdrawal from exogenous IL-2. However, only when TIL were cultured on solid-phase anti-CD3 (in the absence of rIL-2) did we observe optimal proliferation in response to 50 units/ml rIL-2. These results imply that CD3 activation altered p75 expression such that more high-affinity IL-2 receptor was expressed following this treatment, and additional work is in progress to clarify this issue. These data are consistent with a model of TIL activation which, under standard conditions, depends on high concentrations of rIL-2 in order to induce proliferation of TIL which bear little high-affinity IL-2 receptor. Smith et al. (25) have shown that the p75 chain of the IL-2

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**Table 5 Comparison of cytotoxicity following long-term culture in rIL-2 or anti-CD3**

<table>
<thead>
<tr>
<th>Tumor</th>
<th>CD4/CD8 ratio</th>
<th>Daudi</th>
<th>K562</th>
<th>Autologous</th>
<th>Heterologous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal</td>
<td>&lt;1</td>
<td>32</td>
<td>5</td>
<td>36</td>
<td>7</td>
</tr>
<tr>
<td>Ovarian</td>
<td>&lt;1</td>
<td>2</td>
<td>2</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>Melanoma</td>
<td>&lt;1</td>
<td>2</td>
<td>2</td>
<td>36</td>
<td>8</td>
</tr>
<tr>
<td>Renal</td>
<td>&lt;1</td>
<td>91</td>
<td>50</td>
<td>86</td>
<td>89</td>
</tr>
<tr>
<td>Ovarian</td>
<td>&gt;1</td>
<td>47</td>
<td>13</td>
<td>49</td>
<td>6</td>
</tr>
<tr>
<td>Breast</td>
<td>&gt;1</td>
<td>25</td>
<td>5</td>
<td>72</td>
<td>21</td>
</tr>
</tbody>
</table>

*a* Histology of tumor from which TIL were isolated.

*b* The CD4:CD8 ratio was determined by flow cytometric analysis.

*c* TIL were grown in 1000 units/ml rIL-2.

*d* TIL were activated with solid-phase anti-CD3.

*Percentage of specific release at an effector:target ratio of 20:1.*
association constant of IL-2 for the p75 chain is 2-3 orders of magnitude lower than that of the p55 chain and as a consequence, much higher concentrations of rIL-2 are required to drive proliferation. The observation that culture of TIL in reduced concentrations of IL-2 following anti-CD3 activation results in greater cell proliferation, is compatible with our observations that the bulk population expresses high-affinity IL-2 receptor (as judged by p55 chain expression). As expected, these cell populations which express high-affinity IL-2 receptor require lower concentrations of IL-2 for growth.

Culture of TIL on solid-phase anti-CD3 also resulted in the secretion of IL-2 into the culture supernatant. TIL from relatively short-term bulk cultures grown in 1000 units/ml rIL-2 were divided into 2 groups and resuspended in RPMI-1640 without exogenous IL-2. One group was cultured on anti-CD3-coated surfaces and the other was cultured on noncoated surfaces. After 48 h supernatants were removed and assessed for IL-2. TIL cultured on solid-phase anti-CD3 produced IL-2 in agreement with results observed by others (17). If cells were cultured under similar conditions without anti-CD3 spontaneous production of IL-2 was not observed. These findings suggest that anti-CD3 activation of TIL bulk cultures which include CD4+ cells may also serve as an efficient means of activating noncytotoxic populations of TIL whose role may be as important as cytotoxic populations for successful immunotherapy.

In agreement with others, we find that highly cytotoxic cells can be isolated from solid tumor and expanded with rIL-2 (26). However, activation of bulk populations by high concentrations of exogenous rIL-2 typically results in an early phase of antitumor cytotoxicity that closely resembles that of LAK cell-mediated cytotoxicity because of its lack of tumor specificity (27). With time a second pattern emerges in which cytotoxicity against targets such as Daudi, K562, as well as autologous and heterologous tumor declines and eventually becomes difficult to demonstrate against any fresh tumor or tumor cell line. Thus, TIL unresponsive to rIL-2-induced growth and cytotoxicity were cultured on solid-phase anti-CD3 and tested for cytotoxicity against a variety of tumor targets including autologous tumor. We found that cells cultured under conventional conditions which eventually failed to demonstrate cytotoxicity against autologous tumor retained tumoricidal capability following anti-CD3 activation. In some cases the cytotoxic responses were relatively confined to autologous tumor. Similar long-term TIL bulk cultures composed of CD4+ TIL failed to mount tumoricidal activity following anti-CD3 activity. These observations are consistent with earlier findings where antibody redirected lysis of target cells utilizing CD4+ TIL and WT-31 (anti-T-cell antigen receptor) antibody failed to induce lysis of Daudi (28). The relatively modest levels of in vitro autologous tumor cell killing by TIL following anti-CD3 activation indicates that the bulk population contains cells capable of mounting antitumor cytotoxic responses even though cytotoxicity in parallel cultures grown in rIL-2 alone are difficult to demonstrate. This suggests that conventional cytotoxicity assays may not necessarily predict the tumoricidal capacity of CD8+ bulk populations since antibody redirected lysis, but not 4-h chromium release assays, demonstrated the lytic potential of long-term cultures of TIL maintained in IL-2, following interaction with either anti-CD3 or WT-31 (28). The observations reported here are similar to those of studies which used soluble anti-CD3 antibodies to induce augmented cytotoxicity in peripheral blood mononuclear cell populations activated with IL-2 in humans and mice (29–31).

We have also investigated the therapeutic potential of murine TIL bulk cultures which also possess the cytotoxic/suppressor (Lyt-2+) phenotype. We have found no evidence for suppressor activity, despite variability in in vitro cytotoxicity, since such populations are reproducibly effective in mediating regression of metastatic pulmonary disease. Collectively, these results suggest that TIL activation by solid-phase antibodies to CD3 prior to adoptive transfer may provide one important link to improved adoptive immunotherapy, since immunologically active cells, in terms of cell growth and cytotoxicity, are available for infusion. In addition, the activation of TIL on anti-CD3-coated surfaces early in the life of a bulk culture may facilitate the selective expansion of tumor-specific cytotoxic T-cell precursors at the expense of tumor nonspecific NK cells which have been reported to expand in certain TIL bulk cultures under conditions of high-dose rIL-2 (32). The use of solid-phase anti-CD3 is useful because it provides a simple and reproducible stimulus for T-cell growth and does not require the procurement and utilization of accessory cells and an often limited supply of autologous tumor which are required for the serial activation of TIL.

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REFERENCES


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