**ABSTRACT**

Lymph nodes draining the progressively growing, weakly immunogenic MCA 105 sarcoma contained tumor-sensitized but not fully functional pre-effector T-cells. These cells could further differentiate to acquire full antitumor effector function for adoptive therapy in an established in vitro sensitization (IVS) procedure. In this study, we utilized selective depletion with antibodies of lymphocyte subsets bearing the L3T4 (CD4) or Lyt-2 (CD8) antigen and of cells bearing the asialo-GM1, (ASGM-1) glycosphingolipid to identify the phenotype of pre-effector cells elicited during progressive tumor growth. Cells from lymph nodes draining a progressive MCA 105 tumor in the footpad were treated with antibodies plus complement prior to IVS. The antitumor efficacy of resulting IVS cells was assessed in adoptive therapy of 3-day established pulmonary MCA 105 metastases. Depletion of Lyt-2+ cells eliminated in vivo antitumor reactivity with concurrent elimination of in vitro cytotoxic activity against the MCA 105 tumor, whereas depletion of L3T4+ cells did not have an impact on either in vivo or in vitro antitumor reactivities. Treatment with ASGM-1 antiserum plus complement was also found to abrogate therapeutic efficacy. However, the in vitro cytotoxic activity was not affected. These results indicate that the pre-effector cells were Lyt-2+, L3T4+, and ASGM-1+. We next examined whether the sensitization of pre-effector cells in vivo required the participation of L3T4+ helper cells. To approach this, mice were depleted of L3T4+, Lyt-2+, or ASGM-1+ cells by antibody injections before tumor inoculation. Treatment with Lyt-2 monoclonal antibody abrogated the pre-effector cell response in the draining lymph nodes, as evidenced by failure to generate therapeutically effective cells following IVS. On the other hand, neither L3T4 nor ASGM-1 antibody treatment affected the generation of pre-effector cells. Thus, sensitization of Lyt-2+ pre-effector cells in response to progressive tumor occurred in the absence of L3T4+ helper cells.

**INTRODUCTION**

The most critical component of successful adoptive immunotherapy of cancer is the identification and isolation of lymphocytes with potent antitumor effects. In studies with transplantable tumors, lymphocytes from hyperimmunized syngeneic animals represent such cell populations and provided a convenient source of antitumor effector cells (1–5). However, this immunization technique of obtaining immunologically reactive lymphocytes, although helpful in demonstrating the potential of cellular therapy, is poorly suited for use in humans. It has, therefore, been predicted that the application of adoptive immunotherapy for human cancer will require the development of culture techniques that can allow stimulation and expansion of antitumor lymphocytes from cancer patients.

The discovery of T-cell growth factor, now termed IL-2, and subsequent availability of large quantities of purified recombinant material seemed to have provided a means for in vitro expansion of sensitized T-cells (6, 7). Early experiments using the highly immunogenic FBL-3 leukemia have demonstrated the therapeutic efficacy of IL-2-expanded immune lymphocytes (8, 9). More recently, we have established another culture system utilizing IL-2 for propagating immune T-lymphocytes with reactivity against the weakly immunogenic MCA 105 and MCA 106 sarcomas (10). The development of this IVS technique has helped to establish conditions necessary for expanding T-lymphocytes while maintaining their antitumor reactivity. This method was originally thought to facilitate clonal expansion of previously sensitized immune effector cells. However, phenotype analyses of cells before and after IVS revealed that, in addition to cellular expansion, the culture system induced differentiation of antitumor effector cells (11).

Because of these observations, an attempt was made to explore the feasibility of generating therapeutically effective lymphocytes from mice bearing progressive tumors. Unlike lymphoid cells from tumor-immunized animals, freshly isolated lymphocytes from tumor-bearing mice exhibited little or no antitumor effects when tested in adoptive immunotherapy experiments. However, after secondary IVS, recovered cells demonstrated potent in vivo antitumor activity (12–14). Since similar cells could not be generated from normal lymphocytes, progressive tumor growth must have triggered the production of cells that were sensitized to the tumor but not fully functional immunologically. These cells were then referred to as "pre-effector" cells.

In a previous study, the antitumor effector cells generated by IVS have been identified as belonging to the Lyt-2 phenotype of T-lymphocytes (12). However, the nature of pre-effector cells elicited in response to tumor growth was poorly defined. In this study, we have used the MCA 105 sarcoma to analyze the phenotype of pre-effector cells and the cellular requirement during IVS for induction of functional effector lymphocytes. More importantly, we utilized a previously established method of in vivo depletion of lymphocyte subsets to investigate the immunological mechanisms underlying the sensitization of pre-effector cells during progressive tumor growth.

**MATERIALS AND METHODS**

Mice. Female B6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Caged in groups of 6 or fewer, they were maintained in a specific pathogen-free environment and were used for experiments at age 8 to 12 weeks.

Tumor. The MCA 105 sarcoma is a 3-methylcholanthrene-induced tumor of B6 origin (5). A large number of vials of this tumor from the first in vivo passage were cryopreserved. After thawing, the tumor was maintained in syngeneic mice by serial s.c. transplantation. The MCA 105 sarcoma used for this study was in the fourth to seventh transplantation generation. Another similarly induced syngeneic tumor, MCA 102 sarcoma, was used as control tumor in the cytotoxicity assay.

Single-cell suspensions were prepared from solid tumors by digestion with a mixture of DNase, collagenase, and hyaluronidase, as previously described (12). For establishing solid tumor growth, B6 mice were
inoculated s.c. in the footpad with 2 to 4 × 10^5 MCA 105 tumor cells in 0.05 ml of HBSS (GIBCO, Grand Island, NY). The growth of tumors became evident on day 3 and all animals eventually succumbed to the progressive tumor, with a median survival time of 23 days.

**LN Cell Preparation.** Popliteal LN draining the progressive tumor were removed from mice 12 to 15 days after tumor inoculation, when the footpad tumor reached a thickness of 6 to 8 mm. Single-cell suspensions were prepared mechanically by pressing LN with the blunt end of a 10-ml plastic syringe in HBSS. The resulting cell suspensions were filtered through a layer of no. 100 nylon mesh (Nitex; Lawshe Industrial Co., Bethesda, MD), washed, and resuspended for IVS.

IL-2. The recombinant human IL-2 was kindly supplied by the Cetus Corporation (Emeryville, CA). The biological and immunological properties of IL-2 have been described in detail (7). Purified material had a specific activity of 4 to 8 × 10^6 units/mg protein (Cetus units). The endotoxin content was <0.1 ng/10^6 units IL-2, as assessed by the Limulus assay.

**IVS Procedure.** The procedure for IVS of tumor-draining LN cells has been previously described (13). In brief, 2 × 10^6 responding LN cells and 4 × 10^5 irradiated (2000 rad) MCA 105 stimulator tumor cells were cultured in 2.0 ml CM containing 10 units/ml IL-2 in wells of 24-well tissue culture plates (Costar, Cambridge, MA). The composition of CM has been described elsewhere (15). The cultures were incubated at 37°C in 5% CO2 and were fed with 1.0 ml of CM with 10 units/ml IL-2 on day 5. The IVS cells were routinely harvested on days 8–9, when they grew to a high density. These cells were washed 3 times before resuspending in HBSS for adoptive immunotherapy or in CM for cytotoxicity assay.

**Adoptive Immunotherapy.** In *vivo* antitumor effects of IVS cells were assessed by adoptive therapy of established pulmonary MCA 105 metastases. B6 mice were given i.v. injections of 2 to 3 × 10^6 MCA 105 tumor cells in 1.0 ml HBSS, to establish pulmonary metastases. In all adoptive immunotherapy experiments, recipient mice were immunosuppressed by i.v. injections of 100 mg/kg cyclophosphamide (Sigma Chemical Co., St. Louis, MO) 6 h prior to tumor inoculation (16). Although not necessary, this pretreatment facilitated the establishment of consistent numbers of pulmonary tumor nodules between individual animals. In addition, our previous findings clearly demonstrated that host immunosuppression had no impact on the ability of transferred cells to mediate the regression of pulmonary MCA 105 metastases (17).

On day 3, IVS cells were given i.v. and, from days 3 through 5, the mice received i.p. injections of 7500 units IL-2 every 12 h. This regimen of IL-2 alone had little or no antitumor effect but could enhance the therapeutic efficacy of transferred IVS cells (10–12). On days 12 to 14 after tumor inoculation, the mice were sacrificed for enumeration of pulmonary metastases. Counter-staining of the lung with 15% India ink solution, as described (18). Lungs with metastases too numerous to count on autopsy were assigned an arbitrary value of 250, because this was the largest number of tumor nodules that could be reliably counted in each lung.

**Cytotoxicity Assay.** The 4-h 51Cr-release assay was carried out as previously described (13). Briefly, 1 × 10^4 51Cr (Na251CrO4; New England Nuclear, Boston, MA)-labeled target cells and various numbers of effector cells were incubated together in 0.2 ml CM in a round-bottomed microtiter well at 37°C for 4 h. Cell-free supernatant was harvested with the Titertech Collecting System (Flow Laboratories, Inc., McLean, VA). The percentage of 51Cr release was calculated by using the following formula: (experimental release – spontaneous release)/maximal release × 100.

**Depletion of Lymphocyte Subsets in *In Vivo* and *In Vitro*.** Two hybridomas producing rat IgG2b mAb against the mouse L3T4 (GK1.5) and Lyt-2 (2.43) T-cell antigens were obtained from American Type Culture Collection (Rockville, MD). The mAb were produced as ascites fluid from sublethally irradiated (500 rad) DBA/2 mice. The rabbit antiserum was used at 1/50 dilution and there was no significant cytotoxicity observed (25). The Titertech Collecting System (Flow Laboratories, Inc., McLean, VA) was used to determine the significance in differences in numbers of pulmonary tumor nodules between groups. Two-sided P values are presented.

**RESULTS**

Effect of *In Vitro* Antibody and Complement Treatment of Tumor-draining LN Cells on Subsequent Generation of Antitumor Effecter Lymphocytes by IVS. It has previously been demonstrated that the predominant antitumor effecter cells generated by IVS expressed the Lyt-2 phenotype (12). To investigate the cellular requirement during IVS for effector cell generation, tumor-draining LN cells were treated with antibodies and complement to deplete T-cell subsets prior to IVS with irradiated MCA 105 tumor cells and IL-2. Cells recovered after 8 to 9 days of culture were analyzed for expression of Thy-1.2, L3T4, and Lyt-2 antigens by FMF (Fig. 1). Consistent with previous findings, LN cells treated with complement only gave rise to a population of cells predominantly (≥72%) Thy-1+, Lyt-2+, and L3T4+, with <5% L3T4+ cells. This phenotype was not changed by pre-IVS depletion of L3T4+ or ASGM-1+ cells, indicating the IVS procedure preferentially stimulated the growth of Lyt-2+ cells. Interestingly, LN cells depleted of Lyt-2+ cells did not result in the generation of a significant proportion of L3T4+ cells. In fact, L3T4+ cells, in three independent experiments, constituted only 4 to 15% of the total cells recovered. Instead, a cell population that was Thy-1- but L3T4+ and Lyt-2- was predominant (≥80%). These cells appeared to be heavily granulated and considerably larger (diameter = 25 μm) than typical T-lymphoblasts. The morphology of these cells, as well as Thy-1+, Lyt-2+, and L3T4+ lymphoblasts, are depicted in Fig. 2.
Table 1  Effect of antibody and complement treatment of tumor-draining LN cells prior to IVS on subsequent generation of therapeutically effective lymphocytes

<table>
<thead>
<tr>
<th>IVS cells*</th>
<th>Pre-IVS treatment</th>
<th>In vivo IL-2</th>
<th>No. of pulmonary metastasesa</th>
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<td></td>
<td></td>
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<td>Exp. 1</td>
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<td>~</td>
<td>250</td>
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<td>~</td>
<td>Complement</td>
<td>~</td>
<td>241 ± 9</td>
</tr>
<tr>
<td>+</td>
<td>Anti-L3T4 +</td>
<td>+</td>
<td>7 ± 3</td>
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<tr>
<td></td>
<td>complement</td>
<td>+</td>
<td>1 ± 1*</td>
</tr>
<tr>
<td>+</td>
<td>Anti-Lyt-2 +</td>
<td>+</td>
<td>250</td>
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<tr>
<td></td>
<td>complement</td>
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a Numbers of IVS cells given for each mouse were 4 x 10⁶ for experiment 1 and 3 x 10⁶ for experiments 2 and 3.
* Mean ± SE.

Fig. 1. FMF analysis of IVS cells generated from MCA 105 tumor-draining LN cells depleted of lymphocyte subsets by antibody and complement (C) treatment prior to IVS. A~C, fluorescence profiles of medium or NRS controls by indirect immunofluorescence staining. Relative cell number is measured on the ordinate. Each frame consists of 10,000 cells.

Fig. 2. Morphology of IVS cells derived from tumor-draining LN cells depleted of lymphocyte subsets by antibody and complement treatment prior to IVS. A, complement only; B, anti-L3T4 plus complement; C, anti-Lyt-2 plus complement; D, anti-ASGM-1 plus complement. Geimsa-Wright stain. Original magnification, x400.

any treatment (average, 250). IVS cells generated from LN cells treated with complement only demonstrated potent antitumor effects, as evident by reducing numbers of tumor nodules to averages of 7, 25, and 17 in three independent experiments. This antitumor efficacy of IVS cells was not affected if LN cells were depleted of L3T4+ cells by antibody and complement treatment prior to IVS, indicating that the development of Lyt-2+ effector cells during IVS was independent of the presence of L3T4+ lymphocytes. On the other hand, IVS cells generated from Lyt-2- cell-depleted LN cells could not mediate significant reduction of pulmonary metastases in all three experiments, indicating that Thy-1+ granulated large cells lacked antitumor reactivity. Also interesting is that tumor-draining LN cells depleted of ASGM-1+ cells by treatment with rabbit antiserum and complement eliminated pre-effector cell activity. IVS cells generated from treated cells did not exhibit antitumor efficacy, despite their Lyt-2+ phenotype (Fig. 1). These findings thus indicate that pre-effector cells were apparently a subpopulation of Lyt-2+ T-lymphocytes bearing the ASGM-1 antigen.

The cytotoxic reactivities of IVS cells generated from tumor-draining LN cells treated with various antibodies and complement were assessed by the 4-h ⁵¹Cr-release assay. Fig. 3 shows the results of one of three such experiments; IVS cells generated from control (complement-treated) LN cells displayed potent cytolytic effects. This cytolytic activity appeared to be preferential toward MCA 105 tumor cells, because the MCA 102 tumor cells were only minimally lysed. The ability of IVS cells to lyse MCA 105 tumor cells was not affected by pretreatment of tumor-draining LN cells with anti-L3T4 or anti-ASGM-1 antibodies and complement before culture. On the other hand, treatment of LN cells with anti-Lyt-2 mAb and complement abrogated the subsequent generation of cytotoxic effector cells by IVS. Therefore, it appeared that the cytotoxic reactivity was mediated by Lyt-2+ IVS cells. The Lyt-2+ IVS cells, although Thy-1+, did not mediate in vitro cytotoxicity. Also included in Fig. 3 is the finding that both MCA 105 and MCA 102 target cells could be lysed by LAK cells and that freshly harvested tumor-draining LN cells, which contained pre-effector cells, were not cytotoxic.
Effect of in Vivo treatment with Anti-L3T4 mAb, Anti-Lyt-2 mAb, or Anti-ASGM-1 Serum on Pre-effector Cell Generation. The antitumor pre-effector cells in the draining LN were apparently T-lymphocytes of the Lyt-2 phenotype. The sensitization and differentiation of Lyt-2+ T-cells often require the participation of L3T4+ cells for helper function (23–25). To analyze whether eliciting the Lyt-2+ pre-effector cells in vivo during tumor growth also required the sensitization of L3T4+ lymphocytes, we gave mice injections of specific antibody to deplete lymphocyte subsets shortly before tumor inoculation. In our early reports, treatment of B6 mice with one dose of L3T4 or Lyt-2 mAb produced a dramatic reduction of respective lymphocytes in the spleen (20). Treatment of mice with rabbit anti-ASGM-1 serum resulted in reduced capacity for generating LAK cells and depletion of NK activity in the spleen (20). FMF analysis of popliteal LN cells draining the intrafootpad MCA 105 tumor after 14 days revealed a >90% specific depletion of L3T4+ cells in mice treated with L3T4 mAb and L3T4 mAb and Lyt-2+ cells in mice treated with Lyt-2 mAb (Fig. 4). The depletion of L3T4+ cells in mice treated with L3T4 mAb and pad MCA 105 tumor after 14 days revealed a >90% specific depletion of L3T4+ cells in mice treated with L3T4 mAb and Lyt-2+ cells in mice treated with Lyt-2 mAb (Fig. 4), the onset of in vivo depletion by mAb of T-cell subsets was rapid, since similar results were obtained by analyzing spleen cells 1 day after antibody injection (data not shown). Of note, the rate of tumor growth did not seem to be affected by the antibody injection.

The effects of in vivo antibody treatment on subsequent generation of IVS cells were first analyzed by FMF. Similar to in vitro depletion with antibody and complement, LN cells from Rlg-, anti-L3T4 mAb-, NRS-, or anti-ASGM-1 serum-treated mice gave rise to a population of cells that phenotypically were >96% Thy-1+ and Lyt-2+ (Table 2). These IVS cells contained virtually no L3T4+ cells. IVS cells generated from LN of anti-Lyt-2 mAb-treated, tumor-bearing mice revealed that 96% of cells were Thy-1+, L3T4+, and Lyt-2-. The morphology of these two phenotypically distinct IVS cell types was very similar to that depicted in Fig. 2.

The antitumor efficacy of these IVS cells was again tested in adoptive immunotherapy experiments (Table 3). In three independent experiments, significant antitumor effects were demonstrated in mice receiving IVS cells generated from control animals (Rlg- or NRS-treated). In vivo depletion of L3T4+ cells during tumor growth did not affect the generation of pre-effector cells, since IVS cells derived from LN of these mice display potent in vivo antitumor effects. This finding thus indicates that sensitization for eliciting pre-effector cells in response to progressive tumor growth was independent of the participation of L3T4+ T-lymphocytes. Depletion of Lyt-2+ cells during tumor growth profoundly affected the generation of antitumor pre-effector cells. IVS cells derived from their LN did not demonstrate antitumor efficacy when tested in vivo. Thus, Lyt-2+ lymphocytes must have been precursors for pre-effector cells that gave rise to functional antitumor effector cells by IVS. Eliminating this population of T-lymphocytes at any stage of manipulations resulted in abrogation of antitumor efficacy.

Of interest was the finding that in vivo administration of rabbit anti-ASGM-1 serum on the day of tumor initiation did not have any impact on the subsequent sensitization of pre-effector cells, in spite of the demonstration that pre-effector cells expressed ASGM-1 (Table 1). Therefore, the precursors for pre-effector cells were probably ASGM-1- and the expression of this glycosphingolipid must have been acquired during sensitization.

The in vivo lymphocyte subset depletion also had an impact on the subsequent cytolytic activity expressed by IVS cells. As depicted in Fig. 5, IVS cells generated from LN of control animals (Rlg- or NRS-treated) anti-L3T4 mAb- or anti-ASGM-1 antibody-treated animals demonstrated significant cytolytic

![Fig. 4. FMF analysis of tumor-draining LN cells from mice 14 days after treatment in vivo with the monoclonal L3T4 or Lyt-2 antibody or rabbit anti-ASGM-1 serum. Relative numbers of cells are measured on the ordinate; 10,000 cells were analyzed in each frame.](image-url)
activity against the MCA 105 target cells. All these IVS cell preparations shared a common phenotype of Thy-1+, Lyt-2+, L3T4− (Table 2). IVS cells derived from anti-Lyt-2 mAb-treated mice, on the other hand, did not contain Lyt-2+ cells, even though they were Thy-1+. These IVS cells did not demonstrate cytotoxic reactivity. Therefore, it became apparent that cytotoxicity was mediated by IVS cells with the Lyt-2 phenotype.

Discussion

Immune response to syngeneic tumors represents complex biological events involving sequential T-cell activations leading to the acquisition of effector function in the T-cell repertoire. For years we have been studying the mechanisms of T-cell sensitization for the generation of antitumor effector cells. The T-lymphocytes that are of particular interest to us are cells capable of mediating the regression of established tumors when systemically transferred to the tumor-bearing host.

Our earlier studies of the host immune response to progressive growth of the newly induced MCA 105 sarcoma have demonstrated that, although lacking antitumor effector activities, lymphoid cells were sensitized to the growing tumor (12). These lymphocytes, functionally termed pre-effector cells, could be developed into fully mature antitumor effector cells by the IVS culture procedure. It has also been defined that the antitumor reactivity of the IVS cells was attributable to lymphocytes predominately expressing the Lyt-2 phenotype (12). It is thus conceivable that Lyt-2+ cells must have constituted one part of if not the entire pre-effector cell population. In the current study, we have analyzed the cellular requirement during IVS for the generation of Lyt-2+ effector cells. It appeared that the Lyt-2+ cells alone in the tumor-draining LN gave rise to the antitumor effector cells. Elimination of L3T4+ lymphocytes prior to IVS did not have any impact on subsequent effector cell generation. It is possible that the presence of IL-2 in the IVS culture might have negated the requirement for L3T4+ helper cells. However, the fact that in the absence of IL-2 IVS culture could not support the development of effector cells (10) makes this possibility unlikely.

It is anticipated that, by eliminating Lyt-2+ T-lymphocytes in the tumor-draining LN, L3T4+ cells would become dominant during IVS. Our experimental findings indicate that this was not the case. Instead, an unusual cell population overwhelmed the culture wells. Morphologically, the cells appeared to be quite large (approximate diameter of 25 μm) and granulated. Although Thy-1−, they expressed neither the L3T4 nor the Lyt-2 antigen on their surface. Therefore, it was suspected that this cell population might represent nonspecific LAK cells. However, in repeated experiments, we failed to demonstrate cytolytic activity against the MCA 105 or MCA 102 tumor target cells. Since they did not express antitumor reactivity in vivo and in vitro, the origin and nature of these cells were not further pursued. Our experimental results do not reveal the antitumor reactivity of L3T4+ cells. The possibility of generating L3T4+ IVS cells by employing methods of positive selection is currently under investigation. If successful, the antitumor efficacy of L3T4+ IVS cells can be tested in adoptive immunotherapy experiments.

In addition to expressing the Lyt-2 phenotype, the antitumor pre-effector cells expressed ASGM-1 antigens on their surface. Although unlikely, it is also possible that ASGM-1+ cells represented a separate population that promoted the homing of immune effector cells. The ASGM-1 molecules were originally thought to be associated with NK cells but not with cytotoxic T-cells or B-lymphocytes (26–28). However, recent studies revealed the presence of ASGM-1 on both in vivo and in vitro generated effector T-cells (29, 30). Interestingly, precursors of these immune effector T-cells did not seem to be susceptible to anti-ASGM-1 treatment in vivo. Thus, the expression of ASGM-1 on T-cells may be associated with their activation stages. Consistent with these observations, we found that in vivo anti-ASGM-1 treatment of mice on the day of tumor initiation did not affect the subsequent production of tumor-sensitized pre-effector cells. In addition, a more specific mAb (NK1.1) against NK cells in the mouse (31) was similarly tested in vivo and in vitro with complement in our system. Although NK activity, as revealed by lysis of YAC lymphoma cells, was impaired, the antitumor pre-effector cell function was not affected (data not shown). These findings indicate that the sensitization of pre-effector cells did not involve NK cells.

The pre-effector cells in the tumor-draining LN apparently were T-cells of the Lyt-2 phenotype. This lineage of T-lymphocytes has been defined in a variety of immune reactions to be associated with functions such as cytotoxicity and suppression. Recognition of antigens by Lyt-2+ immune T-cells is often restricted and uniquely associated with class I histocompatibility antigens. In many studies, sensitization of Lyt-2+ precursor cells requires the participation of L3T4+ lymphocytes for helper function (23–25). However, in the analysis of in vivo T-cell requirements for eliciting pre-effector cells in response to tumor growth, we found that sensitization occurred in the absence of L3T4+ helper lymphocytes. This conclusion was confirmed by a more vigorous procedure of L3T4+ cell depletion, where mice were thymectomized followed by two injections of mAb prior to tumor inoculation (data not shown). Since the pre-effector cells did not exhibit overt in vivo antitumor reactivity before IVS (12), it is conceivable that the lack of an L3T4+ cell response could be one of the contributing factors to the failure to progress beyond this pre-effector stage in vivo. To support this hypothesis, our previous work demonstrated that it was possible to generate immune lymphoid cells in vivo against the MCA 105 tumor by immunization with tumor cells admixed with a bacterial adjuvant, Corynebacterium parvum (5). Analysis of cellular interactions for eliciting antitumor effector cells after immunization revealed the necessity for an L3T4+ lymphocyte response for sensitization of Lyt-2+ cells (24). Thus the mechanisms of the host immune response to a progressively growing tumor and to active immunization with tumor cells plus adjuvant are characterized differently.

Another aspect of immunological reactivity of IVS cells examined was their in vitro cytolytic activities. Because IVS cells were generated in a low concentration (10 units/ml) of IL-
2, the cytotoxic activity to the MCA 105 tumor did not reflect non-specific LAK cell activity (13). Recent data also demonstrate that the in vivo antitumor efficacy of IVS cells was mediated exclusively through the reactivity of Lyt-2* cells (12). Therefore, it appears that cytotoxic T-lymphocytes might directly contribute to the in vivo therapeutic efficacy. In this study, we have found by both in vitro and in vivo lymphocyte depletions that the cytotoxicity against the MCA 105 tumor was indeed correlated with the presence of Lyt-2* T-cells in IVS cell preparations.

However, the antitumor reactivity, as demonstrated in adoptive immunotherapy, did not seem to parallel the ability to lyse MCA 105 tumor cells in vitro, because treatment of tumor-draining LN cells with anti-ASGM-1 serum and complement prior to IVS abrogated subsequent production of therapeutically effective cells, despite their high in vitro cytotoxic reactivity. These findings document the presence of cytotoxic T-lymphocytes that were not reactive in vivo. Therefore, it remains to be determined whether cytotoxic reactivity is one of the characteristics associated with the ability to mediate in vivo tumor regression.

In summary, the experiments described in this study delineate some aspects of the cellular immune response to the progressively growing MCA 105 sarcoma. The finding that Lyt-2* T-lymphocytes could be sensitized in the absence of L3T4* cells was intriguing. Considering the state of activation and the need for further sensitization in vitro to become effector cells, the pre-effector cells described here may represent an unique population of lymphocytes that has not been previously defined. Since these cells were generated in tumor-bearing hosts, further characterization of their immunological reactivities and conditions of their activation may help the design of clinical strategies for cellular therapy of cancer patients.

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Phenotype Analyses and Cellular Mechanisms of the Pre-effector T-Lymphocyte Response to a Progressive Syngeneic Murine Sarcoma

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