ABSTRACT

Mice with severe combined immunodeficiency (scid) provide an excellent model for studying interactions between human tumor cells and effector cells of the immune system. Because these animals lack functional B and T lymphocytes, they can accept human tumor xenografts and transfer of human effector cells. Here, we determined the ability of a human melanoma-specific, cytotoxic T-cell line (CTL) in suppressing the growth of spontaneously metastasizing human melanoma cells M24 met (HLA-A11, A33) in scid mice. This CTL line was highly cytotoxic and restricted by HLA-A11 against M24 met melanoma cells in vitro but poorly cytotoxic when tested against a human melanoma cell line that did not express HLA-A11. In order to evaluate the efficacy of this CTL line against M24 met melanoma cells in vivo, randomized groups of animals were given injections of either RPMI culture medium, interleukin 2 (IL-2), CTLs, or CTLs + IL-2. IL-2, per se, did not significantly reduce tumor metastases; however, injection of melanoma-specific, HLA-A11 restricted CTLs into scid mice, 1 day postexcision of the previously induced primary tumor, markedly reduced the number of metastatic foci in the lung and decreased metastatic involvement in lymph nodes. The combination of these CTLs with IL-2 proved even more effective, since almost all lung metastases were eradicated and metastatic involvement in both axillary and inguinal lymph nodes was substantially reduced. Our results indicate that these human CTLs maintain their ability for specific killing of metastasizing melanoma cells in scid mice. Our data suggest that reconstitution of scid mice with a specific group of effector cells (step-wise scid/hu) may be helpful for in vivo evaluation of potentially useful cancer immunotherapy modalities.

INTRODUCTION

The major aims of tumor immunotherapy are to slow the progress of neoplastic disease or eradicate it by augmenting the host's antitumor defense mechanisms. This objective was facilitated by the availability of recombinant human IL-2, which induces the T-cell growth and differentiation that lead to tumor suppression. For immunotherapy, IL-2 can be administered alone, in combination with chemotherapy or other cytokines, or with tumor-reactive cells that are activated and expanded in vitro. A variety of lymphoid cells with the ability to lyse tumor cells have been described (1-6). These include NK cells, LAK cells, A-LAK cells, CTLs, and tumor-infiltrating lymphocytes. Recent efforts have focused on the use of tumor-specific CTLs to improve the efficacy of LAK IL-2 therapy (1-4). Several studies have indicated that tumor-specific CTLs can express either helper or cytotoxic functions and are thus able to mediate significant antitumor effects when transferred into tumor-bearing hosts (5-6). This type of immunotherapy is likely to be most effective in a clinical setting when applied as adjuvant therapy to small tumors. Therefore, it has definite limitations because of the difficulty in obtaining sufficient quantities of cells from such small autologous tumors for anti-gen-specific stimulation of CTLs. However, recent studies demonstrated that allogeneic melanoma cells, matched at only one allele of the HLA-A locus, can substitute for autologous tumor cells in stimulating the generation of tumor-specific CTLs (7-10). Thus, with partially HLA-matched allogeneic melanoma cell lines one can generate sufficient numbers of tumor-specific CTLs for therapy, irrespective of the size of the tumor and the stage of patients' disease.

The efficacy of melanoma-specific CTLs in killing tumor cells in vitro has been well established (11-14). Mice with severe combined immunodeficiency disease (C.B-17 scid/scid) were shown to be useful models for studying lymphocyte function at the cellular and molecular levels (15-18). This is because of a lack of functional T and B lymphocytes (19), caused by a mutation on chromosome 16 (20), and consequent rearrangement of genes encoding antigen-specific receptors on these cells (21-22). In view of these findings, these animals have been utilized for a number of successful xenografts with either fresh human tumor specimens or tumor cell lines (23-29).

Here, we describe the first step-wise scid/hu model for a spontaneously metastasizing human melanoma (M24 met) featuring adoptively transferred melanoma-specific CTLs. This model made it possible to study interactions between human tumor cells and human T-lymphocytes, which sustained their function with or without the addition of exogenous IL-2. This step-wise scid/hu model also facilitated a critical evaluation of the efficacy with which melanoma-specific CTLs can suppress the growth of allogeneic, spontaneously metastasizing human melanoma cells in vivo.

MATERIALS AND METHODS

Animals. C.B-17 scid/scid mice were obtained from the rodent breeding colony of The Scripps Research Institute. Six- to 8-week-old female mice were used for metastasis experiments. These animals were housed under specific pathogen-free conditions and were handled in a laminar flow air cabinet. Experiments were performed according to the guidelines set down in the NIH Guide for the Care and Use of Laboratory Animals.

Cell Lines. The human melanoma cell line, M24, was derived from a biopsy of a lymph node metastasis and found to be tumorigenic in nude mice (30). This cell line was kindly provided by Dr. D. L. Morton (UCLA, Los Angeles, CA). A total of 10⁶ M24 cells (passage 30) was injected s.c. into the flanks of 6-week-old nude mice. These animals (3 of 3) grew large s.c. tumors and had multiple metastatic foci in their lymph nodes when sacrificed eight weeks after the injection of the tumor cells. A cell line was established from an invades lymph node of a nude mouse and designated M24 met (26).

M24 met cells were grown in RPMI 1640 and supplemented with 10% FBS, 2 mM glutamine, and 50 µg/ml gentamicin sulfate. The cells were detached from tissue culture plastic with 0.05% trypsin, 0.02 mM EDTA, and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, washed once, and used as a single-cell suspension. Human melanoma cell lines D1M4, 92, and 154 were generously supplied by Dr. Hilliard Seigler (Duke University Medical Center). These cells were cultured in 75-cm² flasks (Corning) with Eagle's minimal essential medium (GIBCO, Grand Island, NY) supplemented with 10% FBS (GIBCO), 100 units/ml penicillin, 100 µg/ml streptomycin, and 50 µg/ml gentamicin. An EBV-transformed B cell line was kindly provided by Dr. F. V. Chisari (The Scripps Research Institute). The human neuroblastoma cell line NMB-7 was a gift from Dr. S. K. Lia (McMaster University, Hamilton, Ontario, Canada). These cells were grown in RPMI 1640 (Whittaker Bioproducts, Walkersville, MD) supplemented with 10% fetal bovine serum (Whittaker Bioproducts).
Spontaneous Metastasis in Mice. M24 met cells (2 × 10⁶), in 100 µl phosphate-buffered saline, were injected s.c. into the flank of scid mice. Tumor sizes were measured with micropenelope, and their volumes were calculated by the formula: (a² × b)/2 with a being the width and b the length of the tumor. s.c. M24 met tumors routinely reached a size of 700-900 mm³ within 14-18 days postinjection. At that time, mice were anesthetized with i.p. injections of 2.5 mg ketamine, and tumors were excised under aseptic conditions. Typically, 22 days after this procedure or when animals were moribund, they were sacrificed and examined for metastases. The difference between the extent of metastasis among control and experimental groups was determined by the nonparametric Wilcoxon rank sum test. Lungs were fixed in Bouin's fixative and examined under a low magnification microscope for the presence of tumor foci on the surface. Enlarged lymph nodes were excised, weighed, and fixed.

Adoptive Immunotherapy Model. One day after the excision of M24 met tumors, the mice were randomized into four groups: (a) RPMI (0.2 µl); (b) IL-2, 10,000 units/day in 0.2 µl injected i.p. for 10 days; (c) CTLS (1.2 × 10⁷); and (d) CTLS plus IL-2. Animals treated with melanoma-specific CTLS received 12 × 10⁶ cells i.v. Group 4 was given i.v. injections of CTLS followed by i.p. injections of 5000 units each of IL-2 or 0.2 ml of RPMI twice daily for 10 days. Animals given only injections of IL-2 received a similar dosage. After 1 month, all animals were sacrificed and their lungs were examined for metastatic nodules. Their enlarged lymph nodes were then excised, weighed, and fixed in Bouin’s solution.

Establishment of Cytotoxic T-Cell Line. Lymphocytes were obtained from the peripheral blood of an EBV-negative healthy donor, positive for HLA-A11, by centrifugation over a Ficoll/Hypaque mixture. These cells were cultured in 24-well plates at an initial density of 1.2 × 10⁶ cells/cm² surface area in RPMI 1640 (GIBCO) supplemented with 10% FBS, 100 units/ml penicillin, 100 µg/ml streptomycin, 0.25 µg/ml amphotericin B, and 50 µg/ml gentamicin. Recombinant human IL-2, kindly supplied by Dr. K. Langley (Amgen, Thousand Oaks, CA), was added at a final concentration of 20 units/ml. The isolated T-cells were cocultured with allogeneic M24 met melanoma cells expressing the restricting MHC Class I antigens HLA-A11, A33. Stimulating melanoma tumor cells (5 × 10⁶) were incubated in 1 ml of complete culture medium containing 100 µg of mitomycin-C (Sigma) for 45 min at 37°C. After washing twice, these mitomycin-C treated tumor cells were added to a lymphocyte/tumor ratio of 10:1. CTLS were restimulated with the M24 met cell line every 10-14 days. The culture medium containing 20 units/ml recombinant human IL-2 was replenished every 3 to 5 days, and the cells were maintained at a density of 1-2 × 10⁶/cm² surface area.

Cytotoxicity Assays. Tumor cells (5 × 10⁶) were labeled for 2 h at 37°C with 0.5 mCi of sodium ⁵¹Cr (10-35 mCi/ml) (Amersham, Arlington Heights, IL) in 0.5 ml RPMI 1640. After washing these cells 3 times, samples of ⁵¹Cr-labeled tumor cells (5 × 10⁶) were plated in 96-well U-bottomed microtiter plates (Corning Laboratory Science, Corning, NY). Effector cells were placed into microtiter wells in a total volume of 100 µl at effector:target cell ratios of 100:1, 50:1, 25:1, and 12.5:1. The plates were subsequently centrifuged at 200 g for 5 min and incubated for 4 h at 37°C in 5% CO₂. A 100-µl aliquot of each supernatant was harvested and analyzed for radioactivity in a Flow Cytometer (Pharmacia LKB Biotechnology, Gaithersburg, MD). Total ⁵¹Cr release was measured by lysing the tumor cells with 2 N HCl. Spontaneous ⁵¹Cr release was determined in wells that contained only tumor cells.

Experimental ⁵¹Cr release = mean spontaneous ⁵¹Cr release measured in triplicate samples.
Mean maximal ⁵¹Cr release = mean spontaneous ⁵¹Cr release × 100

The data are presented as the mean percentage of specific ⁵¹Cr release of triplicate samples.

Inhibition of Cytotoxicity by mAb. W6/32, a mAb directed against a monomorphic determinant on HLA Class I antigens, and L227, a mAb against a monomorphic determinant on HLA Class II antigens, were purified from mouse ascitic fluid by protein A affinity chromatography. Anti-CD3 which recognizes a human T-lymphocyte antigen was purchased from Becton Dickinson, San Jose, CA. For antibody blocking studies, radiolabeled target cells (50 µl) were preincubated with 50 µl of the antibody (10 µg/ml) for 45 min at 37°C, before adding the effector T-cells. Effector cells (100 µl) were also preincubated with anti-CD3, in the same manner, as mentioned above to block cytotoxicity.

Phenotyping of the Killer Cells by Two-Color Analysis. The CTLS were incubated for 60 min at 0°C with 20 µl of each of the following mAbs labeled with either fluorescein isothiocyanate or phycoerythrin: Leu4 (CD3); Leu3a (CD4); Leu2a (CD8); and Leu11a (CD16) (Becton-Dickinson). These cells were washed twice with Dulbecco’s phosphate-buffered saline and two-color analysis was performed by flow cytometry using a Laser flow cytometer (FCM-1D).

CTL Sorting. CD4+ CD8+ cells were sorted by FACS (FACS IV, Becton Dickinson, Mountain View, CA). Sorted cells were cultured in CTL medium and their cytolytic activity against M24 met cells was assessed in a 4-h ⁵¹Cr release assay.

RESULTS

In Vitro Specificity of CTL Line. An HLA-A11 restricted CTL line was established by repeated stimulation of HLA-A11 positive peripheral blood lymphocytes with M24 met melanoma cells (HLA-A11, A33) at a lymphocyte/tumor ratio of 1:10, followed by coculture with human recombinant IL-2 for 14 days. To determine the specificity of this CTL line, its cytotoxicity was tested against several allogeneic tumor target cells. These included three HLA-A11 positive melanoma cell lines, one HLA-A11 negative melanoma cell line, one HLA-A11 positive EBV-transformed B cell lymphoma line, and the neuroblastoma NMB-7 cell line completely lacking HLA-class I antigens. As evident from the data presented in Table I, HLA-A11 positive CTLs that were specifically stimulated with M24 met melanoma cells produced the highest level of cytotoxicity against these tumor cells. Both tumor specificity and HLA-A11 restriction were demonstrated by the considerably less effective lysis of EBV-transformed B lymphoma cells (HLA-A11, A33) and HLA-A11-negative DM92 melanoma cell lines (HLA-A11, A3), respectively. As expected, the neuroblastoma cell line NMB-7, which lacked HLA-class I antigens, was resistant to CTL-mediated lysis. Since the same cells are extremely susceptible to NK/LAK-mediated lysis (31) it is evident that these effector cells did not contribute to tumor cell lysis in our experiments.

Inhibition of Cytotoxicity of the Melanoma-specific CTLs by mAb Directed against Class I and Class II MHC Antigens. To distinguish whether Class I or Class II MHC antigens were involved in the cytotoxic reactivity of the melanoma-specific CTL line against M24 met cells, blocking studies were done with mAb directed against the framework of either MHC Class I (W6/32) or MHC Class II antigens (L227). W6/32 blocked 80% of the cytotoxic reaction of all CTL lines against M24 met, suggesting that our tumor-specific CTL line was MHC Class I restricted. The monoclonal antibody L227, directed against MHC Class II framework antigens, had little or no such inhibitory effect (Table 2).

Inhibition of Cytotoxicity of HLA-A11 Restricted CTLs by mAb against CD3. To determine whether anti-CD3 antibody inhibited lysis of M24 met cells by HLA-A11 positive CTLs, CD3 antigens expressed by these cells were blocked by preincubation with anti-CD3 antibody, Leu 4, prior to addition of target cells and analysis by the ⁵¹Cr release assay. This anti-CD3 antibody significantly inhibited

| Target | HLA class 1 | % of lysis
<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>M24 met melanoma</td>
<td>HLA-A11, A33</td>
<td>65.4 ± 2.0</td>
</tr>
<tr>
<td>DM14 melanoma</td>
<td>HLA-A11, A28</td>
<td>37.1 ± 1.3</td>
</tr>
<tr>
<td>DM92 melanoma</td>
<td>HLA-A1, A3</td>
<td>14.8 ± 1.3</td>
</tr>
<tr>
<td>DM154 melanoma</td>
<td>HLA-A11, A29</td>
<td>27.5 ± 0.45</td>
</tr>
<tr>
<td>EBV B lymphoma</td>
<td>HLA-A24, A11</td>
<td>15.3 ± 0.9</td>
</tr>
<tr>
<td>NMB-7 neuroblastoma</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

a HLA class 1 antigens are not expressed on the surfaces of these cells.
b The data represent the means ± SD of triplicates in a 4-h ⁵¹Cr-release assay. The effector to target ratio was 50:1.

4934

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The growth of M24 met cells in vivo, scid mice received 2 × 10^6 M24 met. To determine the efficacy of human CTLs in suppressing CTL subpopulations. the CD4+ cell population which exhibited little or no cytotoxicity. significantly greater cytolytic activity against this tumor cell line than CD4+ cells (32, 33). As indicated in Fig. 1, CD8+ cells showed were assessed in view of recent reports indicating cytotoxicity of types. Their respective cytotoxicities against M24 met melanoma cells were found to be predominantly CD3+ (97%), CD4+ (60%), and CD8+ (30-40%).

Phenotypic Analysis of the HLA-A11 CTL Line. The phenotype of the HLA-A11 positive CTL lines was established by FACS analysis and found to be predominantly CD3+ (97%), CD4+ (60%), and CD8+ (30-40%).

Phenotype of CTLs Responsible for M24 met Killing. To determine whether CD4+ or CD8+ cells were responsible for killing M24 met, HLA-A11 positive CTLs were sorted to isolate these two cell types. Their respective cytotoxicities against M24 met melanoma cells were assessed in view of recent reports indicating cytotoxicity of CD4+ cells (32, 33). As indicated in Fig. 1, CD8+ cells showed significantly greater cytolytic activity against this tumor cell line than the CD4+ cell population which exhibited little or no cytotoxicity. However, the original CTL line containing both subsets exhibited more cytolytic activity against M24 met cells than either of the two CTL subpopulations.

Adaptive Immunotherapy for Spontaneously Metastasizing M24 met. To determine the efficacy of human CTLs in suppressing the growth of M24 met cells in vivo, scid mice received 2 × 10^6 M24 met s.c. The previously induced primary tumors were excised after 14–18 days of growth and animals were randomly divided into four groups. It should be pointed out, however, that there was no detectable enlargement of lymph nodes or macroscopic metastatic nodules in these animals at the time of excision. Individual groups were given i.p. injections of either RPMI or IL-2 alone (10,000 units) as controls, with CTLs (12 × 10^6) alone, or CTLs plus IL-2. The data shown in Table 3 indicate that all control animals had metastatic nodules in the lung, as well as lymph node involvement. Injection of IL-2 alone affected neither the number of metastatic foci in the lung nor the involvement of lymph nodes. However, scid mice that received melanoma-specific CTLs showed a significant reduction in the number of metastatic lung foci and in the involvement of inguinal lymph nodes when compared to the control animals, even without the addition of exogenous IL-2. The combination therapy of CTLs plus IL-2 drastically decreased the number of metastatic nodules in the lung in comparison to control animals and also substantially decreased metastatic involvement, in both axillary and inguinal lymph nodes (Table 3). Fig. 2 illustrates the effect of these treatment regimens on the lungs of such mice.

**DISCUSSION**

In the step-wise scid/hu melanoma model described here, reconstitution of these immunologically deficient animals with human CTLs suppressed the growth of spontaneously metastasizing human melanoma M24 met cells. The results of our studies presented here indicate that melanoma-specific, HLA-A11-restricted CTLs can be induced that are most effective in lysing allogeneic, spontaneously metastasizing M24 met melanoma cells (HLA-A11, A33); however, these same CTLs are less effective in lysing allogeneic melanoma cells that express an HLA-A antigen which is cross-reactive with HLA-A11, e.g., HLA-A1. Similar to these findings, Hayashi et al. (34) also observed that HLA-A-restricted CTLs are less effective in lysing allogeneic melanomas expressing HLA-A antigens that are cross-reactive with those expressed by CTLs. Interestingly enough, the same investigators found that HLA-A11 restricted, melanoma-specific CTLs killed allogeneic melanomas with which they shared only HLA-A11, equally as well as an autologous melanoma (34). Other reports published previously indicated CTLs to be effective as cytotoxic effector cells for adoptive immunotherapy (1-3, 6). Unlike LAK cells, which require large numbers of cells for effective immunotherapy /', unlike LAK cells, which require large numbers of cells for effective immunotherapy in vivo, smaller numbers of CTLs are more potent and produce high specific cytotoxic activity. Tumor specific CTLs have several advantages over LAK and tumor-infiltrating lymphocyte therapy, since they can be readily generated from peripheral blood lymphocytes by stimulation with autologous tumor cells or cultured HLA-A-matched allogeneic tumor cells. Thus, at the clinical level, CTLs could potentially be generated from stage I and II patients with small tumors that lend themselves more readily to adjuvant therapy. Documentation of the effectiveness with which tumor-specific CD3+ HLA-class I-restricted

---

**Table 2** Inhibition of CTL-mediated M24met melanoma cell lysis by specific monoclonal antibodies

<table>
<thead>
<tr>
<th>mAb</th>
<th>Lysis (%)</th>
<th>Inhibition of lysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W6/32</td>
<td>63.6 ± 6</td>
<td>10.7 ± 2.2</td>
</tr>
<tr>
<td>L27</td>
<td>80.8 ± 3.2</td>
<td>74.7 ± 3.2</td>
</tr>
<tr>
<td>Anti-CD3</td>
<td>84.5 ± 4</td>
<td>36.1 ± 5</td>
</tr>
</tbody>
</table>

*Concentration of all mAbs was 10 μg/ml. The data represent the mean ±SD of triplicates in a 4-h 51Cr-release assay. The effector to target ratio was 50:1.*

**Table 3** Adoptive immunotherapy of M24met human melanoma in scid mice with human CTLs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals with metastasis</th>
<th>Ipsilateral/auxiliary</th>
<th>Ipsilateral/inguinal</th>
<th>No. of metastatic foci in the lung</th>
<th>P valuesa</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPMIb</td>
<td>13/13</td>
<td>12/13</td>
<td>5/13</td>
<td>(22, 22, 26, 26, 70, 79, 80, 80, 153, 184, 190, 275, 300)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>12 × 10^6 CTLs</td>
<td>12/12</td>
<td>10/12</td>
<td>2/12</td>
<td>(6, 10, 11, 12, 13, 17, 17, 20, 21, 32, 58)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>12 × 10^6 CTLs + IL-2</td>
<td>7/8</td>
<td>0/8</td>
<td>2/8</td>
<td>(0, 1, 1, 1, 1, 2, 2)</td>
<td>NS</td>
</tr>
<tr>
<td>IL-2</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>(60, 44, 500, 160, 156, 64)</td>
<td></td>
</tr>
<tr>
<td>RPMIb</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>(83, 110, 155, 153, 133, 96)</td>
<td></td>
</tr>
</tbody>
</table>

*a P values versus controls. A comparison of CTL versus CTL + IL-2 = P < 0.005. The significance of the values was determined by the Wilcoxon signed rank test.

b Control groups were treated with 0.2 ml RPMI i.p. or 10,000 units IL-2 in 0.2 ml RPMI daily for 10 days. NS, not significant.

c One-day post-tumor excision scid mice received either i.v. injection of 12 × 10^6 CTLs alone or this same number of CTLs followed by 10 daily injections of 10,000 units IL-2.
GROWTH SUPPRESSION OF MELANOMA METASTASES BY HUMAN CTLs

Fig. 2. Typical appearance of lungs from control mice receiving RPMI (A); scid mice treated with tumor-specific CTLs (B); or scid mice treated with CTLs plus exogenous IL-2 (C).

CTLs kill tumor cells in both human and animal systems has stimulated interest in the respective role of MHC class I and CD3 antigens in the recognition of tumor cells by CTLs.

The melanoma specificity of our HLA-A11 restricted CTLs was clearly demonstrated when they lysed HLA-A11 positive EBV-transformed B lymphoma cells less effectively than HLA-A11 positive melanoma cells (Table 1). Furthermore, the killing by CTLs of M24 met cells was markedly suppressed by mAbs that blocked HLA class I antigens, suggesting a cross-reactivity for HLA-matched antigens depending on structural similarities.

Moreover, the anti-CD3 antibody also inhibited cytolytic of M24 met melanoma. Thus, blocking by either anti-MHC class I (W6/32) or anti-CD3 (Leu 4) antibody suggests that recognition of tumor cells may occur through specific interactions of the CD3 cell antigen/T-cell receptor complex with antigens presented on the tumor cells in the context of MHC class I products. In this regard, FACS analysis of our melanoma-specific CTL line indicated that more than 97% of these cells were CD3+, with a higher distribution of CD4+ than CD8+ cells. Although T-cell mediated cytotoxicity is usually associated with CD8+ lymphocytes and most CD4+ T-cells represent the so-called helper/inducer subsets, CD4+ CTLs were reported to specifically lyse melanoma cells in vitro (32, 33). Our experiments with either sorted CD4+ or CD8+ cell populations clearly demonstrated that CD8+ cells have higher levels of cytotoxicity (Fig. 1) than CD4+ cell populations. However, the combination of both cell populations resulted in a much higher lytic activity. In view of these and others' (33) results the cytolytic activity of CD8+ cells appears to be amplified by CD4+ cells. In this regard, others reported that synthesis of IL-2 by CD4+ cells enhances cytolytic activity (33). Consequently, the CD4+ cells in our experiment may also synthesize IL-2 when stimulated with M24 met cells.

In evaluating the efficacy of these melanoma specific CTLs in vivo by adoptively transferring them to scid mice after excision of their primary melanoma tumors, we found a significant reduction in the number of metastatic foci in the lungs, as well as a partial reduction of lymph node involvement. Furthermore, our tumor-specific CTLs, together with IL-2, proved even more effective, since they practically eradicated metastatic lung foci and drastically reduced metastases in both axillary and inguinal lymph nodes. To the best of our knowledge, these animal studies demonstrate for the first time the efficacy of CTLs in reducing spontaneous melanoma metastases in vivo without the administration of exogenous IL-2. These results clearly indicate that IL-2 alone failed to decrease metastatic spread to either the lungs or lymph nodes of these scid mice. Furthermore, in subsequent studies (data not shown) the inherent NK/LAK cytolytic activity of scid mice per se was not further enhanced by IL-2 treatment. Thus, splenocytes derived from either M24 met tumor-bearing controls or from tumor-bearing scid mice treated with IL-2 lacked cytolytic activity against M24 met cells in vitro.

In summary, we developed a novel step-wise scid/hu model which proved useful in evaluating the efficacy of HLA-A11 restricted human CTLs in reducing spontaneous metastases. This animal model allows one to populate scid mice with cells of the human immune system, thereby facilitating studies on the interactions of specific effector cells with metastatic tumors and on potential adoptive immunotherapy for cancer.

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GROWTH SUPPRESSION OF MELANOMA METASTASES BY HUMAN CTLs


Human Cytotoxic T-Cells Suppress the Growth of Spontaneous Melanoma Metastases in SCID/hu Mice

Helen Sabzevari and Ralph A. Reisfeld


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