Systemic Induction of Cells Mediating Antibody-dependent Cellular Cytotoxicity following Administration of Interleukin 2

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ABSTRACT

We have previously demonstrated that incubation of murine cells in vitro in interleukin 2 (IL-2) induced antibody-dependent cellular cytotoxicity (ADCC) and that these cells were derived from the NK/LAK, FeR+ cell population. In the present study we show that in vivo administration of IL-2 to mice induces cells which exhibit ADCC activity in the peritoneal cavity, liver, lungs, and to a lesser degree in the bone marrow, spleen, mesenteric lymph nodes, and thymus. A gradual increase in ADCC activity and the number of Fe receptor-positive cells was seen 1 to 3 days after starting IL-2 treatment. The cells mediating ADCC are closely related to LAK cells since they expressed Thy1.2 antigens and are derived from asialo GMI-positive, Lyt2/L3T4-negative, radioresistant cells. These results demonstrate that IL-2 can systemically induce cells with ADCC activity and that this ability may be useful in the establishment of therapeutic models against disseminated cancer when combined with specific antitumor monoclonal antibodies.

INTRODUCTION

Antibody-dependent cellular cytotoxicity is mediated by cytoxic cells which express FeR on the cell surface (1). Several immunomodulators such as Bacillus Calmette-Guérin and Corynebacterium parvum induce ADCC in vitro (2–4), whereas other cytokines, such as interferon and IL-4, induce in vivo (5) and in vitro (6–9) FeR which are necessary for ADCC. The incubation of murine (10–13) or human cells (14) in IL-2 in vitro can induce ADCC activity. This effect was attributed to the ability of IL-2 to generate lytic (LAK) cells (15–16), and to expand cells which express FcR (17). In vivo administration of IL-2 could induce cells in the local area with ADCC activity (13, 18). Similar to the cells generated by exposure to IL-2 in culture (17), the cells induced by IL-2 in vivo were derived from Thy1−, asialo GMI+, presumably NK/LAK cell populations (18). It has not been shown that the systemic administration of IL-2 could induce ADCC although this has been suggested from our previous in vivo studies which demonstrated that systemic IL-2 administration could increase the antitumor efficacy of anti-B16 melanoma Mab against established B16 liver metastases (19). In the present study we show that systemic administration of IL-2 can induce ADCC in various organs including the lungs and liver and that the phenotype of these cells and their precursors are similar to the cells induced in vitro. The potential of these findings for the establishment of therapeutic models based on combined treatment of IL-2 and antitumor antibody is discussed.

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2Abbreviations used are: ADCC, antibody-dependent cellular cytotoxicity; IL-2, interleukin 2; IL-4, interleukin 4; LAK cells, lymphokine-activated killer cells; NK, natural killer; PEC, peritoneal exudate cells; MLN, mesenteric lymph nodes; MNC, lung mononuclear cells; CTL, cytotoxic T-lymphocytes; ASGMi, asialo GMI; FeR, Fc receptors; Mab, monoclonal antibody; CM, complete medium; Fc, [(C57BL/6 x C3H/Hen)F1]; FITC, fluorescein isothiocyanate; LYT, lytic units; HBSS, Hanks' balanced salt solution; HEPEES, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid.

MATERIALS AND METHODS

Animals. C57BL/6, C3H/Hen, and C57B6 × C3H/Hen F1, female mice were obtained from the Small Animal Section, Veterinary Resources Branch, NIH, Bethesda, MD, and were used when they were 8–16 weeks old.

Tumors. Weakly immunogenic B16 melanoma tumor cells syngeneic to C57BL/6 mice were obtained from Dr. Ovejera of Frederick Cancer Research Center, Frederick, MD, and were serially transplanted s.c. in syngeneic mice. The EL4 lymphoma syngeneic to C57BL/6 mice and the P815 mastocytoma syngeneic to DBA/2 mice were passaged in vivo as ascites tumors.

Antibodies. Anti-B16 melanoma Mab of the IgG2a isotype was produced and purified as previously described (19). B10.A A/J F1, anti-B10 (anti-H-2b) and (B10xA)F1, anti-B10.D2 (anti-H-2d) allosera, were produced as described elsewhere (20) and kindly supplied by Dr. David Sachs (Immunology Branch, National Cancer Institute). A FITC-conjugated rat anti-Fc receptor monoclonal antibody (2.4G2) (21) was a gift from Dr. David Segal (National Cancer Institute). Rabbit antiserum against asialo GMI, from Wako Chemicals (Dallas, TX) was screened to select an antisera capable of eliminating splenocytes with NK activity without affecting the ability to generate in vivo allocytic T-lymphocytes against allogenic cells. Anti-Thy1.2 monoclonal antibody was purchased from New England Nuclear (Boston, MA).

Hybridomas producing rat IgG2a Mab against J3T4 (GK1.5) and Lyt-2 (2.43) T-cell antigens were obtained from American Type Culture Collection (Rockville, MD). The Mab were harvested as ascites from sublethally irradiated (500 rads) DBA/2 mice.

Recombinant Interleukin 2. Purified recombinant human IL-2 (22), was kindly provided by the Cetus Corporation (Emeryville, CA) and had a specific activity of 3–4 × 106 units/mg. One Cetus unit is equivalent to 2–3 Biological Response Modifier Program standard IL-2 units.

Generation of LAK Cells. 2 × 106 cells/ml were placed in 24-well tissue culture plates (Costar, Cambridge, MA; 2 ml/well) in CM together with 1000 units IL-2/ml. The plates were then incubated at 37°C in a moist atmosphere with 5% CO2 for 3 or 4 days. The cells were then harvested, washed in CM and resuspended to be used as effector cells in a 4-h chromium release assay.

Preparation of Single Cell Suspensions from Solid Tumors. Single cell suspensions from B16 melanoma were prepared as previously described (19). Briefly, a s.c. tumor was excised, minced with scissors into 1- to 2-mm cubes, washed through 100-gauge nylon mesh (Nitex), washed three times with HBSS and resuspended in CM.

14Cr Release Assay of Cytotoxicity. Fresh tumor cells were labeled with 100 μCi 14Cr (New England Nuclear, Boston, MA), for 1 h at 37°C, washed three times with CM and recounted before dilution and incubation in triplicate with effector cells at varying ratios in 96-well round-bottom plates (Costar) (11). To test ADCC, 10 μl/well of the allantoinsera at the appropriate dilution was incubated with the target for 20–30 min at 37°C before the addition of effector cells. The final concentrations of the allantoinsera to achieve maximal target lysis were determined in titration experiments (between 1:30 to 1:80 for all antiserum) (13). Spontaneous release of 14Cr was measured after incubation of cells with CM only and total release was measured after...
induction of cells in 0.1 N HCl. Plates were harvested as previously described (13) and radioactivity counted. The percent cytotoxicity was determined by:

\[
\frac{\text{Observed cpm} - \text{spontaneous cpm}}{\text{Total release cpm} - \text{spontaneous cpm}} \times 100 = \% \text{ lysis}
\]

Spontaneous release was <35% of maximal release in all experiments presented.

Isolation of Cells from Various Organs of IL-2 Primed Mice. Liver and lungs were excised, minced into 1–3-mm fragments and stirred in the triple enzyme mixture described above for 2–3 h. The cells were then transferred to lympholyte M gradient, centrifuged at room temperature for 20 min and the interphase collected and the erythrocytes lysed by resuspending the cell pellet in 10% buffered ammonium chloride solution (NIH Media Section). The cells were then washed three times in HBSS and resuspended in CM.

Spleen, thymus, and mesenteric lymph nodes were excised, crushed with the hub of a syringe, passed through 100-gauge nylon mesh (Nitex), and washed three times after lysing the erythrocytes as described above.

Bone marrow cells were harvested from the tibia and femora into HBSS, filtered through nylon mesh, washed three times in HBSS after lysing the erythrocytes and resuspended in CM. Peritoneal exudate cells were isolated by washing the peritoneal cavity with RPMI followed by lysis of erythrocytes. The cells were then washed in HBSS and resuspended in CM.

In Vitro anti-Thy1.2 Depletion. 4 × 10⁷ mononuclear lung cells prepared as previously described were incubated with 1 ml of anti-Thy 1.2 sera at 1:200 dilutions. After 45 min on ice, cells were washed once in cold RPMI 1640 (Biofluids Inc., Rockville, MD) supplemented with 0.3% bovine albumin (Path-O-Cyte 4; ICN Immunobiological, Lisle, IL) and 25 mM HEPES (HEPES Buffer, 1 M, Biofluids, Rockville, MD). Freshly prepared 1:10 diluted low tox-m-rabbit complement (Cedarlane Laboratories Limited, Hornby, Ontario, Canada) was then added, and the cells were cultured in 37°C for 20 min. The complement treatment was repeated and cells were then washed twice in the above medium and resuspended in IL-2 containing CM.

In Vivo Depletion of Lymphocyte Subpopulations by Mab or Antiserum. For in vivo depletion of lymphocyte subpopulations mice received one i.v. injection of 0.2 ml of the ascitic fluid of anti-Lyt-2 and anti-L3T4 monoclonal antibodies diluted in 1 ml with HBSS. This procedure has been shown to be effective in inducing long-term depletion of T-cell subpopulations in vivo (23). Injection of purified rat IgG served as a control.

One-half of a 1:50 dilution of rabbit antiserum reactive with the asialo Gm₁ (24) was injected into the tail vein 24 h prior to treatment of mice with anti-B16 monoclonal antibody (19). Injection of pooled normal rabbit serum served as a control. In immunotherapy experiments with high dose IL-2, antibody was given 1–2 days before beginning of IL-2 injections.

Flow Microfluorimetric Analysis. The number of FcR-bearing cells was determined using FITC-conjugated rat anti-FcR Mab (17). The optimal concentration of the antibody to achieve maximal binding was determined in titration experiments with FcR-bearing spleen cells. Control samples were stained with FITC-conjugated Leu-3 monoclonal antibody (Becton-Dickinson, Mountain View, CA), at 1–2 µg/10⁶ cells, a concentration calculated from titration of human Thy 1.2 lymphocytes. The number of Thy 1.2-bearing cells after anti-Thy 1.2 depletion was determined using FITC-conjugated mouse anti-Thy 1.2 Mab at 2–5 µg/10⁶ cells (17). After 45 min on ice, cells were washed three times and analyzed on a Becton-Dickinson FACScan Flow Cytometer (Mountain View, CA).

Mice Irradiation. Mice were given 5 Gy whole body irradiation from a ¹³¹Cs source (Gamamcell 40; Atomic Energy Canada Limited) 8 h prior to IL-2 injections.

Induction of Liver and Lung Metastases. Liver and lung metastases were induced as previously described (19). Briefly, to induce liver metastases 5–7 × 10⁴ freshly prepared B16 melanoma cells were injected under the splenic capsule. After 1 min tumor cells flushed into the portal circulation and the spleen was excised. Lung metastases were induced by i.v. injection of 1–2 × 10⁶ B16 melanoma cells.

 Enumeration of Metastases. The number of liver and lung metastases was determined in a blinded fashion on ear-tagged mice without knowledge of the prior treatment of that animal. Black nodules of B16 melanoma could easily be identified on the surface of the excised organs.

Statistical Analysis. The significance of differences in the number of liver and lung metastases among groups was determined by the Wilcoxon Rank Sum test (25). Two-tailed P values are presented.

RESULTS

Correlation between in Vitro and in Vivo ADCC Induced by IL-2. We have previously shown that LAK cells derived from splenocytes taken from various murine strains mediated ADCC (11, 17). In these experiments C3H LAK cells exhibited the highest ADCC activity whereas C57BL LAK cells showed little if any of this activity. We thus compared the induction of ADCC in vitro and in vivo in various mouse strains. Both C3H and C3H × C57BL/6 F₁, splenocytes cultured in 1,000 units/ml IL-2 for 4 days (Fig. 1) or mononuclear cells from the lungs of mice of these strains primed in vivo for 3 days with 200,000 units of IL-2 (Fig. 2), exhibited ADCC activity whereas C57BL/6 splenocytes did not show any ADCC activity when exposed to IL-2 either in vitro or in vivo. C57BL/6 cells exposed to IL-2 either in vitro or in vivo did not exhibit ADCC as there was no increase in lysis by adding antibodies to the targets during the cytotoxicity assay. This indicates that the lysis observed was due to a direct antibody-independent lysis by LAK cells. In addition, the difference in the extent of lysis observed between the upper and lower panel in Fig. 2 could be the result of employing two different targets (EL4, P815) which express a different number of epitopes recognized by the two antisera (anti-H₂₉ and anti-H₂⁰) used. Based on these results we employed both C3H and F₁, mice for our next experiments.

Induction of ADCC in Various Organs in IL-2-primed Mice. We previously demonstrated that cells taken from various lymphoid organs and then incubated in IL-2 generated cells...
INDUCTION OF CELLS MEDIATING ADCC FOLLOWING IL-2 ADMINISTRATION

The highest activity was seen in the peritoneal cells, followed by the liver and lungs. Less ADCC activity was induced in the spleen and bone marrow followed by the lowest activity in the MLN and thymus cells.

IL-2 Kinetics and Dose-Dependent Induction of ADCC. To optimize the conditions for the in vivo induction of ADCC in lungs we primed mice with various doses of IL-2 for 3 days and then tested the MNC for ADCC activity. As shown in Fig. 4, the induction of ADCC activity appeared to be directly related to the dose of IL-2 administered. We next treated mice with 200,000 units IL-2 for 1, 2, and 3 days. The lungs were removed and their MNC tested for ADCC activity. As illustrated in Fig. 5, a gradual increase in ADCC activity over time was seen in both murine strains. We thus selected the 200,000 units dose of IL-2 given for 3 days for the following experiments.

Effector Cells which Exhibit ADCC Activity are Mainly Thy1+ Cells. We have previously demonstrated that the administration of high doses of IL-2 induced LAK cells in the lungs of treated mice (26) and that these LAK cells exhibited the Thy1 marker (27). To determine whether the IL-2 induced cells in the lungs which exhibited ADCC activity carried the same phenotype we isolated lung MNC from IL-2 primed mice and depleted Thy1+ cells prior to testing ADCC activity. As shown in Fig. 6, depletion of the Thy1+ cell population (confirmed by fluorimetric analysis) significantly reduced ADCC activity to less than 1 LU/10⁷ cells compared to the complement treated and untreated groups which had activities of 156 and 200 LU/10⁷ cells respectively. The possibility that anti-Thy 1 Mab could interfere with ADCC by binding to the FcR seems unlikely since this antibody is of the IgM isotype and is not expected to bind to the FcR which is involved in ADCC (11).

Treatment of Mice with IL-2 Increases the Number of FcR+ Cells in Lungs. We have found that culture of cells in the presence of IL-2 expanded the number of FcR+ cells and that this correlated with the induction of ADCC (17). We have also shown that the FcR which is recognized by the anti-FcR Mab (2.4G2) is involved in ADCC since blocking of this receptor with 2.4G2 Mab completely abrogates ADCC (11). To study the effect of the administration of IL-2 on the number of FcR+ with ADCC activity (17). To test whether in vivo treatment with IL-2 would have a similar effect we primed mice with 200,000 units IL-2 given i.p. for 3 days and then tested cells from the bone-marrow, thymus, MLN, spleen, peritoneal cavity, liver and lungs for ADCC activity against fresh tumor targets. As shown in Fig. 3 the systemic administration of IL-2 induced ADCC in cells derived from many of these organs.

![Fig. 2. Induction of ADCC in the lungs mononuclear cells (MNC) of IL-2 primed mice. C3H, C57BL, and F1 mice were injected with 200,000 units IL-2 i.p. three times daily for 3 days. The lung MNC were then isolated and tested against EL4 (top) and P815 (bottom) in the presence or absence of anti-H2* and anti-H2* allosera, respectively.]

![Fig. 3. ADCC induced in various organs in IL-2-primed C3H and F1 mice. C3H and F1 mice were injected with 200,000 units IL-2 i.p. three times daily for 3 days. The lungs, liver, bone marrow, thymus, spleen, and peritoneal exudate cells were harvested and tested for ADCC activity against EL4 targets in the presence or absence of anti-H2* allosera. Top, cells from F1 mice were employed; middle and bottom, cells from C3H mice were used.]

![Fig. 4. ADCC induced in the lung MNC of IL-2-primed mice. C3H (top) and F1 (bottom) mice were treated for 3 days with various doses of IL-2. The lung MNC were then isolated and tested for ADCC against EL4 targets in the presence or absence of 1:50 diluted anti-H2* allosera.]

6955
INDUCTION OF CELLS MEDIATING ADCC FOLLOWING IL-2 ADMINISTRATION

**Fig. 5.** Kinetics of ADCC induction in the lung MNC of IL-2-primed mice. C3H (top) and F1 (bottom) mice were treated with 200,000 units of IL-2 for 1, 2, and 3 days. The lung MNC were isolated and tested for ADCC activity against EL4 targets in the presence or absence of anti-H-2^d^ allosera. The treatment of the different groups was started on different days and the ADCC assay was performed on the same day.

**Fig. 6.** The in vivo IL-2-induced cells which mediate ADCC are Thy1^-^ cells. Lung MNC of mice primed with 200,000 units IL-2 were treated with a 1:200 dilution of anti-Thy1.2 antibody followed by two cycles of C^+^ treatment. Control groups were either treated with C^+^ or were untreated. The cells were then tested for ADCC activity against EL4 targets in the presence or absence of anti-H-2^d^ allosera.

cells in vivo we treated C3H mice for 3 days with 200,000 units IL-2 or HBSS as a control. Lung MNC were then tested for ADCC activity and the number of FcR^+^ cells were determined using a fluorescein-conjugated rat anti-FcR monoclonal antibody (21). As shown in Table 1, 8% of the cells from the HBSS-treated group expressed FcR and showed no ADCC activity (<1 LU/10^7^ cells) whereas 31% of the cells from the IL-2-treated group expressed FcR and exhibited ADCC activity (303 LU/10^7^ cells). In addition, the increase in the number of FcR^+^ cells was accompanied by a small increase in the FcR density on the cell surface (mean fluorescent channel HBSS, 556; IL-2, 591).

**Irradiation Abrogates Induction of ADCC in Lungs of IL-2 Primed Mice.** We have shown that the precursor of cells induced in vitro that mediate ADCC were radiosensitive (17). To test whether radiation could affect the in vivo IL-2 induced ADCC activity we irradiated mice at 500 rads prior to IL-2 priming for 3 days. The lung MNC were then removed and tested for ADCC activity. As shown in Table 2, irradiation completely abrogated the induction of ADCC in lung MNC compared to the nonirradiated control group which exhibited both LAK and ADCC activities.

**Precursor of IL-2-induced ADCC Cells in Vivo is Lyt 2^-^- L3T4^-^- ASG^+^+.** We have previously shown that the precursor of cells mediating ADCC induced by IL-2 in vitro was Thy1^-^- (17). We thus tested the involvement of Thy1+ lymphocytes in the in vivo induction of ADCC. In preliminary experiments we found that anti-Thy1 Mab administration in vivo was relatively ineffective in depleting Thy1^+^ cells. We thus treated mice with both anti-Lyt2 and L3T4 Mab at a dose previously shown to be effective in depleting T-lymphocytes in vivo (23). Control mice were either treated with normal rat immunoglobulin or were untreated. The mice then received IL-2 for 3 days, and their lung MNC were tested for ADCC activity. A representative experiment is shown in Table 3. Depletion of Lyt2 and L3T4 cells was confirmed both by a fluorimetric analysis (<1% Thy1.2+ cells) and by functional assay, i.e., complete abrogation of allo cytotoxic T-lymphocytes generation in vitro. Depletion of Lyt2+ and L3T4+ cells had no significant effect in the induction of ADCC when compared to the control groups.

We next tested the possibility that the precursor of ADCC cells induced in vivo was ASG^+^, similar to the cells induced in vitro (11, 17). For this purpose we treated mice with anti ASG^+^ serum at a dose which was previously found to be effective in eliminating the NK/LAK precursors from the spleen (24, 27). Mice were then treated with 200,000 units IL-2 for 3 days and the lung MNC tested for ADCC activity. The deple-

### Table 1 Induction of ADCC and FcR^+^ cells in IL-2 primed mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No Ab</th>
<th>+ Ab</th>
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<tbody>
<tr>
<td>HBSS</td>
<td>8 (556°)</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>200,000 units of IL-2</td>
<td>31 (591)</td>
<td>33.3</td>
</tr>
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</table>

* Mean fluorescent channel.

### Table 2 Irradiation abrogates the induction of ADCC in the lungs of IL-2-primed mice

<table>
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<tr>
<th>Treatment</th>
<th>LU/10^7^ Effectors</th>
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<tr>
<td>No treatment</td>
<td>500 rads</td>
</tr>
<tr>
<td>No Ab</td>
<td>10.4</td>
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<tr>
<td>+ Ab</td>
<td>&lt;1.0</td>
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</table>

<table>
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<tr>
<th>Treatment</th>
<th>LU/10^7^ Effectors</th>
</tr>
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<tbody>
<tr>
<td>No treatment</td>
<td>500 rads</td>
</tr>
<tr>
<td>No Ab</td>
<td>10.4</td>
</tr>
<tr>
<td>+ Ab</td>
<td>&lt;1.0</td>
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Induction of cells mediating ADCC following IL-2 administration

Table 3 Precursor of IL-2-induced ADCC cells is a Lyt2/L3T4-negative cell

<table>
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<tr>
<th>Treatment</th>
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<th>L3T4+</th>
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<tr>
<td>Anti-Lyt2/L3T4 antibody</td>
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<td>1</td>
</tr>
<tr>
<td>Normal rat immunoglobulin</td>
<td>34</td>
<td>39</td>
</tr>
<tr>
<td>HBSS</td>
<td>52</td>
<td>43</td>
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Generation of allo CTL from splenocytes of in vivo-treated mice

<table>
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<tr>
<th>Treatment</th>
<th>Targets: P815</th>
<th>EL4</th>
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<tr>
<td>Anti-Lyt2/L3T4 antibody</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Normal rat immunoglobulin</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>HBSS</td>
<td>12.9</td>
<td>&lt;1.0</td>
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</tbody>
</table>

ADCC of Lung MNC

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LU/10^7 Effectors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Lyt2/L3T4 antibody</td>
<td>27.7</td>
</tr>
<tr>
<td>Normal rat immunoglobulin</td>
<td>17.8</td>
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<tr>
<td>HBSS</td>
<td>13.8</td>
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</table>

No Effect of Combined Treatment of F1 Mice with IL-2 and Anti-B16 Mab on Established B16 Melanoma Lung Metastases

We have previously failed to demonstrate any antitumor effect of anti-B16 Mab combined with IL-2 in the treatment of established B16 melanoma lung metastases in C57BL/6 mice. As F1 mice showed a substantially increased ADCC activity compared to C57BL/6 mice (Fig. 2) we tested the effect of the combined treatment with anti-B16 Mab and IL-2 on established B16 metastases in F1 mice. F1 mice were injected with anti-B16 Mab given i.v. either during IL-2 treatment on days 3 and 6 (Experiments 1 and 3) or after IL-2 treatment (day 6, Experiment 2). IL-2 was given i.p. three times a day at 25,000 units in Experiments 1 and 2 or 100,000 units twice a day in Experiment 3. On day 14 the mice were sacrificed and the number of liver and lung metastases enumerated. As shown in Table 5, combined treatment with Mab and IL-2 significantly reduced the number of liver metastases but had no significant effect on lung metastases. The inability to affect lung metastases was seen when mice were injected with the antibody before or after IL-2 priming and when either 25,000 or 100,000 units of IL-2 were employed.

Discussion

Interleukin 2 can induce or augment various types of lymphocyte-mediated cytotoxicity. Thus, besides its ability to generate LAK cells in vitro (15, 16), to activate NK cells (8) and monocytes expressing IL-2 receptor (28), IL-2 can also induce ADCC activity both in murine (11-13, 18) as well as human cells (14). The cells mediating ADCC appeared to be similar to LAK cells both in their pattern of generation as well as in their phenotypic markers (17). The administration of high doses of IL-2 in vivo in mice could generate cells which exhibit LAK activity in various organs (26) and were phenotypically related to LAK cells induced in vitro (27). Based on these findings we have studied the ability of IL-2 to induce ADCC activity in vivo as the basis for developing an immunotherapeutic model using specific antitumor antibodies along with IL-2 administration. We showed that treatment of mice with IL-2 for 3 days induced cells in vivo with LAK and ADCC activities, similar to the cells generated in vitro (Figs. 1 and 2). Correlations existed between the in vivo and in vitro induced cells. IL-2-cultured splenocytes taken from C3H and F1 mice exhibited relatively high ADCC activity compared to cells from C57BL mice; lung MNC isolated from IL-2-prime mice of these two strains (Figs. 1 and 2) exhibited ADCC activity paralleling that induced in vitro.

The ability to induce ADCC in IL-2 primed mice was shown in two other studies where cells with this activity were generated locally at the site of IL-2 injections (13, 18). However, in these studies using 3000 BRMP units of IL-2 for treatment (18) no ADCC was generated in a distant organ (spleen). Using significantly higher IL-2 doses (25,000-200,000 units) we noted dose-dependent generation of cells with ADCC activity in the peritoneal cavity, liver, and lungs. Lower ADCC activity was seen in the splenocytes and bone marrow cells and the lowest activity was found in the MLN and thymus. The difference in ADCC in these organs could be due to differences in IL-2 concentration of ASGM1+ cells was confirmed by testing the NK activity of cells isolated from the spleens of other mice injected with anti-ASGM1 serum at the same time. The control group received normal rabbit serum. As shown in Table 4 anti-ASGM1 serum administration completely abrogated the NK activity of the splenocytes of the treated mice when compared to the control group and reduced ADCC from 119 to 2.5 LU/10^7 cells.

No Effect of Combined Treatment of F1 Mice with IL-2 and Anti-B16 Mab on Established B16 Melanoma Lung Metastases

B16 melanoma liver and lung metastases were induced in F1 mice. On day 3 mice received IL-2 given i.p. three times a day for three days (Exp. 2) and 5 days (Expt. 1.3). On day 3 (Exp. 3), day 6 (Exp. 1) and days 3.6 (Exp. 2) mice were injected i.v. with 1 mg of anti-B16 Mab given in 1 ml HBSS. On day 14 the mice were sacrificed, the lungs and liver removed and the number of metastases enumerated in a blinded fashion.

Table 5 Effect of combined treatment with anti-B16 Mab and IL-2 on established B16 melanoma liver and lung metastases

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment</th>
<th>Mean number of metastases (SEM)*</th>
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<tbody>
<tr>
<td>1</td>
<td>HBSS</td>
<td>4671 (23.8)</td>
</tr>
<tr>
<td></td>
<td>Mab</td>
<td>307 (24.0)</td>
</tr>
<tr>
<td></td>
<td>IL-2</td>
<td>420.0 (15.2)</td>
</tr>
<tr>
<td></td>
<td>Mab + IL-2</td>
<td>42.6 (14.2)*</td>
</tr>
<tr>
<td>2</td>
<td>HBSS</td>
<td>135.8 (69.4)</td>
</tr>
<tr>
<td></td>
<td>Mab</td>
<td>12.8 (4.6)</td>
</tr>
<tr>
<td></td>
<td>IL-2</td>
<td>8.1 (3.2)</td>
</tr>
<tr>
<td></td>
<td>Mab + IL-2</td>
<td>0.4 (0.1)*</td>
</tr>
<tr>
<td>3</td>
<td>HBSS</td>
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<tr>
<td></td>
<td>Mab</td>
<td>247.5 (2.3)</td>
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<td></td>
<td>IL-2</td>
<td>238.1 (7.4)</td>
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<td>Mab + IL-2</td>
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* SEM, mean of six mice in each group, ± SEM. P value < 0.05 compared to the HBSS, IL-2, and Mab alone treated group.

ND, not determined.
distribution to various organs or in the number of cells in these organs which could be activated by IL-2. We, as well as others, have previously shown that cells induced in vitro by IL-2 which mediate ADCC were related to NK/LAK cell populations (17, 18). In this study we found that the ADCC induced in vivo in the lungs resided in the ASCGM™, Lyt2/LYT4—cell population. In addition, the effector cells were mainly Thy1.2+ (Fig. 6), similar to LAK cells induced in vitro, and were enriched in FcR+ cells as previously found in vitro where a correlation between the number of FcR+ cells and ADCC was seen (17). NK cells, which are known to express FcR (8) and mediate ADCC (8, 29), have been described in various organs including the lungs, liver, spleen, bone marrow, and peritoneal exudate cells (30—32).

Several studies demonstrated the ability of immunopotentiators such as Bacillus Calmette-Guérin (2), C. parvum (3, 4), and IL-2 (13, 18, 34) to induce or enhance antibody mediated antitumor effects though, in these studies systemic antitumor effects were seen only in s.c. tumors (3, 4, 18) when treatment was started at the day of tumor inoculation (13) or 2 days later (34). We and others have demonstrated that IL-2 administration can mediate antitumor effects on established metastases (33, 35), and that the combined therapeutic effects of anti-B16 Mab and IL-2 could be demonstrated on established B16 melanoma liver micrometastases (19). The nature of cells involved in this antitumor effect in vivo remains to be elucidated.

Our results suggest that the systemic administration of IL-2-induced cells in vivo which mediate ADCC, expressed NK/LAK cell markers similar to the cells induced in the peritoneal cavity of IL-2-treated mice (13, 18, 34), and showed a similarity to LAK cells in both the kinetics and IL-2 dose dependence of their induction (Figs. 4 and 5). In vivo exposure of cells to IL-2 Explained FcR+ cells, similar to the effect observed in vitro (17), but also increased the FcR density on the cell surface. Similar to our results, others have shown that CSF-1 and α,β, and γ interferon upregulates the number of FcR on murine bone marrow derived macrophages (36). As expected, the inability of IL-2 to induce ADCC in C57BL/6 mice in vivo (Fig. 2) correlated with the absence of an antitumor effect when IL-2 was coadministered together with antibody into C57BL/6 mice bearing B16 lung metastases (37). Moreover, even in (C57BL—xCH)F1 mice in which IL-2 induced, both in vitro and in vivo, significantly higher ADCC activity than in C57BL/6N mice (Figs. 1 and 2), the combined treatment with IL-2 and anti-B16 Mab reduced the number of liver metastases but had no effect on the number of lung metastases (Table 5). This may be a result of the existence of fewer cells mediating ADCC in the lungs of F1 mice when compared to the liver or to a difference in antibody traffic to these two organs (38).

In this study we demonstrated the ability of IL-2 to systemically induce cells with ADCC activity. The role of other cytokines as well as their therapeutic potential against established metastases are currently being studied.

REFERENCES

Systemic Induction of Cells Mediating Antibody-dependent Cellular Cytotoxicity following Administration of Interleukin 2

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