TOXICITY AND THERAPEUTIC EFFICACY OF HIGH-DOSE INTERLEUKIN 2

In Vivo Infusion of Antibody to NK-1.1 Attenuates Toxicity without Compromising Efficacy Against Murine Leukemia

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High concentrations of IL-2 in vitro can induce phenotypically heterogeneous subpopulations of lymphocytes to mediate nonspecific lysis of a broad array of malignant cells (1-4). Although heterogeneous in phenotype, and thus potentially heterogeneous in character and function, IL-2-induced cytolytic effector cells have been generically termed lymphokine-activated killer (LAK)\(^1\) cells (1). Administration of high-dose IL-2 in vivo has been shown to similarly induce the activation of LAK cells in vivo (5, 6), as well as to induce the proliferation of host lymphocytes (7), the secretion of lymphokines (8, 9), and the regression of some malignant tumors (5, 6, 10). The cell populations and precise cellular mechanisms responsible for tumor regression in vivo are ill defined. However, it is likely that the mechanisms by which IL-2 induces tumor regression will prove to vary according to the clinical circumstances, and will include the direct lysis of tumor cells by LAK cells, the primary or secondary induction of cytokines such as interferon or tumor necrosis factor, and the augmentation of autochthonous tumor-specific immunity.

Regardless of the mechanism of tumor regression, toxicity has been a major limitation for the therapeutic use of IL-2 against human malignancy (10, 11). The most pronounced toxicity has been a vascular leak syndrome (10-13). However, hematological, renal, hepatic, and neurological abnormalities have also been observed and limit the amount of IL-2 that can be administered (10-13). The proximate cause of toxicity is poorly defined but may include lymphoid hypertrophy with impingement upon adjacent tissue, secretion of deleterious lymphokines, and/or direct lysis of normal tissue by promiscuously cytolytic cells (14-17).

Determination of the cell populations and clarification of the precise mechanisms responsible for both tumor regression and toxicity might suggest measures to augment efficacy and limit toxicity. For example, if toxicity is largely attributable to

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This work was supported by grants CA-43081, CA-30558, and CA-33084 from the National Cancer Institute, and IM-304 from the American Cancer Society. D. J. Peace is the recipient of Clinical Investigator Award CA-01299 from the National Cancer Institute, Department of Health and Human Services. Please address correspondence to David J. Peace, M.D., Division of Oncology, RK-25, HSB BB1015, University of Washington, 1959 N.E. Pacific Street, Seattle, WA 98195.

\(^1\) Abbreviations used in this paper: ASGM-1, asialoGM-1; LAK, lymphokine-activated killer cells; poly I:C, polyinosinic-polycytidylic acid.
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a distinct lymphocyte subset, suppression or deletion of that subset might abrogate toxicity without impeding antitumor activity.

Although the phenotype of LAK cells mediating in vitro cytolytic activity has been extensively evaluated, few studies have examined the phenotype of cells mediating in vivo efficacy and toxicity, and general principles as to the mechanism(s) of effect of high-dose IL-2 in vivo have yet to evolve. In vitro, in most circumstances LAK cells with an NK-like phenotype are the predominant effector cells of LAK cell-mediated cytolytic reactivity (18–20). However, in vivo, two reported studies assessing the phenotype of cells mediating the therapeutic efficacy of high-dose IL-2 have demonstrated that lymphocytes expressing the Lyt-2+ T cell phenotype are the predominant effector cells against an established MCA sarcoma and a B16 melanoma cell transfected with class I MHC antigens (21, 22). AsialoGM1 (ASGM-1+) cells have been shown to contribute to the therapeutic efficacy of IL-2 against MCA sarcoma soon after implantation, indicating a potential role for cells with an NK phenotype. However, once tumor had become established, ASGM-1+ cells had no detectable role in mediating tumor regression induced by IL-2 (21). By contrast, in the single reported study assessing the phenotype of cells mediating toxicity of high-dose IL-2 in vivo, toxicity was diminished by in vivo depletion of ASGM-1+ cells (23). These results suggest that the toxicity of IL-2 may be mediated primarily by NK cell populations expressing the ASGM-1 determinant (24). However, ASGM-1 has also been identified on lymphocyte populations other than NK cells, including cytotoxic T cells (25), thymus cells (26), and activated macrophages (27), and a substantial role for such cells was not ruled out.

The current studies were designed to further delineate which lymphocyte populations are responsible for the lethal toxicity of high-dose IL-2 and which lymphocyte populations are responsible for therapeutic efficacy. The model chosen for study was the therapy of an established, disseminated Friend virus–induced leukemia, termed FBL-3, in syngeneic C57BL/6 mice. We had previously demonstrated that established FBL-3 leukemia could be cured by high-dose IL-2 (6), but that the therapeutic efficacy was greatly compromised by IL-2-induced toxicity. The initial current studies revealed that high-dose IL-2 administered in vivo induced the proliferation of heterogeneous lymphocyte populations including Lyt-2+ T cells, L3T4+ T cells, NK cells, and B cells. The results of subsequent toxicity and therapy studies revealed that the lethal toxicity of high-dose IL-2 was attenuated by the in vivo infusion of antibody to NK-1.1 but not Lyt-2 or L3T4. By contrast, the therapeutic efficacy of high-dose IL-2 was markedly reduced by infusion of antibody to Lyt-2 and L3T4 but not NK-1.1. Thus, for the therapy of established, disseminated murine leukemia with high-dose IL-2, the lymphocyte subpopulation that mediates toxicity is distinct and separable from the lymphocyte subpopulations required for tumor therapy. The ramifications of these results for IL-2 therapy of human malignancy are discussed.

Materials and Methods

Interleukin 2. Human rIL-2, generously provided by Hoffmann-LaRoche, Inc. (Nutley, NJ), was diluted in HBSS containing 1% pathogen-free syngeneic mouse sera to provide a specific activity of 10^6 units of IL-2 per milliliter as determined by a standard CTLL-2 proliferative assay (28).

Mice. Female C57BL/6 (B6) and BALB/c mice, 6–12 wk old, were obtained from The
Jackson Laboratories (Bar Harbor, ME) and BALB/c- nu/nu mice, 6-12 wk old, were obtained from the Fred Hutchinson Cancer Research Center (Seattle, WA).

**Tumor Cell Lines.** FBL-3 (FBL), a Friend virus-induced leukemia of B6 origin was subcloned and maintained by in vivo passage. YAC-1, an NK-sensitive Moloney virus-induced lymphoma of A/Sn origin was maintained by in vitro passage in our laboratory.

**Hybridomas and Antibody Preparations.** Ascites containing mAb against NK-1.1 (from the hybridoma PK136, a gift of Gloria Koo of Merck Sharp and Dohme, Inc., Rahway, NJ [29]), Lyt-2 (from the hybridoma 2.43, obtained from American Tissue Culture Collection, Rockville, MD [30]), L3T4 (from the hybridoma GK1.5, a gift of Frank Fitch, University of Chicago, Chicago, IL [31]), and murine IL-2-R (from the hybridoma 7D4, a gift of Tom Malek, University of Miami, Miami, FL [32]) were prepared by injecting hybridomas intraperitoneally into pristane-primed BALB/c- nu/nu mice. FITC-conjugated anti-Lyt-2 and anti-Thy-1.2 mAb were purchased from Becton Dickinson Immunocytochemistry Systems (Mountain View, CA); FITC-conjugated goat anti-rat IgG was purchased from Tago, Inc. (Burlingame, CA); and F(ab’)2 fragments of FITC-conjugated rabbit anti-mouse IgG were purchased from Zymed Laboratories, Inc. (San Francisco, CA).

**Immunofluorescent Cell Staining and Flow Cytometry.** Spleen cells depleted of RBC by hypotonic lysis were washed and resuspended at 10⁷ cells/ml in HBSS. All staining steps were performed at 4°C for 45 min. Thy-1 cells were labeled with FITC-conjugated anti-Thy-1 mAb. Lyt-2' cells were labeled with FITC-conjugated anti-Lyt-2 mAb. L3T4' cells were labeled with mAb GK1.5 and a second-step FITC-conjugated goat anti-rat IgG. SIg' cells were labeled with FITC-conjugated rabbit anti-mouse IgG. NK-1.1' cells were labeled with anti-NK-1.1 and a second-step FITC-conjugated rabbit anti-mouse IgG. IL-2-R-bearing cells were labeled with FITC-conjugated anti-IL-2-R mAb 7D4. Cytometric analyses were performed with a cytofluorograph (model 50H) interfaced to a model 2150 computer (Ortho Diagnostics Systems, Westwood, MA). Excitation was achieved with a laser power of 400 mW at 488 nm. FITC fluorescence was measured at 520 to 540 nm (DF 575; Omega Optical Co., Brattleboro, VT). Cells were distinguished as either small or large cells on the basis of the degree of forward light scatter.

**In Vivo Lymphocyte Subset Depletion.** Ascites preparations of PK136, 2.43, and GK1.5 mAb were titered in vivo for their ability to deplete NK-1.1', Lyt-2', and L3T4' lymphocyte subsets, respectively, and amounts found to be effective were used in subsequent experiments. The ability of antibodies to deplete the designated lymphocyte subset was established by immunofluorescent labeling and functional assay of the targeted lymphocyte subset 2 to 3 d after intraperitoneal administration of antibody. In vivo depletion with mAb GK1.5 ascites preparation (200 µl i.p. on two successive days) markedly reduced cells, which could be identified by two-step immunofluorescence. In vivo depletion with mAb 2.43 (100 µl i.p.) markedly reduced cells that could be identified with a FITC-conjugated anti-Lyt-2 antibody that binds to the Lyt-2 determinant in the presence of the mAb 2.43. In vivo depletion of Lyt-2' cells with mAb 2.43 was also shown to abrogate subsequent in vitro generation of allospecific CTL as demonstrated by the failure of Lyt-2-depleted B6 splenocytes stimulated for 5 d with irradiated BALB/c splenocytes to lyse BALB/c Con A-induced lymphoblasts during a 4-h ³¹Cr-release assay. Depletion of NK-1.1' cells with mAb PK136 (100 µl i.p.) reduced cells, which could be identified by FITC-conjugated PK136 and markedly reduced the ability of spleen cells from normal mice or mice primed for 24 h with 100 µg poly(I:C) to lyse the NK-sensitive target YAC-1 in an 18-h ³¹Cr-release assay.

**Results**

High-Dose IL-2 Induces the Proliferation of Heterogeneous Lymphocyte Subsets In Vivo. Normal C57BL/6 (B6) mice were treated with IL-2 200,000 U i.p. three times a day for 5 d and the number and phenotype of resultant lymphocytes determined. Similar to previous reports (7), high-dose IL-2 induced marked hypertrophy of the spleen and lymph nodes as well as lymphocytic infiltration of the liver and lungs. As an example, in a representative experiment depicted in Fig. 1, IL-2 induced a 3-fold
increase in the total number of spleen cells and a 12-fold increase in the number of large spleen cells, which presumably represented a measure of activated and proliferating lymphoid cells. The phenotypes of the cells expanded by IL-2 were examined and shown to be heterogeneous. Thus, Thy-1$^+$ and Thy-1$^-$ spleen cells each increased in number, 4.6-fold and 2.5-fold, respectively (Fig. 1A). Within the enlarged cell population expanded by IL-2 (dark area in Fig. 1), the majority of cells (89%) expressed the Thy-1 determinant. Increases occurred in all lymphocyte subsets examined, including: both Lyt-2$^+$ and L3T4$^+$ T cells; mature NK cells (NK-1.1$^+$); and B lymphocytes (Ig$^+$), with the largest proportionate increase occurring within the NK-1.1$^+$ and Lyt-2$^+$ subsets of enlarged lymphocytes (Fig. 1 B).

It is unknown as to what extent cellular proliferation was a result of direct IL-2 stimulation of IL-2-R-bearing cells versus indirect effects of IL-2 consequent to the induction of the secretion of additional growth factors. The latter is a possibility since only a minority of cells recovered from the spleens of IL-2-treated animals expressed receptors for IL-2 (Table I).

Lymphocyte Subset Depletion in Mice Treated with High-Dose IL-2. To determine the contribution of specific lymphocyte subsets to the toxicity and the therapeutically efficacy of IL-2, subsets were selectively depleted in vivo by administering antibody to specific subsets before administering IL-2. Preliminary experiments confirmed previous reports (21, 33) that antibody to Lyt-2, L3T4, or NK-1.1 could specifically deplete the nominated subset from normal mice. Efficacy of depletion was confirmed by use of both phenotypic and functional analyses as detailed in Materials and Methods.

Before examining the effect of lymphocyte subset depletion on the toxicity and efficacy of IL-2, it was necessary to establish that subsequent IL-2 administration did not substantially reverse the effects of subset depletion. Thus, cohorts of antibody-
TABLE I

Induction of IL-2 Receptors by High-Dose IL-2 Treatment

| IL-2-R*+1 | Small cells | Large cells |
|-----------|-------------|-------------|
| -         | 0.7         | 1.2         |
| +         | 5.1         | 18.1        |

* B6 mice were treated with no IL-2 or high-dose IL-2, 200,000 U i.p. three times per day for 7 d.
+ Spleen cells were harvested and analyzed by FACS for IL-2-R expression as described in Materials and Methods.

The Lethal Toxicity of High-Dose IL-2 Is Attenuated by the In Vivo Depletion of NK-1.1+ Cells but not Lyt-2+ Cells or L3T4+ Cells. The contribution of Lyt-2+, L3T4+, and NK-1.1+ lymphocyte subsets to the toxicity of high-dose IL-2 was determined by selectively eliminating lymphocyte subsets before the administration of a potentially toxic dose of IL-2. This was achieved by injecting mice intraperitoneally with antibodies directed against Lyt-2, L3T4, or NK-1.1 before treatment with IL-2.

TABLE II

Lymphocyte Subset Depletion in Mice Treated with IL-2

| Antibody in vivo* | Lymphocyte subset | Percent positive† |
|------------------|-------------------|-------------------|
| None             | Lyt-2+            | 9.6               |
| Anti-Lyt-2       | Lyt-2+            | 0.8               |
| None             | L3T4+             | 35.6              |
| Anti-L3T4        | L3T4+             | 3.1               |
| None             | NK-1+             | 19.6              |
| Anti-NK-1.1      | NK-1+             | 5.5               |

* B6 mice were injected intraperitoneally with the indicated antibody as described in Materials and Methods then treated with IL-2, 200,000 U i.p. three times per day for 7 d.
† After IL-2 therapy animals were killed and spleen cells were stained for expression of the indicated determinant and analyzed by FACS as described in Materials and Methods. Results are presented as the percent of positive cells.
lethal regimen of high-dose IL-2. Thus, B6 mice were injected with antibody against the Lyt-2, L3T4, or NK-1.1 determinants. Mice were then treated with IL-2 200,000 U three times per day until all mice in an undepleted control groups had expired from the toxic effects of IL-2. Results (Fig. 2) demonstrated that by day 10 all control mice had died from IL-2. Depletion of NK-1.1 cells before IL-2 treatment markedly reduced the number of mice that expired from an otherwise lethal regimen of IL-2 and allowed 44% of mice to survive the LD100 of IL-2. By contrast, prior depletion of L3T4+ or Lyt-2+ cells did not detectably affect the toxicity in mice treated with an identical regimen of IL-2. Mice that survived high-dose IL-2 as a result of depleting NK-1.1+ cells developed signs of toxicity during IL-2 administration manifested by varying degrees of ascites, tachypnea, and ruffled hair, but appeared well within 3 to 4 d after stopping IL-2 and continued to thrive during a 60-day post-therapy observation period. Although antibody to NK-1.1 decreased the toxicity of high-dose IL-2, the attenuation of lethal toxicity was not absolute and could be overcome by continued injections of high-dose IL-2 for two to four additional days (data not presented). The phenotype of cells mediating toxicity in this latter circumstance was not analyzed.

The Therapeutic Efficacy of High-Dose IL-2 Against Disseminated FBL-3 Leukemia Is Reduced by In Vivo Depletion of either Lyt-2+ Cells or L3T4+ Cells but not NK-1.1+ Cells. The role of Lyt-2+, L3T4+, and NK-1.1+ lymphocytes for the therapy of FBL-3 leukemia with IL-2 was similarly examined. We had previously shown that high-dose IL-2 injected from days 5 to 9 into C57BL/6 mice after injection on day 0 with 5 x 10^6 FBL-3 cells (as few as 10 FBL-3 cells will kill 100% of mice) could cure approximately 50% of mice (6). The optimum dose of IL-2 varied slightly from experiment to experiment; and, the therapeutic effect of IL-2 was dose dependent with decreasing doses curing a proportionately smaller percentage of mice. Increasing the dose of IL-2 above the optimum level resulted in increased deaths from the toxicity of IL-2 and thus could not increase the proportion of mice surviving long-term tumor free and never cured 100% of mice.

In the current study (Fig. 3), B6 mice were injected on day 0 with 5 x 10^6 viable FBL-3 cells. On day 3, mice were injected with anti-NK-1.1, anti-Lyt-2, anti-L3T4, or nonimmune sera as a control. On day 5, mice had palpable ascites and disseminated FBL and a total body burden of ~10^9 FBL cells. Mice were then left untreated.

![Figure 2. The lethal toxicity of high-dose IL-2 is attenuated by in vivo depletion of NK-1.1+ cells but not Lyt-2+ cells or L3T4+ cells. B6 mice were treated with anti-Lyt-2 mAb (n = 16), anti-L3T4 (n = 10), anti-NK-1.1 mAb (n = 16), or with nonimmune mouse sera indicated as "control" (n = 22), as described in Materials and Methods. 2 d after initial administration of antibody, mice were treated with rIL-2, 200,000 U in 0.2 ml HBSS i.p., three times per day until all mice in the "control" group died from the toxic effects of high-dose IL-2. IL-2 treatment was then terminated and the percent of surviving animals in each experimental group was monitored for a follow-up period of 2 mo. The graph represents the combined results of four experiments.](image-url)
or were treated with IL-2 (50,000 U three times a day) from days 5–9. Mice receiving no therapy all died by day 14, whereas 78% of mice treated with high-dose IL-2 alone were cured. Depletion of NK-1.1+ cells did not reduce the ability of IL-2 to cure mice of disseminated leukemia. By contrast, depletion of either Lyt-2+ or L3T4+ cells completely abrogated the curative effect of high-dose IL-2.

Discussion

The current study demonstrates that the NK-1.1+ lymphocyte subset contributes substantially to the lethal toxicity of high-dose IL-2. The finding that specific in vivo depletion of NK cells with the anti-NK-1.1 antibody attenuates the lethal toxicity of IL-2, corroborates the demonstration by Gately et al. that depletion of ASGM-1+ cells reduces the toxicity of IL-2 (23), since both ASGM-1 and NK-1.1 are expressed by murine NK cells (24, 29). However, the use of antibody to NK-1.1 provides more direct evidence that phenotypic NK cells are major mediators of toxicity, since the NK-1.1 antigen appears to be more specific for NK cells than ASGM-1, which has been identified on a variety of other cell types, including subsets of activated T cells (25), thymocytes (26) and macrophages (27).

The diminished toxicity resulting from the depletion of NK cells was not merely the consequence of a decrease in total lymphocyte number, since attenuation of toxicity achieved by in vivo removal of NK-1.1+ cells occurred despite a concurrent compensatory increase in the numbers of cells in nondepleted lymphocyte subsets. Thus, IL-2-stimulated NK-1.1+ cells appear to be intrinsically more toxic than the other IL-2 responsive cell types examined in vivo.

The distinguishing attributes that account for the particularly toxic effects of NK cells are undefined but potentially include both an increased propensity to lyse normal tissue and/or propensity to secrete deleterious lymphokines. LAK cells have been considered to be selectively lytic for malignant tumor cells (1). However, in addition to lysing malignantly transformed cells, LAK cells have been reported to lyse a variety of nontransformed cells, including Con A-stimulated lymphocytes (16), cultured fibroblasts (15), primary explants of epithelial cells (14), endothelial cells (17), as well as hapten-modified self (19).

LAK cells are heterogeneous in respect to precursor and effector phenotype, and
thus are likely to be heterogeneous in respect to target spectrum and the propensity to lyse normal tissue. The two major phenotypic categories of LAK effector cells derived from murine spleens are NK-like (NK-1\(^+\)) and T cell-like (Lyt-2\(^+\)) cells that are derived from Lyt-2\(^-\) and Lyt-2\(^+\) precursor cells, respectively (19, 20). Ballas et al. (19) demonstrated that for lysis of haptenated self, differences exist between the lytic efficacy of murine T cell-like LAK cells and NK-like LAK cells. In this circumstance, T cell-like LAK cells rather than NK-like LAK cells were most lytic. However, the results demonstrated that differences in lytic spectrum exist. In humans, Sondel et al. (16) have confirmed that LAK cells derived from T cell-depleted PBL cells, and therefore presumably highly enriched for NK-like LAK cells, are lytic towards autologous lymphocytes, whereas Damle et al. (17) have shown that NK-like LAK cells can lyse endothelial cells. Thus, NK-like LAK cells evidently can lyse normal cells. However, differences in the spectrum of lytic activity against normal tissues between LAK cell subsets have not yet been extensively evaluated, and whether such differences are responsible for differences in toxicities is uncertain.

An alternative explanation for the predominant toxicity of the NK subset is the potential preferential secretion of deleterious cytokines by IL-2-activated NK cells. In vivo administration of high-dose IL-2 results in elevated levels of secondary cytokines, such as TNF-\(\alpha\) and IFN-\(\gamma\) (8, 9), which are each capable of mediating lethal toxicity. Combined administration of IFN-\(\gamma\) and TNF-\(\alpha\) has been shown to induce toxic manifestations that are similar to those of high-dose IL-2 therapy, including fever, hypotension, leukopenia, and renal, hepatic, and neurologic dysfunction (34). Moreover, TNF-\(\alpha\) has been implicated as the primary mediator of endotoxic shock (35), a condition characterized by vascular instability, fluid extravasation, and hypotension similar to the vascular leak syndrome induced by IL-2.

The primary in vivo source of IL-2-induced IFN-\(\gamma\) or TNF-\(\alpha\) is unknown but may be inherent within the NK cell population. In vitro, NK cells have been shown to produce both IFN-\(\gamma\) and a TNF-\(\alpha\)-like factor that is serologically crossreactive with macrophage-derived TNF-\(\alpha\) (36, 37). Furthermore, NK cells, but not T cells, are induced by IL-2 to produce IFN-\(\gamma\) during short-term culture in vitro (36). Thus, NK cells may be preferentially stimulated by IL-2 in vivo to produce IFN-\(\gamma\) and TNF-\(\alpha\); and reduction of the toxicity of IL-2 by elimination of NK cells may result from the removal of the major source of IFN or TNF or other, as yet undetermined, cytokines. The in vivo ability of neutralizing antibody directed against specific cytokines to attenuate IL-2-induced toxicity is being evaluated currently.

Depletion of NK cells with anti-NK-1.1 increased the LD-100 of IL-2 but did not completely abrogate lethal toxicity, since animals treated with longer courses of high-dose IL-2 all eventually died (data not shown). Gately et al. (23) similarly observed that anti-ASGM-1 delayed, but did not prevent, the occurrence of lethal toxicity in mice that received extended courses of IL-2. The nature of effector cells that mediate the toxic effect of IL-2 in this circumstance was not examined. Either additional cell subsets or reconstituted NK cells may be responsible for the delayed toxicity of IL-2 following NK cell depletion. In vitro studies have demonstrated that IL-2 can promote the maturation of NK-1.1\(^+\) cells from NK-1.1\(^-\) precursors (33). Thus, protracted IL-2 therapy may eventually reconstitute depleted populations of toxic NK cells in vivo. On the other hand, cells potentially activated by IL-2 that are outside of the NK cell lineage, such as B cells, T cells, or monocytes may mediate the lethal effect of protracted IL-2 in the absence of substantial numbers of NK cells.
Both Lyt-2* and L3T4* cells, which were not dominant mediators of lethal toxicity, were required for expression of IL-2-induced therapy in the FBL-3 model. Others have demonstrated that Lyt-2* cells are required for IL-2-induced regression of established macrometastatic MCA-induced sarcomas (21), and regression of B16 melanoma—expressing class I MHC antigens (22). The mechanism by which Lyt-2* cells contribute to the therapeutic effects of IL-2 in the aforementioned models is undetermined. In vitro, IL-2 can induce Lyt-2* cells to become LAK effectors capable of lysing a broad spectrum of tumors in a non-MHC-restricted manner, but also can facilitate the generation and function of specifically immune Lyt-2* cells capable of mediating destruction of tumor through specific recognition of tumor-associated antigen presented by class I MHC antigens. Although both specific and nonspecific mechanisms might be operative during IL-2-induced tumor eradication, it is likely that autochthonous tumor-specific T cell immunity is a predominant, if not essential component. Evidence for this view is taken from several sources. First, the current observation that depletion of NK-1.1+ cells did not diminish therapeutic efficacy of IL-2 implies that the nonspecific component of IL-2 therapy might be weak. Second, in both the FBL-3 model and the MCA sarcoma model, delayed IL-2 therapy after tumor implantation has been shown to be significantly more effective than therapy immediately after tumor implantation (5, 6), implying that the evolution of a host–tumor interaction is essential for successful therapy with IL-2. One likely host–tumor interaction would be the induction of specific host antitumor immunity. Accordingly, mice cured of FBL-3 by high-dose IL-2 have been shown to contain T cells specifically immune to FBL-3 (6). Third, in the MCA sarcoma model, Mulé (21) has demonstrated that for the therapy of established MCA sarcoma, weakly immunogenic sarcomas respond to IL-2, whereas nonimmunogenic sarcomas are refractory to the therapeutic effects of IL-2 despite the fact that immunogenic and nonimmunogenic MCA sarcomas are lysed equivalently by LAK cells in vitro (21).

The principle difference identified between nonimmunogenic and weakly immunogenic MCA sarcomas by Mulé was the expression of class I MHC antigens by the immunogenic tumors but not by the nonimmunogenic tumors (21). Neither expresses class II MHC antigens. Therefore, only the weakly immunogenic MCA sarcomas have the potential to be lysed by tumor antigen–specific MHC-restricted T cells. The importance of expression of MHC antigens by tumor cells for elicitation of therapeutic IL-2-induced responses was further established by Weber et al. (22) in studies involving transfection of MHC genes into B16 melanoma. They demonstrated that expression of class I MHC rendered established B16 melanoma sensitive to IL-2 therapy. In this circumstance, Lyt-2* cells were also required for therapy.

In the current experiments, L3T4* cells, as well as Lyt-2* cells, were shown to be required for the therapeutic effects of IL-2. During the generation of most normal immune T cell responses, L3T4* T cells respond to antigen presented by class II MHC antigen and provide T cell help, and Lyt-2* T cells respond to antigen presented by class I MHC antigens and provide for specific cytolytic reactivity (38, 39). Similarly, in the FBL-3-specific T cell response, the L3T4* subset has been shown to provide the majority of help required for the generation of FBL-3 tumor-specific Lyt-2* cytolytic T cells; and direct cytolytic reactivity is mediated exclusively by Lyt-2* T cells (40). It is noteworthy that FBL-3 does not express class II MHC antigens nor can such antigens be induced by IFN-γ (41). Thus, L3T4* T cells that are restricted to recognizing antigen presented by class II MHC antigens
are incapable of directly recognizing FBL-3 cells; and, stimulation of L3T4+ T cells by tumor requires that tumor antigen be processed and presented by APCs, such as macrophages (40). Since L3T4+ T cells cannot directly lyse FBL-3 in an antigen-specific fashion, and since IL-2-activated L3T4+ cells constitute a minor subset of nonspecific LAK cell effectors, as determined in in vitro studies, it is possible that L3T4+ cells in the current therapy study were requisite for the evolution of the initial host antitumor immunity rather than for the effector phase of IL-2-induced therapy. Alternatively, immune L3T4+ T cells might not be required for the initial IL-2-induced antitumor response, but might be required for mediating a specific antitumor response to eradicate the last remaining tumor cells potentially present long after IL-2 therapy.

The implication in murine models that autochthonous tumor-specific T cell immunity may be a predominant component of successful therapy with high-dose IL-2 may be especially germane to the use of IL-2 for the treatment of human malignancies. The observation that most fresh human tumors can be nonspecifically lysed by LAK cells in vitro, whereas only a modest proportion of tumors can be induced to regress by IL-2 in vivo implies that non-MHC-restricted lysis of tumors may not be the essential component of IL-2-induced tumor regression in vivo. Moreover, tumor types that have responded most consistently to IL-2-based regimens in humans are those that have long been considered to be immunogenic in nature (i.e., melanoma and renal cell carcinoma [10, 11]). Therefore, autochthonous host antitumor immunity may be required for response to high-dose IL-2 in humans. In the case of human malignant melanoma, the tumor most extensively assessed for responsiveness to IL-2, lymphocytes obtained from melanoma tumor sites either before or after therapy with IL-2 predominantly express T cell determinants (42, 43). Moreover, T lymphocytes that specifically lyse autologous tumor cells have been cloned directly from melanoma tumors, confirming the existence of autochthonous tumor-specific T cells at the site of tumor that might be activated or induced to proliferate by administration of IL-2 in vivo (44). In the small number of patients examined to date, only melanoma tumors expressing class II MHC antigens have responded to IL-2 (43), adding further credence to the possibility that MHC-restricted T cell responses are necessary for effective therapy with IL-2.

The implication that human tumors responding to IL-2 therapy may be immunogenic in the autochthonous host serves to focus attention on potential methods to augment specific aspects of IL-2 therapy, as opposed to the current emphasis on developing methods to augment nonspecific aspects. Finally, regardless of whether or not T cells are required for the efficacy against the majority of human tumors, for those human tumors in which the therapeutic effects of IL-2 are predominantly mediated by T cells, the current study predicts that it should be possible to reduce toxicity and increase the therapeutic index of IL-2 by selectively depleting NK cell subsets.

Summary

In the current study we used the therapy of established murine leukemia to identify the lymphocyte subsets responsible for toxicity and for therapeutic efficacy of high-dose IL-2. Initial results confirmed that high-dose IL-2 induces marked proliferation of a variety of host cells, including NK cells, Lyt-2+ T cells, L3T4+ T cells,
and B cells. Infusion of antibody to NK-1.1 depleted NK-1.1+ cells in vivo and greatly reduced the toxicity of IL-2, but did not decrease therapeutic efficacy. By marked contrast, depletion of host T cells, either Lyt-2+ or L3T4+, had no effect on toxicity but greatly reduced therapeutic efficacy. The requirement for host T cells for the curative effect of IL-2 gives credence to the possibility that substantial efficacy of high-dose IL-2 against established malignancy may require existent host antitumor immunity. Since the human tumors that have been shown to have the most substantial responses to IL-2 (i.e., malignant melanoma and renal cell carcinoma) are those long considered to be immunogenic in the autochthonous host, the current study predicts that for these, as well as other immunogenic human tumors, it should be possible to decrease the toxicity and thus increase the therapeutic index of IL-2 by selectively depleting NK cells in vivo.

The authors are especially grateful to S. Emery, K. Slaven, and J. Smith for their expert technical help; and to W. Robertson, A. Rogers, and L. Wilson for their assistance in the preparation of this manuscript.

Received for publication 19 September 1988.

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