Monoclonal Antibody Therapy of Murine Lymphoma: Enhanced Efficacy by Concurrent Administration of Interleukin 2 or Lymphokine-activated Killer Cells


ABSTRACT

Lymphokine-activated killer (LAK) cells have recently been shown to be very efficient effector cells for antibody-dependent cellular cytotoxicity. Thus, we explored, in a murine lymphoma model, administration of LAK-inducing doses of interleukin-2 (IL-2) or adoptive transfer of LAK cells as a means of enhancing therapy with tumor-specific monoclonal antibody (mAb). AKR/Cum (Thy-1.2*) hosts were inoculated on day 1 s.c. with the SL-2 thymoma of AKR/J origin (Thy-1.1*) and developed palpable tumor on day 4. Tumor-specific anti-Thy-1.1 IgG2a mAb, 1A14, was given on days 4 and 8 with 50,000 units/day IL-2 i.p. divided in two doses on days 4–12. Therapy with IL-2 or mAb alone had minimal activity, prolonging control median survival of 22 days to 25 and 29 days, respectively, whereas therapy with IL-2 plus mAb significantly prolonged median survival to 40 days. However, combined therapy did not result in cures and long-term survival. The efficacy of combined therapy did not result from alterations in the biodistribution of mAb by concurrent IL-2 infusions, as determined by studies with radiolabeled mAb.

The combined effect of in vitro generated LAK (10^6 cells) adoptively transferred i.v. with 1A14 on days 4 and 8 following SL-2 inoculation was also evaluated. This regimen had no detectable toxicity, and treatment of mice with LAK and mAb resulted in 60% long-term survival compared with 17% or 0% for mice treated with mAb or LAK alone. Thus, the therapeutic effects of tumor-specific mAb was enhanced by in vitro administration of IL-2 or by adoptively transferred LAK, which may represent means to provide the host with increased antibody-dependent cellular cytotoxicity effector cells. Adoptively transferred LAK has the additional benefit of augmenting mAb therapy of tumor without the toxicity associated with the induction of such cells in vivo with high dose IL-2.

INTRODUCTION

mAbs have been generated against many human tumor-associated antigens, the majority of which are differentiation- or lineage-associated antigens expressed in high density on tumor cells. Therapy using such mAbs has caused tumor regression in patients with lymphoma, melanoma, neuroblastoma, and colon carcinoma but rarely results in complete tumor eradication or improves the long-term survival of treated patients. Mechanisms by which mAbs can eradicate tumor cells in vivo are complement-dependent cytotoxicity and ADCC. Studies in animal models using complement-deficient or depleted hosts have suggested that the primary mechanism of in vivo lysis of tumor involves ADCC rather than complement-dependent cytotoxicity. Therefore, factors limiting the efficacy of mAb therapy may include inadequate number, suboptimal activation, or poor availability at tumor sites of the ADCC effector cells involved in the lysis of tumor. The purpose of this study was to attempt to improve the efficacy of mAb tumor therapy by augmenting the activation and availability of effector cells capable of mediating ADCC.

Potential ADCC effector cell populations include macrophages, NK cells, neutrophils, eosinophils, and platelets. Of these, the primary ADCC effectors in mice appear to be both the macrophage and NK cell populations, whereas in humans NK cells appear to be the principle effector cells. Recently another cell population, termed LAK cells, have been described as potential ADCC effectors.

LAK cells are a heterogeneous cell population induced by exposure of lymphocytes to high concentrations of IL-2 and include cells of the NK phenotype. The adoptive transfer of in vitro generated LAK cells or the in vivo induction of LAK cells by high doses of recombinant IL-2 in tumor-bearing hosts can result in regression of tumors. Previous studies have shown that a subpopulation of LAK effector cells express FcR and, in vitro studies have confirmed that FcR-bearing LAK effector cells can mediate ADCC. Moreover, administration of high, LAK-inducing doses of IL-2 has improved the efficacy of antidiotype mAbs in the treatment of mice with a B-cell lymphoma, and mAbs in combination with high doses of IL-2 have been shown to augment regression of a LAK-resistant tumor. Thus, LAK cells generated by IL-2 may mediate ADCC in vivo and enhance the efficacy of mAb therapy for the eradication of tumor.

We have previously shown that a spontaneous T-cell lymphoma, SL-2, can be completely eradicated in AKR/J mice (Thy-1.1*) with a mAb reactive with the differentiation antigen, Thy-1.1. However, therapy in this setting results in depletion of Thy-1.1 normal T-cells. Thus, a model was developed using the AKR/Cum mouse (Thy-1.2*), which is congenic to AKR/J at the Thy-1 locus, as the host for the treatment of SL-2 (Thy-1.1) with anti-Thy-1.1 mAb. Under these conditions, the mAb is tumor specific and does not result in depletion of possible effector cells. Efforts to increase the efficacy of LAK cells by in vivo administration of very high doses of IL-2 have been limited by capillary injury and vascular permeability resulting in severe pulmonary edema. In this study, we examined whether the administration of lower nontoxic doses of IL-2 or adoptive transfer of in vitro generated LAK effector cells could augment the therapeutic efficacy of mAb in this tumor model. The results suggest that in vivo induction or adoptive transfer of IL-2-activated ADCC effectors can improve mAb therapy with regimens that have no significant toxicity to the host.
MATERIALS AND METHODS

Mice and Tumor Targets. Six- to 10-week-old male AKR/Cum mice (Thy-1.2, H-2S), congenic to AKR/J (Thy-1.1, H-2K), mice at the Thy-1 locus, were obtained from Cumberland Farms (Clinton, TN). An AKR/J spontaneous T-cell lymphoma, SL-2 (Thy-1.1), was established and maintained as described earlier (25). SL-2 does not express the IL-2 receptor, produce IL-2, or proliferate in response to exogenous IL-2. FBL-3 is an NK-sensitive, LAK-sensitive, Friend virus-induced erythroleukemia of C57BL/6 origin maintained in tissue culture (26). YAC-1 is an NK-sensitive, Moloney virus-induced lymphoma of A/Sn origin, also maintained in tissue culture.

Recombinant IL-2 and Reagents. Recombinant IL-2 was obtained from Hoffmann-LaRoche, Inc. (Nutley, NJ) at a concentration of 10° biological response modifier units/ml and diluted in HBSS. Anti-Thy-1.1 monoclonal antibody 1A14 is an IgG2a class-switch variant, with an association constant of \( K = 3.0 \times 10^9 \text{M}^{-1} \), that was derived from the IgG3-producing parent line 31C5.31 as previously described (27). 1A14 can mediate lysis of SL-2 tumor targets in both complement-dependent cytotoxicity and ADCC assays in vitro and can cause regression of SL-2 in vivo. The serum half-life of 1A14 is approximately 72 h.

Generation of LAK Activity. In vitro LAK cells were generated by culturing \( 2 \times 10^6 \) AKR/Cum spleen cells/ml in medium (RPMI 1640 containing 10% FCS, L-glutamine, 2-mercaptoethanol, and penicillin-streptomycin) supplemented with 1,000 units of IL-2/ml for 5 days at 37°C in 5% CO₂ (14). Control effector spleen cells were cultured under identical conditions without the addition of IL-2. In vivo LAK activity was generated by injection of 25,000 units of IL-2 i.p. twice/day. Spleen cells were removed after 4 or 8 days, suspended in 5% FCS, and assayed after 4 or 8 days against the congenic (Thy-1.1*) SL-2 tumor target to be used in subsequent therapy studies with anti-Thy-1.1 mAb 1A14. Controls were generated by injection of 25,000 units of IL-2 OR ADOPTIVE LAK AND MONOCLONAL ANTIBODY THERAPY

\( \text{IL-2 OR ADOPTIVE LAK AND MONOCLONAL ANTIBODY THERAPY} \)

\( \text{(Thy-1.2, H-2S) mice and tested against the congenic (Thy-1.1*) SL-2 tumor target to be used in subsequent therapy studies with anti-Thy-1.1 mAb 1A14. Controls were generated by injection of 25,000 units of IL-2 OR ADOPTIVE LAK AND MONOCLONAL ANTIBODY THERAPY} \)

\( \text{SL-2 tumor cell lysis. Incubation of 1A14 with tumor targets and no effector cells caused no lysis of tumor.} \)

Radioiodination of mAb. The concentration of purified antibody 1A14 was determined by a Coomassie blue binding assay (Bio-Rad, Richmond, CA) (28). Purified 1A14 was then labeled with \( ^{125}\text{I} \) (ICN, Irvine, CA) with Iodogen (Pierce Chemical Co., Rockford, IL), producing antibody with a specific activity of \( 2 \times 10^9 \text{cpm/µg} \). Activity constants of 1A14 after labeling were unchanged after analysis by Scatchard analysis of the binding of \( ^{125}\text{I}-\text{labeled 1A14 to } 2 \times 10^8 \text{ live SL-2 (Thy-1.1*) cells or AKR/J thymocytes (Thy-1.1*) for 1 h at 37°C (5)}. \)

Assessment of Biodistribution of Radiolabeled Monoclonal Antibody. SL-2 cells \( (5 \times 10^6) \), suspended in phosphate-buffered saline, were injected s.c. into AKR/Cum mice (day 1). HBSS (control) or IL-2 was administered on day 4 i.p. after s.c. tumor was palpable. Radiolabeled 1A14 \( (100 \text{µg/mouse}, \text{containing } 10^6 \text{cpm of } ^{125}\text{I}) \) was injected i.v. on day 8 (4 days later) in the tail vein. Mice were sacrificed at 8, 48, and 168 h after injection of 1A14, and blood, brain, lymph node, lung, liver, kidney, muscle, spleen, stomach, salivary gland, and s.c. tumor were collected, weighed, and counted on a Packard Auto-gamma scintillation spectrometer (Autogamma model 5330 spectrometer; Packard Instruments, Downers Grove, IL) for 1–2 min. The remaining carcass was homogenized in water and assayed. Blood levels were checked at 1 h after injection of 1A14 in each mouse in order to calculate the actual amount of mAb delivered per mouse. Distribution in organs was calculated by the formula:

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\text{% Injected dose/g of tissue = \frac{\text{cpm/g of tissue}}{\text{total cpm injected/g of mouse}}}\]

Therapy Experiments. AKR/Cum mice were given injections of \( 10^7 \) SL-2 cells s.c. on day 1. After development of a palpable tumor by day 4, experimental groups were treated with 50,000 units of IL-2/day divided into two doses injected i.p. from days 4–12, 100 µg 1A14 mAb in 0.2 phosphate-buffered saline infused i.v. in the tail vein on days 4 and 8, and/or 0.2 ml of LAK cells at \( 5 \times 10^5 \text{cells/ml} \) infused in the tail vein on days 4 and 8. Response to therapy was evaluated by measurement of s.c. tumor volume (width × length × thickness) expressed in mm³ and by survival. All mice alive at 80 days remained apparently tumor-free at 6 months.

Statistical Analysis. Tumor size data were analyzed using Student's \( t \)-test. Survival data were analyzed with a life table analysis program, using the Mantel-Haenzel \( \chi^2 \) test (29).

RESULTS

Establishment of a Model for Therapy with mAb and IL-2. A therapy model was developed to evaluate the combination of IL-2 and mAb on eradication of the Thy-1.1* thymoma SL-2 in AKR/Cum (Thy-1.2*) mice. A dose of \( 10^5 \) SL-2 tumor cells was found to induce a s.c. primary tumor measurable within 7 days and distant metastasis. Mice were treated with 100 µg of 1A14 anti-Thy-1.1 mAb given on days 4 and 8, since this dose was expected to produce an antitumor effect and potentially result in long term survivors in 10–30% of the treated mice. Because IL-2 had not previously been used for therapy of SL-2 in AKR/Cum mice, experiments were done to establish a dose to be used in therapy that did not cause lethal toxicity. Mice were given doses from 0 to 400,000 units of recombinant IL-2 divided into two doses/day for a total of 8 days. The highest dose of IL-2 that did not result in fatal toxicity was 50,000 units/day. Therefore, studies were performed to evaluate whether this IL-2 dose induced in vivo LAK and augmented ADCC activity and NK activity.

In vivo spleen effector cell function was evaluated after 4 and 8 days of IL-2 administration. NK activity in spleen cells (Fig. 1), as measured by lysis of YAC-1 cells, increased significantly from a control level of 12% in mice given HBSS to 42% after 8 days of IL-2. LAK activity, measured against the NK-resistant, LAK-sensitive target FBL-3 (14), increased minimally from

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Therapeutic Efficacy of mAb as an Adjunct to IL-2. To assess whether IL-2 could augment mAb eradication of tumor in vivo, AKR/Cum mice were inoculated with 10^7 SL-2 s.c. (day 1) and were not treated (HBSS), treated with either mAb or IL-2 alone, or treated with both IL-2 and mAb. Mice received IL-2 i.p. on days 4–12 and 100 μg of anti-Thy-1.1 mAb were given on day 4 and again on day 8. The therapeutic response for each therapy group was evaluated by measurement of the volume of the primary tumor and determination of median and long term survival. Treatment with antibody alone had no effect on tumor growth, and treatment with IL-2 alone resulted in transient regression of s.c. tumor (Fig. 2A), but the difference was not statistically significant when compared to untreated control mice (P > 0.05). When mAb was used as an adjunct to IL-2, there was significant regression (P < 0.05) of the s.c. tumor, with total eradication of tumors in some mice. Similarly, median survival (Fig. 2B) in mice treated with IL-2 plus mAb was significantly prolonged to 40 days, compared to mice receiving either IL-2 alone (25 days), mAb alone (29 days), or no therapy (22 days). However, long term survival (>80 days) was not significantly different in control mice (0%) or any of the therapy groups (range, 7 to 20%) (Fig. 2B). Thus, while therapy with either mAb or IL-2 alone had only a minimal effect on regression of primary tumor or survival, the combination of IL-2 in addition to mAb was able to induce significant regression of the primary tumor and prolongation of the median survival.

Effect of the Treatment Dose of IL-2 on mAb Biodistribution. One of the major toxic effects of in vivo administered IL-2 is the induction of increased permeability of capillaries, resulting in extravasation of intravascular albumin, proteins, and fluid (30). Potentially, IL-2 could affect the penetration of mAb into tumor when IL-2 and mAb are given together in therapy, resulting in increased tumor regression. To evaluate the effect of the IL-2 dose used in therapy on the biodistribution of mAb into tumor, 5 × 10^6 SL-2 cells were given s.c. (day 1) to AKR/Cum mice and allowed to develop into a palpable tumor (about 0.5-cm diameter at day 4). IL-2, 50,000 units/day divided into two doses/day, was started on day 4 and an anti-Thy-1.1 mAb, 1A14, labeled with ^111I was given 4 days later on day 8. The concentration of mAb was assessed in normal tissues (lung and liver) and the s.c. tumor of mice at 8, 48, and 168 h after injection of mAb. IL-2 was continued until sacrifice of the mouse. Anti-Thy-1.1 mAb levels in lung were 4.0 ± 0.5% (mean ± SD) of the injected dose of 1A14/g of lung in IL-2-treated mice versus 3.8 ± 0.8% for control mice at 48 h (Fig. 3). Similarly, mAb levels in tumor were 9.2 ± 7.1% of the injected dose of 1A14/g of tumor in IL-2-treated mice, compared to 9.6 ± 3.0% for HBSS-treated mice at 48 h. Other time points (8 and 168 h) and other normal tissues (liver) also demonstrated no significant difference due to IL-2 therapy on the biodistribution of anti-Thy-1.1 mAb (Fig. 3). Thus, the nontoxic dose of IL-2 used in these experiments did not significantly alter the biodistribution of anti-Thy-1.1 mAb in either tumor or normal tissues.

Ability of Adoptively Transferred LAK to Augment mAb Eradication of Tumor in Vivo. Since mice were receiving the highest nontoxic dose of IL-2 and the dose could not be increased further, in vitro generated LAK were evaluated as an alternative approach to increase in vivo ADCC activity in therapy. Before examining the combination of adoptively transferred in vitro generated LAK with mAb in therapy, we first determined the in vitro lytic activity against SL-2 lymphoma of spleen cells cultured for 5 days with LAK-inducing concentrations of IL-2 (1000 units IL-2/ml). Control splenocytes cultured in medium for 5 days in the absence of IL-2 demonstrated only background lysis of SL-2, either with or without anti-Thy-1.1
cells on days 4 and 8 of therapy with each dose of 1A14 mAb. Therapy included
infusion of tumor.
crease in long term survival from 17% (mAb therapy alone) to
complete regression of s.c. tumor. In this experiment, 50% of
against anti-Thy-1.1 mAb-coated SL-2.
and 5:1, respectively, when evaluated alone and demonstrated
24%, 3%, and 1% lysis of SL-2 at the
and 5:1, respectively, when evaluated alone and demonstrated
a significantly increased lysis of 78%, 60%, and 42% at the
each effector to target (E:T) ratio is expressed in the graph.

Fig. 4. AKR/Cum spleen cells were incubated for 5 days at 2 × 10⁶ cells/ml
either with or without IL-2 (1000 units/ml) and evaluated for cytolytic activity in
a chromium release assay. The lytic activity of spleen cells (cultured without IL-2)
or LAK cells (cultured with IL-2) was assayed in the presence of (+) or absence
of (—) anti-Thy-1.1 antibody. The anti-Thy-1.1 mAb was assayed with the target
at an initial concentration of 10 μg/ml followed by serial dilutions. The maximum
lysis for each effector to target (E:T) ratio is expressed in the graph.

Fig. 5. AKR/Cum mice were given injections of 10⁸ in vitro generated LAK
cells on days 4 and 8 of therapy with each dose of 1A14 mAb. Therapy included
mice given HBSS (control), LAK cells, or mAb alone or LAK in combination
with mAb infused in the tail vein (arrows on the x-axis) in the same dosing
schedule as in previous experiments.

mAb-coated target (Fig. 4). By contrast, LAK effectors induced
by 5 days of in vitro culture with 1000 units/ml IL-2 caused
24%, 3%, and 1% lysis of SL-2 at E:T ratios of 100:1, 25:1,
and 5:1, respectively, when evaluated alone and demonstrated
a significantly increased lysis of 78%, 60%, and 42% at the
same E:T ratios when LAK were used as ADCC effectors
against anti-Thy-1.1 mAb-coated SL-2.

To evaluate whether in vitro generated LAK effectors could
increase tumor regression in vivo in conjunction with mAb,
mice were inoculated s.c. with 10⁷ SL-2 cells and given LAK
(10⁸) cells i.v. on days 4 and 8, alone or with 100 μg of purified
1A14 mAb (Fig. 5). LAK cell infusion caused no observable
toxicity. Control mice given HBSS (control) all died by day 45.
Mice treated with LAK alone had a statistically similar survival
pattern, with all dead by day 62. None of these mice had
complete regression of s.c. tumor. In this experiment, 50% of
mice treated with LAK alone had complete regression of s.c.
tumor, but only 17% exhibited long term survival. By contrast,
LAK given in conjunction with mAb infusions caused an
increase in long term survival from 17% (mAb therapy alone) to
60%. Thus, ex vivo generated LAK cells given in conjunction
with mAb can augment therapeutic efficacy without demonstra-
table toxicity, presumably by enhancing ADCC-mediated eradica-
tion of tumor.

DISCUSSION

In this report, we demonstrate that a nontoxic IL-2 regimen
or adoptively transferred in vitro generated LAK cells used in
conjunction with tumor-specific mAb can augment the efficacy
of mAb therapy of tumor. In addition, neither approach caused
the toxicity observed with the use of high dose IL-2. Although
previous reports have shown a therapeutic augmentation in
tumor regression by the combination of IL-2 and mAb (18, 19),
this paper further describes that infusion of in vitro generated
LAK with mAb can augment the therapeutic response, presum-
ably by increasing in vivo ADCC effector function. Both ap-
proaches may induce tumor regressions, through either similar
or different mechanisms, and may be used clinically to over-
come different clinical therapeutic obstacles.

High dose IL-2, which generates in vivo LAK activity, likely
induces tumor regression in combination with tumor-specific
mAb through the mechanism of ADCC. This is supported by
the fact that in vitro mAb-labeled tumor targets are lysed more
efficiently by LAK (Fig. 4), that LAK cells express FcR and
can mediate ADCC (11), and that IL-2 had little effect when
given alone. In addition, there is greater selection for tumor
cells that are negative for the antigen recognized by mAb after
therapy with IL-2 plus mAb (19), compared to those treated
with mAb alone. This latter observation suggests that the tumor
cells recognized by mAb rather than antigen-loss variants are
preferentially lysed in vivo even in the presence of IL-2-induced
LAK effector cells. In this study, complement-mediated cyto-
toxicity is not likely to contribute significantly to tumor eradica-
tion, since AKR mice are deficient for the fifth component
of complement. Although a humoral anti-Thy-1.1 antibody
response by the AKR/Cum host to the congenic SL-2 tumor
could potentially contribute to the tumor regressions observed
in IL-2-treated hosts, the minimal antitumor responses ob-
served in mice not receiving exogenous anti-Thy-1.1 mAb (and
the high dose of mAb necessary to achieve a therapeutic effect)
suggest that a host antibody response is probably not significant
during therapy of this tumor.

This study suggests that adoptively transferred LAK cells
represents an effective alternative therapeutic approach when
the use of LAK activity in conjunction with tumor-specific mAb
is considered. As shown in this study, IL-2 toxicity may limit
the use of higher doses of IL-2 in attempts to further augment
LAK activity in vivo. The use of in vitro generated LAK cells
has the potential to increase in vivo LAK function without
toxicity and possibly to improve therapeutic outcome. It may
also be possible to manipulate LAK cells in vitro prior to
infusion to augment LAK effector ADCC function. Culture of
lymphocytes with other cytokines, such as α interferon, β inter-
feron, γ interferon (31), IL-2, or tumor necrosis factor α (32),
in combination with IL-2 in vitro, may increase LAK ADCC
function without the toxicity associated with these combina-
tions in vivo. Other strategies for ex vivo manipulation of LAK
effectors include conjugation of tumor-reactive mAb to the FcR
on LAK cells (33, 34), conjugation to CD3⁺ LAK cells of an
anti-CD3 heteroconjugated to a mAb reactive to the tumor (35),
or conjugation of palmate-derivatized mAb to all LAK cell
populations (36).

In conclusion, this study demonstrates that nontoxic doses
of IL-2 used therapeutically in conjunction with antitumor mAb
can induce regression of tumor. Combined therapy utilizing in
vitro generated LAK cells as an adjunct to mAb was also able
to cause tumor regressions without toxicity. Thus, adoptively
transferred LAK cells may represent an approach to further
augment in vivo LAK-mediated ADCC activity without the risk
of increased toxicity induced by continuous infusion of high
dose IL-2.
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REFERENCES


5425
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