Interleukin 2 and Lymphokine-activated Killer Cell Therapy: Analysis of a Bolus Interleukin 2 and a Continuous Infusion Interleukin 2 Regimen

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ABSTRACT

Several groups have described the efficacy of interleukin 2 (IL-2) plus lymphokine-activated killer (LAK) cells in the treatment of cancer patients with significant response rates noted in patients with renal cell cancer and malignant melanoma; however, the optimum regimen remains undefined. The Biological Response Modifiers Program of the National Cancer Institute conducted two consecutive Phase II/III studies evaluating the toxicity and clinical efficacy of different methods of IL-2 and LAK cell therapy. In the first trial, we modified the standard Rosenberg regimen by decreasing the duration of priming in an attempt to reduce the toxicity related to this phase of the therapy and thereby administer more IL-2 doses with the LAK cells. In the second trial, we used a continuous i.v. infusion IL-2 regimen and altered both the leukapheresis procedure and the LAK cell culture techniques based on our in vitro and preclinical studies suggesting that 2-day LAK cells were superior. Thirty cancer patients received i.v. bolus IL-2 at 100,000 units/kg every 8 h for 3 days during priming and for 5 days during LAK cell administration. A second group of 22 cancer patients received IL-2 by continuous i.v. infusion at $3 \times 10^6$ units/m² for 5 days during priming and an additional 5 days of IL-2 with the LAK cell phase of the treatment. The timing of the start of the leukapheresis procedures, their duration and number, and the LAK cell culture techniques differed in the two trials. Overall, 52 patients with various cancers were treated. The toxicities associated with each regimen were similar to those seen in other IL-2 plus LAK cell trials. Four patients (one each with melanoma and diffuse large cell lymphoma and two with renal cell cancer) exhibited partial responses lasting 2, 4, 10, and 15+ mo. Serial tumor biopsies from treated patients demonstrated that therapy can produce a marked monocellular infiltrate and an increase in HLA-DR expression on tumor cells. There was no difference in the overall response rate between the two regimens, but toxicity was less with continuous i.v. infusion IL-2. The 5-day continuous i.v. infusion regimen resulted in significantly higher rebound lymphocytosis, cell yield from leukapheresis, and number of LAK cells harvested from culture.

INTRODUCTION

The initial report in 1985 of tumor responses after adoptive immunotherapy with IL-2 and LAK cells prompted multiple confirmatory trials evaluating the toxicity and efficacy of various schedules of high-dose IL-2 plus LAK cell therapy in the treatment of cancer (1-8). Rosenberg et al. (1, 2) used high-dose i.v. bolus IL-2 (100,000 units/kg/8 h) for the priming and LAK cell administration phases of treatment. Although this approach resulted in an overall response rate (partial and complete responses) of 22%, toxicity was significant, and a majority of patients required admission to an intensive care unit some time during treatment. West et al. (3) administered IL-2 at $3 \times 10^6$ units/m²/day by continuous i.v. infusion for the priming and LAK cell phases. This change in dose and schedule significantly reduced toxicity without decreasing the overall response rate.

We conducted two sequential Phase II/III studies evaluating the toxicity and clinical efficacy of different methods of IL-2 and LAK cell therapy. In our first trial, we modified the standard Rosenberg regimen by decreasing the duration of IL-2 priming from 5 to 3 days in an attempt to reduce the toxicity related to this phase of the therapy and thereby administer more IL-2 doses with the LAK cells. In our second study, we used a continuous i.v. infusion IL-2 regimen and altered both the leukapheresis procedure and the conditions of LAK cell culture. We began leukapheresis 36 h after IL-2 priming ended and reduced the number of leukapheresis from five to three based on our own observations and data from other trials (2, 9), indicating that the peak lymphocyte count occurs 48 h after cessation of IL-2 and the yield of LAK cells decreases dramatically on Days 4 and 5 of pheresis. The length of in vitro culture of cells with IL-2 was shortened to 2 days because of data suggesting that these cells produce more cytokines than cells cultured for longer periods (10) and because of animal experiments suggesting that cells cultured for shorter periods of time traffic better to tumor sites (11). This paper documents the toxicity, immunological effects, and the antitumor activity of these two different IL-2 plus LAK cell regimens.

PATIENTS AND METHODS

All patients were treated at the Biological Response Modifiers Program, National Cancer Institute, Frederick, MD. Both protocols described below were approved by the Institutional Review Boards of the Clinical Oncology Program, National Cancer Institute, and the Frederick Cancer Research and Development Center. All patients voluntarily gave written informed consent prior to treatment.

Patients. Eligibility criteria for both protocols were the following: (a) documented cancer with evaluable or measurable disease; (b) failed standard treatment; (c) age ≥15 yr; (d) expected survival of greater than 3 mo; (e) diffusion capacity ≥75% of predicted; (f) no central nervous system metastases; (g) white blood cell count ≥2000/mm³; (h) no antineoplastic therapy within the month prior to study entry; and (i) no active systemic infection, coagulation disorders, or major cardiovascular or pulmonary disease.

Prestudy evaluation included history and physical examination, complete blood cell count, serum chemistry profile, prothrombin time and
partial thromboplastin time, urinalysis and culture, serum iron binding capacity, hepatitis B surface antigen, antibodies to human immunodeficiency virus 1, chest X-ray, CT scan of the brain, other appropriate imaging studies to measure disease, and pulmonary function tests including carbon monoxide diffusing capacity and arterial blood gases. Many of the patients also underwent an exercise treadmill test to assess cardiac capacity.

Patient characteristics are listed in Table 1, and diagnoses are listed in Table 4. The characteristics of the patients enrolled in the two studies are similar except for a higher percentage of patients who received any prior treatment on the first trial (80% versus 59%) as well as a higher percentage of patients who received chemotherapy and/or radiation therapy (73% versus 50%). Initial patients enrolled onto the first schedule primarily had renal cell carcinoma, malignant melanoma, or colon carcinoma. After treating 19 patients, efforts focused on entering patients with relapsed Hodgkin’s disease or relapsed non-Hodgkin’s lymphoma. Patients were enrolled onto the second trial without regard to their diagnosis.

Treatment Schema. The two treatment regimens are depicted in Figs. 1 and 2. The recombinant IL-2 used in these trials was produced in Escherichia coli transfected with the gene from the Jurkat cell line and was generously supplied by Cetus Corporation, Emeryville, CA. Its specific activity was approximately $3 \times 10^6$ units/mg of protein (Cetus units).

Schedule I is identical to the regimen reported by Rosenberg et al. (1) except that the initial IL-2 priming period was 3 days instead of 5, and a repeat cycle of leukapheresis not preceded by IL-2 priming was administered in Schedule II. IL-2 was either given in every 8 h for a maximum of 9 doses as priming therapy and likewise given. Recombinant IL-2 (100,000 units/kg) was diluted in 50 ml of 5% dextrose in water and administered as an i.v. bolus over 15 min every 8 h for a maximum of 9 doses as priming therapy and likewise with the LAK cells for a planned 5 to 7 days. IL-2 was administered by continuous i.v. infusion at $3 \times 10^6$ units/m$^2$ for 120 h as priming and for 120 h with the LAK cells, and an identical course was repeated after 2 wk of rest (Fig. 2). The timing of leukapheresis in Schedule II was 36 h after IL-2 priming ended, rather than the 56-h interval in Schedule I. The number of leukaphereses was reduced to three, but their duration was increased to 5 h using the same machine and technique as previously described.

LAK Cell Generation. For Schedule I, patients’ mononuclear cells were separated by centrifugation through a Ficoll-Hypaque density gradient and washed with Hanks’ balanced salt solution. Cells were cultured in RPMI-1640 medium containing 50 µg of streptomycin, 5 units/ml of penicillin, 2 µg/ml of gentamicin, 2% heat-inactivated human AB serum (M. A. Bioproducts, Walkersville, MD), and 1000 units/ml of IL-2 at a concentration of 1 to 2 x $10^6$ cells/ml. Penicillin was excluded from cultures of cells from penicillin-allergic patients. Cells were cultured in 2-liter roller bottles at 1 rpm at 37°C for 3 or 4 days. LAK cells were collected by centrifugation, washed in Hanks’ salt solution, and resuspended in 200 ml of normal saline with 5% human serum albumin and 75,000 units of IL-2. This mixture was filtered through Nytex filters (Tetko, Inc., Zurich, Switzerland) and transferred to a 600-ml transfer unit pack. Aliquots were examined microscopically, cultured for bacterial and fungal contamination, and tested for LAK activity. LAK activity was assessed in vitro using Daudi cells as targets in a 4-h chromium release assay that we have described previously (12).

Autologous LAK cells were administered in Schedule I in three infusions on Days 10 (cells from Days 6 and 7 leukaphereses), 11 (cells from Day 8 leukaphereses), and 13 (cells from Days 9 and 10 leukaphereses). LAK cells were infused i.v. over 30 to 60 min starting within 45 min from the end of cell harvest.

LAK cell generation in the second trial differed in several ways. Patients’ mononuclear cells were not centrifuged over Ficoll-Hypaque because a detailed analysis of the cells obtained from leukapheresis revealed that less than 5% of the cells were granulocytes and that this level of granulocyte contamination did not adversely affect LAK cell generation. Instead of using culture medium containing human AB serum, serum-free medium (AIM V; Gibco) with glutamine, streptomycin, and gentamicin was used. This medium supported the growth of LAK cells as well as the serum-containing medium for the 2-day period of culture. Cells from patients treated on Schedule II were cultured at a density of 2.5 to 5.0 x $10^6$ cells/ml instead of 1 to 2 x $10^6$ cells/ml, because experiments indicated similar LAK cell yields and activity under these conditions. Lastly, the duration of culture was shortened to 2 days.

For patients treated according to Schedule II, autologous LAK cells were administered in six infusions on Days 10, 11, and 12 with cells

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![Fig. 1. First IL-2/LAK clinical protocol.](7344)
from Days 8, 9, and 10 leukaphereses, respectively, and on Days 38, 39, and 40 with cells from Days 36, 37, and 38 leukaphereses (see Fig. 2).

In Schedule I, patients routinely underwent IL-2 priming on a regular inpatient oncology floor but received their LAK cells in a unit with the capability for continuous ECG and pulse oximeter monitoring and intensive care unit-level nursing care. Patients treated according to Schedule II were transferred to the monitoring unit only when clinically indicated.

Patients were evaluated for response 4 wk after the completion of therapy. Any objective antitumor response, even if it did not meet the criteria for a partial response, qualified the patient for another treatment cycle.

Response Criteria. Standard response criteria were used. A complete response was defined as the disappearance of all tumor for at least 4 wk without any new lesions appearing. Progressive disease was defined as disease not meeting the above criteria for response or progression.

Supportive Care. In both treatment regimens, patients received indomethacin (25 to 50 mg p.o. or per rectum every 8 h), ranitidine (150 mg p.o. twice a day or 50 mg i.v. every 6 h), and acetaminophen (650 mg p.o. or per rectum every 4 h). In addition, meperidine for chills, hydroxyzine hydrochloride or diphenhydramine for pruritus, low-dose dopamine for oliguria, phenylephrine for blood pressure support, and antiarrhythmic and antiemetic treatment were administered as needed. The first nine patients without a diagnosis of Hodgkin's disease or lymphoma treated on Schedule I received dexamethasone (4 mg) p.o. every 6 h during the LAK cell infusion phase of the protocol because it was thought at that time that steroids would reduce IL-2-related toxicity and allow more IL-2 to be administered. Subsequently, concern emerged that the steroids were interfering with the antitumor efficacy of the regimen, so no other patients on either study received steroids.

Immunopathological Studies. Histopathological and immunophenotypic analyses of sequential tumor biopsy specimens from three patients with accessible metastatic lesions were performed according to the techniques described previously (13).

RESULTS

Twenty-seven of 30 patients who began treatment according to Schedule I are evaluable for toxicity and response assessment. Three patients were removed from study during priming because of intercurrent medical problems [hypercalcemia (one patient), vertebral metastasis (one patient)] requiring treatment or because of patient refusal (one patient). Twenty-three of the 27 evaluable patients completed the entire 30 days of treatment; four patients received treatment through their first LAK cell phase only, because of toxicity described below.

All patients are evaluable for toxicity, and 21 of 22 are evaluable for response on Schedule II. One patient completed IL-2 priming on Schedule II but refused to continue. Four patients did not receive their second round of IL-2/LAK treatment (Days 29 to 43); two because of progressive disease and two because of toxicity.

Number of IL-2 Doses and Cells Administered. The mean total number of IL-2 doses administered on Schedule I was 29. Patients received an average of 8.6 of the planned 9 IL-2 priming doses (27 patients), a mean of 10 doses (range, 1 to 15) with the first LAK cell infusions (27 patients), and a mean of 10.4 doses (range, 4 to 23) with the second round of LAK cell infusions (23 patients). Only three patients received more than 15 doses of IL-2 with LAK cells (one patient, 16 doses; one patient, 17 doses; one patient, 23 doses). The number of IL-2 doses administered with the LAK cells was not significantly different in the patients who also were treated with steroids (total of 21 with steroids versus 22.3 without). One of 9 patients (11.1%) treated with steroids and 3 of 18 patients (17.6%) treated without steroids did not receive the second half of their planned therapy due to the toxicity of treatment.

The mean lymphocyte rebound, number of cells obtained from leukapheresis and placed into culture, and the number of LAK cells infused per leukapheresis cycle for both schedules are listed in Table 2. Despite the fact that IL-2 usually ended 5 to 7 days before the second set of leukaphereses in Schedule I, the lymphocyte count on the first day of that leukapheresis, the leukapheresis yield, and the number of LAK cells harvested were essentially the same as those observed during the first set of leukaphereses after IL-2 priming. The number of LAK cells harvested after culture in vitro represented 48% of the cells (lymphocytes and monocytes) obtained from leukapheresis.

Patients treated with continuous infusion IL-2 on Schedule II received a mean of 119.5/120 h (99.6%) of IL-2 priming during the first half of their treatment course (22 patients) and 117.5 of 120 h (98%) of IL-2 priming during the second half (17 patients). They received a mean of 116 of 120 h (96.7%) with their first set of LAK infusions (21 patients) and 112.6 of 120 h (93.8%) with their second set (17 patients).

The lymphocyte rebound, yield from leukapheresis, and number of LAK cells harvested for Schedule II are listed in Table 2. The results in each of these areas were approximately 2 times greater than with Schedule I (P < 0.0001). In Schedule II, the number of LAK cells harvested represented 64% of the cells obtained from leukapheresis.

Toxicity. The toxicities associated with treatment are listed in Table 3. These are very similar to those reported for other
high-dose IL-2 plus LAK cell therapy treatment regimens (1–6, 13–16). On both schedules, all patients had flu-like symptoms; fevers; chills; malaise; and fatigue. The majority of patients also had fluid retention with weight gain, erythematous rashes; fevers; chills; malaise; and fatigue. The majority of patients also had fluid retention with weight gain.

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Schedule I, respiratory distress requiring intubation occurred in two patients, and a grand mal seizure and angina were observed in one patient each. On Schedule II, no patients required intubation, although one had severe respiratory distress, and one each had a seizure and angina. One patient on Schedule II developed transient cortical blindness lasting less than 24 h. Extensive neurological workup including a magnetic resonance imaging scan of the brain was negative. The only death during treatment was a patient with Hodgkin’s disease on Schedule I who had parenchymal lung lesions and had been heavily pretreated with chemotherapy, radiation therapy, and an autologous bone marrow transplant. This patient required intubation for respiratory distress after the third dose of LAK cells and could not be extubated. Bronchoscopic biopsies on two occasions showed marked pulmonary fibrosis which led to her demise. Although the toxicity on Schedule II was overall qualitatively very similar to that on Schedule I (Table 3), quantitatively it was much less severe as shown by the lower incidence of Grade III or Grade IV toxicity. All toxicities on both treatment regimens resolved within 2 wk of stopping treatment except for occasional patients who had persistent fatigue, myalgias, or arthralgias as long as 4 wk after stopping treatment.

Clinical Response. The diagnosis of patients and the response to treatment for both trials are listed in Table 4. Two partial responses (melanoma and DLCL) were seen in patients following treatment according to Schedule I, and two partial responses
(both RCC) were seen in patients treated according to the second schedule. The patient with melanoma received only 10 doses of IL-2 and one infusion of LAK cells because of the development of angina accompanied by ischemic changes on ECG. Her followable disease consisted of four subcutaneous nodules and a pulmonary nodule. When treatment was stopped due to angina, she had the four subcutaneous nodules (that had not changed in size) resected for evaluation of immunohistochemical changes (described below). At her return evaluation 1 mo later, the nodule on chest X-ray had resolved and was not detectable by CT scan. She had previously received radiation therapy for a left humoral metastasis with a residual bone scan abnormality in that area which persisted unchanged throughout her course. Her response persisted for 10 mo when she relapsed with a subcutaneous metastasis without recurrence of her lung nodule or the development of any new pulmonary lesions.

The patient with DLCL had progressive disease post chemotherapy. He had complete resolution of two subcutaneous nodules during the IL-2 priming phase of his treatment without any change in a left paraaortic lymph node. The response lasted for 2 mo before disease progression occurred, manifested by the development of a new 1-cm x 1-cm subcutaneous nodule, an increase in the size of the paraaortic lymph node, and the development of a new paraaortic lymph node.

On Schedule II, one patient with RCC had a partial response consisting of nearly complete regression of a large parenchymal lung mass and disappearance of a second pulmonary nodule without any change in paratracheal lymphadenopathy. An additional treatment cycle failed to improve his partial remission which lasted 4 mo when he had progression of disease in his largest lung metastasis. The second patient with RCC had a 30% reduction in the size of a large biopsy-proven paraaortic lymph node metastasis after one cycle of therapy and a further reduction to >50% after a second treatment cycle (Table 4). The patient removed himself from treatment after approximately 85 h of IL-2 during a third treatment cycle. CT scans 9 mo post therapy continue to show nearly total resolution of his retroperitoneal adenopathy.

Evidence of a transient minor response was seen during the IL-2 priming portion of the bolus regimen in two patients (disappearance of a lymph node in a patient with follicular small cleaved cell lymphoma and decrease in the size of subcutaneous lesions in a patient with melanoma), but both of these patients had progressive disease at the time of their evaluation 1 mo after treatment. One patient with melanoma treated on the continuous infusion regimen had a mixed response with >50% reduction in subcutaneous metastases with progression of pulmonary metastases. He was retreated with further response of his cutaneous disease but not of his pulmonary disease.

Characteristics of Renal Cell Cancer Patients. Six of the nine patients with RCC treated according to Schedule I had prognostic features associated with a poor response (less than or equal to 10%) to LAK plus IL-2 in other studies (4). Three patients still had their primary tumor in place (two were > than 100 cm² in the product of longest perpendicular diameters), and three had liver metastases. One of the remaining three was 65 yr old, and three patients overall were 63 y old or older. This is older than any reported responders from other IL-2 plus LAK trials, although the ages of most responders has not been stated in these reports.

The four RCC patients treated with continuous infusion IL-2 (two of whom responded) had relatively good prognostic factors for response (metastases to lung plus bone in one, lung plus subcutaneous tissue in a second, lung only in the third, and retroperitoneal lymph nodes in the fourth).

Immunopathological Changes. One patient with malignant melanoma treated according to Schedule I had subcutaneous nodules that were biopsied before treatment, 1 wk after treatment, and at relapse more than 1 yr later. Pathological examination showed a marked T-cell infiltrate and an increased number of cells bearing the monocyte marker Leu M3 in the biopsy obtained 1 wk after treatment. No Leu 19-positive cells were detected in any of the biopsies, suggesting that the adoptively transferred LAK cells did not traffic to these tumor sites. This patient’s tumor cells showed a marked increase in HLA-DR expression from <20% of the cells showing weak (1+) positivity to all of the cells showing strong (3+) positivity after treatment (Fig. 3). Strong expression of HLA-DR was also noted on the biopsy obtained at relapse 10 mo later. The patient was not offered another course of LAK + IL-2 at relapse because of the treatment-induced myocardial ischemia documented on the first cycle.

Two other patients with malignant melanoma treated according to Schedule I had serial biopsies. One patient who had a minor tumor regression had a small T-cell and monocyte infiltrate pretreatment that increased slightly after therapy. No Leu 19+ cells were noted before or after therapy. This patient’s biopsies before, during, and immediately post treatment all stained strongly (3+) positive for HLA-DR, but the biopsy obtained 10 days post treatment showed less than 10% of the tumor cells positive for HLA-DR. The other patient did not respond to treatment and had no T-cell infiltrate before or after treatment. An infiltration of Leu M3- and Leu M5-positive cells was noted pretreatment, but did not increase post treatment. Less than 10% of this patient’s tumor cells were HLA-DR positive before and after treatment.

DISCUSSION

A number of studies have shown that high-dose IL-2 alone or in combination with LAK cells has antitumor activity against RCC, melanoma, NHL, and to a lesser extent, colon cancer. A variety of schedules of IL-2 administration have been used, but it is still not clear what the optimum treatment schema is. To determine if modifications in the regimen might improve efficacy, 52 patients with various cancers were treated with either a bolus IL-2 regimen in which the IL-2 priming phase consisted of 9 doses versus 15 in the Rosenberg regimen (1) and with a repeat cycle of IL-2 plus LAK cells without priming (30 patients) or a continuous infusion IL-2 regimen using 3 days of leukapheresis and a shorter duration of in vitro LAK cell culture with IL-2 than that reported by West et al. (22 patients) (3).

Toxicities of these two regimens were very similar to those reported with other IL-2 plus LAK regimens. Although qualitatively similar, toxicity was quantitatively significantly less severe with continuous infusion IL-2 than with bolus administration. This difference in toxicity was due to the fact that considerably less IL-2 was given on a daily basis during continuous infusion as opposed to bolus injection. Patients on the continuous infusion IL-2 regimen received only one-fourth to one-third the daily dose of IL-2 compared with the patients on the bolus injection regimen. The continuous infusion method of administration is actually more toxic; the maximum tolerated dose for IL-2 is much lower for a 24-h continuous i.v. infusion than for the same dose given by i.v. bolus (8).
Fig. 3. Tumor biopsy specimens of a patient with malignant cutaneous melanoma before (A, C, E, and G) and after (B, D, F, and H) treatment with IL-2/LAK. Staining (H & E) shows malignant melanoma cells without a significant lymphoid infiltrate before therapy (A). Four days after the end of treatment, a marked mononuclear cell infiltrate is obvious with H & E (B). Before therapy, Leu-M3* cells (C) and CD3* cells (E) were rare, but after treatment they became more numerous (D and F). Before therapy, less than 20% of the tumor cells stained positively for HLA-DR (G). After treatment (H), all the tumor cells stained strongly positive. This patient had a partial response to therapy. (Original magnification: A, x 100; B, x 200; C to H, x 100).
An aspect of our second schedule that differed from other IL-2/LAK regimens was the use of LAK cells that had been cultured for only 2 days instead of 3 to 4 days. We showed in this study that large numbers of these 2-day LAK cells could be given on 3 consecutive days with acceptable toxicities.

One of the unique features of the first regimen was a 3-day IL-2 priming period which we hoped would produce comparable rebound lymphocytosis and LAK cell yield with less toxicity and thereby permit more IL-2 to be given with the LAK cells. Unfortunately, shortening the IL-2 priming to 3 days did not permit delivery of significantly more bolus IL-2 with the LAK cell infusions as was hoped. The mean total number of bolus IL-2 doses (29 doses) in this study is greater than that administered by the Extramural IL-2/LAK Working Group (20 to 24 doses) and by Rosenberg (20 doses); however, our doses were given over 30 days in 2 cycles, instead of 16 days in one cycle. The mean number of LAK cells infused per pheresis cycle in Schedule I (4.6 x 10^9) is lower than that reported by other investigators (7.6 x 10^9); however, the mean total dose of LAK cells given (9.1 x 10^9) is comparable, although it was spread over two cycles instead of one.

The mean rebound lymphocytosis, leukapheresis yield, and number of LAK cells harvested were approximately 2 times greater for the continuous IL-2 infusion regimen compared with the bolus IL-2 regimen (P < 0.0001) (Table 2). While differences in baseline lymphocyte counts and prior treatment with chemotherapy and/or radiation therapy could all contribute to differences in lymphocytosis or LAK cell yields, it probably does not account for the magnitude of the difference between the two schedules. Boldt et al. (9) noted that the peak rebound lymphocyte count following IL-2 priming correlated with age, number of IL-2 priming doses, prior treatment, and baseline lymphocyte count. The patients without prior chemotherapy or radiation therapy had a mean rebound lymphocytosis of 5444 cells per µl versus 4084 cells per µl in patients with prior treatment. A higher percentage of patients treated in our first study had prior chemotherapy or radiation therapy (73%) versus our second trial (50%); however, the difference in mean rebound lymphocytosis in the two trials (7623 versus 3577 cells per µl, P < 0.0001) is greater than the difference expected from prior treatment alone. Eliminating the patients with non-Hodgkin’s lymphoma or Hodgkin’s disease who were treated according to Schedule I from the analysis did not change the magnitude or significance of the difference between the two schedules in any of the above-mentioned parameters.

It appears that 3-day bolus IL-2 priming does not produce either as pronounced a rebound lymphocytosis or LAK cell yield as do 5 days of continuous IL-2 infusion priming; however, other factors may be influencing this difference. One is the timing of leukapheresis (20 h earlier and 5 h/day for 3 days on Schedule II). The other is differences in culture technique (the use of serum-free medium, the omission of Ficoll-Hypaque, and the shorter duration of culture with Schedule II). The LAK cell culture efficiency increased from 48% [similar to the 53% efficiency noted by others (9)] in Schedule I to 64% in Schedule II. The lytic activity per cell of the LAK cells from both regimens was similar and equivalent to that of cells generated by 5 days of bolus IL-2 priming (data not shown).

The contribution of the reinfused ex vivo-generated LAK cells to the antitumor effect of IL-2 plus LAK is still not clear, since responses to high-dose IL-2 alone have been reported and there has not been a correlation between the number of LAK cells infused and response in most studies (1–3, 5, 9). This was also true in our study where three of the four responders received equal to or less than the mean number of LAK cells of their respective regimens. Because there are extensive animal data suggesting the importance of LAK cells in the IL-2 response, it would appear desirable to attempt to maximize LAK cell number and activity in those regimens using ex vivo-generated LAK cells. Therefore, 5-day priming with either continuous infusion or bolus IL-2 would appear preferable to 3-day priming.

The continuous infusion IL-2 regimen produced a marked increase (approximately 2-fold) in rebound lymphocytosis and LAK cell yield. Because of the many differences between the two schedules, it is difficult to define the contribution of any one change. Recently, a randomized trial was reported comparing IL-2 given i.v. by continuous infusion versus bolus, both with LAK cells, in patients with renal cell carcinoma (14). The start of leukapheresis (36 h after IL-2 priming) was earlier than previous trials, and the technique was changed from prior trials to one where they collected for 5 h/day for 4 days using a centrifuge speed of 1400 rpm, WBC collection rate of 4 ml/min, and flow rate of 80 ml/min, yielding a 1-liter final volume of the Leukopak. LAK cell culture was performed without Ficoll-Hypaque separation of cells and with serum-free medium for 3 to 4 days. The LAK cell harvest in the bolus arm was considerably higher (2-fold) than earlier studies using the same bolus IL-2 regimen (100,000 units/kg every 8 h) despite the fact that patients received a mean of 10.8 bolus boluses, less than the number of priming doses reported in earlier trials by the same group [12.9 doses (4) and 14 doses (6)]. This indicates that the timing and duration of pheresis, elimination of Ficoll-Hypaque centrifugation, and use of serum-free medium probably account for most of the increase in LAK cell harvest observed in their trial as well as ours.

The striking T-cell and macrophage infiltrate and the absence of cells bearing natural killer or LAK phenotypic markers noted in the tissue biopsies of the responding patients are consistent with the observations of Cohen et al. (13), who noted a correlation between such an infiltrate and response to treatment. These investigators also suggested that HLA-DR expression on tumor cells, whether it was present on the tumor before therapy or was induced as a result of treatment, correlated with tumor regression clinically. The data from our patients support this finding also; one patient with a partial response to therapy had high levels of HLA-DR induced by treatment, another patient with a minor tumor regression had high levels present before treatment, and a nonresponding patient had almost no HLA-DR expression on tumor cells before and after therapy. These observations suggest that the tumor regressions noted in vivo are not mediated directly by LAK cells; instead, they could be ascribed to an indirect effect of the IL-2 or LAK cells (induction of secondary cytokines) or be a result of the influx of T-cells or macrophages.

The diseases that responded to LAK and IL-2 in our two studies (melanoma, RCC, DLCL) are the same tumors in which responses were seen with other IL-2 plus LAK regimens. Although the small number of patients, potential differences in patient characteristics between different trials, and the lack of information from randomized trials make the comparison of response rates between different trials problematic at best, the 20% response rate (one of 5) of the melanoma patients on Schedule I is comparable to that seen in other IL-2 plus LAK studies (1–3, 5–7). Among the nine patients with melanoma treated with continuous infusion IL-2, seven had progressive disease, one had stable disease, and one had a mixed response.
Although the marked reduction in cutaneous metastases seen in one patient with melanoma indicates antitumor activity, no complete or partial responses were seen in melanoma patients treated on the continuous infusion regimen. This result is consistent with the experience of the Extramural IL-2/LAK Working Group (14, 15) and Thompson et al. (16), where continuous infusion IL-2/LAK regimens produced negligible response rates in melanoma (one of 39 and zero of 11, respectively). The reason for the difference in response rates of melanoma to bolus versus continuous infusion IL-2 regimens remains unexplained.

Two of four RCC patients treated with continuous infusion IL-2 had a partial response, a third patient had a slight reduction in the size of pulmonary lesions while his overall disease remained stable, and the fourth patient had to be removed from study after he had received only one of the two planned courses of IL-2 plus LAK cells because he developed a febrile metastasis requiring radiation. In contrast, although two of the RCC patients treated on the bolus regimen had stable disease for a prolonged time (14.5 and 8.5 mo), there were no responses. It is difficult to know whether this is related to the treatment regimen or to other factors. Six of the nine patients treated with the bolus regimen had one or more of the features associated with a ≤10% response rate (liver metastases, primary in place, or mass >100 cm³) when patients from other bolus IL-2 plus LAK cell trials were analyzed (4). Four of these patients received steroids during the LAK cell portion of their treatment including one of the three patients without the poor prognostic factors listed above. Although there has been no randomized trial testing the effect of steroids, there are limited data suggesting that steroids may have adverse effects on the clinical efficacy of treatment (17). Finally, three of these patients were 63 yr of age or older and received 14, 21, and 24 total doses of IL-2, respectively, which are all less than the mean number of IL-2 doses given on this regimen (29). Therefore, there are a number of possible explanations for the absence of response in these patients. In contrast to the experience in melanoma patients, there has not been a significant difference in response rates for renal cell carcinoma patients treated with bolus versus continuous infusion IL-2 in other trials (2–4, 7, 16) and in the one randomized trial noted earlier (14).

These trials confirm the antitumor activity of IL-2 plus LAK against RCC, melanoma, and NHL, cancers previously reported to be responsive to this immunotherapy. Although not conclusive, these studies indicate that 5 days of IL-2 priming by continuous i.v. infusion produced a significantly greater lymphocyte rebound, leukapheresis yield, and number of LAK cells harvested than 3 days of bolus IL-2 and did so with substantially less toxicity. Efforts at improving IL-2/LAK therapy by combining it with other active agents such as α-interferon, monoclonal antibodies, and chemotherapy against these diseases are ongoing.

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