duced and protein-enhanced bending of DNA has been observed for the origins of replication of plasmid R6K (*6*) and phage λ (*7*), as well as for the cyclic adenosine monophosphate gene-activating protein interaction with its binding site on the *lac* DNA, which shows limited homology to the RepC-enhanced bend site (*1*). These induced bends may have functional significance, such as bringing the protein into the proper spatial relation with its recognition sequence (*1, 4*), or opening the helix to allow replication (*6*). The static bends may act as recognition sites for protein-DNA interactions.

REFERENCES AND NOTES

1. H. Wu and D. M. Crothers, *Nature (London)* **308**, 509 (1984).
2. S. Diekmann and J. Wang, *J. Mol. Biol.* **186**, 1 (1985).
3. K. Zahn and F. R. Blattner, *Nature (London)* **317**, 451 (1985).
4. K. Ryder, S. Silver, A. DeLucia, E. Fanning, P. Tegtmeyer, *Cell* **44**, 719 (1986).
5. L. Bossi and D. Smith, *ibid.* **39**, 643 (1984).
6. S. Mukherjee, P. Indravadan, D. Bastia, *ibid.* **43**, 189 (1985).
7. K. Zahn and F. Blattner, *EMBO J.* **4**, 3605 (1985).
8. R. Koepsel *et al.*, *J. Biol. Chem.* **260**, 8571 (1985).
9. R. Koepsel, R. Murray, S. Khan, *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 5484 (1986).
10. S. Khan and R. Novick, *Plasmid* **10**, 251 (1983).
11. S. Khan, G. Adler, R. Novick, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4580 (1982).
12. R. Koepsel *et al.*, *ibid.* **82**, 6845 (1985).
13. H-S. Koo, H.-M. Wu, D. M. Crothers, *Nature (London)* **320**, 501 (1986).
14. H. Drew *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2179 (1981).
15. P. Hagerman, *ibid.* **81**, 4632 (1984).
16. C. Calladine, *J. Mol. Biol.* **161**, 343 (1982).
17. R. Dickerson, *ibid.* **166**, 419 (1983).
18. J. Zyskind, N. Harding, Y. Takeda, J. Cleary, D. Smith, in *The Initiation of DNA Replication*, ICN-UCLA Symposia on Molecular and Cellular Biology, D. S. Ray, Ed. (Academic Press, New York, 1981), vol. 22, pp. 13–25.
19. G. W. Gough and D. M. J. Lilley, *Nature (London)* **313**, 154 (1985).

20. We thank B. Baum and B. Rooney for secretarial services, R. Fields for photographic services, and T. Steck for critical reading of the manuscript. Supported by NIH grant GM 31685 (to S.A.K.) and Institutional Grant IN-58W (to R.R.K.).

27 May 1986; accepted 1 August 1986

A New Approach to the Adoptive Immunotherapy of Cancer with Tumor-Infiltrating Lymphocytes

STEVEN A. ROSENBERG, PAUL SPIESS, RENE LAFRENIERE

The adoptive transfer of tumor-infiltrating lymphocytes (TIL) expanded in interleukin-2 (IL-2) to mice bearing micrometastases from various types of tumors showed that TIL are 50 to 100 times more effective in their therapeutic potency than are lymphokine-activated killer (LAK) cells. Therefore the use of TIL was explored for the treatment of mice with large pulmonary and hepatic metastatic tumors that do not respond to LAK cell therapy. Although treatment of animals with TIL alone or cyclophosphamide alone had little impact, these two modalities together mediated the elimination of large metastatic cancer deposits in the liver and lung. The combination of TIL and cyclophosphamide was further potentiated by the simultaneous administration of IL-2. With the combination of cyclophosphamide, TIL, and IL-2, 100% of mice (n = 12) bearing the MC-38 colon adenocarcinoma were cured of advanced hepatic metastases, and up to 50% of mice were cured of advanced pulmonary metastases. Techniques have been developed to isolate TIL from human tumors. These experiments provide a rationale for the use of TIL in the treatment of humans with advanced cancer.

THE TREATMENT OF HUMANS WITH advanced metastatic cancer represents a major therapeutic challenge. One new approach to treating metastatic cancer is adoptive immunotherapy, a treatment in which immune cells with antitumor reactivity are transferred to the tumor-bearing host (*1*). The major obstacle to the use of adoptive immunotherapy has been the inability to generate, from cancer patients, immune cells with antitumor reactivity in numbers sufficient for cancer treatment. Virtually all prior experimental applications of this approach have utilized lymphocytes from highly immunized syngeneic animals, a cell source not available in the human (*2*).

In 1980, we described the lymphokine-activated killer (LAK) cell phenomenon, a method for generating cells capable of lysing fresh tumor by the incubation of lymphocytes in interleukin-2 (IL-2) (*3*). We reported that the adoptive transfer of LAK cells in conjunction with IL-2 was capable of mediating the regression of a variety of advanced metastatic cancers in humans (*4*). Of 55 patients treated with this approach, objective regression of cancer has been achieved in 21 patients, with complete regression of malignancy in five individuals (*4, 5*). Extensive testing of LAK cell therapy in vivo was conducted in various murine tumor models before this treatment was attempted in humans (*6*). These studies showed that tumor regression was optimally induced when both LAK cells and IL-2 were systemically administered and that LAK cells proliferated in vivo under the influence of IL-2 and maintained their cytolytic activity. Although highly effective in mediating tumor regression in some individuals, this treatment approach is cumbersome and can be toxic. Very large numbers of cells are required to mediate cancer regression (about 10^8 cells in a mouse and 3×10^{10} to 3×10^{11} cells in a human). In addition, the high doses of IL-2 required (approximately 100,000 units per kilogram every 8 hours) mediate toxic side effects, the most common of which is a capillary permeability leak syndrome that results in major fluid retention. We have thus sought means of generating cells with more antitumor reactivity and reducing the requirement for the administration of high doses of IL-2.

We have now identified a cell population that can be obtained from tumor-bearing patients and that appears to be 50 to 100 times more potent than LAK cells when used for adoptive immunotherapy. These cells, which constitute a subpopulation of lymphocytes that infiltrate into growing cancers, can be expanded in IL-2 to numbers sufficient to mediate the regression of large metastatic tumors. The adoptively transferred cells are effective in the absence of administered IL-2, although low doses of IL-2 can enhance their therapeutic efficacy. Successful therapy with these tumor-infiltrating lymphocytes (TIL) is dependent upon immunosuppression of the host at the time of treatment with either a high dose of cyclophosphamide or total body irradiation. TIL are highly effective in mediating the regression of advanced pulmonary and hepatic metastases in several mouse model tumors and are effective in curing mice with tumor burdens unaffected by the LAK/IL-2 adoptive immunotherapy system. We have recently developed techniques for isolating these TIL from human tumors (*7*). The experimental results presented in this report provide the rationale for testing this treatment approach in humans with advanced cancer.

A transplantable murine sarcoma and a murine colon adenocarcinoma in syngeneic C57BL/6 mice were used in these studies. The MCA-105 sarcoma was induced in our

Surgery Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

Table 1. Comparison of IL-2 and LAK cells in the therapy of mice bearing MCA-105 sarcoma micrometastases. Treatment was begun 3 days after tumor induction by the intravenous injection of MCA-105 tumor cells. TIL or LAK cells were injected intravenously on day 3 and IL-2 was started on day 3 at doses of 7,500 to 20,000 units intraperitoneally three times daily. Values are mean ± SEM from two to eight experiments for each treatment group, with at least five mice in each group in each experiment. The number of metastases per lung were counted on days 15 to 18 in blind fashion as previously described (*6*).

Number of transferred cells ($\times 10^6$)	Percent reduction in established day-3 micrometastases	
	TIL	LAK
0.4–0.5	16 ± 8	
1–2	74 ± 12	
4–5	96 ± 4	
8–10	100	12 ± 1
30		28 ± 4
60		59 ± 26
100		65 ± 13
200		97 ± 2

laboratory by the intramuscular injection of 3-methylcholanthrene. The tumor was used in the first six transplant generations, at which time a new vial from the first transplant generation was thawed. The MC-38 murine colon adenocarcinoma was induced by the subcutaneous injection of dimethylhydrazine in syngeneic C57BL/6 mice and was passaged subcutaneously.

TIL were prepared as follows. Tumors were harvested aseptically, minced into 1- to 2-mm pieces, and stirred in 40 ml of saline containing 4 mg of deoxyribonuclease, 40 mg of collagenase, and 100 units of hyaluronidase (Sigma) for 1 to 2 hours at room temperature. The resulting cell suspension was filtered through Nitex mesh, washed twice, and suspended in complete medium containing human recombinant IL-2 (1000 unit/ml). The cell suspension was cultured at 2.5×10^5 cells per milliliter in 24-well culture plates. After several days, small colonies of lymphoid cells could be seen among the tumor cells. The number of lymphoid cells increased and that of tumor cells decreased until about day 8 when nearly all of the remaining cells were lymphocytes. The wells were then replated, and cells were grown for several weeks until sufficient numbers of cells were obtained for adoptive transfer. Approximately 100-fold expansion of the original number of cells was obtained during the first 15 days. At 15 days of culture, no tumor cells remained as assessed by cytologic examination, and no pulmonary tumors were induced by injection of these cells.

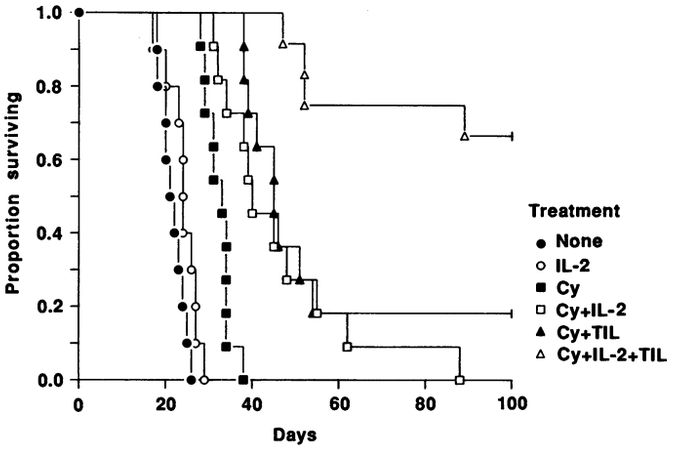
We first explored the use of TIL in the treatment of mice with established pulmonary micrometastases by using our previously established tumor models (*6*). Three days after the injection of the MCA-105 sarcoma, therapy was instituted with either TIL or LAK cells in combination with the systemic administration of IL-2 (Table 1). In accord with our previous observations, the adoptive transfer of approximately 2×10^8 LAK cells was required to mediate the elimination of established micrometastases (*6*). The therapeutic effect was highly dependent on the number of cells transferred, and 10^7 LAK cells had virtually no therapeutic effect. In contrast, the adoptive transfer of 4×10^6 to 5×10^6 TIL in conjunction with IL-2 eliminated 96% of these micrometastases, and as few as 10^6 TIL reduced metastases by 74%. Thus, the adoptive transfer of TIL was 50 to 100 times more potent, on a per cell basis, than the adoptive transfer of LAK cells. In this micrometastasis model, similar results were obtained with the MC-38 colon adenocarcinoma, the B16 melanoma, and the MCA-106 sarcoma in C57BL/6 mice and with the 1660 bladder carcinoma in BALB/c mice (*8*).

Because of the high therapeutic potency of TIL compared to LAK cells, we next explored the treatment of large established pulmonary and hepatic metastases; we tested mice with tumor burdens for which LAK cells had proved ineffective. In preliminary experiments, we found no impact of TIL on large established metastases. We found, however, that cyclophosphamide (Cy) administered at 100 mg/kg resulted in substantial therapeutic benefit when used in combination with TIL, but no benefit when used in combination with LAK cell therapy. Two experiments in which TIL were used to treat mice with advanced liver metastases from the MC-38 colon adenocarcinoma are shown in Table 2. Hepatic metastases were induced by the intrasplenic injection of tumor cells, as we have previously described (*6*); 8 days later, at a time when tumor metastases were visible on the surface of the liver, treatment was initiated. Treatment with TIL alone, IL-2 alone, or the combination of TIL and IL-2 had no impact on survival of mice. The use of Cy improved survival slightly [from median survival time (MST) of 18 and 17 days in control mice to 29 ($P < 0.01$) and 28 days ($P < 0.01$) in mice treated with Cy in experiments 1 and 2, respectively]. The combination of Cy and IL-2 provided a possible survival benefit in experiment 1 ($P = 0.09$ compared to Cy

Table 2. Treatment of advanced liver metastases from the MC-38 colon adenocarcinoma with TIL. Treatment was begun 8 days after tumor induction by intrasplenic injection of MC-38 tumor cells. The IL-2 dose was 25,000 units three times daily for 5 days (days 8 to 12). The cyclophosphamide (Cy) dose was 100 mg/kg intravenously (6 hours before starting IL-2 with or without TIL). The TIL dose was 1.4×10^7 cells and 1.2×10^7 cells intravenously in experiments 1 and 2, respectively.

Treatment			Experiment 1				Experiment 2			
Cy	IL-2	TIL	No. of mice		Survival (days)	Median survival time (days)	No. of mice		Survival (days)	Median survival time (days)
			Total	Cured			Total	Cured		
0	0	0	6	0	17,17,18,18,19,22	18	6	0	16,16,16,17,18,18	17
0	0	+	6	0	18,19,20,22,23,24	21	5	0	16,17,18,19,19	18
0	+	0	5	0	19,20,21,22,28	21	6	0	18,18,18,18,20,21	18
0	+	+	5	0	21,21,22,22,24	22	5	0	17,17,17,18,19	17
+	0	0	12	0	24,25,25,26,27,28 29,29,29,30,32,86	29	5	0	24,25,28,28,29	28
+	+	0	6	2	27,27,40,44,>100,>100	42	6	0	24,25,28,28,29,30	28
+	0	+	6	2	45,51,67,91,>100,>100	79*	6	0	30,30,35,41,43,62	38*
+	+	+	6	6	>100,>100,>100, >100,>100,>100	>100*	6	6	>100,>100,>100, >100,>100,>100	>100*
+	+	LAK†	5	0	28,29,29,29,30	29				

*$P < 0.01$ compared to Cy alone; $P = 0.02$ and $P < 0.01$ with Cy + TIL compared to Cy + TIL + IL-2 in experiments 1 and 2, respectively. In this and subsequent experiments, the survival distributions of different groups were evaluated using the Kruskal-Wallis test as proposed by Breslow (*9*). In this and subsequent experiments, mice were arbitrarily considered cured if they survived longer than 100 days since we rarely saw deaths due to tumor after this time. †The LAK cell dose was 10^8 cells intravenously.



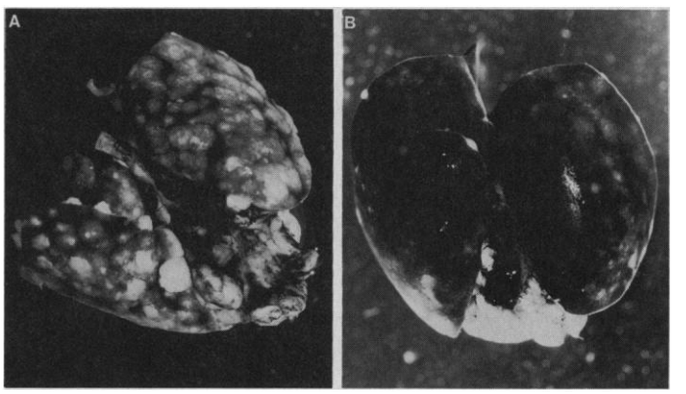
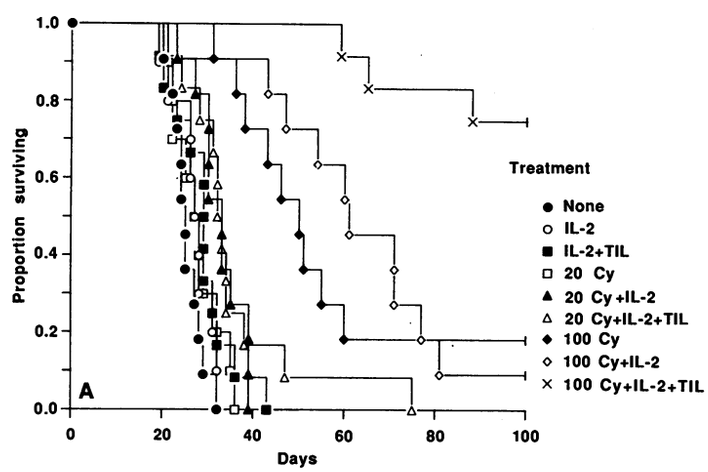


Fig. 1 (left). Treatment of mice with advanced pulmonary metastases from the MC-38 colon adenocarcinoma. MC-38 tumor cells (5×10^5) were injected intravenously. This figure summarizes two experiments in which treatment was begun 12 and 14 days, respectively, after intravenous injection of tumor cells. Mice received cyclophosphamide (Cy) at 100 mg/kg intravenously 6 hours before therapy was begun with IL-2 or TIL or with IL-2 plus TIL. The dose of IL-2 was 20,000 units intraperitoneally every 8 hours for 5 days. The TIL dose was 2.4×10^7 cells in experiment 1 and 2.0×10^7 cells in experiment 2. The results in the two experiments were very similar and they are therefore combined in this figure. In each experiment, there were five or six mice in each group. Although Cy could mediate a modest improvement in survival, Cy combined with either IL-2 or TIL could improve survival slightly ($P = 0.004$ and $P = 0.002$ in experiments 1 and 2, respectively). Substantial improvements were seen, however, when Cy was combined with both TIL and IL-2 ($P = 0.002$ versus Cy and $P = 0.04$ versus Cy and TIL in experiment 1; $P = 0.002$ versus Cy and $P = 0.03$ versus Cy and TIL in experiment 2). Fig. 2 (right). (A) Typical appearance of MC-38 pulmonary metastases in mouse lungs when therapy was begun on day 14 after tumor cell injection. Metastases appear white on a black background after insufflation of the lung with India ink. Metastases are 1 to 2 mm in diameter and replace about 80% of the lung surface. (B) Typical appearance of mouse lungs 6 days after instituting therapy with Cy, IL-2, and TIL.

alone) but not in experiment 2. Use of TIL with Cy improved survival in both experiments compared to the use of Cy alone [to 79 ($P = 0.002$) and 38 ($P = 0.002$) days, respectively]. However, the combination of Cy given on day 8 along with TIL and IL-2 resulted in the long-term cure of all mice in both experiments ($P < 0.001$ compared to mice treated with IL-2 alone, Cy alone, Cy plus IL-2, or Cy plus TIL). Experiment 1 also shows that Cy plus IL-2 added to LAK therapy had no impact on 8-day hepatic metastases, in accord with many other experiments. In addition to this substantial therapeutic difference between TIL and LAK cells, the lytic reactivity of TIL and LAK cells in vitro differed as well. As shown in Table 3, LAK cells exhibited similar lysis of MCA-102 and MC-38 tumor cells (7.7 and 9.1 lytic units (L.U.) per 10^6 cells, respectively). In contrast, TIL obtained from the MC-38 tumor exhibited specific lysis of the MC-38 tumor (0.4 and 125.0 L.U. per 10^6 cells versus MCA-102 and MC-38, respectively).

Similar experiments conducted to explore the use of TIL in the treatment of mice bearing advanced pulmonary metastases from the MC-38 colon adenocarcinoma are shown in Fig. 1 which summarizes the results of two experiments in which treatment was begun 12 and 14 days after the intravenous injection of MC-38 tumor cells.



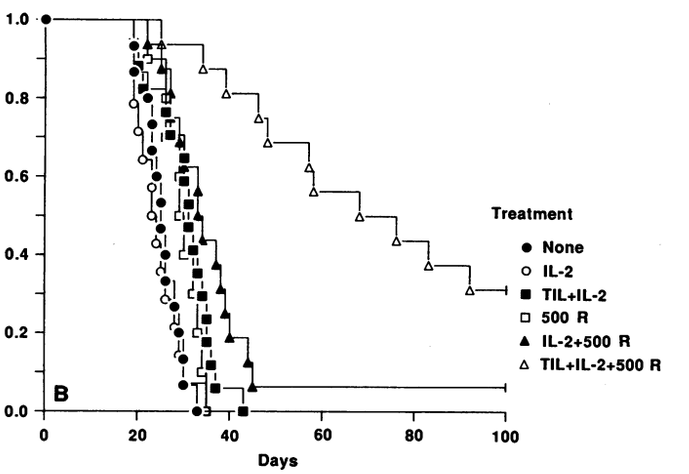


Fig. 3. (A) Treatment of mice with advanced pulmonary metastases from the MC-38 adenocarcinoma. MC-38 pulmonary metastases were induced as in Fig. 1 and treatment was begun 14 days later. This figure summarizes two experiments. Cy at 20 mg/kg or 100 mg/kg was given intravenously, and 6 hours later mice received 2×10^7 TIL followed by IL-2 at either 7500 units every 8 hours or 100,000 units every 8 hours in these two experiments. Results were similar in the two experiments. Cy at 20 mg/kg had little impact either alone or in combination with other treatments. However, treatment with 100 mg/kg resulted in improved survival ($P < 0.001$) which could be markedly enhanced when combined with TIL and IL-2 ($P < 0.001$ versus Cy and $P = 0.001$ versus Cy + IL-2). Five or six animals were used in each treatment group in each experiment. (B) Treatment of mice with advanced pulmonary metastases from the MC-38 adenocarcinoma. Pulmonary metastases were induced as described in Fig. 1 and therapy was initiated 13 to 17 days later. Three separate experiments are summarized. Mice received 500 rads of total body irradiation, and 6 hours later treatment was begun with 7500 units of IL-2 intraperitoneally every 8 hours or IL-2 combined with 1×10^7 to 2×10^7 TIL given intravenously. As with the use of Cy in previous experiments, marked synergy was seen when irradiation was combined with the use of IL-2 and TIL ($P < 0.001$ versus no treatment, irradiation alone, or irradiation plus IL-2).

At this time, tumor nodules were 1 to 2 mm in diameter and were grossly visible on the surface of the mouse lung (Fig. 2). Five to six mice were included in each treatment group in each experiment. As in the treatment of established hepatic metastases Cy improved survival to a modest extent [from MST of 21 to 31 days ($P = 0.004$) in experiment 1 and from 23 to 34 days ($P = 0.002$) in experiment 2]. The addition of TIL administration to Cy increased MST to 45 days in experiment 1 ($P = 0.007$ versus Cy alone) and to 46 days in experiment 2 ($P = 0.002$ versus Cy alone). However, dramatic improvement with long-term cure in 75% of all mice was seen when Cy treatment was combined with the administration of TIL and IL-2 [experiment 1, MST >100 days ($P = 0.002$ versus Cy and $P = 0.04$ versus Cy + TIL); experiment 2, MST >100 days ($P = 0.002$ versus Cy and $P = 0.03$ versus Cy + TIL)]. Thus, in the therapy of advanced liver and lung metastases, treatment with Cy plus TIL was of value and could be enhanced by the administration of IL-2.

High-dose Cy was required to produce this synergistic effect. An evaluation of low- and high-dose Cy (20 mg/kg and 100 mg/kg) in mice treated 14 days after the intravenous injection of MC-38 tumor is shown in Fig. 3A (summary of two experiments). Treatment with Cy at 20 mg/kg had little effect alone, nor could it synergize with IL-2 or TIL plus IL-2 therapy. Treatment with Cy at 100 mg/kg caused an improvement in survival (from MST of 25 days to 50 days, $P < 0.001$), enhanced the effect of treatment with IL-2 (MST of 61 days, $P < 0.001$), and mediated marked synergy when combined with TIL and IL-2; 75% of mice in the group treated with Cy plus TIL and IL-2 survived long term [MST > 100 days ($P < 0.001$ versus Cy and $P = 0.001$ versus Cy + IL-2)].

Because the therapeutic benefit of Cy when used alone was modest, it appeared that the major effect of Cy was on host components required for the success of therapy with TIL. The importance of eliminating suppressor cells to optimize adoptive immunotherapy had been well demonstrated in other studies (*2*). We thus tested whether a different form of immunosuppression, 500 rads of total body irradiation, could similarly synergize with therapy with TIL. In three experiments, mice bearing day-13 to day-17 pulmonary metastases from the MC-38 colon adenocarcinoma were treated with 500 rads of total body irradiation and then with either IL-2 alone or IL-2 plus TIL (Fig. 3B). A marked synergy was seen when irradiation was combined with the use of IL-2 and TIL ($P < 0.001$ versus no treatment, irradiation alone, or irradiation plus IL-2).

Table 3. Specificity of lysis with TIL obtained from the MC-38 colon adenocarcinoma. One lytic unit is defined as the number of effector cells required to cause 25% lysis of 10^4 fresh tumor target cells in a 4-hour chromium-51 release assay.

Effector	No. of experiments	Lysis of target (L.U./10^6 cells)	
		MCA-102	MC-38
LAK*	6	7.7	9.1
TIL from MC-38	6	0.4	125.0

*Syngeneic C57BL/6 LAK cells generated by 3 days of culture in IL-2.

The treatment of advanced pulmonary metastases from the MCA-105 sarcoma produced results similar to those obtained with treatment of the MC-38 adenocarcinoma, showing marked synergy of Cy with administration of TIL and IL-2. In one experiment, for example, mice bearing large 19-day established pulmonary metastases from the MCA-105 sarcoma treated with Cy, TIL, and IL-2 had an MST of 84 days (five of ten mice cured) compared to 21 days (none of nine mice cured) in untreated mice and 49 days (two of nine mice cured) in mice treated with Cy and IL-2. Similar synergy between Cy, TIL, and IL-2 were seen in two other experiments with the MCA-105 sarcoma.

Additional experiments have revealed that mice cured of these tumors by combined treatment are immune to subsequent challenge with the same tumor. Analysis using cell depletion with antibody and complement showed that TIL grow from Thy^+ precursors in the tumor. More than 95% of the cells that grow in IL-2 are Lyt-2^+ as determined by fluorescence-activated cell sorting analysis.

The use of TIL for adoptive immunotherapy offers several potential advantages over the use of LAK cells. TIL appear to have up to 100 times the antitumor efficacy of LAK cells and can mediate antitumor effects in animals with tumor burdens unaffected by LAK cell therapy. Adoptive transfer of TIL, unlike that with LAK cells, can mediate antitumor effects in the absence of IL-2 administration, although low doses of IL-2 can enhance the effects of TIL. This lower requirement for IL-2 administration substantially reduces the side effects of therapy. The antitumor effects of TIL but not of LAK cells are substantially enhanced by the administration of Cy. Cy itself may have antitumor effects but probably acts mainly by the elimination of suppressor factors that can inhibit the effectiveness of transferred immune cells. As an added benefit, we have recently shown that Cy reduces the toxicity of IL-2 administration in animal models.

Finally, we have recently developed techniques for the isolation of TIL from a variety of human tumors, including melanoma, renal cell cancer, and various other adenocarcinomas (*7*). These human TIL can be grown for up to 2 months with substantial increases in cell numbers. In three of six patients with malignant melanoma, these expanded TIL exhibited killing in vitro that was specific for the tumor cells from which they were derived. TIL could be expanded from resected tumors obtained from 23 of 28 patients with renal cell cancer and from 18 of 19 tumor specimens from patients with a variety of adenocarcinomas and sarcomas.

Clinical trials in the human will test the therapeutic efficacy of TIL plus IL-2 in combination with Cy in the treatment of humans with advanced cancer.

REFERENCES AND NOTES

1. S. A. Rosenberg and W. D. Terry, *Adv. Cancer Res.* **25**, 323 (1977); S. A. Rosenberg, *Cancer Treat. Rep.* **68**, 233 (1984); A. Fefer *et al.*, *Ann. N.Y. Acad. Sci.* **276**, 573 (1976); E. Keder and D. W. Weiss, *Adv. Cancer Res.* **38**, 171 (1983).
2. H. Borberg, H. F. Oettgen, K. Choudry, E. J. Beattie, Jr., *Int. J. Cancer* **10**, 539 (1972); M. A. Cheever, P. D. Greenberg, A. Fefer, *J. Immunol.* **125**, 711 (1980); E. Fernandez-Cruz, B. Halliburton, J. D. Feldman, *ibid.* **123**, 1772 (1979); H. G. Smith *et al.*, *J. Natl. Cancer Inst.* **58**,1315 (1977); M. J. Berendt and R. J. North, *J. Exp. Med.* **151**, 69 (1980); S. Shu and S. A. Rosenberg, *Cancer Res.* **45**, 1657 (1985).
3. S. A. Rosenberg, *J. Natl. Cancer Inst.* **75**, 595 (1985); I. Yron, T. A. Wood, Jr., P. J. Spiess, S. A. Rosenberg, *J. Immunol.* **125**, 238 (1980); M. T. Lotze, E. A. Grimm, A. Mazumder, J. L. Strausser, S. A. Rosenberg, *Cancer Res.* **41**, 4420 (1981); S. A. Rosenberg, E. A. Grimm, M. T. Lotze, A. Mazumder, in *Lymphokines*, S. B. Mizel, Ed. (Academic Press, New York, 1982); E. A. Grimm, A. Mazumder, H. Z. Zhang, S. A. Rosenberg, *J. Exp. Med.* **155**, 1823 (1982); M. Rosenstein, I. Yron, Y. Kaufmann, S. A. Rosenberg, *Cancer Res.* **44**, 1946 (1984).
4. S. A. Rosenberg *et al.*, *N. Engl. J. Med.* **313**, 1485 (1985).
5. S. A. Rosenberg, unpublished data.
6. J. J. Mulé, S. Shu, S. L. Schwarz, S. A. Rosenberg, *Science* **225**, 1487 (1984); J. J. Mule, S. Shu, S. A. Rosenberg, *J. Immunol.* **135**, 646 (1985); R. Lafreniere and S. A. Rosenberg, *Cancer Res.* **45**, 3735 (1985); S. A. Rosenberg, in *Important Advances in Oncology*, V. T. DeVita, S. Hellman, S. A. Rosenberg, Eds. (Lippincott, New York, 1986); S. E. Ettinghausen, E. H. Lipford, J. J. Mule, S. A. Rosenberg, *J. Immunol.* **135**, 1488 (1985); *ibid.*, p. 3623.
7. L. M. Muul, P. J. Spiess, E. P. Director, S. A. Rosenberg, in preparation; A. Belldegrun, L. M. Muul, S. A. Rosenberg, in preparation; L. Topalian, L. M. Muul, S. A. Rosenberg, in preparation.
8. P. J. Spiess, J. C. Yang, S. A. Rosenberg, in preparation.
9. N. Breslow, *Biometrika* **57**, 579 (1970).
10. Human recombinant IL-2 was kindly supplied by the Cetus Corporation, Emeryville, CA.

10 July 1986; accepted 15 August 1986