REGRESSION OF A DISSEMINATED SYNGENEIC SOLID TUMOR BY SYSTEMIC TRANSFER OF LYMPHOID CELLS EXPANDED IN INTERLEUKIN 2

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Specific adoptive immunotherapy is a theoretically attractive approach to the treatment of tumors, although few examples exist of the effective treatment of established syngeneic solid tumors by this approach (1). Early reports described the use of large numbers of thoracic duct lymphocytes from immunized animals (2) as well as lymphocytes from immunized allogeneic and xenogeneic animals (3) in an attempt to eradicate solid tumors. Borberg et al. (4) treated Meth A sarcomas with up to 4×10^9 immunized syngeneic lymphocytes and succeeded in causing regression of established tumors. Using the Meth A tumor, but an alternative method of immunization, Berendt and North (5) demonstrated that the intravenous infusion of sensitized T cells from immune donors could cause complete regression of large established tumors growing in T cell-deficient hosts. They also showed that infusion of splenic T cells from tumor-bearing donors could inhibit this regression of established tumor, suggesting that failure to reject this tumor was the result of suppressor T cells in the tumor-bearing host. Fernandez-Cruz et al. (6) showed that intravenous infusion of immune lymphocytes was capable of curing irradiated rats bearing a subcutaneous tumor.

A common factor in these adoptive immunotherapy investigations was the large number of sensitized lymphocytes that were required to cause the regression of established solid tumors. Because the expansion of lymphoid cells in interleukin 2 (IL-2)[1] is capable of generating large numbers of specifically immune cells, we have explored the possible use of these cells for the adoptive immunotherapy of a solid tumor. T lymphocytes expanded in IL-2 retain cytotoxic as well as other functional immunologic properties, and individual lymphocytes can be cloned to large cell numbers without loss of immunologic specificity (7–11). Using these techniques, we have demonstrated that specifically sensitized cells expanded in lectin-free IL-2 (LF-IL-2) were capable of mediating allogeneic skin graft rejection (12) when adoptively transferred to normal hosts. More recently, we have established long-term T lymphoid lines specifically lytic for the syngeneic FBL-3 lymphoma (13) and have successfully used these cells in the adoptive chemoimmunotherapy of a disseminated micrometastatic tumor (14).

[1] *Abbreviations used in this paper:* CM, conditioned medium; Con A, concanavalin A; FCS, fetal calf serum; HBSS, Hanks' balanced salt solution; IL-2, interleukin 2; IVS, in vitro sensitization; LF, lectin-free; LSM, lymphocyte separation medium.

In this paper we have extended these observations to the treatment of an established palpable syngeneic tumor in the footpad of C57BL/6 mice at a time when the tumor is also disseminated throughout the host. We have demonstrated that immune lymphocytes, in vitro sensitized and expanded in LF-IL-2 as a sole treatment, are effective in curing mice of both local tumors and disseminated metastases after one intravenous adoptive transfer.

Materials and Methods

Animals. 16-wk-old C57BL/6 mice were used in these experiments. They were obtained from The Jackson Laboratory, Bar Harbor, ME.

Tumors. FBL-3 (kindly supplied to us by Dr. C. C. Ting, National Cancer Institute) is a Friend virus-induced lymphoma/leukemia transplanted in C57BL/6 mice in its ascitic form. This tumor bears tumor specific and/or viral antigens that cross-react with other tumors induced by the Friend, Moloney, and Rauscher viruses (15) and grows well in the ascitic form in syngeneic mice. This tumor will grow and regress in a normal C57BL/6 mouse if injected intramuscularly or subcutaneously, although it grows progressively at these sites in irradiated mice.

MCA-103 is a methylcholanthrene-induced fibrosarcoma induced in C57BL/6 mice in our laboratory as previously described (16). It was maintained by serial intramuscular passage.

In Vivo Immunization. 8–12-wk-old C57BL/6 female mice were immunized with one intramuscular injection of 10^7 live FBL-3 suspended in 0.05 ml sterile Hanks' balanced salt solution (HBSS). An intramuscular tumor will grow for ~2 wk and then completely regress. All animals used as immune spleen donors had no evidence of tumor at the time of spleen harvest. Less than 10% of the immunized animals grew tumors that resulted in the death of the animal.

In Vivo Assay of Adoptive Immunotherapy. Mice were given 500 rad whole-body irradiation (Cs 137), and 2–4 h later 10^7 live FBL-3 tumor cells were injected into the right hind footpad in 0.05 cc of sterile HBSS.

By day 5 tumors were readily palpable and the footpad diameter measured between 2.5 and 3 mm. Also by day 5 either the right popliteal lymph node or 0.75 ml of blood from tumor-bearing mice could cause progressive tumor growth when injected intraperitoneally into a normal mouse that had received 500 rad. If left untreated, both the footpad tumor and its metastases grew and eventually killed the animal by 20 d after the initial tumor injection.

5 d after tumor was induced, when the tumor was clearly measurable in all animals, mice were randomly assigned to treatment groups and injected intravenously with experimental or control cells in 1 cc of sterile HBSS. Each mouse was ear tagged and measured every 2nd or 3rd d in a blinded fashion without knowledge of the previous treatment or of the previous measurements for that mouse.

Spleen Cell Suspension. Spleens were aseptically removed, pooled, and crushed gently with the blunt end of a 10-cc syringe plunger in HBSS with 1% fetal calf serum (FCS). The cells were then centrifuged at 500 *g* for 5 min. The pellet was resuspended in buffered ammonium chloride solution (National Institutes of Health [NIH] Median Unit) for one minute at room temperature to lyse red blood cells. The cells were then washed three times in sterile HBSS and resuspended in complete medium (CM) before determination of cell viability using trypan blue. CM was composed of RPMI 1640 (Grand Island Biological Co., Grand Island, NY) with 10% heat-inactivated FCS (Grand Island Biological Co.), 1 μM sodium pyruvate (Microbiological Associates, Walkersville, MD), 0.1 mM nonessential amino acids (Microbiological Associates), 0.03% fresh glutamine (NIH Media Unit), 5×10^{-5} M 2-mercaptoethanol, 100 U/ml penicillin, and 100 μg/ml streptomycin.

In Vitro Sensitization (IVS): Conditions for IVS have been previously described (13, 14). In brief, 6×10^7 viable responder cells and 10^6 irradiated stimulator cells were placed in upright flasks (3013; Costar, Data Packaging, Cambridge, MA) in 20 ml of CM. Stimulator cells were irradiated in a gamma irradiator (Cs 137) with 2000 rad for normal C57BL/6 lymphocytes or 10,000 rad for fresh FBL-3 tumor. Cells were harvested on day 5 and tested in vitro for cytotoxicity, used in experiments or placed in IL-2. If the cells were injected in vivo, they were first placed on a 5 ml lymphocyte separation medium (LSM) gradient (Cedarlane Laboratories,

Ontario, Canada) at a concentration of 10^7 cells/ml and spun at 1000 *g* for 20 min. The interface was then removed using a pasteur pipette, and the cells were washed three times in HBSS, passed over 100-mesh nylon, counted, and injected. This technique resulted in cell viability >95%.

Production of LF-IL-2. Optimal conditions for the production of IL-2 have been previously described (11). Briefly, exbreeder DBA/2 or BALB/c spleens were aseptically harvested, minced, washed, and then incubated with 10 μg/ml concanavalin A (Con A) (Miles Laboratories, Elkhart, IN) for 2 h. The cells were washed three times in HBSS and resuspended in CM for 24 h at 37°C and 5% CO_2. The resulting culture supernatants were harvested, centrifuged, and poured through 0.45-μm filters (Millipore Corp., Bedford, MA). This LF-IL-2 was >95% Con A free as determined by the absence of mitogenic activity on fresh lymphocytes and by measurement of removal of radiolabeled Con A (11).

Expansion of IVS Lymphoid Cells. Cells were aseptically harvested from the IVS flasks, centrifuged, and resuspended in fresh CM. Cell viability was assessed using trypan blue dye exclusion. The sensitized cells were then adjusted to a concentration of 5×10^4 viable cells/ml in an equal volume of CM and LF-IL-2. This final suspension was placed in 24-well flat-bottomed plates (3524; Costar), 2 ml/well. The cells expanded a minimum of 8.5 times the original number in 7 d. If adoptively transferred, the cells were washed in HBSS three times, passed over 100-mesh nylon, counted, and adjusted to the desired concentration for injection. All injections were in 1 ml vol of HBSS.

In the experiment using cells expanded multiple times in IL-2, the cells were harvested every 5–7 d, centrifuged at 500 *g* for 5 min, and readjusted to 5×10^4 viable cells/ml in fresh CM:LF-IL-2. 10^5 irradiated FBL-3 tumor cells/ml (10,000 rad) were present in the culture from IVS until the final 10 d before adoptive transfer. Cells expanded in IL-2 were not placed on LSM gradients before adoptive transfer, and always had >95% viability.

Chromium Release Cytotoxicity Assay. An 18-h chromium release assay was used as previously described (8). Briefly, varying ratios of effector cells were plated in 96-well round-bottomed plates (Linbro Chemical Co., Hamden, CT) with 10^4 viable ^{51}Cr-labeled tumor targets per well. The plates were then centrifuged at 80 *g* for 5 min, incubated at 37° for 18 h, and recentrifuged at 400 *g* for 10 min, and the supernatants were harvested using the Titertek Collecting System (Flow Laboratories, Rockville, MD).

Fresh FBL-3 was harvested from the ascites of a tumor-bearing mouse, washed, and labeled with ^{51}Cr. MCA-103 tumor was minced with fine scissors, trypsinized for 7 min, passed through double-layer 100-mesh nylon, and washed three times before ^{51}Cr label. Spontaneous release for these experiments was ~40%. In previous studies (13) MCA-103 was shown to be at least as lysable as FBL-3 by an allogeneic effector. 18-h assays revealed substantially higher and more reproducible levels of specific tumor lysis than were seen in 4-h ^{51}Cr-release assays.

Cytoxicity is expressed as lytic units/10^6 cells. A lytic unit is defined as the number of effector cells that causes 50% lysis of 10^4 ^{51}Cr-labeled target cells.

Statistical Methods. Survival of mice in these experiments was computed using the methods of Peto et al. (17). To compare survival curves, mean survival time was MST calculated and Student's *t*-test used to determine *P* values. No animals were excluded from statistical evaluation.

Results

Treatment of Disseminated FBL-3 with In Vivo Immunized Lymphoid Cells. Adult C57BL/6 female mice were immunized to FBL-3 with one intramuscular injection of live FBL-3. Approximately 3–4 wk later, their spleens were harvested and tested in vitro and in vivo. In every experiment, these immune cells showed no in vitro cytotoxicity for FBL-3 tumor in an 18-h ^{51}Cr-release assay (data not shown). When these same cells were adoptively transferred to mice with disseminated footpad tumors, however, they conferred significant survival benefit when compared with no treatment or treatment with similar numbers of lymphocytes from normal syngeneic mice. Fig. 1 is a representative experiment of four experiments, each with a similar result. The left panel shows the mean footpad tumor size of each group and the right panel the

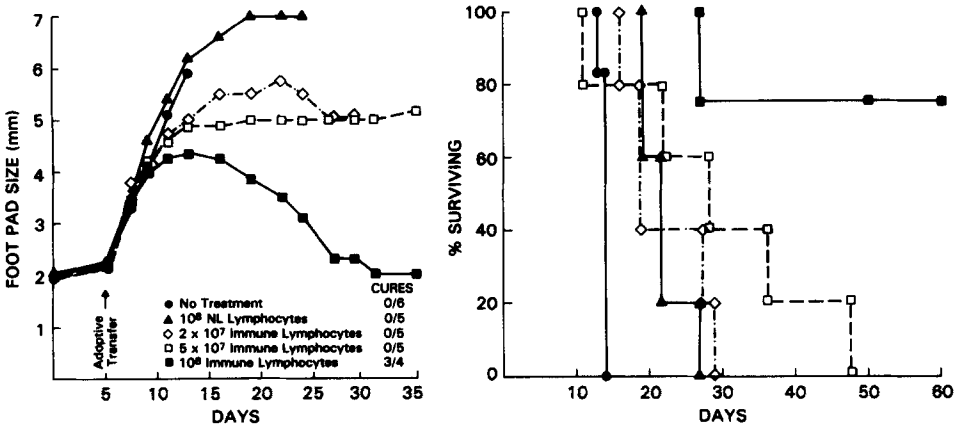

FIG. 1. Footpad tumor size (left) and survival (right) of mice with disseminated solid FBL-3 lymphoma treated with in vivo immunized lymphocytes. Treatment with 10^8 immune cells was necessary to completely eradicate footpad tumors and cure animals. 10^8 normal lymphocytes or fewer immune lymphocytes were not capable of impacting significantly on tumor growth or survival.

corresponding survival curve. As seen in Fig. 1, 10^8 immune cells given intravenously were required to cure mice. In no experiment were 5×10^7 immune cells effective. Combining all four experiments, 26 of 28 mice given 10^8 immune cells were cured of tumor, whereas 3 of 17 mice with normal lymphocytes were cured ($P < 0.0005$). All 20 untreated animals died of disseminated FBL-3 tumor ($P < 0.0005$ compared to treatment with immune cells).

Adoptive Transfer of In Vitro Sensitized Lymphoid Cells before Expansion in LF-IL-2. In vivo immunzed lymphocytes were resensitized to irradiated FBL-3 tumor in vitro. Simultaneously, nonimmune lymphocytes were co-cultured with normal C57BL/6 irradiated stimulators in vitro. After 5 d in culture, the cells were harvested, washed, placed on an LSM gradient, rewashed three times, counted, and adoptively transferred to mice that had been injected with tumor in the footpad 5 d earlier. No more than 5×10^7 viable cells were transferred in any experiment. Before transfer, an aliquot of the cells was tested for in vitro cytotoxicity.

Fig. 2 is a representative experiment (one of three) showing the levels of cytotoxicity attained after in vitro sensitization. Only the immune cells resensitized to FBL-3 tumor in vitro showed specific lysis of fresh FBL-3 (91 lytic units). Nonimmune cells co-cultured with normal lymphocytes showed nonspecific lysis, <5 lytic units, for both fresh FBL-3 and fresh MCA-103 targets. Fig. 3 shows two of the three experiments using treatment with in vitro sensitized cells. In all three experiments, 11 of 14 mice treated with the immune cells resensitized to FBL-3 were cured of tumor, compared with 0 of 16 mice treated with nonimmune lymphocytes resensitized to normal lymphocytes ($P < 0.0005$). Once again, none of the 18 untreated controls survived ($P < 0.0005$).

Adoptive Transfer of Lymphoid Cells Expanded in LF-IL-2. Cells were harvested from IVS flasks, washed, counted, and placed in LF-IL-2 at 5×10^4 cells/ml. There was a minimum 8.5-fold expansion of cell number in 7 d. The cells were harvested from the expansion plates, washed three times, and adjusted to the appropriate cell number in 1 cc of sterile HBSS. Fig. 4 shows the results of one of three similar experiments

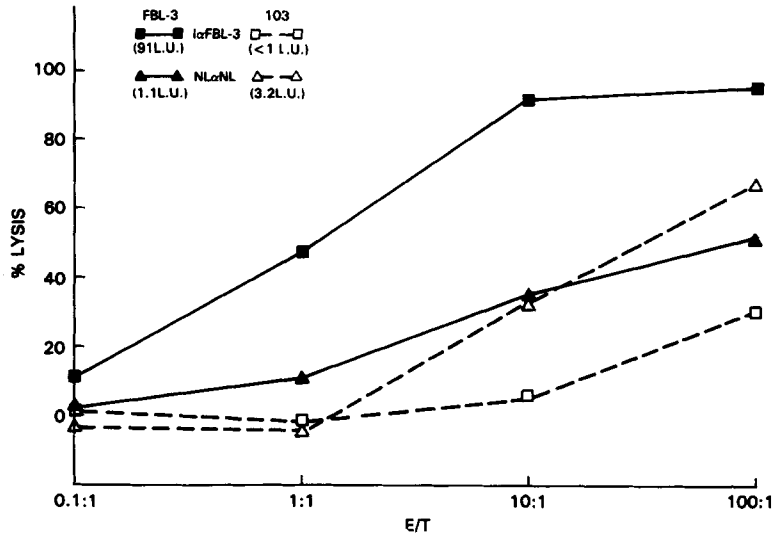

FIG. 2. In vitro cytotoxicity of immune cells co-cultured with FBL-3 tumor for 5 d or nonimmune cells in vitro sensitized to normal C57BL/6 lymphocytes for 5 d. Resensitization of immune cells to FBL-3 tumor conferred specific cytotoxicity at the higher effector/target ratio (E/T). L.U., lytic units.

demonstrating a high degree of specific lysis of fresh FBL-3 tumor. Immune lymphocytes previously co-cultured with FBL-3 tumor and then expanded 8.5-fold in LF-IL-2 showed 2,000 lytic units against fresh FBL-3 and <15 lytic units against fresh MCA 103. Nonimmune, in vitro sensitized lymphocytes cultured in IL-2 for an equal length of time showed <5 lytic units when tested against either fresh tumor target. Nonimmune lymphocytes sensitized in vitro to FBL-3 tumor also demonstrated no lysis of either tumor both before and after expansion in LF-IL-2 (data not shown).

Fig. 5 shows the results of the first experiment with the adoptive transfer of expanded cells in this disseminated footpad tumor model. Immune lymphocytes resensitized to FBL-3 tumor for 5 d and then expanded 8.5-fold in LF-IL-2 for 7 d were capable of curing 11 of 12 mice treated with one intravenous injection of either 5×10^7 or 2.5×10^7 cells ($P < 0.0005$). The only mouse in these groups that died did so on day 42 without evidence of tumor, and this animal received a lower dose of effector cells (2.5×10^7). There was no significant difference either in footpad size (Fig. 5, left) or survival (Fig. 5, right) between mice receiving 5×10^7 or 2.5×10^7 lymphocytes. Normal lymphocytes expanded in LF-IL-2 for 7 d failed to have any impact on either footpad tumor size or survival.

In a repeat experiment, the adoptive transfer of cells occurred on day 6 after injection of tumor in the footpad. Once again, immune lymphocytes co-cultured with FBL-3 tumor for 5 d and then expanded in LF-IL-2 for 7 d conferred significant survival benefit ($P < 0.0005$), with all but one footpad tumor returning to normal by day 17. There was one death on day 31 in a mouse with disseminated tumor. When these cells received 2,000 rad just before adoptive transfer (open squares, Fig. 6), the survival benefit was abrogated. When irradiated cells were used, no animals were cured, and the survival time was the same as in the untreated control group. Thus, it

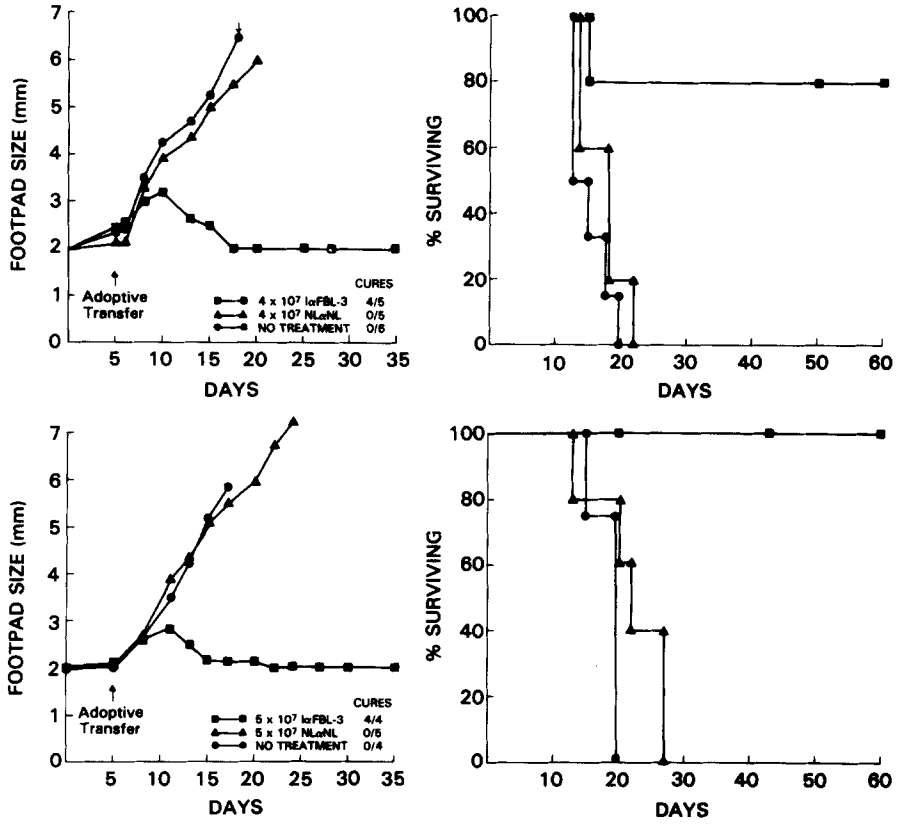

FIG. 3. Footpad tumor size (left graphs) and survival (right graphs) of mice with disseminated solid syngeneic tumors treated with cells in vitro sensitized for 5 d in CM. Immune lymphocytes resensitized to FBL-3 tumor cured 9 of 10 animals in these two experiments. Nonimmune lymphocytes cocultured to normal C57BL/6 lymphocytes were no more effective than no treatment.

appeared that a cell capable of dividing in vivo was necessary to mediate an increase in survival.

Table I shows the combined result of all three experiments performed with adoptive transfer of cells expanded in IL-2 for 1 wk. 5×10^7 immune sensitized cells expanded in IL-2 cured 93% of all animals treated. 2.5×10^7 cells cured 80% of the animals, and the one animal that died did so without evidence of tumor. A dose of 5×10^6 immune expanded cells cured three of eight animals treated. Irradiation at 2,000 rad of these same cells totally abolished their effectiveness in vivo. Similarly, nonimmune sensitized and expanded cells cured none of the 11 animals so treated, and all of the 17 untreated control animals died.

The immune cells in Fig. 5 were expanded 3,500-fold in LF-IL-2 for ~1 mo as described in Materials and Methods. These cells maintained a high degree of specific lysis of fresh FBL-3 tumor (Fig. 7) when tested in an 18-h ^{51}Cr release assay on the day of adoptive transfer (3,571 lytic units). These same cells conferred significant survival benefit (Fig. 8) when adoptively transferred. Five of nine animals receiving 5×10^7 cells were cured, and three of seven animals were cured at a dose of 10^7 transferred cells per mouse ($P < 0.01$). Nonimmune lymphocytes resensitized to

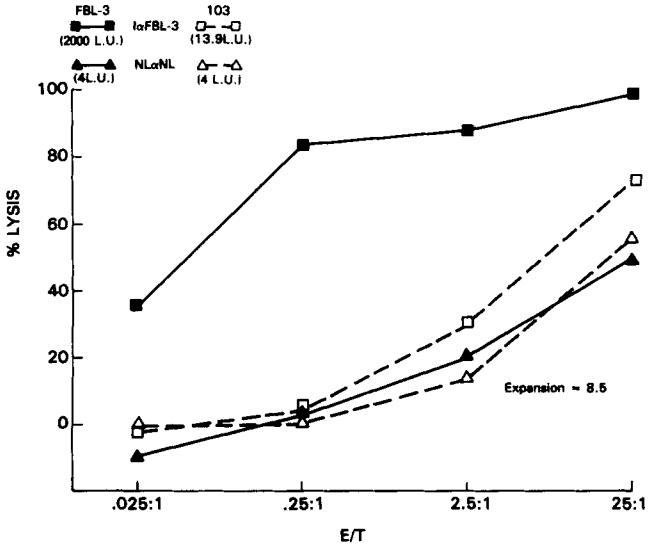

FIG. 4. In vitro cytotoxicity of cells expanded for 7 d in LF-IL-2. Immune cells sensitized to FBL-2 tumor and expanded in IL-2 showed increased specific lysis when compared to nonimmune lymphocytes sensitized to normal C57BL/6 lymphocytes and expanded in IL-2 for a similar period of time. L.U., lytic units.

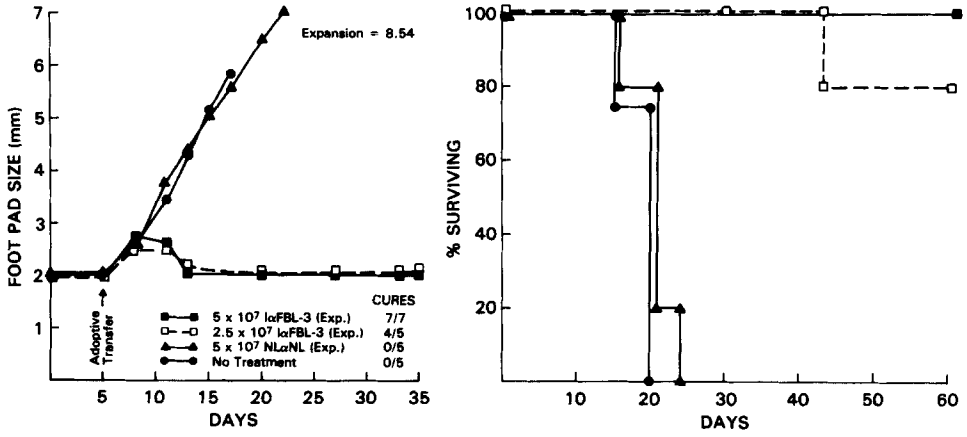

FIG. 5. Footpad tumor size (left) and survival (right) of mice with disseminated solid tumors treated with cells expanded in IL-2 over 7 d. Immune cells sensitized to FBL-3 and expanded for 7 d in IL-2 cured all mice treated, and cured 80% when a reduced dose of these cells was given. Nonimmune cells sensitized and then expanded in IL-2 for a similar period were no better than the no-treatment control group.

normal C57BL/6 stimulators and expanded in LF-IL-2 for the same length of time could not mediate the shrinkage of footpad tumors (Fig. 8, left) or impact on survival (Fig. 8, right).

Discussion

In the experiments reported in this paper, we have demonstrated that mice bearing a palpable local and disseminated tumor could be cured by the systemic adoptive

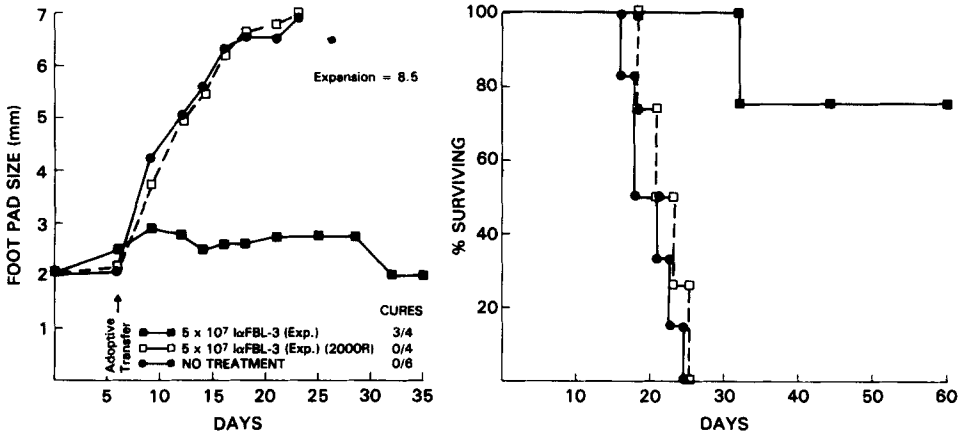

FIG. 6. Footpad tumor size (left) and survival (right) of mice with disseminated solid syngeneic tumors treated on day 6 with cells expanded in IL-2. Immune cells sensitized to FBL-3 tumor and then expanded in IL-2 for 7 d cured three of four mice treated; however, if these same cells were given 2,000 rad before transfer, their in vivo effectiveness was abolished.

TABLE I

	Footpad cures	Survival	Percent survival
I α FBL-3 expanded*			
5×10^7	13	13/14	93%
2.5×10^7	5	4/5	80%
10^7	2	2/4	50%
5×10^6	3	3/8	37.5%
5×10^7 (2,000 rad)	0	0/4	0%
NL α NL expanded‡			
5×10^7	0	0/11	0%
No treatment	0	0/17	0%

* Immune lymphocytes resensitized to FBL-3 tumor in vitro and expanded in LF-TCGF for 7 d.

‡ Nonimmune lymphocytes resensitized to normal lymphocytes in vitro and then expanded for 7 d in LF-TCGF.

transfer of a single injection of in vivo immunized cells, in vitro sensitized cells, or sensitized cells expanded >3,000-fold in IL-2. These experiments are the first to demonstrate cure of a syngeneic solid tumor using cells expanded for many generations in IL-2. These results also extend our previous observations of the treatment of intraperitoneal FBL-3 tumor. We previously demonstrated (14) that a combination of chemotherapy and adoptive transfer of sensitized cells expanded in IL-2 was capable of curing mice with disseminated and intraperitoneal FBL-3 tumor. Similar results were obtained by Cheever et al. (18, 19).

However, the interpretation of our previous studies (14) and those of others (18, 19) using the adoptive transfer of cells expanded in IL-2 to treat intraperitoneal FBL-3 tumor were confused by the need for cytoreductive treatment of the tumor-bearing mouse with cyclophosphamide before cell transfer and by the need to inject the transferred cells intraperitoneally at the site of major tumor growth. These problems led us to develop the present model, in which expanded lymphoid cells injected

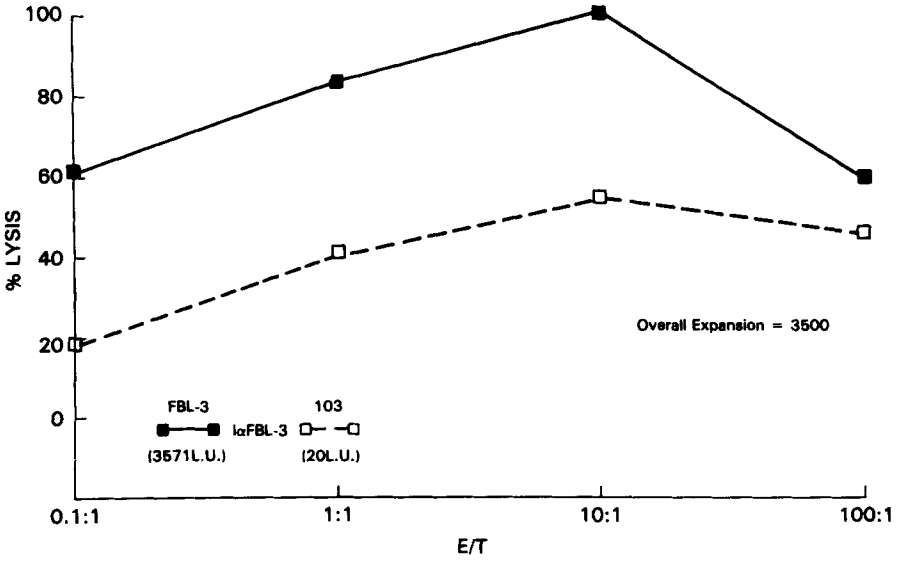

FIG. 7. In vitro cytotoxicity of cells expanded ~1 mo in IL-2. Immune cells sensitized to FBL-3 tumor and expanded 3,500-fold in LF-IL-2 maintained specific lysis of fresh FBL-3 tumor. L.U., lytic units.

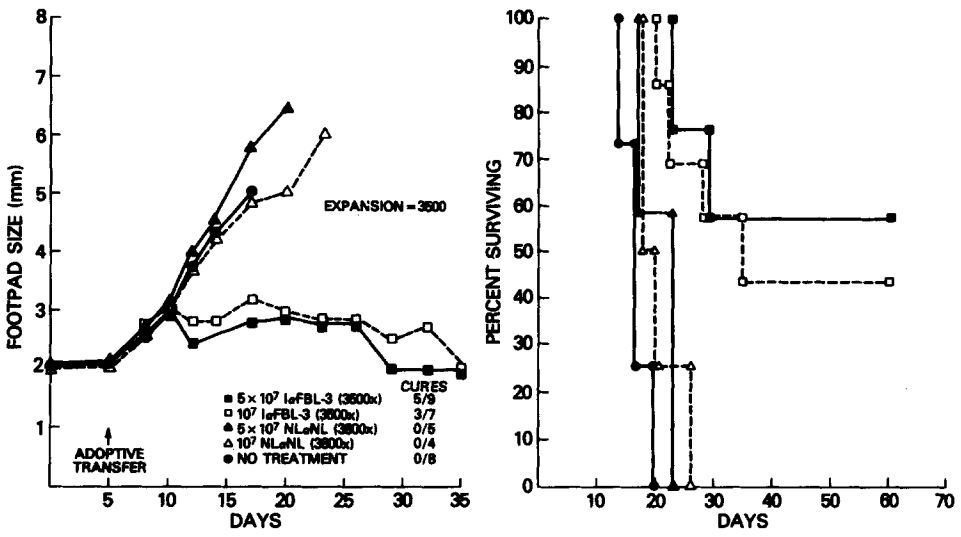

FIG. 8. Footpad tumor size (left) and survival (right) of mice with disseminated solid FBL-3 tumors treated with cells expanded in IL-2 for ~1 mo. Immune cells sensitized to FBL-3 tumor and expanded 3,500-fold cured a significant number of mice at the high dose of cells transferred and low dose. No dose of similarly expanded control cells was capable of cure.

intravenously are used as sole therapy to test the ability of cells expanded in IL-2 to cure mice of syngeneic palpable and disseminated tumor.

This model has several important advantages. The tumor being treated by adoptive transfer of cells is a palpable solid tumor in the footpad of mice, and the regression of this palpable tumor can be easily followed by direct measurement. On the day of

treatment, tumor is disseminated both in the blood and in the lymphatics, and thus treatment effects on local and distant tumor can be evaluated. Tumor-bearing mice receive treatment with cells alone, and thus the potentially confusing cytoreductive effects of cyclophosphamide are avoided. Another feature of our new model is that adoptive treatment with cells is administered intravenously and not into the site of the tumor. In previous studies of the treatment of intraperitoneal tumor, we (14) and others (18, 19) injected effector cells intraperitoneally. The possibility existed, therefore, that adoptively transferred cells acted mainly in local tumor neutralization, rather than systemic immunotherapy.

Whole body preirradiation of mice is essential to enable growth of the FBL-3 tumor in the footpad. The effect of this irradiation on the therapeutic effectiveness of the adoptively transferred lymphocytes is not known, but may be an important factor by eliminating suppressor cells. Berendt and North (5), showed that adoptive transfer of fresh immune lymphoid cells could cause the complete regression of large established tumors only if the tumor bearers were T cell deficient. Furthermore, these authors showed that infusion of splenic T cells from tumor-bearing donors could inhibit the regression of established tumor in T cell-deficient recipients, confirming the importance of host suppressor mechanisms in these tumor systems.

In this paper, we have shown that lymph nodes and/or blood from a mouse bearing the FBL-3 tumor 5 d after tumor injection are capable of transferring tumor to a normal, irradiated, C57BL/6 syngeneic recipient. The tumor is thus widely disseminated by day 5. Treatment of this tumor with in vivo immunized cells, on day 5, when it is also clearly palpable, resulted in cure of 93% of all mice treated in four experiments. As seen in Fig. 1, however, $\sim 10^8$ cells were required to cure a mouse. When these in vivo immunized cells were resensitized to FBL-3 in vitro for 5 d, cures could be achieved with smaller doses of cells (Fig. 3). This finding emphasizes the value of in vitro sensitization in the activation/reactivation of cells that are necessary for successful adoptive immunotherapy (20).

When appropriately sensitized cells were expanded in LF-IL-2, they were highly lytic for fresh FBL-3 tumor in vitro (Figs. 4 and 7) and capable of curing mice of disseminated footpad tumors (Figs. 5 and 6). Indeed, in three experiments (as seen in Table I), expanded cells adoptively transferred to mice with disseminated footpad tumors cured mice when as few as 5×10^6 cells were transferred. Cells grown in IL-2 for almost 1 mo (3,500-fold expansion) were also capable of curing a significant number of animals with disseminated footpad tumors (Fig. 8). Thus, through the use of IL-2, we have been able to expand the therapeutically effective cell to sufficient numbers to cure mice.

The type of cell effecting the cure of tumor in this model is not known. It appears that a cell capable of proliferation in vivo is necessary, and that this proliferation takes 7–10 d before a critical mass of effector cells is reached to impact on tumor growth. Although the in vitro sensitized and expanded effector populations capable of curing animals in this model are cytotoxic for fresh FBL-3 tumor in vitro, there is a potential problem in selecting cells for use in adoptive immunotherapy based solely on in vitro reactivity. The primary effector cells in vivo in this model may not be the cytotoxic cell measured in our in vitro assays. Fernandez-Cruz et al. (6) showed that

the subset of T cells most effective in eradicating rat solid tumors in vivo were noncytotoxic in an in vitro ^{51}Cr release assay. Supporting the hypothesis that a helper cell is the primary effector cell in mediating tissue rejection is the work of Loveland et al. (20, 21), who showed that skin and tumor allograft rejection in vivo was dependent on the presence of Lyt-1^+ cells. Greenberg et al. (22) reported similar findings used in vivo immunized cells depleted of Lyt-2^+ cells to cure mice of disseminated FBL-3 tumor. Similar findings were obtained by Fernandez-Cruz (23) in a syngeneic rat solid tumor model. Regardless of which cell is effective in our tumor model, we were able to increase the number of cells available for adoptive transfer using IL-2.

The demonstration that intravenously injected lymphoid cells expanded in IL-2 are capable of mediating local and disseminated tumor regression has important implications for the adoptive immunotherapy of tumors. The abnormal traffic patterns seen when cells expanded in IL-2 are reinjected into mice and humans (24) have led to concern that these expanded cells would not be functional when injected in vivo. Our previous demonstration that intravenous injection of cells expanded in IL-2 was capable of accelerating the rejection of allogeneic skin grafts (12), and the present demonstration that syngeneic tumors could be made to regress following intravenous injection of expanded sensitized cells indicate that cells expanded in IL-2 can distribute appropriately and mediate immunologic tissue destruction following intravenous injection. Our current efforts are directed at sensitizing autologous lymphoid cells to human tumors in the hope that expansion of these cells will be useful in the adoptive immunotherapy of human tumors.

Summary

We have studied the ability of immunized lymphoid cells expanded in IL-2 to mediate the cure of mice with localized and disseminated syngeneic lymphoma. Mice received 500 rad total-body irradiation before injection of tumor into the footpad. Mice were treated 5 d later when a palpable local tumor and disseminated metastases were present. Intravenous injection of in vivo immune lymphocytes cured 93% of all mice, significantly better than any control group ($P < 0.0005$). Immune cells, secondarily sensitized to the FBL-3 tumor in vitro, also conferred significant survival benefit ($P < 0.0005$) when injected intravenously, curing 79% of the animals treated. When these in vitro sensitized cells were expanded in IL-2, 8–10-fold over 7 d, 93% of the animals thus treated were cured, ($P < 0.0005$). When these cells were grown for multiple generations in IL-2 they retained their ability to cure mice (56% cured, $P < 0.01$). This is the first demonstration that intravenous injection of sensitized cells grown in long term culture in IL-2 is capable of curing mice of established local and disseminated syngeneic tumor.

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