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Analysis of interleukin 2 and various effector cell populations in adoptive immunotherapy of 9L rat gliosarcoma: Allogeneic cytotoxic T lymphocytes prevent tumor take

(brain tumor/glioma/killer cells)

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ABSTRACT **Recombinant interleukin 2 (rIL-2) and various effector cell populations were used for adoptive immunotherapy in the Fischer strain 9L rat gliosarcoma model. The *in vivo* cytotoxicities of nonspecifically activated lymphocytes and specifically activated cytotoxic T lymphocytes (CTLs) were assessed in a modified *in vivo* neutralization (Winn) assay. Effector cells (10^6) and 9L tumor cells (10^5) were combined with 10^4 units of rIL-2 and stereotactically implanted into the right frontal centrum semiovale of the Fischer (F344) rat. At 7 and 14 days, additional effector cells (10^6) and rIL-2 (10^4 units) were administered through the same burr hole. Nonspecifically activated splenocytes were lymphokine-activated killer (LAK) cells, both plastic-adherent and nonadherent, whereas specifically activated CTLs were either syngeneic (genetically identical) or allogeneic (genetically dissimilar). Syngeneic CTLs were T lymphocytes from Fischer rats primed *in vivo* with 9L cells and restimulated *in vitro*. Allogeneic CTLs were generated by exposing DA rat lymphocytes either to irradiated Fischer lymph node cells or to 9L Fischer tumor cells *in vitro*. Control groups included rats bearing 9L tumor who were untreated, those who received peripheral (i.p. or s.c.) administration of rIL-2, or those who received syngeneic unstimulated T lymphocytes and rIL-2. For a set of animals given the same inoculum of 9L tumor, significantly improved survival was shown for groups treated with nonadherent or adherent LAK cells ($P \leq 0.0003$), syngeneic CTLs ($P = 0.0327$), or allogeneic CTLs ($P = 0.0025$) over untreated control animals by using Mantel–Haenzel nonparametric logrank equations. Only treatment with allogeneic CTLs prevented tumor take.**

The rat 9L gliosarcoma model provides an efficient and rapid means to explore the efficacy of lymphokine and cellular therapy for brain tumors. It is derived from an inbred strain of Fischer rats, with a major histocompatibility complex haplotype of $RT1^{lvl}$ (1, 2). Intracranially implanted 9L glioma cells grow in a predictable fashion in the syngeneic Fischer 344 rat (3). Rats bearing 9L tumor and given systemic recombinant interleukin 2 (rIL-2) therapy show a small increase in survival time compared to untreated control animals (4).

Adoptive immunotherapy has been investigated in other brain tumor models. Lymphokine-activated killer (LAK) cells and rIL-2 were combined with the F98 rat glioma tumor 18 hr before implantation into the rat cerebrum and the rats exhibited an increased survival (5). Similarly, a clone of syngeneic tumor-sensitized cytotoxic T lymphocytes (CTLs) was partially effective in the immunotherapy of 203 glioma in an animal model (6). The *in vivo* antitumor activity of the clone has been demonstrated both in a Winn neutralization assay against 203 glioma cells inoculated intracranially and when administered intravenously 7 days after intracranial inoculation of 203 glioma. In rats bearing T9 gliosarcoma, LAK cells administered intravenously and intratumorally increased survival, but immune spleen cells did not (7). Likewise, LAK cells administered to Wistar rats bearing C6 glioma showed antitumor activity *in vitro* and *in vivo* (8).

The implication that the brain is immunologically privileged was based on demonstrations that allogeneic and xenogeneic glioma cells were maintained intracranially (9). More recent studies have revealed some immunological response to these tumors (10, 11); thus, the brain is now considered to be a semiprivileged immune site. On that premise and because it has been shown that CTLs from an allogeneic source are more effective against tumor than those from a syngeneic source (12), we have investigated whether brain tumors in rats could be cured by local adoptive transfer. The advantage of using allogeneic CTLs for therapy of glioma patients is that the cells are from a healthy donor. This circumvents collecting the patients' own cells for therapy, which would exacerbate their already immunocompromised state (from steroid treatment or chemotherapy), and avoids the use of their inherently immunodeficient cells, expressed as a $CD4^+$ lymphopenia (13, 14). This paper describes, using the 9L rat gliosarcoma model, the *in vitro* and *in vivo* efficacy of rIL-2 with various preparations of nonspecifically and specifically activated effector cells, which were derived from allogeneic (DA) or syngeneic (Fischer) sources.

MATERIALS AND METHODS

Animals. Fischer ($RT1^{lvl}$) rats, male 200–250 g, were obtained from Sasco (Omaha, NB). DA ($RT1^{avl}$) rats were obtained from the Trudeau Institute (Saranac Lake, NY).

Maintenance of Tumor. A low passage number of 9L gliosarcoma was obtained from Stanley Geyer (Seattle, WA). Tumor cells were cultured in Dulbecco's modified Eagle's medium containing 15% (vol/vol) fetal calf serum. To ensure reproducibility in the mixture of tumor cells given in the animal trials, 9L tumor was propagated in tissue culture and aliquots of tumor were frozen. 9L was cultured for 4–5 days before use in an adoptive transfer experiment.

Generation of LAK T Lymphocytes. Splenocytes, isolated from aseptically removed spleens, were prepared by pressing

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Abbreviations: rIL-2, recombinant interleukin 2; LAK, lymphokine-activated killer; CTL, cytotoxic T lymphocyte; HBSS, Hanks' balanced salt solution; TDL, thoracic duct lymphocyte; MST, mean survival time; E:T ratio, effector/target ratio.

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through a wire mesh screen in the presence of Hanks' balanced salt solution (HBSS) as described (15). The cells were then dispersed mechanically with a modified Bellco Tenbroeck tissue homogenizer. Cells were washed twice with HBSS and suspended at a concentration of 3–5 × 10^6 cells per ml in Iscove's modified Dulbecco's medium containing 10% (vol/vol) fetal bovine serum (Hazelton, Kansas City, MO), gentamicin (5 μg/ml), penicillin/streptomycin (100 units/ml and 100 μg/ml, respectively), and rIL-2 (1000 units/ml). After incubation at 37°C in humidified 95% air/5% CO_2 for 1 hr, the nonadherent cells were transferred to another culture flask for 2 days. Excess erythrocytes were then lysed by treating the pelleted cell suspension with buffered 0.14 M ammonium chloride. The cell suspension was washed twice with HBSS and placed back into the original cell supernatant and cultured an additional 2 days. The nonadherent cells were washed twice, counted, and suspended in HBSS. For adherent LAKs, cells were scraped from the plastic, washed twice, counted, and suspended in HBSS.

Collection of Rat Lymphocytes. Thoracic duct lymphocytes (TDLs) were obtained from rats by drainage through a surgically implanted thoracic duct fistula (16). Lymphocytes were also obtained from excised cervical and mesenteric lymph nodes by dispersing them in a Bellco tissue homogenizer in HBSS. Cells were centrifuged at 200 × *g* for 10 min. The supernatant was removed and the cells were washed two times with HBSS.

Generation of Tumor-Sensitized CTLs. For *in vitro* generation of allogeneic CTLs, TDLs from DA rats were collected. Stimulator cells were inactivated by ^{60}Co γ irradiation (5000 rads for 9L tumor or 2000 rads for Fischer lymph node; 1 rad = 0.01 Gy) before exposure to the TDLs. TDLs and inactivated stimulator cells were cultured, 10:1 for TDL-9L or 1:1 for TDL/Fischer lymph node, in Iscove's medium containing 10% (vol/vol) fetal bovine serum for 5 to 7 days (17). Column-purified Con A supernatant factor prepared as described (17) from rat spleen cell cultures (10% vol/vol) was added. Generation of syngeneic CTLs involved collection of TDLs from Fischer rats primed i.p. at least 3 wk earlier with mitomycin C-inactivated (50 μg/ml per 10^6 cells at 37°C for 1 hr) 9L tumor (10^7 cells). TDLs from these animals were then stimulated *in vitro* with 9L tumor as discussed for *in vitro*-generated CTLs. The TDLs were monitored for cytotoxic activity against 9L tumor or Con A blast targets at various effector/target ratios.

Targets Used in Cytotoxicity Experiments. Tumor targets included the 9L gliosarcoma, the natural killer-sensitive F4 (adenovirus-transformed rat embryo fibroblast line; ref. 17), and YAC-1 (mouse T-cell leukemia) tumor lines and the natural killer-resistant P815 (mouse mastocytoma) cell line. They were maintained in RPMI 1640 culture medium containing 10% fetal bovine serum. Con A-stimulated Fischer blasts were prepared by incubating lymph node cells at a final concentration of 2 × 10^6 per ml in Iscove's medium containing Con A at 5 μg/ml for 2–3 days.

Cytotoxicity Assay. The *in vitro* cytotoxicity of rat effector cells was assessed with a 4-hr chromium release assay as described (16). Effectors included splenocytes, TDLs, nonadherent and adherent LAK effectors derived from splenocytes, and specifically stimulated syngeneic Fischer and allogeneic DA CTLs. These effectors were tested against some or all of the following cell targets: (*i*) F4 and YAC-1 cells, (*ii*) P815 cells, and (*iii*) 9L tumor. Briefly, 5 × 10^3 ^{51}Cr-labeled tumor targets or Con A blasts were incubated with various dilutions of effectors in 0.1-ml volumes in Greiner 96-well V-bottomed microtiter plates for 4 hr. After centrifugation, 50% of the volume was harvested and radioactivity was measured. Values reported are mean specific release of triplicate wells (the standard error did not exceed 10%).

Surgical Protocol for Inoculation of Cells into Rats. Cells were implanted intracranially into an anesthetized F344 adult male rat (250–300 g). The rat was positioned in a stereotactic head frame; the head was shaved and prepped, and a linear sagittal scalp incision was made. A small retractor was inserted and the coronal, sagittal, and lambdoidal sutures were identified. At a point 2 mm anterior and 2 mm lateral to the bregma on the right side, a small burr hole was drilled and the dura was opened. A 27-gauge needle of a 50-μl Hamilton syringe, filled with rIL-2 and 9L gliosarcoma, with or without effector cells at appropriate concentrations, was advanced 4 mm from the top of the cranium, into the frontal lobe. It was withdrawn 1 mm and 10–12 μl of the cell mixture was slowly injected. The needle was then withdrawn and the burr hole was sealed with bone wax. The scalp was closed using stainless steel Michel clips. At 7 and 14 days, additional effectors and rIL-2, suspended in 10–12 μl, were placed stereotactically through the previously placed burr hole into anesthetized rats as described. The treatment protocol did not involve additional injections of effectors because by the 3rd week the animals were experiencing morbidity and mortality associated with combined tumor growth and anesthesia.

Histological Study. Brains were fixed with 10% formalin for 1 week, then were embedded in paraffin and sectioned. Tumor volume, necrotic foci, and infiltrating lymphocytes were estimated on hematoxylin/eosin-stained sections. For tumor volumes, diameters were measured from hematoxylin/eosin-stained coronal sections (5–6 μm) that were taken every 0.25 mm. The largest diameter measured was used and tumor growth was assumed to be spheroidal.

Statistical Analysis. Mean survival times were calculated by the method of Geran *et al.* (18). Survival data were also analyzed by the University of Colorado Cancer Center Statistics Core Facility using Mantel–Haenzel nonparametric logrank (uniformly treats data) and Wilcoxon (weights early failure) tests. P values < 0.05 were considered significant.

RESULTS

***In Vitro* Cytotoxicity of Effector Cells.** At given effector/target ratios (E:Ts), adherent LAK cells demonstrated efficient kill against 9L, natural killer-sensitive (F4 and YAC-1), and natural killer-insensitive (P815) tumor targets; rat nonadherent LAK cells showed minimal kill (Table 1).

The cytotoxic capability of two types of CTLs generated against 9L tumor targets, allogeneic CTLs (DA anti-Fischer) and syngeneic CTLs (Fischer anti-9L), was compared to that of nonspecifically activated adherent and nonadherent LAK cells (Fig. 1). The *in vitro* cytotoxicity of both CTL populations against the 9L tumor was significant. Overall, adherent LAK cells were most cytolytic but the cell yields were quite

Table 1. Cytotoxicity by rat adherent and nonadherent LAK cells to tumor

Tumor target	E:T ratio	% specific lysis of tumor	
		Adherent	Nonadherent
9L	33:1	77.6 ± 6.4	9.3 ± 0.8
	10:1	50.5 ± 1.4	4.0 ± 2.4
F4	33:1	61.8 ± 2.2	13.6 ± 1.2
	10:1	37.2 ± 2.9	5.8 ± 0.5
YAC-1	33:1	64.2 ± 4.1	14.1 ± 0.7
	10:1	50.3 ± 0.2	9.2 ± 0.8
P815	33:1	44.8 ± 4.4	−5.4 ± 2.4
	10:1	21.6 ± 5.9	4.5 ± 3.7

Results are mean ± SEM.

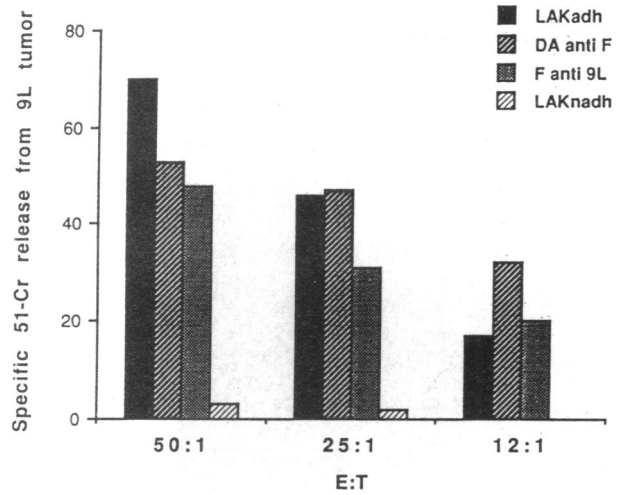

FIG. 1. Cytolytic activity of syngeneic adherent LAK cells (LAKadh), nonadherent LAK cells (LAKnadh), and Fischer anti-9L CTLs (F anti-9L) and of allogeneic DA anti-Fischer CTLs (DA anti-F) against a 9L tumor target at various E:T ratios.

low (1–3% of the total number of bulk splenocytes). Allogeneic CTLs were the next most cytolytic and large numbers of them could be generated.

Two types of DA allogeneic CTLs were generated: (*i*) CTL sensitized against Fischer antigens (DA anti-Fischer) and (*ii*) CTLs sensitized against 9L Fischer tumor (DA anti-9L). Compared with unstimulated DA lymphocytes, the *in vitro* cytotoxicity of both DA anti-Fischer and DA anti-9L CTLs against Fischer Con A blasts and against the 9L tumor was significant (Table 2). However, the total cell yields from DA anti-9L cultures were routinely 10% or less of those observed for DA anti-Fischer cultures.

Survival Data. Untreated control rats injected with 10^5 9L glioma cells had a mean survival time (MST) of 20.2 days (Table 3). When rIL-2 (250,000 units, three times a day) was given for 5 days peripherally (i.p. or s.c.) to rats, 7 days after 9L tumor implantation, it was ineffective in extending MST from the untreated control (MST = 20.3 days). Survival of 9L tumor-bearing control rats who received unstimulated, syngeneic Fischer lymphocytes and intracranial rIL-2 by adoptive transfer was not extended (MST = 21.9 days). Treatment with syngeneic nonadherent LAK cells generated from splenocytes and intracranial rIL-2 extended that group's survival to 26.0 days. 9L tumor-bearing rats treated with adherent LAK cells and intracranial rIL-2 showed an increased MST (27.0 days). Syngeneic Fischer anti-9L CTLs extended the MST of rats treated only twice (2×10^6 effectors and 2×10^6 units of rIL-2) to 23.8 days. Treatment with allogeneic CTLs (DA anti-Fischer) along with rIL-2 extended the survival time almost 100% (MST = 37.7 days) with 2 of the 13 animals surviving 85 days when the animals were arbitrarily sacrificed. For a homologous set of animals given the same 9L inoculum, Mantel–Haenzel nonparametric logrank equations (which included censored observations at 85 days for survival animals treated with allogeneic CTLs) demonstrated a significantly improved survival for groups treated with either nonadherent or adherent LAK cells ($P \leq 0.0003$, $n = 8$), with allogeneic CTLs ($P = 0.0025$, $n = 6$), or with syngeneic CTLs ($P = 0.0327$, $n = 14$) over the untreated controls ($n = 5$). Wilcoxon equations also showed significance for the same preparations.

Table 2. Cytotoxicity by allogeneic effectors

Effector	E:T ratio	% specific release of ^{51}Cr	
		Fischer Con A blasts	9L tumor
DA anti-Fischer CTL	50:1	38.4	44.6
	25:1	30.4	34.6
	12:1	23.3	21.4
DA anti-9L CTL	50:1	53.9	54.0
	25:1	40.5	45.5
	12:1	26.3	28.2
Unstimulated DA lymphocytes	50:1	1.1	0.5
	25:1	−0.4	−2.4
	12:1	−0.5	1.5

Table 3. Survival of rats with 9L gliosarcoma

Treatment	MST, days (*n*)
None (control)*	20.2 (12)
rIL-2†	20.3 (12)
Syngeneic lymphocytes‡	21.9 (14)
Nonadherent LAK‡	26.0 (23)
Adherent LAK‡	27.0 (8)
Syngeneic CTL§	23.8 (23)
Allogeneic CTL‡¶	37.7 (13)

*Control rats were given an intracranial inoculation of 10^5 9L cells.
†Seven days after inoculation with 9L tumor, rats were given rIL-2 (250,000 units, three times a day) for 5 days. Survival was identical whether rIL-2 was given s.c. or i.p.
‡9L and effector cells (10^5 and 10^6, respectively; E:T = 10:1) and 10^4 units of rIL-2 were implanted. One and 2 weeks later treatment was repeated; totals of 3×10^6 effector cells and 3×10^4 units of rIL-2 were given.
§Rats were treated twice with Fischer anti-9L CTLs for totals of 2×10^6 effector cells and 2×10^4 units of rIL-2; the 3rd week, *in vitro* cytotoxicity assays showed no activity against 9L cells.
¶Rats were treated with DA anti-Fischer CTLs. The experiment was arbitrarily ended by sacrificing two survivors at day 85.

To reproduce the survival findings with the allogeneic DA anti-Fischer CTLs at an improved E:T ratio, 9L tumor was titrated in the F344 rat model and survival was determined. A dose of 5000 9L implanted tumor cells predictably killed Fischer rats in about a month (MST = 36.5 days, $n = 10$). Using 5000 9L cells as an inoculum, we repeated the Winn assay survival experiment as before. The group treated with DA anti-Fischer CTLs again resulted in three long-term (85 days) survivors (MST = 43.6 days, $n = 19$).

Histological Examination of Brain. Hematoxylin/eosin-stained axial slices of brain from an untreated control animal bearing intracranial 9L tumor (Fig. 2) show the interface (arrows) of normal brain to a well-demarcated tumor by gross section (Fig. 2*A*) and by histologic section (Fig. 2*B*). The largest tumor area on both axial and coronal sections measured between 21 and 25 mm^2. The tumor was composed of spindle cells with moderate amounts of amphophilic cytoplasm, elongated vesicular nuclei with one to three inconspicuous nucleoli, and numerous mitotic figures. The tumor contained numerous blood vessels with little or no endothelial proliferation. The presence of necrosis was variable. There was no diffuse infiltration of brain by neoplasm. A cavity in the center of the tumor was usually identified and was presumably secondary to the tumor instillation.

Histologically, those animals treated i.p. with rIL-2 were essentially identical to untreated controls. No animals demonstrated extensive tumor necrosis surrounding the central cavity. The brain of an animal treated with nonadherent LAK cells and having extended survival (34 days) over untreated control animals showed extensive central necrosis. More LAK-cell-treated animals exhibiting extended survival would be needed to determine whether the massive necrosis observed resulted from LAK cell therapy.

Fig. 3 shows a hematoxylin/eosin-stained axial gross section (Fig. 3*A*) and a histologic section (Fig. 3*B*) from long-term survivor allogeneic DA anti-Fischer CTL-treated animals. The point of needle penetration through tissue is at the

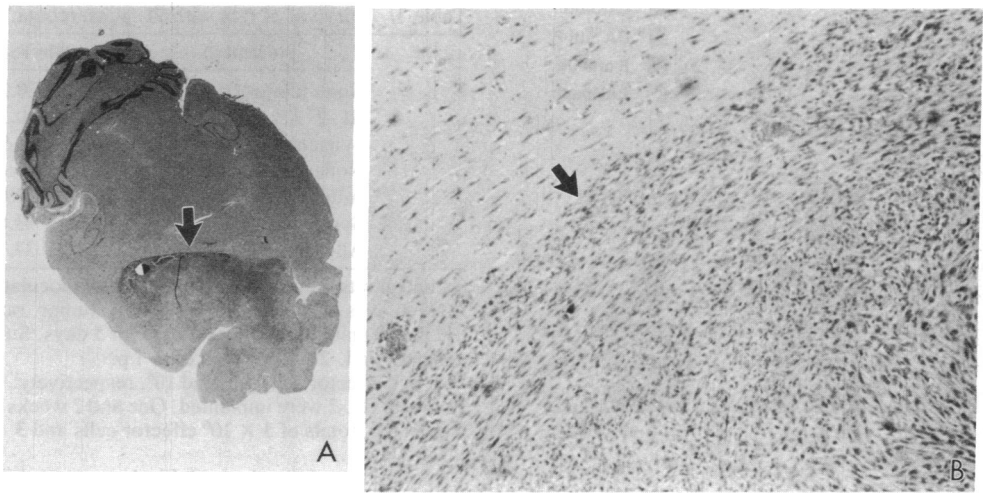

FIG. 2. Hematoxylin/eosin-stained gross axial section (*A*) and histologic section (*B*) of brain from a rat given 9L tumor and untreated. (*A*, ×3; *B*, ×60.)

center of the photograph. Allogeneic CTL-treated animals sacrificed 85 days after tumor (10^5 9L) inoculation and treatment showed a small, slit-like lesion in the brain (arrow), surrounded by small hemosiderin deposits, but no widespread brain destruction and no viable tumor. Neither large numbers of lymphoid-like cells, cellular reaction, nor adjacent neuronal necrosis was apparent.

DISCUSSION

Two types of LAK cell preparations, nonadherent and adherent, were investigated. Vujanovic *et al.* (19) have reported the conversion of rat large granular lymphocytes, in response to IL-2, to plastic-adherent LAK cells. They showed that adherent LAK cells produce significant tumor kill. We tested this observation and reproduced their findings; the adherent population is indeed highly cytotoxic against 9L tumor as a target (Fig. 1). The yield of adherent LAK cells from the splenocyte population, however, is quite small and obtaining therapeutically significant numbers of such cells is a problem. Nonadherent LAK cells showed little kill relative to the adherent LAK cells *in vitro* against murine tumor (Table 1) and 9L tumor (Fig. 1) targets. However, in the *in vivo* neutralization assay both preparations of LAK cells gave similar extensions in rat survival. In this instance, there appears to be no correlation between *in vitro* and *in vivo* cytotoxicity.

Allogeneic CTLs, versus syngeneic CTLs, also are very cytolytic (Fig. 1 and Table 2). These data for the rat system corroborate the findings by Gately *et al.* (20) for the human, which is that allogeneic reactions are substantially stronger than tumor-specific autologous responses. Additionally, therapeutically significant numbers of CTL effectors can be generated. An effector preparation containing CTLs showed an increase in cytotoxicity to tumor between 4 and 18 hr; cytotoxicity by LAK cells, while significant, failed to increase with time (13). Thus, LAK cells may not recycle or may do so inefficiently. Direct contact of LAK cells with tumor cells may be necessary for a lethal hit (21). CTLs, however, can recycle and continue killing tumor with time (22). This implies that kill by CTLs is cumulative as long as they remain in the tumor tissue. Also, because CTLs are T cells, they have the inherent capability to migrate, important in a system where tumor infiltrates normal brain. Overall, CTLs have many desirable characteristics for brain tumor therapy.

To cure a rat of a given tumor burden in the brain both the number and the quality of the effector cells and the frequency of application figure in the theoretical considerations of adoptive therapy. To calculate the tumor volume that caused death (or occupied space enough to produce intracranial

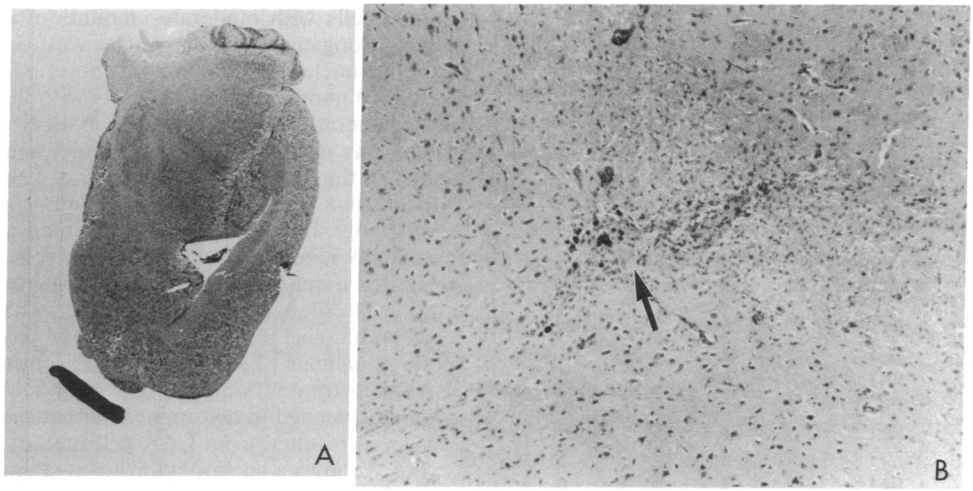

FIG. 3. Hematoxylin/eosin-stained axial gross section (*A*) and histologic section (*B*) from brains of long-term survivor rats, 85 days after 9L tumor implantation and treatment with allogeneic DA anti-Fischer CTLs. (*A*, ×3; *B*, ×60.)

pressure that resulted in death), the largest diameter on slices of brain from untreated control rats bearing 9L tumor was measured. Assuming that the tumor grew spherically, with cell number doubling every 2–3 days, and that 1 cm^3 of tissue contained a billion cells, 7.35×10^7–10^8 tumor cells caused death. *In vitro* cytotoxicity by DA anti-Fischer CTL effectors was maximally 30% at an E:T ratio of 12:1 when measured in 4-hr Cr-release assays. In our animal experiments, at a similar E:T dose (10:1) initially, if the percentage of tumor killed *in vivo* were equivalent to that killed *in vitro* in 4 hr, the tumor burden still would have been significant, such that two more inoculations of 10^6 CTLs a week apart could not have destroyed the remaining tumor. To have obtained cured animals, CTLs must have recycled.

We have performed a clinical trial involving the intratumoral implantation of rIL-2-activated lymphocytes, along with rIL-2, in patients with recurrent primary brain tumors (23, 24). Our clinical protocol (BB IND 2412) allowed for the treatment of children, some of which were too small to safely tolerate removal of the large volumes of blood necessary to generate autologous activated lymphocytes for reimplantation. The alternatives are to find better effector cells or to increase the number of effectors given by considering donors other than self. The most likely fate of allogeneic cells administered systemically would be immediate destruction by the host immune system. However, the use of allogeneic CTLs sensitized to autologous tumor for brain tumor treatment may offer an alternative for this subset of patients. Although major histocompatibility differences may exist on the allogeneic CTLs, because the brain is a semiprivileged immune site, allogeneic effectors may be able to contact and kill tumor before they themselves are destroyed. Histopathology of the brains of long-term survivors treated with allogeneic CTLs did not show large numbers of lymphoid-like cells (Fig. 3*B*), which implies that the effector cells placed in the brain were not localized there permanently, or evidence of a chronic inflammatory response, as would be shown by an infiltration of host lymphocytes.

The significance of the animal studies is to further support adoptive transfer as an alternative form of therapy in the treatment of rapidly fatal intracranial malignancy and provide a rational basis upon which to optimize ongoing clinical trials. In clinical trials to date, the effector populations being tested were nonspecifically activated LAK cells and/or a lectin/rIL-2 autologous-stimulated lymphocyte preparation that contains non-major histocompatibility complex-restricted CTLs (13). At this point only a slight improvement in patient survival has occurred (23–27). One explanation for this could be that suppressor factors, known to be produced by glioma cells (28), could inhibit effector cells *in situ*. It appears that the therapy offers as much survival hope as other regimens involving reoperation with adjuvant treatment (23); it is safe and well tolerated. The experiments which led to the national clinical trials were not performed systematically in an animal model. Although we recognize that there are inherent limitations associated with an animal model, at present, the rat 9L tumor model provides a rapid and efficient mechanism to critically examine various parameters and optimize the conditions employed to test gliomas by local adoptive transfer of activated lymphocytes. The rat is a practical model in which to test this approach preclinically.

One of the primary goals of this study was to determine whether adoptive immunotherapy could lead to a cure of brain tumors in rats. Combining experiments of 9L tumor-bearing rats treated with allogeneic CTLs, 16% of the rats (5 of 32) given a lethal dose of 9L tumor cells have survived. Nonspecifically activated LAK cells were incapable of a cure, although increased survival times were obtained for those rats. Resting T cells (unstimulated lymphocytes) with rIL-2 had no impact on survival, nor did peripherally administered rIL-2. Overall, these data suggest that stimulated lymphocytes with rIL-2 are effective in inhibiting tumor growth and prolonging survival. Only allogeneic CTLs were able to effect a cure. When placed into the immunologically privileged brain, allogeneic CTLs may survive and be cytolytic to tumor long enough to be practical for therapy.

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