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Allogeneic Astrocytoma In Immune Competent Dogs

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Abstract

We have induced in canines long-term immune tolerance to an allogeneic cell line derived from a spontaneous canine astrocytoma. Allogeneic astrocytoma cells were implanted endoscopically into the subcutaneous space of fetal dogs before the onset of immune competency (< 40th gestational day). At adulthood, dogs rendered tolerant successfully serve as recipients of intracranial transplants of their growing allogeneic, subcutaneous tumor. Transplanted dogs subsequently develop a solid brain tumor with histological features similar to the original astrocytoma. This model may allow rapid development and evaluation of new therapies for brain tumors, as well as afford tumor biology studies that are untenable in smaller, immune incompetent, or inbred animals harboring less representative tumors.

Keywords: glioma, animal model, immune tolerance, astrocytoma.

Introduction

Progress in development and testing new anticancer therapies is impeded or delayed by the lack of models accurately manifesting features of spontaneously arising neoplasms. For primary brain tumors, the low incidence in humans [1,2], coupled with the precipitous lethality of this cancer [3,4], leads to a small and inhomogeneous population by tumor location, patient age, and histological characteristics of malignant astrocytoma [5,6]. Because surgery is the single most effective treatment for patients with a brain tumor [7–9], an optimal brain tumor model for testing new treatments would use an animal of sufficient size to allow meaningful surgical resection of the tumor. Additionally, the tumor should manifest histological, immunological, genetic, and therapeutic determinants analogous to those of the spontaneous human counterpart [10].

Canine brain tumors very closely approximate the human disease relative to histopathology, epidemiology, and clinical course [11–16]. Such lesions have proven useful in development of imaging studies of intracranial masses [17,18], as well as in pioneering novel radiation treatment strategies [19–21]. A spontaneous canine anaplastic astrocytoma was developed into a long-term cell line, DL3580c2 [22], which grows anchorage-independently, over-expresses epidermal growth factor receptor, and is tumorigenic in athymic mice. No mutations in exons 4, 5, 6, 7, and 8 of the canine p53 gene [23–25] have been detected in this cell line.

DL3580c2 cells were used to induce allogeneic immune tolerance in outbred beagles according to paradigms and hypotheses exploring this concept [26–30]. It is believed that appropriately timed (fetal or neonatal) exposure to allogeneic cells can induce native immune tolerance. Whereas functional immune tolerance can be induced in experimental animals by the creation of hematopoietic-chimeric animals [31], the intent here was to preserve the constitutive immunological identity of the host while inducing tolerance to allogeneic cells. We decided to deposit the allogeneic astrocytoma cells into the subcutaneous space of fetal dogs because of the rich immune exposure of this tissue, and the immunologically naive or preimmune status of fetal pups [32].

Materials and Methods

Gestational ages of time-dated beagles (Marshall Farms, NY) were determined by serial ultrasound measurements of chorionic sac diameter, crown-rump length, and head diameter [33]. Fifty-microliter implants containing 10^7 DL3580c2 cells were delivered separately to each fetus via endoscope into the subcutaneous space within the flank region. Briefly, on the 37th gestational day, the gravid uterus was exposed through a midline abdominal incision. A self-retaining introducer fitted with a Teflon seal was installed into the uterus through a 4-mm incision. Access to the fetus was gained by use of a 2.8-mm rigid lens endoscope (Karl Storz Endoscopy-America, Inc., Culver City, CA) with a 1.0-mm working channel; illumination was provided with a 150 W tungsten halogen lamp. Cells were implanted through a 30-cm TFE 30TW cannula (Cole-Parmer Instrument Co.) fitted with a 26-gauge sharp needle.

Fetal and placental membranes are potentially significant obstructions to clear viewing of, and access to, the fetal skin. The magnitude of the obstruction is highly dependent on fetal age; at later gestational ages (> 42 days) the membranes retract tightly around the fetus, becoming less entangling to endoscopic approaches. Scheduling the fetal implants before the 40th gestational day was based on reports that later dates were outside the tolerance-induction window for soluble antigens in dogs [32].

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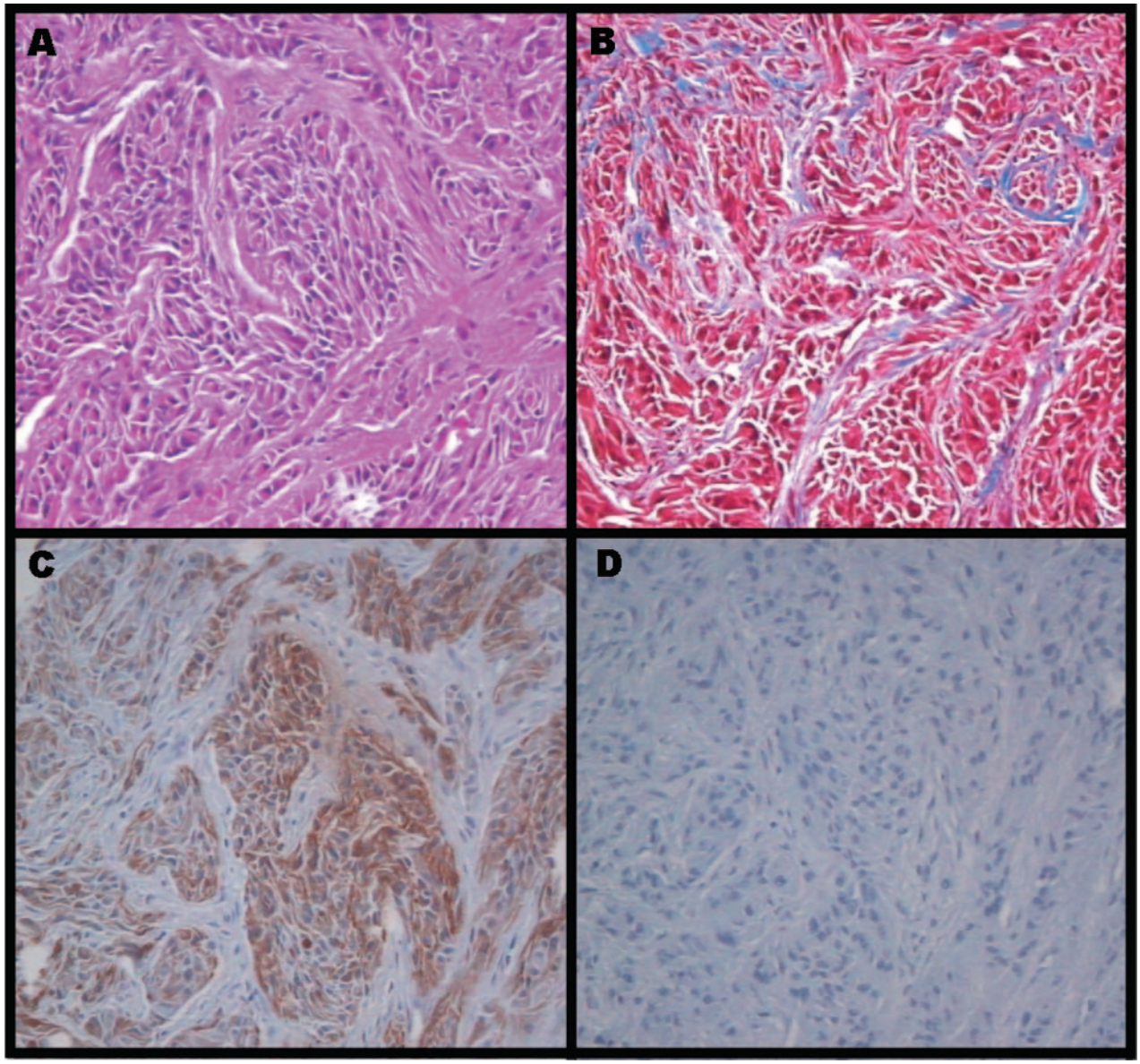

Figure 1. *Histological view of subcutaneous tumor generated from subcutaneous allogeneic astrocytoma implant into fetal dogs. H&E stained section (A) shows bundles of fibrillary malignant cells within a network of connective tissue. Trichrome staining (B) shows a rich collagenous network of fibers around the tumor cell bundles. Immunostaining with anti-GFAP antibodies (DAKO Corp., Carpinteria, CA). (C) portrays a strong presence of this glial-specific intermediate filament protein. (D) The isotype matched negative section. (Original magnification, 40 × objective).*

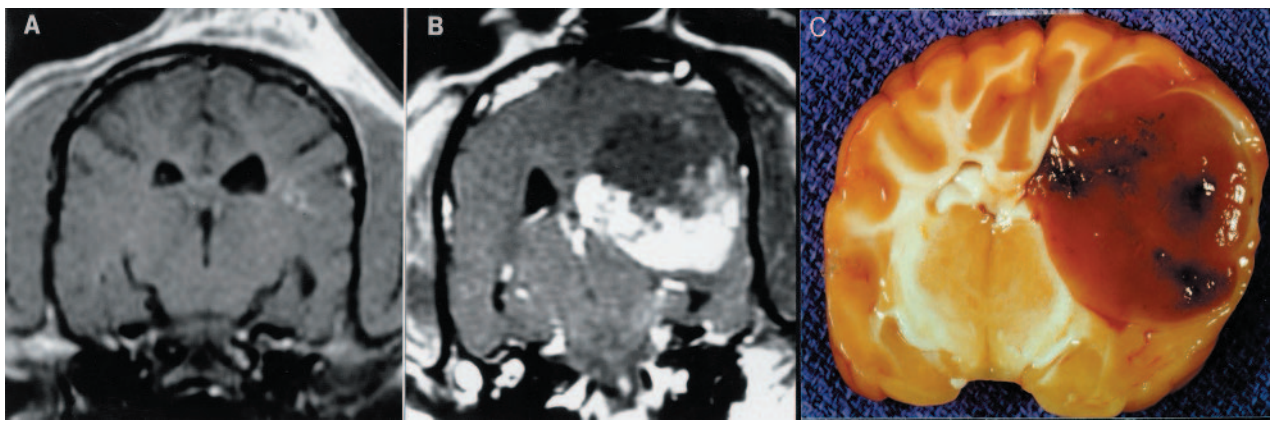

Figure 2. *Clinical and postmortem appearance of transplanted intracranial astrocytoma. T1-weighted MRI images after injection of paramagnetic contrast agent 5 weeks (A) and 10 weeks (B) after intracranial implant. Coronal section of the brain at necropsy confirmed the MRI findings, showing a large, anatomically displacing, hemorrhagic lesion in the left parietal lobe with extension of the mass and edema into the left frontal and temporal lobes.*

Results

Within the first 6 weeks after birth, pups may develop palpable masses at the site of fetal implantation. After 5 months the lesions were resected and processed for histological analysis. Conventional hematoxylin & eosin (H & E) staining showed tightly packed bundles of tumor cells enmeshed in a rich connective tissue network (Figure 1A). Trichrome staining showed the presence of collagenous fibers around the tumor bundles (Figure 1B). Immunohistochemical staining for glial fibrillary acidic protein (GFAP)

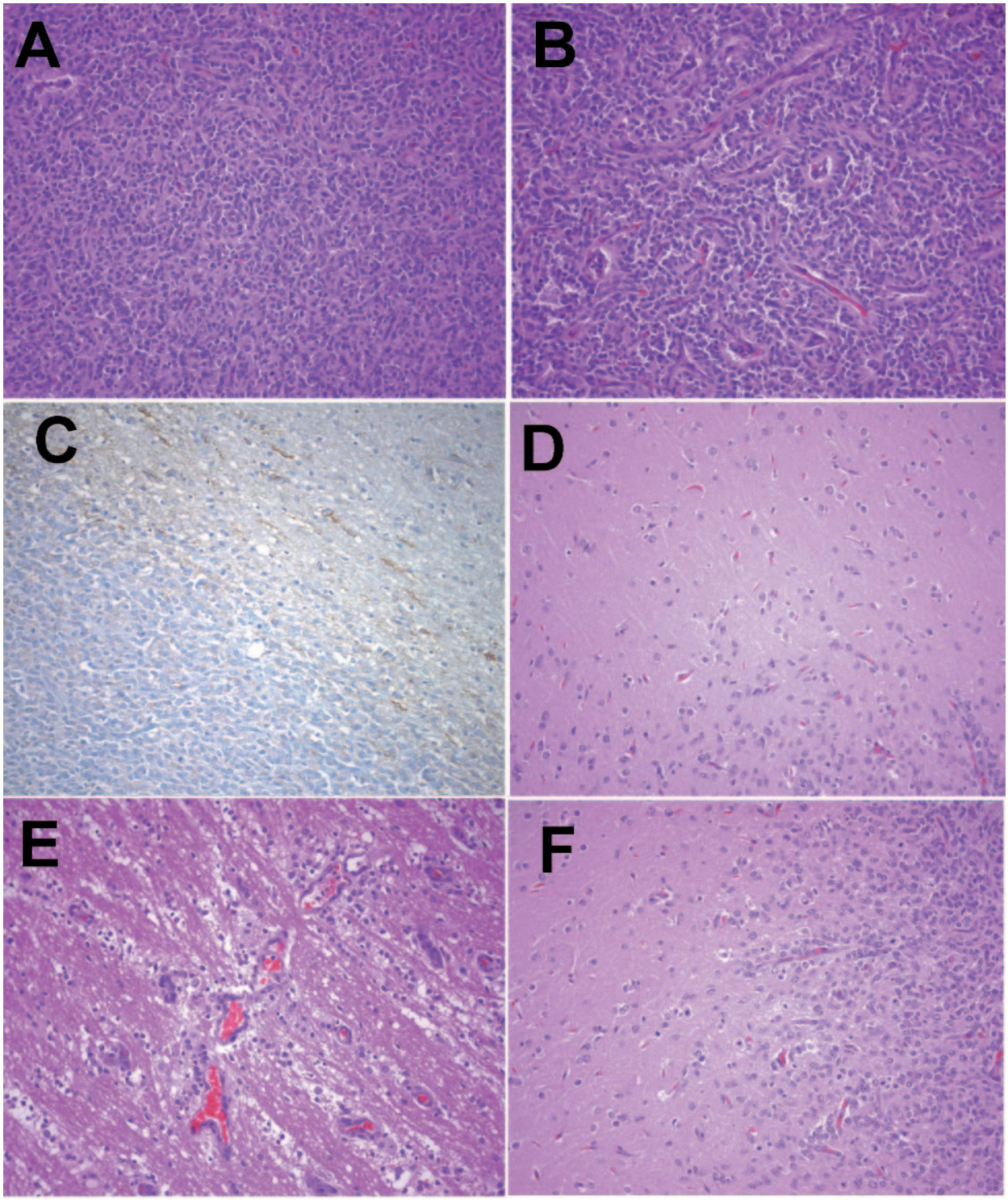

Figure 3. *Histological view of intracranial allogeneic astrocytoma. Regions of the brain specimen shown in Figure 2 were processed in formalin and sectioned from paraffin blocks. The tumor showed heterogeneous hyperdense regions of proliferative, malignant cells with pleomorphic nuclei (A); surrounding areas showed regions of neovascularization (B). The intracranial tumor only stained weakly for GFAP (C). Although grossly the tumor appeared to be well demarcated, there were regions of single astrocytoma cell percolation into white matter (D), conduits of invasion along perivascular structures (E), and wide areas of generalized astrocytoma invasion into adjoining normal brain (F). (Original magnification, 20× objective).*

showed strong expression of this glial cell–specific intermediate filament marker (Figure 1C). Comparative karyotypic analysis confirmed that the subcutaneous tumor was derived from the cells implanted during fetal development (data not shown).

During the same single surgical procedure under isofluorane anesthesia, regions of the resected subcutaneous tumors (glial lineage confirmed on cryostat sections) were mechanically dissociated into a fresh cell brie, and 50 μL were injected over a 2-minute interval intracranially into the left internal capsule by using stereotactic coordinates [34] and an external frame (David Kopf Instruments, Tujunga, CA). Hydrostasis in the cranium after the injection was re-established by using bone wax in the burr hole, and the scalp incision was closed in a conventional fashion. After recovery from anesthesia, the animals were returned to routine kennel stay including circadian photoperiods, daily play intervals and ad libitum dog chow according to IACUC-approved protocol.

Tumor growth was followed by cranial magnetic resonance imaging (MRI) studies every 5 to 7 weeks. MRI of the brain was performed by using T1-, intermediate, and T2-weighted images. The intermediate and T2-weighted images were performed by using a fast-spin echo, dual-echo technique with a TR 300/TE 40/80 ms on a GE Signa (General Electric, Milwaukee, WI). After the intravenous infusion of paramagnetic contrast material (gadolinium-DTPA, Magnevist Abbott Laboratories, Abbott Park, IL), T1-weighted images were collected in the axial and sagittal planes. Five weeks after intracranial transplantation, MRI showed no definitive diagnostic aberrations (Figure 2A). Seven weeks later, a large, anatomically displacing, hemorrhagic mass was evident (Figure 2B). At necropsy, a gross cranial coronal section showed a highly vascular, apparently well-demarcated tumor (Figure 2C). On H&E stained sections, the microscopic features included regions of dense hypercellularity with mitotic figures (Figure 3A, pseudopalisading necrosis, and rich neovascularization (Figure 3B). These are the criteria for a diagnosis of World Health Organization (WHO) grade IV astrocytoma (glioblastoma multiforme) [35–37]. Immunohistochemical studies indicated weak staining by anti-GFAP antibodies (Figure 3C). Modulation of GFAP expression in response to environmental signals, cell density, and malignant transformation has been reported [38–40].

Because local invasion is such a clinical problem in management of patients with astrocytomas [41–43], brain sections adjacent to the tumor were processed to determine patterns of the allogeneic astrocytoma invasion. Solitary tumor cell infiltration into white matter (Figure 3D), perivascular trajectories (Figure 3E), as well as star-burst-like invasion from the rim of the tumor (Figure 3F) were identified. Overall, the tumor showed the typical centrally expansive and peripherally diffusively infiltrative growth that characterizes high grade astrocytomas.

Of 13 attempts to induce allogeneic tolerance by subcutaneous implants into fetal pups (wherein a litter of pups was successfully whelped), seven litters harbored dogs who eventually developed glial tumors. The most successful procedure was a litter wherein 4 of 5 litter mates sustained allogeneic tumor growth. Reasons for failure may include inadequate allogeneic cell inoculum [27], arrested or stunted immune tolerance due to tardiness of the implant [28], or possible incompatible major immunohistocompatibility genotypes between allograft and host [44–46]).

Discussion

Successful modeling of astrocytoma in a large animal that preserves significant pathologic features of the spontaneous disease is likely to afford new opportunities for accelerated and novel therapy development [10,47,48]. Whereas an intracranial xenograft of human gliomas in cats has been reported [49], this system relies on aggressive and persistent immunosuppression of the host, which renders the model unsuitable for immune-based therapies. Even treatments relying on genetic manipulation of the tumor, such as gene therapy, may require a functioning immune system to accurately evaluate the consequences of the intervention [35,50–52]. A transplantable canine brain tumor model in immune competent hosts has been used over the past decade [16,53], but this tumor shows a growth pattern and histological features more consistent with a gliosarcoma.

The brain tumor model described in this report demonstrates the propagation of a spontaneous, allogeneic canine astrocytoma in immune competent dogs. The tumor properties match well the growth patterns, histological characteristics, and molecular pathology of human astrocytomas. Because of the surgically accessible anatomical size of the model and the intact immune status of the host, this allogeneic astrocytoma model in dogs may serve as an effective tool for accelerated cancer therapy development and testing. These may include gene therapy [54], radiation treatment [55], novel treatment delivery strategies such as enhanced convection delivery [56–58] or slow release formulations [59,60] in a system appropriate for prehuman studies. Because novel cancer treatments inevitably must pass toxicity testing in canines [54], it may be advantageous to use the allogeneic astrocytoma model to optimize treatment delivery schemes before human trials.

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References

[1] Berens ME, Rutka JT, and Rosenblum ML (1990). Brain tumor epidemiology, growth and invasion. *Neurosurg Clin N Am* **1**, 1-18.

[2] Surawicz TS, McCarthy BJ, Kupelian V, Jukich PJ, Bruner JM, and Davis FG (1999). The descriptive epidemiology of brain and central nervous system tumors in the United States. Results from CBTRUS 1990-1994. *Neuro-Oncology* **1**, 14-25.

[3] Levin VA, Gutin PH, and Leibel S (1993). Neoplasms of the Central Nervous System. In *Cancer: Principles & Practice of Oncology* (4th ed). VT DeVita, Jr., S Hellman and SA Rosenberg (Eds). J.B. Lippincott, Philadelphia, PA. pp. 1679-1746.

[4] Davis FG, Freels S, Grutsch J, Barlas S, and Brem S (1998). Survival rates in patients with primary malignant brain tumors stratified by patient age and tumor histological type: An analysis based on Surveillance, Epidemiology, and End Results (SEER) data, 1973-1991. *J Neurosurg* **88**, 1-10.

[5] Winger MJ, Macdonald DR, Schold SC, and Cairncross JG (1989). Selection bias in clinical trials of anaplastic glioma. *Ann Neurol* **26**, 531-534.

[6] Kirby S, Brothers M, Irish W, Florell R, Macdonald D, Schold C, and Cairncross G (1995). Evaluating glioma therapies: Modeling treatments and predicting outcomes. *J Natl Cancer Inst* **87**, 1884-1888.

[7] Wisoff JH, Boyett JM, Berger MS, Brant C, Li H, Yates AJ, McGuire-Cullen P, Turski PA, Sutton LN, Allen JC, Packer RJ, and Finlay JL (1998). Current neurosurgical management and the impact of the extent of resection in the treatment of malignant gliomas of childhood: A report of the Children's Cancer Group trial no. CCG-945. *J Neurosurg* **89**, 52-59.

[8] Vecht CJ, Avezaat CJ, van Putten WL, Eijkenboom WM, and Stefanko SZ (1990). The influence of the extent of surgery on the neurological function and survival in malignant glioma. A retrospective analysis in 243 patients. *J Neurol Neurosurg Psychiatry* **53**, 466-471.

[9] Alexander E, and Loeffler JS (1998). Radiosurgery for primary malignant brain tumors. *Semin Surg Oncol* **14**, 43-52.

[10] Knapp DW, and Waters DJ (1997). Naturally occurring cancer in pet dogs: important models for developing improved cancer therapy for humans. *Mol Med Today* **3**, 8-11.

[11] Palmer AC (1976). Comparative aspects of tumors of the central nervous system in the dog. *Proc Royal Soc Med* **69**, 49-51.

[12] Bigner DD, and Swenberg JA (1977). *Experimental Tumors of the Central Nervous System.* First English edition translated from W. Janisch and D. Schreiber. The Upjohn Company, Kalamazoo, MI.

[13] Rubinstein LJ (1977). Correlation of animal brain tumor models with human neurooncology. *NCI Monograph* **46**, 43-49.

[14] Frenier SL, Moore MP, Kraft SL, and Gavin PR (1990). Canine intracranial astrocytomas and comparison with the human counterpart. *The Small Animal Compendium* **12**, 1422-1433.

[15] Heidner GL, Kornegay JN, Page RL, Dodge RK, and Thrail DE (1991). Analysis of survival in a retrospective study of 86 dogs with brain tumors. *J Vet Intern Med* **5**, 219-226.

[16] Zaki FA (1977). Spontaneous central nervous system tumors in the dog. *Vet Clin North Am* **7**, 153-163.

[17] Turrel JM, Fike JR, LeCouteur RA, and Higgins RJ (1986). Computed tomographic characteristics of primary brain tumors in 50 dogs. *J Am Vet Med Assoc* **188**, 851-856.

[18] Kraft SL, Gavin PR, DeHaan C, Moore M, Wendling LR, and Leathers CW (1997). Retrospective review of 50 canine intracranial tumors evaluated by magnetic resonance imaging. *J Vet Intern Med* **11**, 218-225.

[19] Gavin PR, Kraft SL, DeHaan CE, Swartz CD, and Griebenow ML (1994). Large animal normal tissue tolerance with boron neutron capture. *Int J Radiat Oncol Biol Phys* **28**, 1099-1106.

[20] Gavin PR, Kraft SL, Huiskamp R, and Coderre JA (1997). A review: CNS effects and normal tissue tolerance in dogs. *J Neurooncol* **33**, 71-80.

[21] Whelan HT, Schmidt MH, Segura AD, McAuliffe TL, Bajic DM, Murray KJ, Moulder JE, Strother DR, Thomas JP, and Meyer GA (1993). The role of photodynamic therapy in posterior fossa brain tumors. A preclinical study in a canine glioma model. *J Neurosurg* **79**, 562-568.

[22] Berens ME, Bjotvedt G, Levesque DC, Rief MD, Shapiro JR, and Coons SW (1993). Tumorigenic, invasive, karyotypic and immunological characterization of clonal cell lines derived from a spontaneous canine anaplastic astrocytoma. *In Vitro Cell Dev Biol Anim* **29A**, 310-318.

[23] Devilee P, Van Leeuwen IS, Voesten A, Rutteman GR, Vos JH, and Cornelisse CJ (1994). The canine p53 gene is subject to somatic mutations in thyroid carcinoma. *Anticancer Res* **14**, 2039-2046.

[24] Van Leeuwen IS, Hellmen E, Cornelisse CJ, Van den Burgh B, and Rutteman GR (1996). P53 mutations in mammary tumor cell lines and corresponding tumor tissues in the dog. *Anticancer Res* **16**, 3737-3744.

[25] Veldhoen N, and Milner J (1998). Isolation of canine p53 cDNA and detailed characterization of the full length canine p53 protein. *Oncogene* **16**, 1077-1084.

[26] Billingham RE, Brent L, and Medawar PB (1953). Actively acquired tolerance of foreign cells. *Nature* **172**, 603-606.

[27] Ramsdell F, and Fowlkes BJ (1992). Maintenance of *in vivo* tolerance by persistence of antigen. *Science* **257**, 1130-1134.

[28] Fadem BH, Hill HZ, Huselton CA, and Hill GJ (1988). Transplantation, growth, and regression of mouse melanoma xenografts in neonatal marsupials. *Cancer Invest* **6**, 403-408.

[29] Edwards RG, Jauniaux E, Binns RM, Layton M, Jurkovic D, Grillo TA, and Campbell S (1995). Induced tolerance and chimaerism in human fetuses using coelocentesis: A medical opportunity to avert genetic disease? *Hum Reprod Update* **1**, 419-427.

[30] Alferink J, Tafuri A, Vestweber D, Hallmann R, Hammerling GJ, and Arnold B (1998). Control of neonatal tolerance to tissue antigens by peripheral T cell trafficking. *Science* **282**, 1338-1341.

[31] Kim HB, Shaaban AF, Yang EY, Liechty KW, and Flake AW (1998). Microchimerism and tolerance after in utero bone marrow transplantation in mice. *J Surg Res* **77**, 1-5.

[32] Tizard I (1992). *Veterinary Immunology: An Introduction* (4th ed). WB Saunders Co., Philadelphia, PA.

[33] Yeager AE, Mohammed HO, Meyers-Wallen V, Vannerson L, and Concannon PW (1992). Ultrasonographic appearance of the uterus, placenta, fetus, and fetal membranes throughout accurately timed pregnancy in Beagles. *Am J Vet Res* **53**, 342-351.

[34] Lim RK, Liu C, and Moffitt RL (1960). *A Stereotaxic Atlas of the Dog's Brain.* Charles C. Thomas, Springfield, MO.

[35] Sobol RE, Fakhrai H, Shawler D, Gjerset R, Dorigo O, Carson C, Khaleghi T, Koziol J, Shiftan TA, and Royston I (1995). Interleukin-2 gene therapy in a patient with glioblastoma. *Gene Ther* **2**, 164-167.

[36] Ojeda VJ, and Moutaery KR (1998). Neuropathological biopsy diagnoses in clinical practice: How thorough and reproducible are they? *Pathology* **30**, 328-329.

[37] Schiffer D (1998). Classification and biology of astrocytic gliomas. *Forum* (Genova) **8**, 244-255.

[38] Herpers MJ, Budka H, and McCormick D (1984) Production of glial fibrillary acidic protein (GFAP) by neoplastic cells: adaptation to the microenvironment. *Acta Neuropathol* (Berl) **64**, 333-338.

[39] Gomes D, de Nechaud B, Maunoury R, Moura Neto V, Brigaudeau C, Labrousse F, and Dupouey P (1997). Glial fibrillary acidic protein expression in a new human glioma cell line in culture before and after xenogenic transplantation into nude mice. *Acta Neuropathol* (Berl) **94**, 376-384.

[40] Pekny M, Eliasson C, Chien CL, Kindblom LG, Liem R, Hamberger A, and Betsholtz C (1998). GFAP-deficient astrocytes are capable of stellation *in vitro* when cocultured with neurons and exhibit a reduced amount of intermediate filaments and an increased cell saturation density. *Exp Cell Res* **239**, 332-343.

[41] Scherer HJ (1940). The forms of growth in gliomas and their practical significance. *Brain* **63**, 1-35.

[42] Laerum OD, Bjerkvig R, Steinsvag SK, and de Ridder L (1984). Invasiveness of primary brain tumors. *Cancer Metastasis Rev* **3**, 223-236.

[43] Laws ER, Jr., Goldberg WJ, and Bernstein JJ (1993). Migration of human malignant astrocytoma cells in the mammalian brain: Scherer revisited. *Int J Devel Neurosci* **11**, 691-697.

[44] Thomson AW, Lu L, Murase N, Demetris AJ, Rao AS, and Starzl TE (1995). Microchimerism, dendritic cell progenitors and transplantation tolerance. *Stem Cells* (Dayt) **13**, 622-639.

[45] Ruiz P, Nassiri M, Viciana AL, Padmanabhan J, and Streilein JW (1995). Characterization of donor chimerism, alloreactive host T cells and memory cell development in thymi from mice resistant to neonatal transplantation tolerance. *J Immunol* **154**, 633-643.

[46] Ruiz P, Nassiri M, Gregorian S, Viciana AL, and Streilein JW (1996). Neonatal transplantation tolerance is associated with a systemic reduction in memory cells, altered chimeric cell phenotype, and modified eicosanoid and cytokine production. *Transplantation* **61**, 1198-1205.

[47] Crafts D, and Wilson CB (1977). Animal models for brain tumors. *Natl Cancer Inst Monograph* **46**, 11-17.
[48] Peterson DL, Sheridan PJ, and Brown WE (1994). Animal models for brain tumors: historical perspectives and future directions. *J Neurosurg* **80**, 865-876.
[49] Krushelnycky BW, Farr-Jones MA, Mielke B, McKean JD, Weir BK, and Petruk KC (1991). Development of a large-animal human brain tumor xenograft model in immunosuppressed cats. *Cancer Res* **51**, 2430-2437.
[50] Andreansky S, He B, van Cott J, McGhee J, Markert JM, Gillespie GY, Roizman B, and Whitley RJ (1998). Treatment of intracranial gliomas in immunocompetent mice using herpes simplex viruses that express murine interleukins. *Gene Ther* **5**, 121-130.
[51] Herrlinger U, Kramm CM, Johnston KM, Louis DN, Finkelstein D, Reznikoff G, Dranoff G, Breakefield XO, and Yu JS (1997). Vaccination for experimental gliomas using GM-CSF-transduced glioma cells. *Cancer Gene Ther* **4**, 345-352.
[52] Tseng SH, Hwang LH, and Lin SM (1997). Induction of antitumor immunity by intracerebrally implanted rat C6 glioma cells genetically engineered to secrete cytokines. *J Immunother* **20**, 334-342.
[53] Salcman M, Scott EW, Schepp RS, Knipp HC, and Broadwell RD (1982). Transplantable canine glioma model for use in experimental neuro-oncology. *Neurosurgery* **11**, 372-381.
[54] Guidance for Industry (1997). S6 Preclinical Safety Evaluation Of Biotechnology-Derived Pharmaceuticals. Drug Information Branch (HFD-210), Center for Drug Evaluation and Research (CDER), Rockville, MD. pp. 1-14.
[55] Williams JA, Dillehay LE, Tabassi K, Sipos E, Fahlman C, and Brem H (1997). Implantable biodegradable polymers for IUdR radiosensitization of experimental human malignant glioma. *J Neurooncol* **32**, 181-192.
[56] Bobo RH, Laske DW, Akbasak A, Morrison PF, Dedrick RL, and Oldfield EH (1994). Convection-enhanced delivery of macromolecules in the brain. *Proc Natl Acad Sci USA* **91**, 2076-2080.
[57] Kroll RA, Pagel MA, Muldoon LL, Roman-Goldstein S, and Neuwelt EA (1996). Increasing volume of distribution to the brain with interstitial infusion: dose, rather than convection, might be the most important factor. *Neurosurgery* **38**, 746-752.
[58] Groothuis DR, Ward S, Itskovich AC, Dobrescu C, Allen CV, Dills C, and Levy RM (1999). Comparison of 14C-sucrose delivery to the brain by intravenous, intraventricular, and convection-enhanced intracerebral infusion. *J Neurosurg* **90**, 321-331.
[59] Fung LK, Ewend MG, Sills A, Sipos EP, Thompson R, Watts M, Colvin OM, Brem H, and Saltzman WM (1998). Pharmacokinetics of interstitial delivery of carmustine, 4-hydroperoxycyclophosphamide, and paclitaxel from a biodegradable polymer implant in the monkey brain. *Cancer Res* **58**, 672-684.
[60] Garcia-Contreras L, Abu-Izza K, and Lu DR (1997). Biodegradable cisplatin microspheres for direct brain injection: preparation and characterization. *Pharm Dev Technol* **2**, 53-65.