N.Y. Acad. Sci. 636, 28 (1991).
16. H. Jiang, S.-I. Zhang, B. Pernis, *Science* 256, 1213 (1992).
17. D.-R. Koh *et al.*, *ibid.*, p. 1210.
18. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
19. J. W. Kappler, U. Staerz, J. White, P. C. Marrack, *Nature* 332, 35 (1988).
20. O. Leo, M. Foo, D. H. Sachs, L. E. Samelson, J. A. Bluestone, *Proc. Natl. Acad. Sci. U.S.A.* 84, 1374 (1987).
21. J. A. Ledbetter and L. A. Herzenberg, *Immunol. Rev.* 47, 63 (1979).
22. D. P. Dialynas *et al.*, *J. Immunol.* 131, 2445 (1983).
23. We wish to thank G. Ruberti for helpful suggestions and R. Kizer and K. Sturgis for their secretarial support in the preparation of this manuscript. Supported in part by NIH grants AI 27989 and DK 39959 and by the National Multiple Sclerosis Society. A.G. was a fellow of the American Diabetes Association.

22 June 1992; accepted 21 September 1992

Treatment and Prevention of Rat Glioblastoma by Immunogenic C6 Cells Expressing Antisense Insulin-Like Growth Factor I RNA

Jerzy Trojan, Thomas R. Johnson, Susan D. Rudin, Judith Ilan, Mark L. Tykocinski, Joseph Ilan*

Rat C6 glioma cells express insulin-like growth factor I (IGF-I) and form rapidly growing tumors in syngeneic animals. When transfected with an episome-based vector encoding antisense IGF-I complementary DNA, these cells lost tumorigenicity. Subcutaneous injection of IGF-I antisense-transfected C6 cells into rats prevented formation of both subcutaneous tumors and brain tumors induced by nontransfected C6 cells. The antisense-transfected cells also caused regression of established brain glioblastomas when injected at a point distal to the tumor. These antitumor effects result from a glioma-specific immune response involving CD8$^+$ lymphocytes. Antisense blocking of IGF-I expression may reverse a phenotype that allows C6 glioma cells to evade the immune system.

Glioblastoma is the most frequent brain tumor in man and is usually fatal. Both human and rat glioma cells express high amounts of IGF-I. We previously reported that transfection of rat C6 glioma cells (*1*) with an antisense IGF-I cDNA transcriptional cassette driven by the mouse metallothionein-I promoter abrogated their tumorigenicity in syngeneic rats (*2*). This antisense expression vector was episomal and included the Epstein-Barr virus origin of replication and the gene encoding nuclear antigen 1, which together drive extrachromosomal replication (*2, 3*). Southern blot analysis indicated that the episome was stably maintained for at least 6 months in this cell line (*4*). Although the transfected cells differed morphologically from parental (nontransfected) cells, their viability was unaffected by activation of the transgene in vitro (*2, 4*). Rats injected with these transfected glioma cells have remained tumor-free for 13 months. In contrast, rats injected with parental C6 cells consistently developed large tumors within 3 weeks after injection.

We noted an accumulation of large mononuclear infiltrates, consisting predominantly of lymphocytes, at the sites of injection of the transfected glioma cells before the lesions disappeared (*2*). This observation suggested the possibility that antisense-mediated inhibition of IGF-I rendered the glioma cells more immunogenic, and hence that their loss of tumorigenicity might have an immune basis.

To determine whether glioma cells transfected with the antisense IGF-I transcriptional cassette elicit a host immune response against parental glioma cells in vivo, we subcutaneously injected 10^7 parental cells into each of ten syngeneic BDX rats (*1*) at a site above the left hind leg. After 4 to 6 days, when the resulting tumors were less than 1 cm in diameter, we injected 10^7 IGF-I antisense-transfected glioma cells at a site above the right hind leg. In all ten rats the tumors regressed within 3 weeks and the animals have remained tumor-free for 13 months (Table 1). In control animals, including rats secondarily injected with glioma cells transfected with the vector devoid of the IGF-I sequence, tumors persisted and after 1 to 2 months grew to a size that necessitated sacrifice of the animals (Table 1).

We next investigated the effect of the transfected glioma cells on rats from which large, established glioblastomas (2 to 3 cm in diameter, usually observed 3 to 4 weeks after injection) had been surgically excised. Twelve such rats were divided into two groups. Six animals received no further treatment, and, of these, five had a local recurrence of tumor after approximately 3 weeks, reflecting the incomplete excision of the primary tumor. The other group of six animals received an injection of 10^7 IGF-I antisense-transfected glioma cells immediately after surgery, and none of them showed tumor recurrence at the excision site or elsewhere over a 12-month period (Table 1).

To establish whether treatment by IGF-I antisense-transfected cells was effective against glioblastomas established in the brain, we injected a group of rats intracranially with 1×10^6 to 2×10^6 parental or transfected C6 cells. After 1 week several rats were sacrificed and their brain tissue prepared for histopathological examination. Of the remaining rats, six of six that had been injected with IGF-I antisense-transfected cells have survived 4 months with no evidence of tumor development, whereas five of five injected with parental cells either died after 3 to 4 weeks or were sacrificed when tumor growth was evident at the point of injection. A second group of six rats was injected intracranially with 1×10^6 to 2×10^6 parental cells and, 2 to 3 days later, injected subcutaneously above the right hind leg with 10^7 transfected cells. These rats have all remained tumor-free for 4 months (Table 1).

During tumor involution in each of the above experiments, we noted abundant infiltration of mononuclear cells accompanied by neovascularization at sites proximal to persisting glial elements (Fig. 1, A and B). In late stages of tumor involution (2 weeks after injection of the transfected cells), 70 to 80% of the infiltrating cells were CD8$^+$ lymphocytes (Fig. 1C), as deduced by staining with an antibody to CD8. The remaining infiltrating cells were macrophages and CD8$^-$ lymphocytes. Sections of normal rat spleen stained with the same antibody showed the expected proportion of CD8$^+$ cells (~10%).

To determine whether IGF-I antisense-transfected glioma cells could prevent glioblastoma development, we subcutaneously injected 11 rats above the left hind leg with 10^7 parental glioma cells and above the

J. Trojan, INSERM, Paris, and Department of Anatomy–Laboratory of Developmental Genetics and Molecular Biology, Case Western Reserve University, Cleveland, OH 44106.
T. R. Johnson and S. D. Rudin, Department of Anatomy–Laboratory of Developmental Genetics and Molecular Biology, Case Western Reserve University, Cleveland, OH 44106.
J. Ilan, Departments of Reproductive Biology, Anatomy–Laboratory of Developmental Genetics and Molecular Biology, and the Cancer Center, Case Western Reserve University, Cleveland, OH 44106.
M. L. Tykocinski, Institute of Pathology, and the Cancer Center, Case Western Reserve University, Cleveland, OH 44106.
Jo. Ilan, Department of Anatomy–Laboratory of Developmental Genetics and Molecular Biology, and the Cancer Center, Case Western Reserve University, Cleveland, OH 44106.
*To whom correspondence should be addressed.

Table 1. Treatment and prevention of rat glioblastomas with IGF-I antisense-transfected C6 glioma cells. Cells were injected into rats subcutaneously above the right or left hind legs, or directly into the brain, as indicated.

Injection protocol*			Surgical excision	Days between injections†	Tumor development		
Right leg	Left leg	Brain			Right leg	Left leg	Brain
C6T	C6	—	—	0	0/11	0/11	—
C6V	C6	—	—	0	6/6	6/6	—
C6T	C6	—	—	4 to 6	0/10	0/10	—
C6V	C6	—	—	4 to 6	6/6	6/6	—
C6T	C6	—	+	21	0/6	0/6	—
—	C6	—	+	—	—	5/6	—
—	—	C6	—	—	—	—	5/5
—	—	C6T	—	—	—	—	0/6
C6T	—	C6	—	2 to 3	—	—	0/6
—	C6-IR	—	—	—	—	0/4	—
C6-IR	C6	—	—	0	0/4	4/4	—
C6T-IR	C6	—	—	0	0/4	0/4	—
C6T-IR	C6	—	—	3 to 5	0/4	0/4	—

*C6T, C6 glioma cells transfected with the IGF-I antisense expression vector; C6V, C6 glioma cells transfected with the expression vector devoid of IGF-I antisense sequences; IR, cells irradiated with 5000 rads of ^{60}Co. †Simultaneous injections are indicated by a zero.

right hind leg with 10^7 transfected glioma cells (Table 1). No tumors developed in these rats over a 12-month period. Control rats injected with parental glioma cells at both sites developed a tumor at each site, and in some animals the individual tumors fused into one large mass. When glioma cells transfected with the vector devoid of IGF-I antisense DNA were injected at the secondary site, all animals developed tumors (Table 1). Control experiments with parental and transfected cells rendered nonviable by 5000 rads of ^{60}Co irradiation established that the antitumor effects of transfected cells were not lost after irradiation and that irradiated parental C6 cells provided no protection against tumor formation by viable parental cells (Table 1). These results suggest that the protective effect is specifically associated with transfected glioma cells that express IGF-I antisense sequences and that immunogenicity is not lost after irradiation.

To investigate the specificity of the host antitumor immune response, we performed a series of cell-mixing experiments (Table 2) with the B104 neuroblastoma cell line, which is syngeneic with the C6 glioma cell line (*5*). IGF-I transcripts are not detectable in nontransfected B104 cells, and the antisense IGF-I construct is fully active in these cells (*2*). We injected a mixture of 10^7 transfected B104 cells and 10^7 parental C6 glioma cells into five rats above the right hind leg. All five rats developed tumors, which were shown by histological examination and by immunostaining for glioma- and neuroblastoma-specific markers [glial fibrillary acid protein (GFA) and neurofilaments, respectively] to consist of both glioma and neuroblastoma cells (Fig. 1, D and E). In contrast, injection of a mixture of 10^7 IGF-I antisense-transfected glioma cells and 10^7 IGF-I antisense-transfected neuroblastoma cells resulted in tumors, in five of five rats, that consisted solely of neuroblastoma cells (Fig. 1, F and G). Likewise, injection of a mixture of 10^7 parental C6 glioma cells and 10^7 transfected or nontransfected B104 neuroblastoma cells, followed 3 to 5 days later by an injection of transfected glioma cells, yielded tumors consisting solely of neuroblastoma cells (Table 2). The latter tumors were histologically identical with those that developed in the simultaneous injection pro-

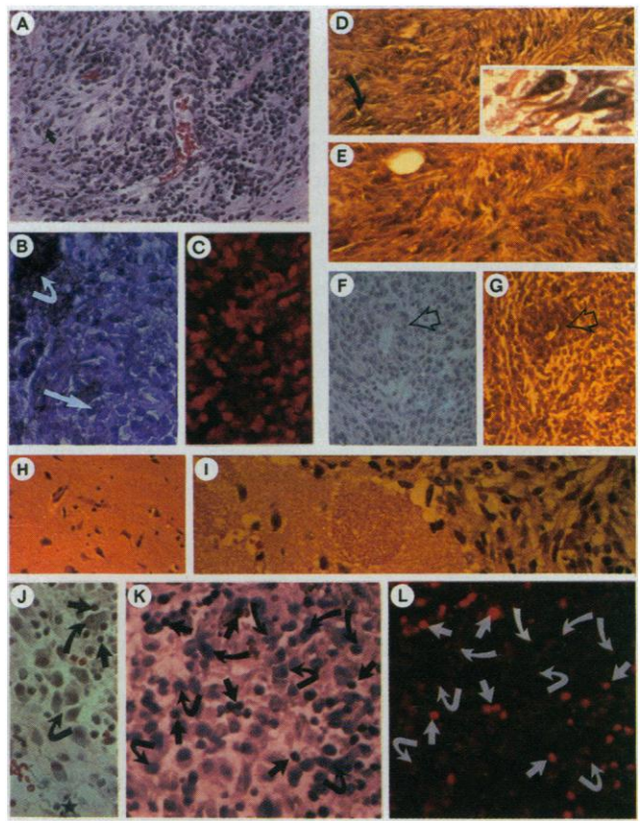

Fig. 1. Immunocytochemical analysis of the host immune response mounted against IGF-I antisense-transfected and parental C6 glioma cells. The glioma cells are indicated by curved solid arrows and the lymphocytes by straight solid arrows. (**A**, **B**, and **C**) Late stage (2 weeks after injection) regression of glioblastoma resulting from subcutaneous coinjection of transfected and parental glioma cells. (A) This section shows neovascularization (center) and lymphocyte accumulation (center and right). Hematoxylin and eosin stain. (B) Immunoperoxidase labeling (*25*) with antibody to GFA shows clusters of persisting glioma cells among mononuclear cells. Hematoxylin stain. (C) Direct immunofluorescence staining of a section adjacent to (B) with an antibody to CD8 coupled to rhodamine. (**D** and **E**) Sections of mixed tumor derived from subcutaneous coinjection of B104 neuroblastoma cells and nontransfected C6 glioma cells. Section (D) was immunoperoxidase-labeled with antibody to GFA and section (E) with antibody to neurofilaments. The inset in (D) is a higher magnification of the indicated glioma cells. Hematoxylin. (**F** and **G**) Sections of neuroblastoma derived from subcutaneous coinjection of B104 neuroblastoma cells and transfected C6 glioma cells. Open arrows show neuroblastic rosettes. (F) was stained with hematoxylin and eosin, and (G) was labeled with antibody to neurofilaments. All cells show positive staining. (**H**, **I**, **J**, **K**, and **L**) Eradication of glioblastoma in brain. (H) Normal brain tissue. Hematoxylin and eosin. (I) Boundary between normal brain tissue (left) and glioblastoma (right), 1 week after injection of parental C6 cells. Hematoxylin and eosin. (J) Boundary between normal brain tissue and glioblastoma, 1 week after subcutaneous injection of transfected C6 cells into a rat that 3 days previously had been injected intracranially with untransfected C6 cells. The normal tissue is marked by a star. Giemsa hematoxylin stain, blue filter. (K) Same as (J), without normal brain tissue. Hematoxylin and eosin. (L) Same microscopic field as (K), but stained for CD8 antigen as in (C). Fluorescence bright field. Final magnifications: (A), ×150; (B), ×250; (C), ×250; (D), ×200; (E), ×200; (F), ×200; (G), ×200; (H), ×250; (I), ×400; (J), ×400; (K), ×400; and (L), ×400.

Table 2. Specificity of immune response in rats elicited by IGF-I antisense-transfected tumor cells. For experiments involving cell injections above both hind legs, the injections were performed simultaneously except where indicated.

Injection protocol*		Tumor development		Histological characterization	
Right leg	Left leg	Right leg	Left leg	Right leg	Left leg
—	C6T and C6	—	0/11	—	—
C6T and C6	C6T and C6	0/6	0/6	—	—
—	C6T and B104	—	8/8	—	Neuroblastoma only
—	C6T and B104T	—	5/5	—	Neuroblastoma only
—	C6 and B104T	—	5/5	—	Glioma and neuroblastoma
—	C6 and B104	—	5/5	—	Glioma and neuroblastoma
C6T	C6 and B104	0/5	5/5	—	Neuroblastoma only
C6T†	C6 and B104T	0/10	10/10	—	Neuroblastoma only

*C6T, C6 glioma cells transfected with IGF-I antisense expression vector; B104T, B104 neuroblastoma cells transfected with IGF-I antisense expression vector. †In this experiment, the C6T cells were injected 5 days after injection of the mixture of C6 and B104T cells.

tocol (Fig. 1, F and G). These data indicate (i) that the host immune response induced by IGF-I antisense-transfected glioma cells is specific for glioma cells and can distinguish between glioma and neuroblastoma cells within a mixed tumor and (ii) that nonspecific bystander effects mediated by other immune cells, such as natural killer cells, are not primarily responsible for tumor regression.

The host immune response elicited by subcutaneous injection of IGF-I antisense-transfected glioma cells also eradicated glioblastoma established in the brain by intracranial injection of 2×10^6 to 3×10^6 parental glioma cells (Table 1 and Fig. 1, H and I). As with the tumors established subcutaneously, accumulation of $CD8^+$ lymphocytes accompanied tumor regression (Fig. 1, J, K, and L).

The progression of somatic cells into a tumorigenic state is influenced by multiple molecular factors, including oncogenes, tumor suppressor genes, and angiogenic factors. Our study focused on another aspect of the tumorigenic phenotype, namely, the capacity to elude host antitumor immune responses. We have demonstrated that the inhibition of IGF-1 expression elicits a highly immunogenic phenotype in glioma cells. The possibility that IGF-I directs the immunogenic phenotype is of special interest from the standpoint of developmental biology. Rat C6 glioma cells originate from adult rat brain astrocytes (5), which do not express IGF-I. However, fetal rat glial cells, like many other embryonic and fetal cells, do express IGF-I. Expression of IGF-I and its receptor in embryonic rat brain is developmentally regulated and IGF-I participates in both proliferation and differentiation (6). The expression in tumors of genes that are active in embryonic or fetal structures may represent reenactment of an early embryonic or fetal program. Such a reversion of glioma cells to an embryonic phenotype may enable them to evade immune recognition (7).

Other tumor types that express high amounts of IGF-I or the related growth factor IGF-II are astrocytomas and meningiomas (8), sarcomas (9), thyroid adenomas (10), estradiol-regulated and nonregulated breast carcinomas (11), Wilms tumor (12), colon carcinomas (13), squamous cell carcinomas (14), small cell lung carcinomas (15), teratocarcinomas (16), and hepatocarcinomas (17). Because IGF-II can act through the IGF-I type I receptor as well as its own specific receptor (18), it will be important to investigate whether inhibition of IGF-II expression also induces an immunogenic phenotype. The present experimental design provides an efficient coupled in vivo–in vitro assay for determining the role of other molecular factors that may be responsible for down-modulating tumor immunogenicity.

Previous studies have explored the potential role of the IGFs as molecules driving the proliferation of tumor cells that produce them. This work has led to attempts to inhibit growth of such tumor cells with exogenously added antibodies to IGF-I or IGF-II (19). Although some growth inhibition was observed with this approach, tumor development in vivo could not be effectively blocked by antibodies to IGF. These experiments were performed in immunologically deficient nude mice, and hence possible alterations in glioma cell immunogenicity were not even considered. Furthermore, because intracrine mechanisms (autocrine mechanisms that bypass growth factor secretion) (20) may well be involved here, antibodies to IGF-I may not parallel antisense IGF-I RNA in altering tumor immunogenicity in an immunologically intact animal.

For reasons that are not understood, many tumors that arise de novo are poorly immunogenic and therefore can evade host antitumor responses (21). This study provides one potential therapeutic approach toward enhancing tumor immunogenicity that is based on antisense gene transfer. Alternative approaches for enhancing tumor cell immunogenicity—for example, by transfection with genes coding for specific antigens (22) or cytokines (23)—have been described. An elegant and successful method for treating established glioblastomas in rats by retrovirus-mediated gene transfer in vivo was recently reported (24). Unlike the latter approaches, which enhance tumor cell immunogenicity by expression of an exogenously introduced foreign gene product, we effected such enhancement by blocking expression of a cytokine that is normally expressed by the tumor cell.

REFERENCES AND NOTES

1. H. Druckrey, S. Ivankovic, R. Preussman, *Z. fur Krebsforschung* **66**, 389 (1965); P. Benda, J. Lightbody, G. Sato, L. Levine, W. Sweet, *Science* **161**, 370 (1968).
2. J. Trojan *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 4874 (1992).
3. G. W. Stuart, P. F. Searle, H. Y. Chen, R. L. Brinster, R. D. Palmiter, *ibid.* **81**, 7318 (1984); J. E. R. Hambor *et al.*, *ibid.* **85**, 4010 (1988).
4. J. Trojan and T. R. Johnson, unpublished data.
5. D. Schubert *et al.*, *Nature* **249**, 224 (1974).
6. E. M. Shooter, in *Modern Concepts of Insulin-like Growth Factors*, E. M. Spencer, Ed. (Elsevier, New York, 1991), pp. 297–307.
7. K. D. Boehm, M. F. Kelley, Jo. Ilan, J. Ilan, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 656 (1989).
8. H. N. Antoniades, T. Galanopoulis, J. Nevile-Golden, M. Maxwell, *Int. J. Cancer* **50**, 215 (1992).
9. P. J. Roholl *et al.*, *Histopathology* **16**, 455 (1990).
10. D. W. Williams, E. D. Williams, D. Wynford-Thomas, *Mol. Cell. Endocrinol.* **61**, 139 (1989).
11. J. A. Foekens, H. Portengen, M. Janssen, J. G. Klijn, *Cancer* **63**, 2139 (1989); N. Brünner, D. Yee, L. Skriver, K. J. Cullen, *Breast Cancer Res. Treat.* **16**, 148 (1990).
12. T. Gansler *et al.*, *Am. J. Pathol.* **130**, 431 (1988).
13. J. M. Colouscou, M. Remacle-Bonnet, F. Garrouste, J. Marvaldi, G. Pommier, *Int. J. Cancer* **40**, 646 (1987); T. C. Hall *et al.*, *Proc. Am. Assoc. Cancer Res.* **29**, A802 (1988).
14. Y. Brewer and M. Reiss, *Proc. Am. Assoc. Cancer Res.* **31**, A247 (1990).
15. V. M. Macaulay *et al.*, *Cancer Res.* **50**, 2511 (1990).
16. N. H. Jing *et al.*, *In Vitro Cell. Dev. Biol.* **27**, 864 (1991).
17. X. X. Fu *et al.*, *J. Virol.* **62**, 3422 (1988); D. Y. Yang and C. E. Rogler, *Carcinogenesis* **12**, 1983 (1991).
18. M. M. Rechler and S. D. Nissley, *Annu. Rev. Physiol.* **47**, 425 (1985).
19. J. Blatt, C. White, S. Dienes, H. Friedman, T. Foley, *Biochem. Biophys. Res. Commun.* **123**, 373 (1984); T. Gansler *et al.*, *Am. J. Pathol.* **130**, 431 (1988); K. K. Huff *et al.*, *Cancer Res.* **46**, 4613 (1986); F. Minuto *et al.*, *ibid.* **48**, 3716 (1988).
20. C.-H. Heldin and B. Westermark, *Eur. J. Biochem.* **184**, 487 (1989).
21. H. B. Hewett, E. R. Blake, A. S. Walder, *Br. J. Cancer* **33**, 241 (1976).
22. E. R. Fearon *et al.*, *Cancer Res.* **38**, 2975 (1988).
23. ———, *Cell* **60**, 397 (1990).
24. K. W. Culver *et al.*, *Science* **256**, 1550 (1992).
25. Immunocytochemical analysis was performed according to methods described in (2) and in J. Trojan *et al.*, [*Dev. Neuroscience* **6**, 251 (1984)]. The antibodies to GFA and neurofilaments were from Sigma, and the CD8 antibody (a monoclonal

antibody to rat non-helper T cells) was from Sera-lab, Sussex, England.
26. We thank S. Emancipator for critical advice in the immunocytochemical studies, U. Roessmann for histopathological examination of tumor specimens, R. Groger for advice on episome analyses, D. Anthony, D. Landis, and F. Abdul-Karim for their valuable input, and C. Porter for expert technical assistance. Supported by grants from the National Institutes of Health (HD-25004 to Jo.I., HD-18271 to J.I., CA-47566 to M.L.T., and CA-43703 to the Cancer Center) and by a grant from the American Diabetes Association to J.I. We also thank the Sypolt family for their generous gift.

8 June 1992; accepted 22 October 1992

Dormancy of Inhibitory Interneurons in a Model of Temporal Lobe Epilepsy

Jonathan W. Bekenstein and Eric W. Lothman*

In humans temporal lobe epilepsy (TLE) is characterized by recurrent seizures, neuronal hyperexcitability, and selective loss of certain neuronal populations in the hippocampus. Animal models of the condition indicate that a diminution of inhibition mediated by gamma-aminobutyric acid (GABA) accounts for the altered function, and it has been hypothesized that the diminution arises because GABAergic basket interneurons are "dormant" as a result of their being disconnected from excitatory inputs. In hippocampal slices, inhibitory postsynaptic potentials (IPSPs) were elicited in CA1 pyramidal cells by activation of basket cells; responses from an animal model of TLE were compared to those from control tissue. IPSPs evoked indirectly by activation of terminals that then excited basket cells were reduced in the epileptic tissue, whereas IPSPs evoked by direct activation of basket cells, when excitatory neurotransmission was blocked, were not different from controls. These results provide support for the "dormant basket cell" hypothesis and have implications for the pathophysiology and treatment of human TLE.

There has been long-standing controversy over whether a diminution of inhibition mediated by the amino acid GABA causes epilepsy and, if so, how this comes about. The premise that a decrease in GABAergic inhibition participates in epileptogenesis arose from observations made decades ago that several drugs that block GABA-mediated inhibition produce acute seizures (*1*). However, proper investigation of this issue requires the study of tissue from a persistently epileptic brain. This approach has been hampered by limited availability of brain tissue from humans with epilepsy. Recently, appropriate animal models of epilepsy, sharing critical characteristics with the condition in human patients, have been developed (*2–4*). These features include neuronal hyperexcitability, distinctive neuropathological changes such as loss of neurons and sprouting of certain axon terminals, and recurrent spontaneous seizures.

Models of epilepsy established with electrical stimulation are associated with an enduring, apparently permanent loss of GABAergic inhibition (*3–5*). Furthermore, in one of these models, an intriguing paradox was reported: physiological studies indicated loss of GABA-mediated inhibition of principal cells in the hippocampus, whereas immunohistochemical studies revealed survival of GABAergic interneurons in that structure (*3*). This led to the concept that the basket cell interneurons that exert an inhibitory effect in the hippocampus by releasing GABA onto principal cells (pyramidal cells in Ammon's horn and granule cells in the dentate gyrus) are "dormant" rather than dysfunctional (*4*). There has been controversy (*6*) over whether GABAergic interneurons do survive in this situation and, if so, how this relates to heightened epileptogenesis.

If the dormant basket cell hypothesis is correct, several predictions can be made. The first two relate to stimuli delivered at a site distant to a CA1 pyramidal cell that indirectly engage basket cells by initially exciting terminals presynaptic to the basket cell (Fig. 1). Under these conditions, intracellular recordings would be predicted to show inhibitory postsynaptic potentials (IPSPs) elicited in CA1 pyramidal cells from epileptic tissue that are smaller than corresponding IPSPs in neurons from control tissue. The second prediction is that under these conditions $GABA_A$ IPSPs and $GABA_B$ IPSPs should be affected to the same degree. The third prediction is that IPSPs evoked in CA1 pyramidal cells by direct near-site stimulation of basket cells, in the presence of agents that block excitatory neurotransmission, should not differ between epileptic and control tissue. Thus, there should be substantial differences between the results with near- and far-site stimulation.

Department of Neurology and Neuroscience Program, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

*To whom correspondence should be addressed.

We have now tested these predictions. Experimental arrangements are schematized in Fig. 1. Intracellular recordings were taken from pyramidal cells in CA1. Slices were obtained either from control animals or from animals that had experienced a previous episode of self-sustaining limbic status epilepticus (*9, 10*), which leads to persistent sequelae of hyperexcitability of hippocampal slices under conditions that precipitate epileptiform discharges (*5*), neuropathological changes like mesial temporal sclerosis (*11*), and recurrent hippocampal seizures (*5*). Stimuli were given at three sites. Near-site stimulation was done in the presence of pharmacological antagonists 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX) and D-2-amino-5-phosphonovalerate (APV) to block excitatory synapses utilizing non-*N*-methyl-D-aspartate (non-NMDA) and NMDA-type glutamate receptors, respectively. Far-site stimulations, done without the antagonists, activated GABAergic basket cells either "antidromically," by first discharging CA1 pyramidal cells, or "orthodromically," by first activating Schaeffer collaterals.

In response to challenges with elevated concentrations of extracellular potassium ($[K^+]_o$), slices from epileptic animals are hyperexcitable relative to slices from control animals, as determined by measurements of population spikes in the stratum pyramidale of CA1 (*5*). However, under conditions with $[K^+]_o$ of 3 mM, the normal concentration in brain and the value used in the current study, population spike responses from the two types of slices are the same, without epileptiform discharges. This latter condition was maintained in the present study. There were no differences in intrinsic membrane properties between neurons studied in slices from normal animals and in those from epileptic animals (Table 1).

In slices from control animals, the two far-site stimuli produced IPSPs in CA1 pyramidal cells (Fig. 2) identical to those previously described (*12–14*). Orthodromic stimulation elicited a biphasic IPSP, with an earlier peak occurring at about 40 ms and a later peak at about 110 ms. Antidromic stimulation evoked a monophasic IPSP, with a peak at about 40 ms. Investigators (*12, 13*) have previously established that (i) all three IPSPs are mediated by GABA; (ii) both the earlier component of the orthodromic IPSP and the antidromic IPSP result from activation of $GABA_A$ receptors that open Cl^- channels, whereas the later component of the orthodromic IPSP arises from activation of $GABA_B$ receptors that open K^+ channels; and (iii) the magnitude of the IPSPs increases with stimulus strength to reach maximal values. Thus, stimulus intensity was adjusted in our