Exp Brain Res (1985) 60: 204–208

Experimental Brain Research

© Springer-Verlag 1985

Research Note

Cyclosporin A increases survival of cross-species intrastriatal grafts of embryonic dopamine-containing neurons

P. Brundin, O.G. Nilsson, F.H. Gage, and A. Björklund

Department of Histology, University of Lund, Biskopsgatan 5, S–223 62 Lund, Sweden

Summary. The survival and function of cross-species (mouse-to-rat) grafts of fetal mesencephalic dopamine (DA) neurons, implanted as a cell suspension in the striatum of rats with lesions of the meso-striatal DA system, have been studied in animals with and without immunosuppression induced by Cyclosporin A (CyA). At 6 weeks after grafting 3 out of 7 non-CyA treated animals showed some degree of graft survival and variable functional compensation. In those three animals an average of 92 DA neurons per graft was counted. In the grafted animals treated with daily CyA injections, all grafts survived and produced partial or complete functional compensation, and they had an average of 557 DA neurons per graft. It is concluded that intracerebral graft survival and function can be greatly improved by CyA treatment and that the immunological protection of neural transplants in the brain is only partial.

Key words: Neuronal transplantation – Cyclosporin A – Cross-species – Dopamine neurons

Introduction

The proposed immunologically privileged status of the brain has reemerged as a concept of interest due to recent successes in intracerebral syngeneic grafting (see Björklund and Stenevi 1984 for review) but the immunological constraints for implantation of embryonic CNS neurons have so far not been fully explored. Several laboratories have reported some success with xenogenic grafts of fetal brain tissue between different rodent species, but the results have generally been substantially inferior to and considerably more variable than the results obtained with comparable syngeneic grafts (Björklund et al. 1982; Bragin and Vinogradova 1981; Daniloff et al. 1984, 1985; Low et al. 1985; Vinogradova et al. 1985). Although it thus seems clear from available data that rejection of immunologically incompatible tissue can take place in the brain, it remains unclear to what extent the highly variable results reported from experiments with cross-species neural grafts in rodents is due exclusively to immunological mechanisms or whether other technical factors, such as graft placement or extent of trauma to the host, are responsible as well.

In the present experiment we have studied the survival and function of cross-species neural grafts in a rat model of Parkinson's disease, using intraparenchymal placement with the cell suspension injection technique. We analysed the effects of the immunosuppressive drug Cyclosporin A (CyA) on graft survival and function. The results show that also with this technique, which causes little damage to the host's vascular system, graft survival and function was poor and variable unless the recipient was treated with CyA.

Material and methods

Twenty-six young adult female Sprague-Dawley rats of an inbred strain (ALAB Stockholm, Sweden) were given unilateral 6-hydroxydopamine (6⁻ OHDA, 8 μg in 4 μl) injections into the ascending nigrostriatal pathway as described previously (Björklund et al. 1983; Schmidt et al. 1983). The completeness of the lesion was assessed by monitoring the intensity of the amphetamine-induced turning behaviour according to Ungerstedt and Arbuthnott (1970). The criterion was set at 6 full turns per min over 90 min in response to 5 mg/kg metaphetamine. This is consistent with a 97% DA-depletion in the ipsilateral neostriatum (Schmidt et al. 1983).

Three to five months after the 6⁻ OHDA lesion, 21 of the rats received cell suspension grafts from the ventral mesencephalon obtained from approximately 45 14-day old (crown-to-rump length

Offprint requests to: P. Brundin (address see above)

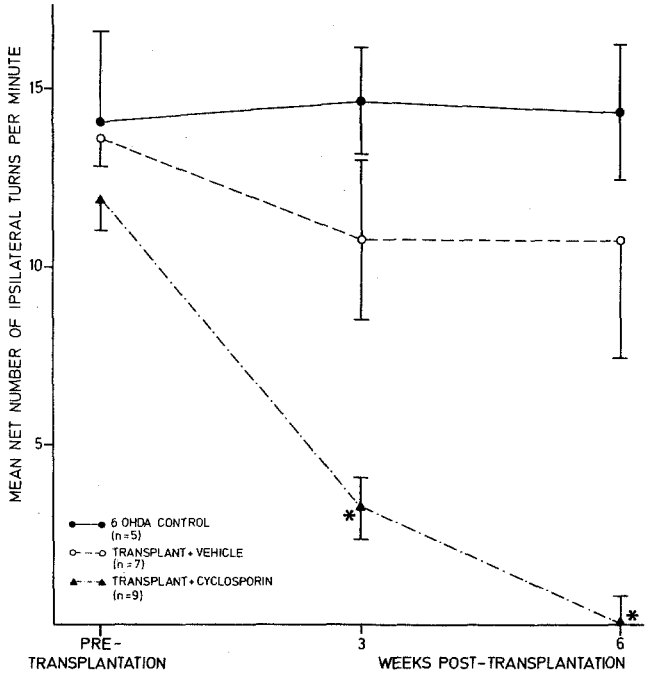

Fig. 1. Effect of mouse nigral suspension grafts, in rats with and without CyA treatment, on motor asymmetry as assessed in the amphetamine-induced rotation test. The compensation of turning bias is significant in the CyA-treated group alone (triangles and dash-dotted line) at 3 and 6 weeks after grafting ($p < 0.01$ in both cases; ANOVA with posthoc Newman-Keuls' test)

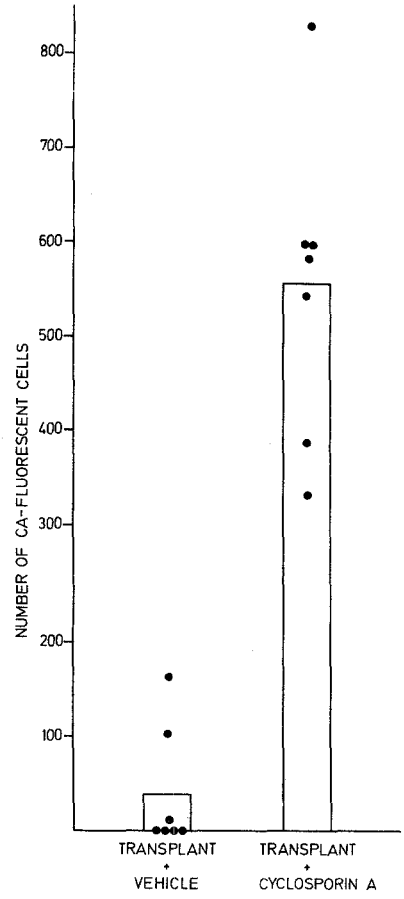

Fig. 2. Number of surviving catecholamine (CA)-containing neurons in mouse-to-rat nigral grafts, at six weeks post-transplantation with and without CyA treatment. Each dot represents one graft, and the bar the group mean value. The difference between the CyA and vehicle-treated group is significant on the $p < 0.01$ level (Mann-Whitney U-test)

9–11 mm) mouse fetuses of A.SW/Kl strain (supplied by the Dept. of Genetics, University of Lund, Sweden). The cell suspension was prepared by enzymatic digestion and mechanical dissociation, as described previously (Björklund et al. 1983), and 2 µl (equivalent to approximately 280000 viable cells, as assessed according to Brundin et al. 1985) were deposited by stereotaxic injection over two sites in the head of the caudate-putamen. The remaining 5 rats received no transplants. The transplant group was divided into two subgroups; one that received daily intraperitoneal injections of 11 mg/kg CyA (n = 14) dissolved by sonication in olive oil to a concentration of 10 mg/ml and one that received olive oil (vehicle) (n = 7). A stock solution, lasting approximately 4–5 days, was made up and stored in the dark at 4° C. The first injection was given eighteen hours prior to transplantation, and injections were then given daily for 6 weeks. The 6- OHDA control rats were given daily vehicle injections. The rats were divided into groups such that the pre-transplantation rotation scores for the three groups were balanced. All rats were tested for rotational asymmetry again at 3 and 6 weeks post-transplantation. During the course of the experiment 5 rats in the CyA-treated group died, possibly due to opportunistic respiratory infection, and were excluded from the analysis. Catecholamine (CA) histochemistry was performed according to the ALFA-method (Lorén et al. 1980).

Results

Three weeks post-transplantation the CyA-treated group showed a significant reduction in ipsilateral amphetamine-induced rotation bias when compared to controls (Fig. 1) (one-way analysis of variance (ANOVA) with post-hoc Newman-Keuls' test, $p < 0.01$) and had significantly lower rotation scores than the vehicle treated rats ($p < 0.01$). At 6 weeks post-transplantation the vehicle injected group did not differ from controls, whereas the CyA-treated rats as a group were completely compensated (mean = 0.03 ipsilateral turns/min) and were significantly different from both the controls and the vehicle-treated grafted rats ($p < 0.01$). All rats in the CyA-treated group showed activation in response to amphetamine and at least 65% reduction of their rotational bias, whereas in the vehicle-treated group only 2 of the 7 rats showed any compensation of rotational bias. Five of the 9 surviving CyA-treated rats showed some degree of "overcompensation" (more contralateral than ipsilateral turns). Two days after the 6-week rotation test all rats that had received grafts, except for two of the rats that showed complete behavioural

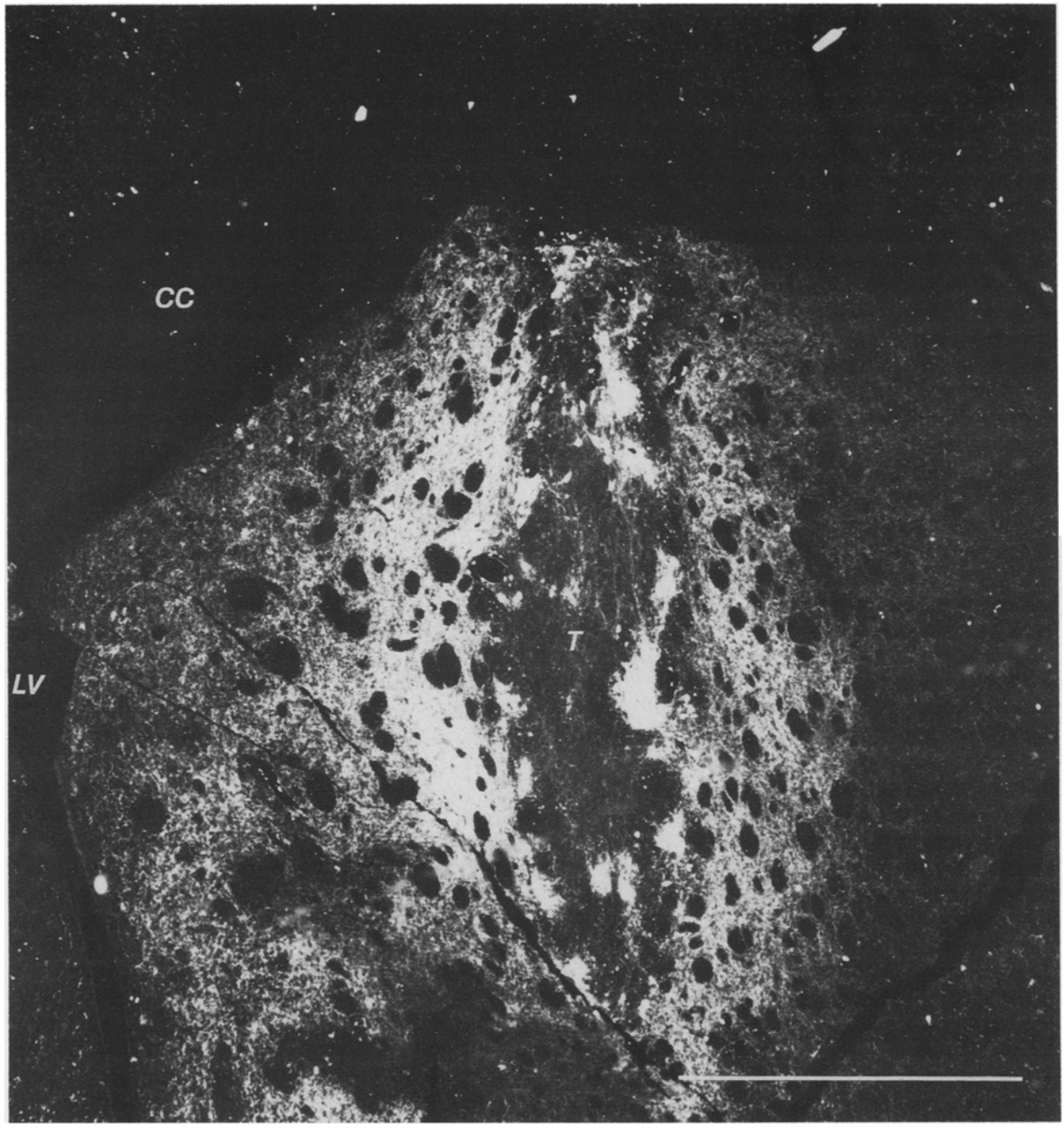

Fig. 3. Catecholamine (CA) histofluorescence picture of a surviving mouse nigral suspension graft from the CyA-treated group, 6 weeks after transplantation (frontal section). The surviving transplant (T) contains large numbers of CA-fluorescent neurons, scattered and in aggregated clusters, and is surrounded by a 1–1½ mm wide halo of a newly formed CA-fluorescent terminal network. LV = lateral ventricle; CC = corpus callosum. Scale bar = 1 mm

compensation in the CyA-treated group, were perfused for CA histofluorescence (Lorén et al. 1980). In the remaining 2 rats the CyA treatment was discontinued. One was rotation tested again at 10 weeks after grafting and then processed for histofluorescence. The other rat was rotation-tested at 10, 13 and 18 weeks after grafting. Both these rats remained compensated in the rotation test to the same degree as before discontinuation of the CyA treatment.

Histochemical analysis revealed surviving grafts in all 9 of the CyA-treated rats, and in 3 out of 7 in the vehicle-treated group. The number of CA-

fluorescent cell bodies was counted in a blind manner in every third section throughout the grafts according to Abercrombie (1946). The mean number of surviving DA neurons in the grafts of the CyA-treated rats analysed 6 weeks after transplantation (557 ± 65) was significantly higher (Mann-Whitney U-test, U (7,7) = 49; $p < 0.01$) than that of the vehicle-treated group (40 ± 24) (Fig. 2). This was also the case when the CyA-treated group was compared to the subgroup of 3 vehicle-rats that showed surviving DA neurons (mean = 92 DA neurons). In fact, the smallest graft in the CyA-group (329 DA neurons) contained twice as many DA neurons as the largest in the vehicle-treated group (161 DA neurons). The proportion of injected viable cells that survived as DA neurons in the CyA-treated group was in the order of 2 in 1000 which is comparable to results previously obtained in rat syngeneic grafts (Brundin et al. 1985). Also, the fiber outgrowth was similar in magnitude to that seen with mesencephalic syngeneic grafts (Björklund et al. 1983) and would in some cases encompass large portions of the dorsal neostriatum (Fig. 3). Orange autofluorescent cell bodies (presumably containing phagocytosed material) were in general much more common around the needle tract and at the injection site in the vehicle-treated rats than the grafts of CyA-treated rats. As in our previous study (Björklund et al. 1982) no marked infiltration of lymphocytes could be detected at the graft site in cresyl violet-stained sections in any of the vehicle or CyA-treated grafted animals.

The rats perfused 4 and 14 weeks after the termination of CyA-treatment also had large surviving grafts (392 and 701 DA neurons, respectively) that fell within the range of the size or grafts found after 6 weeks in the CyA-treated group (Fig. 2).

Discussion

The results thus demonstrate that the survival of intracerebral embryonic neural xenografts is greatly increased in the presence of immunosuppressive treatment. This indicates that immunological rejection mechanisms are the major cause of the poor and variable survival of cross-species neural grafts in the brain. In fact, in the presence of immunosuppression with CyA the survival rate, the number of DA neurons surviving, the extent of DA fiber outgrowth, and the magnitude of the functional effects of the mouse xenografts were all comparable to the results obtained with syngeneic mesencephalic grafts. Similar results have been obtained using CyA with allografts of peripheral nerve (Zalewski and Gulati 1984) and with xenogenic (mouse-to-rat) intraventricular grafts of embryonic neocortical tissue (Inoue et al. 1985).

In their classic study on xenogenic tumor grafts Murphy and Sturm (1923) reported that rejection occurred in the brain only with grafts in extraparenchymal locations, i.e. in the ventricular spaces. The susceptibility of such intraventricularly located grafts has more recently been corroborated by Mason et al. (1985) in studies of allo- and xenografts of embryonic hypothalamic tissue. For mouse-to-rat xenografts of mesencephalic DA neurons, however, neither the placement nor the trauma caused by the grafting procedure seem to play any major role. Thus, without immunosuppressive treatment the survival rate seen in the present series of intraparenchymally injected cell suspension grafts (43%) was similar to that obtained in our previous study (Björklund et al. 1982) on grafts placed extraparenchymally in a surgically prepared transplantation cavity (56%), and the number of DA neurons recovered in the surviving grafts appeared to be of the same magnitude in both cases.

Available data thus support the view that although the brain is immunologically privileged relative to other sites in the body the protection against immunological rejection is only partial. In the strain combination studied by Mason et al. (1985) rejection of intraventricular allografts that were incompatible with the hosts for only major histocompatability antigens was not observed, whereas rejection developed with xenografts and allografts differing for both major and minor antigens. Nevertheless, there are several reports of partial long-term survival (up to about 6–7 months) of cross-species neural grafts in both intraparenchymal and extraparenchymal locations in the brains of rodents (Björklund et al. 1982; Bragin and Vinogradova 1981; Daniloff et al. 1984, 1985; Low et al. 1985; Vinogradova 1985), which suggests that the xenogenic graft tissue in some animals in a group can partly escape rejection even in the absence of immunosuppression. The reason for this variability is not clear. However, since the blood-brain barrier and the poorly developed lymphatic drainage of the brain are likely factors to contribute to the protection of intracerebral grafts against immunological rejection (Barker and Billingham 1977; Mason et al. 1985), it seems possible that variation between animals in the exposure of the graft site to the blood circulation during the first few days after surgery, due to the inevitable surgical damage to the host brain, could play a role. This possibility, which finds some support in the sequential grafting experiments of Raju and Grogan (1977), suggests that there may be a transient critical period (perhaps until the brain has healed and the graft has

become fully integrated with the host) for the activation and/or execution of the rejection mechanism. This, in combination with studies showing that short-term CyA treatment permits prolonged survival of xenogenic heart grafts in the rat (Homan et al. 1981), suggests that it may be sufficient to administer CyA only for a limited period to obtain good long-term survival of the intracerebral neural grafts. Indeed, the two grafted rats in the present study which were left to survive after discontinuation of the CyA treatment provide some tentative support for this idea.

Acknowledgements. We thank Jan Berglund, Ulla Jarl, Yvette Jönsson and Getrude Stridsberg for technical assistance, and Ragnar Mårtensson for the photograph in Fig. 3. This work was supported by grants from the Swedish MRC (04X-3874) and the National Institutes of Health and Aging (AG-3766 and NS-6705). Cyclosporin A was supplied by Sandoz Ltd., Täby, Sweden.

References

Abercrombie M (1946) Estimation of nuclear population from microtome sections. Anat Rec 94: 239–247

Barker CF, Billingham RE (1977) Immunologically privileged sites. Adv Immunol 25: 1–54

Björklund A, Stenevi U, Dunnett SB, Gage FH (1982) Cross-species neural grafting in a rat model of Parkinson's disease. Nature 298: 652–654

Björklund A, Stenevi U, Schmidt RH, Dunnett SB, Gage FH (1983) Intracerebral grafting of neuronal cell suspensions. II. Survival and growth of nigral cells implanted into different brain sites. Acta Physiol Scand (Suppl) 522: 9–18

Björklund A, Stenevi U (1984) Intracerebral neural implants: neuronal replacement and reconstruction of damaged circuitries. Ann Rev Neurosci 7: 279–308

Bragin AG, Vinogradova OS (1981) Homo- and hetero-specific transplantation of embryonal central nervous tissues. Bull Exp Biol Med 10: 486–489 (in Russian)

Brundin P, Isacson O, Björklund A (1985) Monitoring of cell viability in suspensions of embryonic CNS tissue and its use as a criterion for intracerebral graft survival. Brain Res 331: 251–259

Daniloff JK, Wells J, Ellis J (1984) Cross-species septal transplants; recovery of choline acetyltransferase activity. Brain Res 324: 151–154

Daniloff JK, Low WC, Bodony RP, Wells J (1985) Cross-species neural transplants of embryonic septal nuclei to the hippocampal formation of adult rats. Exp Brain Res 59: 73–82

Homan WP, Williams KA, Fabre JW, Millard PR, Morris PJ (1981) Prolongation of cardiac xenograft survival in rats receiving cyclosporin A. Transplant Proc 31: 164–166

Inoue H, Kohsaka S, Yoshida K, Ohtani M, Toya S, Tsukada Y (1985) Cyclosporin A enhances the survivability of mouse cerebral cortex grafted into the third ventricle of rat brain. Neurosci Lett 54: 85–90

Lorén I, Björklund A, Falck B, Lindvall O (1980) The aluminum-formaldehyde (ALFA) method for improved visualization of catecholamines and indoleamines. I. A detailed account of the methodology for central nervous tissue using paraffin, cryostat or vibratome sections. J Neurosci Meth 2: 277–300

Low WC, Lewis PR, Bunch ST (1983) Embryonic neural transplants across a major histocompatability barrier: survival and specificity of innervation. Brain Res 262: 328–333

Low WC, Daniloff JK, Bodony RP, Wells J (1985) Cross-species transplants of cholinergic neurons and the recovery of function. In: Björklund A, Stenevi U (eds) Neural grafting in the mammalian CNS. Elsevier Science Publishers B.V., Amsterdam, pp 575–584

Mason DW, Charlton HM, Jones A, Parry DM, Simmonds SJ (1985) Immunology of allograft rejection in mammals. In: Björklund A, Stenevi U (eds) Neural grafting in the mammalian CNS. Elsevier Science Publishers B.V., Amsterdam, pp 91–98

Murphy JB, Sturm E (1983) Conditions determining the transplantability of tissues in the brain. J Exp Med 38: 183–197

Raju S, Grogan JB (1977) Immunologic study of the brain as a priviledged site. Transplant Proc 9: 1187–1191

Schmidt RH, Björklund A, Stenevi U, Dunnett SB, Gage FH (1983) Intracerebral grafting of neuronal cell suspensions. III. Activity of intrastriatal nigral suspension implants as assessed by measurements of dopamine synthesis and metabolism. Acta Physiol Scand (Suppl) 522: 19–28

Ungerstedt U, Arbuthnott GW (1970) Quantitative recording of rotational behaviour in rats after 6-hydroxydopamine lesions of the nigro-striatal dopamine system. Brain Res 24: 4865–4893

Vinogradova OS, Bragin AG, Kitchigina VF (1985) Spontaneous and evoked activity of neurons in intrabrain allo- and xenografts of the hippocampus and septum. In: Björklund A, Stenevi U (eds) Neural grafting in the mammalian CNS. Elsevier Science Publishers B.V., Amsterdam, pp 409–419

Zalewski A, Gulati AK (1984) Survival of nerve allografts in sensitized rats treated with cyclosporin A. J Neurosurg 60: 828–834

Received April 15, 1985 / Accepted June 10, 1985