Histol Histopathol (1996) 11: 1063-1073

Histology and Histopathology

Invited Review

Neuroectodermal grafting: A new tool for the study of neurodegenerative diseases

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Summary. Transgenic and knockout mice have contributed much to our current understanding of the role played by single genes during development and in pathological processes of the CNS, such as neurodegeneration. However, embryonic lethality resulting from the disruption of important genes has often hindered the interpretation of such experiments. Grafting of immature cells from genetically modified organisms into healthy recipients promises to efficiently bypass this problem. We have used neural transplantation techniques which allow us to keep CNS tissue of knockout and transgenic mice viable for a prolonged period of time in the brain or in the kidney capsule of healthy recipients. We have characterized biological parameters such as growth, proliferation and differentiation and also the formation of an intact blood-brain barrier (BBB) after grafting of wild-type telencephalic anlage in this system. We have also employed this technique to study the long-term properties of neuroepithelial tissue derived from knockout mice. The results of our studies are discussed in the context of neurodegenerative diseases.

Key words: Adhesion molecule on Glia (AMOG/ß2), Alzheimer's disease, Blood-brain barrier, CNS development, Degeneration, Differentiation, Gene knockouts, Immunocytochemistry, Neu differentiation factor (NDF), Neural transplantation (mice), Prion protein (PrP), Proliferation, Scrapie, Transgenic mice

Introduction

More than 100 years have passed by since the first transplantation studies in the CNS of rodents were undertaken (for review see Das, 1990; Woerly and Marchand, 1990; Fisher and Gage, 1993). In a number of studies neural grafting has been used to address questions related to developmental neurobiology (Kromer et al., 1979; O'Leary and Stanfield, 1989; Renfranz et al., 1991; Fisher and Gage, 1993). Several studies investigated the establishment of neuronal organization within grafts and interactions with the host CNS (Lund and Hauschka, 1976; Kromer et al., 1979; Jaeger and Lund, 1980). More recently, grafting studies were aimed at questions related to neural plasticity. For example, it was asked whether and to what extent undifferentiated progenitor cells can integrate into and take part in the formation of the host CNS (Brüstle et al., 1995; Campbell et al., 1995; Gage et al., 1995). Other studies were undertaken to address questions related to tumorigenic potential of various oncogenes by grafting retrovirally transduced cells into the rodent CNS (Aguzzi et al., 1991; Brüstle et al., 1992; Wiestler et al., 1992).

In the field of neurodegenerative disorders, until now grafting studies have been mainly aimed at reconstituting certain pathways or particular functions after surgical or toxic lesions to selected functional systems (Dunnett et al., 1981; Dunnett, 1990; Lindvall, 1991; Fisher and Gage, 1993). In these models, an artificial lesion leads to degeneration of specific neuronal systems. Grafting of neural tissue or genetically engineered cells aims at functional repair of induced lesions (Dunnett, 1990). The vast majority of such experiments were carried out in the rat system which is well suited for developmental studies and allows stereotaxic surgical interventions with appropriate accuracy.

However, with the advent of transgenic techniques, it has become possible to study in more detail the role played by single molecules during development and in pathological processes in mice (for review see Aguzzi et al., 1994, 1995). More recently, the generation of knockout mice by targeted deletion of genes of interest (Thomas and Capecchi, 1987) has further broadened our insight into molecular mechanisms of neural development and pathogenesis of CNS diseases. A number of transgenic and knockout mice have delivered valuable models for neurodegenerative diseases (Büeler et al., 1993; Aguzzi et al., 1994; Games et al., 1995; LaFerla et al., 1995; Schenk et al., 1995). Others, however, show early postnatal (Klein et al.,

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1993; Magyar et al., 1994; Smeyne et al., 1994) or even embryonic (Bladt et al., 1995; Meyer and Birchmeier, 1995) lethal phenotypes which can be difficult to interpret. Although these models provide striking evidence for a crucial role of the respective gene products during development and hint towards an important role of these factors for the determination of cell fates during differentiation (Bladt et al., 1995; Gassmann et al., 1995; Lee et al., 1995; Meyer and Birchmeier, 1995), they do not allow the study of the role these factors play in secondary pathological processes such as neurodegeneration. In an effort to overcome this problem we have employed transplantation approaches for neural tissue derived from such mouse embryos. Using grafting techniques, it has been possible to study neural tissue of mice with premature lethal genotypes at time points exceeding by far the life span of the mutant mice (Isenmann et al., 1995, 1996a).

In this paper, we present a characterization of biological properties such as tissue growth, proliferation and differentiation in wild type neuroepithelial grafts. Special emphasis is laid on the development of the blood-brain barrier (BBB) after grafting. A further section will introduce transgenic and knockout mouse models for neurodegeneration. In these models, embryonic telencephalic tissue anlage has been successfully grafted to the caudoputamen and lateral ventricles of recipient mice in order to elucidate pathogenetic processes, and define molecular characteristics of respective tissue in vivo. We also delineate, as an alternative grafting approach, the technique of neural transplantation under the renal capsule. Finally, consideration is paid to the role of grafting strategies in the emerging field of ex vivo gene therapy for neurodegenerative disorders.

Neuroectodermal grafting to the caudoputamen

The grafting procedure is relatively simple (Isenmann et al., 1995, 1996a). Embryos are harvested from timed pregnant dams at defined stages at mid-gestation. Graft tissue is radiolabelled for later identification by autoradiography (Jaeger and Lund, 1980; Isenmann et al., 1996a) and injected into the caudoputamen or lateral ventricles of recipient mice using a stereotaxic frame (Aguzzi et al., 1991; Isenmann et al., 1996a). If histocompatible strains of mice are used, signs of graft rejection, such as lymphocytic infiltration and tissue necrosis, remain an exceptional finding and are detected in less than 5% of neural grafts (Isenmann et al., 1996a).

Growth, proliferation and differentiation of grafts

In an effort to determine the optimal time for embryonic tissue preparation and transplantation, we compared the final size of grafts resulting from tissue harvested at various embryonic stages. We found murine telencephalic tissue from embryonic day (E) 12.5 to reliably differentiate into large neural grafts which are suitable for detailed graft analysis. Tissue harvested at earlier embryonic stages often resulted in grafts containing non-neural tissue portions, since it was difficult to clearly separate mesenchymal tissue from the neural anlage at E9.5-E11.5. Such tissue portions induce permanent BBB leakage after grafting (Isenmann et al., 1996b) and were thus considered unfavorable. In contrast, neural tissue harvested at later embryonic stages (E13.5-E16.5) was easily separated from the meninges. However, proliferation and growth potential were markedly reduced, resulting in smaller transplants that were only partially accessible to thorough examination. Moreover, when tissue was harvested and transplanted at E12.5, the total number of neural grafts was higher than with tissue harvested at other embryonic stages (Fig. 1; Isenmann et al., 1996a).

Graft cell proliferation, as determined with immunocytochemistry to injected 5-bromo-2'-deoxyuridine (Gratzner, 1982) showed that proliferation indices of graft cells decreased sharply from initially 35% of grafted cells to around 5% during the second week after transplantation and less than 1% after more than seven weeks (Isenmann et al., 1996a). At the same time, differentiation of grafted cells proceeded to the terminal postmitotic state. Thus, mature neuroepithelial grafts contain neurons with myelinated processes and a dense synaptic network, glia (astrocytes, oligodendrocytes, and microglia), and blood vessels four weeks after grafting (projected age of grafted tissue: approx. P20) (Isenmann et al., 1995, 1996a). Taken together, these findings indicate that embryonic neuroepithelial tissue grafted

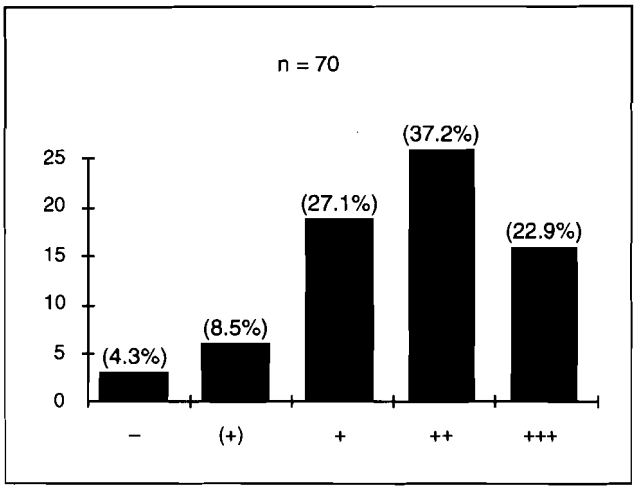

Fig. 1. Distribution of graft size from 70 neuroectodermal tissue anlagen transplanted at E12.5. Bars show the distribution of the graft size at histological analysis. y-axis: absolute numbers of grafts in each group; numbers on bars (in brackets): percentage. Graft size: -, no graft detectable; (+), needle track and/or glial scar and only few grafted cells; +, graft size 100-200 x 200-400 μm, detectable on more than 20 adjacent sections; ++, size between + and +++; +++, graft size larger than 1mm^2, and detectable on more than 100 adjacent sections.

into an adult host brain follows a program of maturation and differentiation similar to the in vivo time course (Isenmann et al., 1996a).

Blood-Brain Barrier (BBB)

The BBB maintains the homeostatic environment in the brain by preventing blood-borne compounds from free entry into the CNS parenchyma. The barrier is formed by tight junctions in the vascular endothelia which are probably induced by astrocytes (Janzer and Raff, 1987; Risau and Wolburg, 1990). A number of pathological CNS processes, such as inflammation, demyelination, tumor or degeneration can induce breakdown of the BBB. In turn, BBB leakage may induce CNS dysfunction caused by blood-borne neurotoxic compounds normally excluded from the brain parenchyma (Svendgaard et al., 1975; Rosenstein and Brightman, 1983).

Various investigators have reported controversial findings on the post-transplantation status of the BBB in rodents. An early, yet most valuable study has suggested that the type of donor tissue determines the characteristics and BBB properties of graft-supplying vessels (Stewart and Wiley, 1981). According to this hypothesis, neural grafts would be expected to induce BBB properties in the supplying blood vessels. In fact, several authors have described a complete BBB reconstitution after neural grafting to the CNS. Some even find no residual BBB leakage as early as one week after grafting (Broadwell et al., 1989, 1990; Swenson et al., 1989; Bertram et al., 1994). Other studies, however, have claimed the BBB to remain permanently disrupted after neural grafting to the CNS (Rosenstein and Brightman, 1986; Rosenstein, 1987). Our group carried out careful studies in the model described using four independent marker molecules to detect damage to the BBB (Isenmann et al., 1996b). The results obtained with various techniques were surprisingly consistent, with minor variations owing to variable sensitivity of individual techniques rather than differing findings. Magnetic resonance imaging (MRI) using a contrast agent in vivo (Fig. 2) indicates, in agreement with the histological techniques employed, that in our paradigm, i.e. grafting of tissue fragments as opposed to single cell suspensions, the BBB is reconstituted in 67% of all grafts after 3 weeks, and in 90% of the grafts seven weeks after grafting (Isenmann et al., 1996b). These findings are particularly important with respect to the exploitation of neurografting techniques in neurodegenerative diseases. They indicate that the grafting procedure as such does not induce permanent BBB leakage that might expose the grafted tissue to a non-physiological environment. Moreover, our data suggest that the genotype of the grafted tissue determines the BBB properties of the graft. Thus, a pathological condition exclusively affecting the graft can result in secondary BBB disruption (our unpublished data).

Grafting in Models of neurodegenerative diseases

Adhesion molecule on glia (AMOG/ß2)

Adhesion molecule on glia (AMOG) was first described as a Ca^{2+} independent recognition molecule expressed mainly by astrocytes (Antonicek et al., 1987).

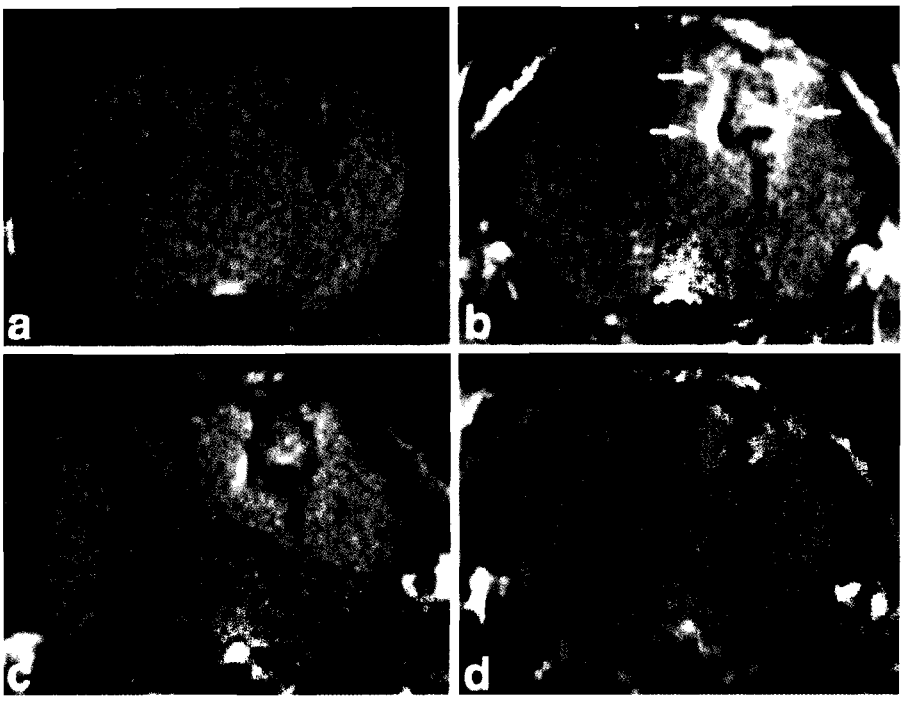

Fig. 2. Repetitive contrast enhanced MRI scanning for the evaluation of the post-transplantation status of the BBB (for technical details see Isenmann et al., 1996b). **a.** T1 weighted image showing tissue graft in the left cortex and striatum 20 days after transplantation. **b-d.** Contrast enhanced T1 weighted images 20 (b), 36 (c), and 70 days (d) after transplantation. Signal enhancement indicating BBB damage is readily detected 20 days after grafting (b), but decreases markedly within the following weeks (c, d). x 10

However, it also represents as the ß2-subunit of the membrane Na,K-ATPase an integral part of an enzyme that regulates ion homeostasis of cells (Gloor et al., 1990). Hence, the term AMOG/ß2 was coined. Knockout mice for the molecule were generated on the assumption that these animals would show defects of cell migration in the CNS, and give a clue as to how cell-cell interaction mechanisms might be coupled to ion homeostasis in the CNS. However, mice deficient for AMOG/ß2 are born normal and do not show any behavioral abnormalities during the first two weeks after birth (Magyar et al., 1994). Surprisingly, at postnatal day P16 they become weak, start to tremble and die shortly thereafter. The predominant neuropathological findings are vacuolation and gliosis in defined parts of the brain (Magyar et al., 1994). These changes were interpreted as tissue alterations leading to death by acute brain stem dysfunction. The aspect of the alterations was reminiscent of early signs of CNS degeneration which could proceed to overt neurodegeneration provided the tissue was allowed to survive for extended periods of time. We transplanted telencephalic anlage derived from AMOG/ß2 deficient mouse embryos into the brain of wild type mice in order to follow the presumptive neurodegenerative process to more advanced states in the graft situation (Isenmann et al., 1995). Surprisingly, such grafts remained viable for 500 days, the end point of the analysis, without showing any histological changes. No spongiosis or pronounced gliosis were apparent and expression of a variety of neural and glial markers was unchanged when compared to neural grafts derived from wild-type littermates. However, we found that graft-borne astrocytes do not express the ß1 nor the AMOG/ß2 subunit of the membrane ATPase at detectable levels (Fig. 3; Isenmann et al., 1995). Thus, these cells must employ an alternative way of maintaining ionic homeostasis without Na,K-ATPase ß subunits. Na,K-ATPase α subunits have been shown to depend on a ß subunit for intracellular processing and enzymatic activity (Geering, 1991). Graft-derived astrocytes expressing neither the ß1 nor the AMOG/ß2 subunit of the Na,K-ATPase might maintain their ion homeostasis by forming a functional ion pump composed of other, yet unidentified combinations of subunits. These findings have implications for the concept of differential regulation of subunits of heteropolymeric transmembrane molecules during development (Becker et al., 1993; Isenmann et al., 1995).

Spongiform encephalopathies

Spongiform encephalopathies such as Creutzfeldt-Jakob Disease in humans or scrapie in sheep and mice are characterized by neuronal loss, spongiosis of the neuropil, astrogliosis and, as a hallmark of the disease, plaques containing PrP^{Sc}, a pathological isoform of the physiologically occurring PrP^{C} (Prusiner, 1991). In contrast to PrP^{C}, which naturally occurs in α-helix conformation, PrP^{Sc} forms ß-sheets and, as a consequence, is protease-resistant. In the past years, a multitude of transgenic mouse models has shed light on the pathogenesis and transmission characteristics of scrapie prions (DeArmond and Prusiner, 1995; Telling et al., 1995). One of the most interesting findings is that PrP-deficient mice are resistant to inoculation with scrapie prions and do not propagate scrapie prions (Büeler et al., 1993; Sailer et al., 1994).

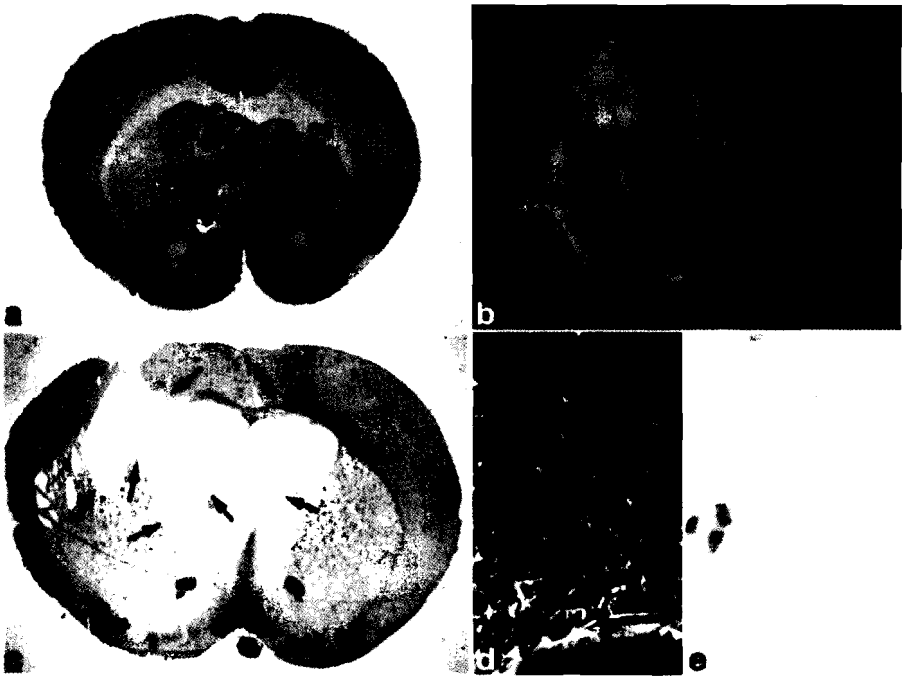

Fig. 3. AMOG/ß2$^{o/o}$ telencephalic graft. **a.** Hematoxylin and Eosin stain of an AMOG/ß2$^{o/o}$ telencephalic graft located in the lateral ventricles and adjacent striatum (*) twelve months after grafting. x 10. **b.** T2 weighted MRI scan 8 months after grafting showing the central graft and a cortical graft portion. x 10. **c.** Immunocytochemistry to AMOG/ß2 showing widespread expression in the host cortex and caudoputamen, but sparing of the graft. x 12. **d and e.** Labeling of the graft for glial fibrillary acidic protein (GFAP, shown in d) and for ß1 (shown in e. x 320. **d.** GFAP immunocytochemistry demonstrates the presence of activated astrocytes within the graft; in situ hybridization for ß1 of the same graft area (e) shows a complete lack of hybridization signal at the sites of the astrocytes; strong signals derive from large graft-borne neurons. co: cortex; cc: corpus callosum; cp: caudoputamen.

However, the mechanisms leading to scrapie pathology are not fully understood. To address this question, we grafted neural tissue of mice over-expressing PrP^C (tga20; Fischer et al., 1996) into PrP-deficient mice. The idea was to examine whether after inoculation of grafts with scrapie prions chronic exposure of PrP^C-deficient host CNS with PrP^{Sc} would provoke scrapie pathology in the knockout tissue (Brandner et al., 1996). As expected, grafts displayed severe histopathological changes. In addition, proteinase-resistant PrP was transported to distant sites of the host brain and formed deposits. Interestingly, no scrapie-associated pathological changes could be detected in the PrP-deficient host brain as late as 16 months after infection. These findings indicate that in addition to being resistant to scrapie infection, CNS tissue devoid of PrP^C is not damaged by chronic exposition to exogenous PrP^{Sc} (Brandner et al., 1996). Furthermore, the grafting approach has allowed us to examine the long-term fate of scrapie-infected neuroectodermal tissue derived from tga20 mice. Normally, such mice die 60 days after infection. In the graft setting, however, it was possible to examine the histopathology after more than seven times this period. The tissue changes found 70 weeks after scrapie inoculation indicated that neuronal damage initially leads to vacuolation and spongiosis. As the pathological process advances, astrogliosis gets more pronounced and results in increased cellularity with basically all cells being of glial origin, and with no neurons left (Brandner et al., 1996).

Alzheimer's disease

Alzheimer's disease is the most common cause of dementia in people aged over 60 years. The histopathological findings are characterized by neuronal loss, amyloid plaques and neurofibrillary tangles. Plaques consist of pathological extracellular deposits of ßA4 protein, a cleavage product of the amyloid precursor protein (ß-APP), which is a physiological transmembrane protein (for review see Selkoe, 1994). Many authors believe plaque deposition to be causally involved in the pathogenesis of the syndrome. This hypothesis is supported by the finding of mutations in the APP gene in familial forms of the disease (Goate et al., 1991). Until recently, no transgenic model was available to remodel the histopathology of the condition in mice.

In an attempt to model plaque formation in a mouse paradigm, Richards and coworkers grafted embryonic hippocampal tissue derived from mice carrying a trisomy 16 into wild type mice (Richards et al., 1991). Several genes located on chromosome 21 in humans, among them APP, map to mouse chromosome 16. In addition, all human individuals with a trisomy 21 come down with Alzheimer's pathology when they reach more than 40 years of age. In order to detect pathology in the CNS tissue of mutant mice, grafting was necessary since the respective mice die during late gestation, before any histopathology can be detected. Interestingly, grafts from trisomy 16 mutant mice show deposits resembling amyloid plaques (Richards et al., 1991). T. Bayer and colleagues have employed a more straightforward approach in rats: they have infected embryonic CNS tissue in vitro with retroviruses coding for a human APP gene carrying a mutation which has been described in a Swedish kindred with a familial form of the disease (Mullan et al., 1992). Grafting such cells into the CNS of adult rats results in neural grafts consisting of transduced cells. In a preliminary study, expression of the transgenic protein was detected for more than six months after transplantation. At this stage, neurons displayed signs of degeneration. Moreover, ßA4 immunoreactive plaque-like extracellular deposits were seen (Bayer et al., 1995). Further studies are underway and might offer more insight into the process of amyloidogenesis and plaque deposition. In addition, transgenic mice have now been described which express yet another mutated allele of the human APP gene isolated from another affected family (V717F; Murrell et al., 1991). These mice develop spontaneous Alzheimer-like pathology (Games et al., 1995). They might be useful for grafting approaches in order to restore function or interfere with amyloidogenesis and plaque deposition using ex vivo gene transfer (Fisher and Ray, 1994; Isenmann et al., 1996c).

In a completely different set of experiments, grafting approaches have been used in attempts to rescue neurons in animal models for neurodegenerative diseases (for review see Dunnett, 1990; Lindvall, 1991). Thus, a surgical lesion to the fimbria fornix leads to degeneration of cholinergic neurons in the medial forebrain due to deprivation of their neurotrophic supply. This process of neurodegeneration is regarded as a rodent model for Alzheimer's disease. In this setting, transplantation studies have aimed at replacing cholinergic neurons (Dunnett, 1990). Alternatively, fibroblasts genetically engineered to produce nerve growth factor (NGF) have been transplanted to replace the surgically ablated neurotrophic supply in an attempt to rescue these neurons (Rosenberg et al., 1988; Fisher et al., 1991; Hoffman et al., 1993).

Parkinson's disease

Parkinson's disease is caused by a loss of dopaminergic neurons in the substantia nigra that leads to a movement disorder characterized by rigor, tremor and hypokinesia. The underlying cause of neurodegeneration is still poorly understood. Hence, genetically-defined animal models are not available. However, administration of neurotoxic substances such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridin (MTPT) causes a syndrome which pathologically and clinically resembles Parkinson's disease in primates and rodents. This model is often used to evaluate therapeutic approaches to Parkinson's syndrome using grafting

techniques (Perlow et al., 1979). A variety of cell types genetically modified to produce tyrosine hydroxylase (TH), the critical enzyme needed for dopamine production, have been used successfully for studies in chemically-induced models of the syndrome (Wolff et al., 1989; Fisher et al., 1991; Jiao et al., 1993). Alternatively, chromaffin cells and dopamine-producing embryonic cells have been used successfully in humans suffering from Parkinson's syndrome (Freed et al., 1992; Spencer et al., 1992; Widner et al., 1992). However, human fetal tissue is available only to a limited extent, and its therapeutic use in Parkinson's has caused ethical controversies, and legal restrictions might follow (Garry et al., 1992; Kassirer and Angell, 1992). Therefore, future therapeutic grafting strategies for neurodegenerative disorders will largely depend on gene transfer techniques. Transfer of appropriate genes to suitable cells ex vivo followed by transplantation or even direct stereotaxic injection of appropriate viral vectors in vivo could offer new therapeutic modalities to a multitude of patients suffering from neurodegenerative disorders (for review see Fisher and Ray, 1994; Isenmann et al., 1996c). To this end, several studies have already been undertaken using neural progenitor cells transduced by recombinant adenoviruses (Le Gal La Salle et al., 1993; Sabatè et al., 1995). As an alternative approach recombinant adenovirus (Akli et al., 1993; Bajocchi et al., 1993; Davidson et al., 1993) or adeno-associated virus (Kaplitt et al., 1994) have been used for direct gene transfer to the rodent brain in vivo. While such strategies can be utilized to produce deficient factors (such as Dopamine or TH) to replace endogenous cell functions, grafts can also be engineered to produce neurotrophic factors such as BDNF and GDNF in order to enhance survival of endogenous dopaminergic neurons (Frim et al., 1994; Tomac et al., 1995).

Grafting under the renal capsule

For studies aiming at examining neurodegenerative phenotypes in animal models, an alternative graft location is underneath the kidney capsule. After a lateral flank incision, the kidney can be easily mobilized and tissue introduced through a glass capillary via a small incision in the capsule (Tam, 1990). Under certain circumstances, this graft location can offer several advantages over the telencephalic location. It is not necessary to pre-label the graft tissue since grafted neural tissue can be easily identified and histologically delineated from the host kidney and perirenal tissue. Moreover, in case of knockout graft tissue that is deficient for soluble factors (e.g. receptor ligands) mainly present in the CNS, graft tissue located distant from the host CNS can be easily prevented from contact with the respective host-borne molecule. We have tested the feasibility of this technique for neural tissue and found it to be suitable for grafting studies where the aim is to keep graft tissue viable without aiming at connections with the host CNS. Grafting of telencephalic anlage resulted in large neuroectodermal grafts showing characteristic cellular composition of CNS tissue (Fig. 4). Moreover, we were able to successfully graft spinal cord tissue, spinal ganglia, and skeletal muscle to this location (not shown).

Neu Differentiation Factor (NDF)

Neu differentiation factor (NDF) is a ligand for members of the epidermal growth factor (EGF) receptor family (Peles et al., 1992). NDF, also known as

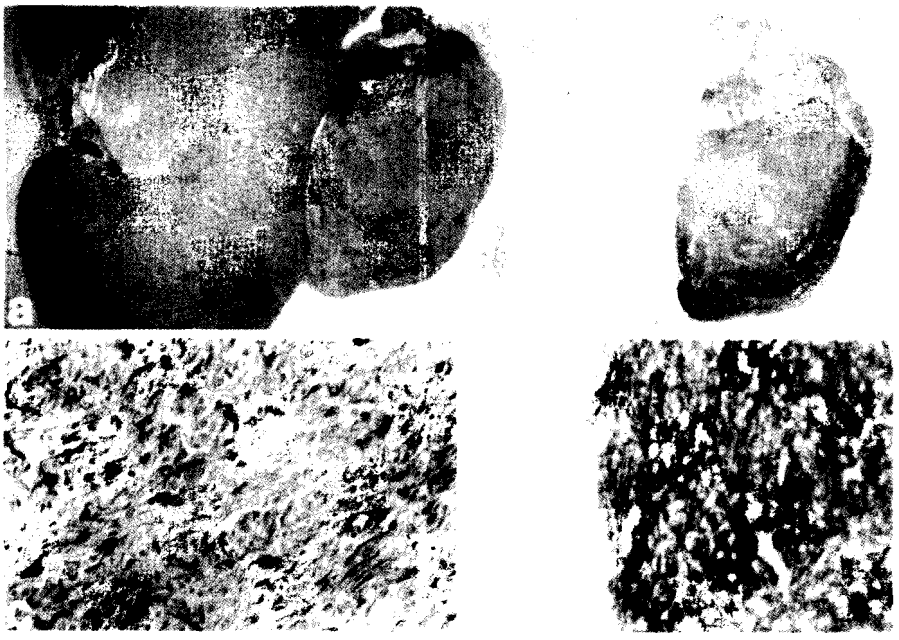

Fig. 4. Mature wild type CNS tissue graft located under the renal capsule. **a.** Hematoxylin and Eosin (H&E) stain of a telencephalic transplant grafted at E 10.0 and analyzed 20 days after grafting. x 8. **b.** Adjacent section stained with an antibody to synaptophysin. Immunoreactivity is restricted to the neural graft. x 8. **c.** Immunocytochemistry to GFAP showing mild astrogliosis in the graft. x 320. **d.** Immunocytochemistry to synaptophysin (detail from (b)). x 320. k: host kidney; g: graft.

neuregulin, heregulin, glial growth factor (GGF), and acetylcholine-receptor inducing activity (ARIA) can induce proliferation and differentiation in cultured epithelial, glial, and muscle cells (Wen et al., 1992; Falls et al., 1993; Marchionni et al., 1993). NDF has been shown to play an important role for survival and differentiation of various glial cell populations in vitro. Thus, it prevents apoptotic death and induces maturation of Schwann cell precursors (Dong et al., 1995), enhances the development of oligodendrocytes from O2A progenitor cells (Vartanian et al., 1994), and is ascribed the role of a survival factor for astrocytes (Pinkas-Kramarski et al., 1994). The factor exerts its biological functions through the tyrosine kinase receptors erbB2, erbB3, and erbB4. Heterodimerization of erbB2 with either of the other two receptors seems to be a prerequisite for NDF signaling (Carraway and Cantley, 1994). To investigate the developmental role of the neuregulin system in vivo, knockout mice have been generated for NDF (Meyer and Birchmeier, 1995), erbB2 (Lee et al., 1995) and erbB4 (Gassmann et al., 1995). Each of the mutations shows an embryonic lethal phenotype at E 10.5. Apparently, embryonic death occurs due to cardiac dysfunction caused by a lack of

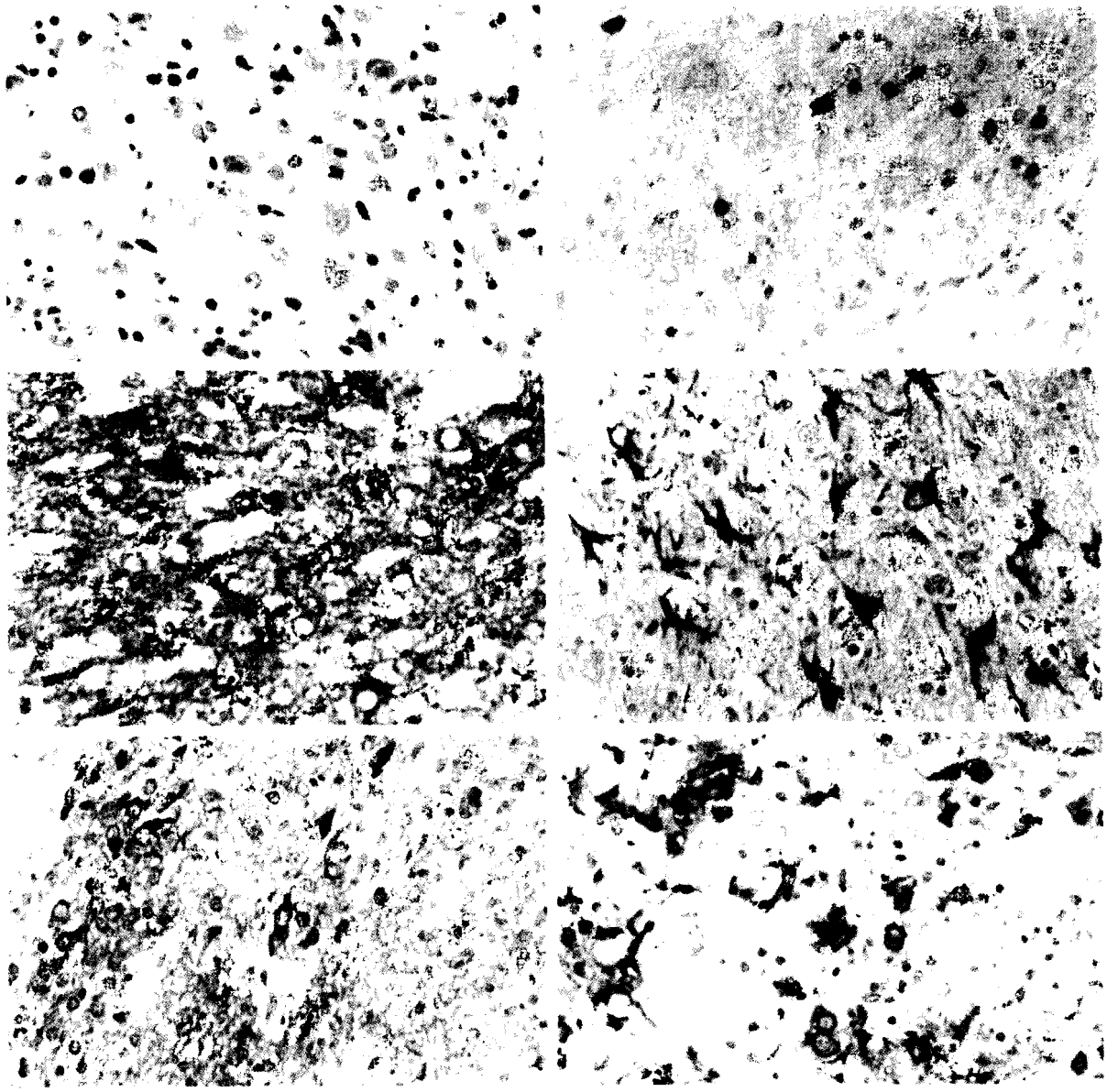

Fig. 5. NDF$^{o/o}$ graft under the renal capsule. **a.** H&E stain of a telencephalic transplant grafted at E10.0 and analyzed 5 weeks after grafting. Morphologically, large neuronal and smaller glial cell nuclei can be differentiated. **b and c.** Synaptophysin (b) and NSE (c) immunocytochemistry reveal mature CNS neurons forming a dense synaptic network (b) and some cytoplasmic staining. **d.** Immunohistochemistry for S-100 showing stained nuclei of glial cells. **e.** Immunohistochemistry for GFAP. Astrocytic density is comparable to that of wild-type grafts (cp. Fig. 4c). **f.** Immunocytochemistry for the MHC class II antigen M5/114 (for details see Isenmann et al., 1996a). Mainly perivascular microglia are stained. x 400

trabeculation in the myocardium (Gassmann et al., 1995; Lee et al., 1995; Meyer and Birchmeier, 1995). Interestingly, all three mutations also result in abnormal neural development. Null mutations of either NDF or erbB2 result in defects in cranial ganglia, where Schwann cells fail to develop (Lee et al., 1995; Meyer and Birchmeier, 1995), whereas erbB4 mutants reveal a role for the factor in axonal guidance (Gassmann et al., 1995).

To test the long-term effects of NDF deficiency on differentiation and survival of glial cells in the CNS in vivo, we have grafted CNS anlage of NDF deficient ($NDF^{o/o}$) embryos isolated at E 10.0 under the renal capsule of wild type mice and analyzed them up to six weeks after grafting (Fig. 5). Both wild type and $NDF^{o/o}$ grafts grew to form large transplants (>4x4x3mm). Using immunohistochemical methods we detected astrocytes and oligodendrocytes, in addition to mature CNS neurons, in $NDF^{o/o}$ grafts (Fig. 5). Grossly, the number and distribution of glial cells did not differ from that observed in wild type grafts (cp. Fig. 4), indicating that glial cell types can differentiate and survive in mature CNS grafts even in the absence of endogenous NDF. These findings suggest that in $NDF^{o/o}$ grafts factors other than NDF itself exert signals to induce glial cell survival and differentiation in neural progenitor cells.

Perspectives

Intracerebral grafting has proved to be a valuable tool for the extended study of neuroectodermal tissue derived from transgenic and knockout mice in which the genetic alteration leads to premature death of such animals. Important biological parameters of such grafts have been carefully characterized to provide baseline information on graft proliferation, differentiation, and the post-transplantation status of the BBB. For grafting to the host CNS, the caudoputamen and lateral ventricles offer a physiological environment for the developing graft tissue. Full exploitation of this technique will offer a chance to examine the role played by single genes in neurodegenerative processes, in addition to the discovery of their function during development, which can more directly be examined in mutant embryos. Moreover, the technique offers the opportunity to study interactions of CNS tissues of differing genotypes in vivo. Under certain circumstances, grafting under the renal capsule can be an attractive alternative to grafting to the caudoputamen for this type of study.

As indicated for Alzheimer's and Parkinson's disease models, in addition to being an "in vivo culture system" for neuroectodermal tissue, grafting techniques will be of even more profound value for establishing ex vivo gene therapy approaches in animal models for neurodegenerative disease. Such approaches might one day lead to the establishment of new therapeutic standards for patients suffering from neurodegenerative diseases (see Fisher and Ray, 1994; Isenmann et al., 1996c).

Acknowledgments. Our work is supported by a postdoctoral fellowship from the Boehringer Ingelheim Foundation to S.I. and by grants of the European Union BIOMED program (BMHI-CT93-1142), by the "Nationales AIDS-Forschungsprogramm der Schweiz" (no. 31-36059.92), the Schweizerischer Nationalfonds (NFP38) and the Schweizer Krebsliga to A.A.

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