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STRATEGIES FOR THE AUGMENTATION OF GRAFTED DOPAMINE NEURON SURVIVAL

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1. ABSTRACT

The percentage of grafted embryonic DA neurons that survive transplantation is low, estimated at 5-20%. Significant agreement has emerged from the work of research groups worldwide that specific conditions associated with the transplant procedure and post-transplantation interval render grafted mesencephalic cells susceptible to apoptotic death. Detrimental triggers including hypoxia/ischemia, trophic factor withdrawal, and oxidative stress appear to exert the most impact on grafted DA neuron survival. Treatment strategies that aim to reduce or eliminate the triggers of grafted cell death appear to be more successful than approaches that target the intracellular apoptotic cascade. In particular, treatment of mesencephalic cell suspensions with isolated neurotrophic factors (GDNF, BDNF, NT 4/5) as well as glial-derived factors, antioxidant therapies and augmentation of graft vasculature have demonstrated consistent survival promoting effects. Caspase inhibition, although initially quite promising, has not been demonstrated to reliably increase grafted cell survival. Bcl-2 overexpression similarly has yet to prove beneficial, although this may be due to biologically irrelevant levels of bcl-2 present during the critical immediate post-grafting interval. Future strategies will target a "cocktail" approach in which effective treatment agents are combined to maximize grafted DA neuron survival. Refinements in *ex vivo* transduction parameters will allow for efficient sustained delivery of survival promoting agents to grafted cells. Once identified, the optimal survival-enhancing treatment of grafted primary embryonic DA neurons should also benefit future transplant therapies utilizing alternatively derived DA neurons.

2. TRANSPLANTATION FOR PARKINSON'S DISEASE

Parkinson's disease (PD) is a chronic neurodegenerative disorder that affects approximately 1-% of the population over the age of 65 (1). The disease is caused by the specific degeneration of the dopaminergic neurons of the substantia nigra pars compacta which is likely due to cumulative effects of genetic and environmental factors. The changes in motor function symptomatic of PD are not usually apparent until dopamine (DA) levels in the striatum have dropped to less than 20% of normal (2).

Treatment strategies for PD can be generally categorized into two types: ablative procedures and augmentative strategies. Ablative procedures include surgical pallidotomy, electrical stimulation therapy, and the use of glutamate receptor antagonist drugs. These deletion approaches are aimed at diminishing the activity of portions of the basal ganglia circuitry that become overactive in response to striatal DA depletion, thereby ameliorating behavioral symptoms. The long-term efficacy of these techniques remains to be demonstrated, and there is no guarantee that the neural plasticity responsible for this overactivity will not reestablish itself to yield further changes that may result in diminished therapeutic benefit.

Augmentative strategies such as gene therapy and transplantation of DA neurons are directed at restoring the DA neurochemistry that is lost in the disease, returning brain function to the state that existed prior to the onset of symptoms of PD. The original augmentative approach is still the standard therapy for PD: oral administration of a combination of levodopa and carbidopa (3). While this pharmacotherapy provides dramatic improvement of symptoms for several years, efficacy wanes as more and more nigral neurons degenerate, and unwanted side-effects of poorly regulated excess DA become problematic (4). In

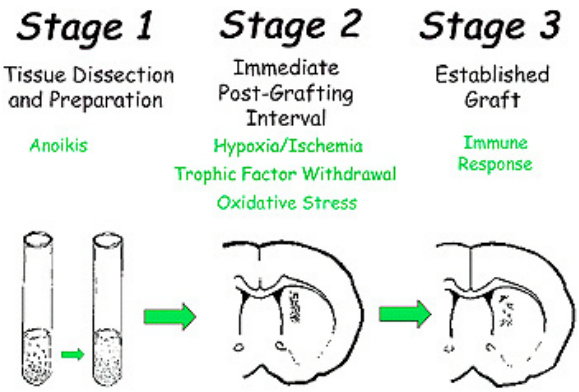

Figure 1. Cell preparation/grafting conditions that can trigger grafted cell death

addition, evidence suggests that loading the system with DA, without replacing lost neurons, may elevate the amount of DA in the microenvironment to toxic levels which contributes to the degeneration of the few remaining nigral neurons (5,6).

Transplantation of embryonic neurons incorporates the advantages of levodopa therapy, replacing DA lost in PD, but performs this function by also replacing DA neurons. Physical replacement of the cellular population lost in the disease provides the potential for regulated release of DA, buffering the toxic effects of DA in the microenvironment by replenishing the complement of DA terminals, and represents a therapy that is theoretically good for the lifetime of the patient. Many years of successful research on neural grafting in animal models of PD (7,8) have led to several clinical trials worldwide (9-11). Findings in animal models have had significant predictive value. The survival of large numbers of grafted DA neurons has been verified in some PD patients (12,13), serial PET scans show long-lasting increases in striatal DA tone following transplants (13-16), and meaningful clinical benefit has been reported (17). However, recent results indicate that transplantation of mesencephalic DA neurons may also produce unwanted side effects (10) and may be of diminished efficacy in older patients. Only patients under 60 experienced significant improvement in standardized measurements of PD and 15% of the grafted patients experienced debilitating “runaway dyskinesias”. This study was unique in terms of its surgical approach and preparation of tissue for grafting. It is possible that these side effects are related to these parameters. Nonetheless, these findings should compel basic researchers to carefully examine potential behavioral side effects after transplantation.

3. GRAFTED DA NEURON DEATH

Despite encouraging findings from the majority of clinical trials for PD, significant problems still exist for the therapeutic application of DA neuron grafting. Problems concerning the biology of these implants center upon survival of the grafted cells. The percentage of grafted embryonic DA neurons that survive transplantation is low, estimated at 5-20% (8,12,18), requiring implantation of tissue from many donors to achieve even moderate therapeutic benefit. Until relatively recently, it was assumed that cell death in embryonic mesencephalic grafts primarily was necrotic, i.e. the result of damage to cells during tissue dissection or transplantation. However, Mahalik and colleagues (19) revealed that apoptosis occurs at early times following grafting in mesencephalic tissue grafts. Apoptosis differs from necrotic cell death in that it is the result of a genetically driven death program, instead of physiologic insult or injury. During apoptotic cell death nuclear chromatin condenses, the plasma membrane blebs, yet remains intact, and neatly compartmentalized cell fragments break down into apoptotic bodies. Biochemically, endonucleases generated during apoptotic cell death produce a particular "laddering" pattern of internucleosomal DNA fragmentation. In contrast, during necrotic cell death the cell membrane is compromised allowing cellular contents to leak out, nuclear chromatin remains unchanged and DNA degradation follows a random pattern (20-22). A variety of signals in the cell’s environment can trigger an intrinsic cell "suicide" program (20).

In our laboratory, we have observed a high rate of apoptosis (TUNEL-positive nuclei) in mesencephalic suspension grafts during the first week after implantation (23-25). Cell death by either apoptosis or necrosis drastically reduces the yield of viable grafted neurons, however, the significant contribution of apoptotic cell death offers specific avenues for intervention. Significant agreement has emerged from the work of research groups worldwide that specific conditions associated with the transplant procedure and post-transplantation interval render grafted cells susceptible to apoptotic death. A summary of these threats to grafted neuron survival is presented in figure 1.

The triggers for apoptosis in grafted mesencephalic cells are best understood if the transplantation process is divided into three stages. During Stage 1 -Tissue dissection and preparation, it has been demonstrated that the dissociation procedure itself is enough to trigger apoptosis in mesencephalic cell suspensions, indicating that a large population of DA neurons appear already committed to die prior to implantation (26). This may include apoptosis induced by disruption of cell-cell interactions or cell-extracellular matrix interactions. Detachment from the extracellular matrix, termed "anoikis", has been demonstrated to induce apoptosis in other cell types (27).

In the Stage 2 - Graft environment, is likely analogous to what is experienced in the brain during ischemic insult, where access to blood-borne nutrients (including oxygen) is denied. During ischemia a slow wave of apoptotic-like, caspase-mediated cell death is observed outside the most severely effected area, within the penumbra (28-32). Indeed, a procedure that maximizes access of grafted cells to host blood vessels, has been reported to enhance grafted DA neuron viability nearly three-fold (33). Immediately following transplantation, grafted cells are dependent upon diffusion of oxygen and

blood borne material from the host vasculature at the graft periphery until they establish circulation with the host brain. Fetal mesencephalic cell grafts are initially avascular. Depending on the methodology, the transplantation procedure partially or completely destroys the immature vascular network and must therefore be reconstructed. In rats, the peak of growth of the host and donor vessels occurs at approximately three days post-transplantation (34). Nevertheless, circulation with the host is not completely developed until more than one week after transplantation (35-38).

During Stage 2, oxidative stress in the graft environment, generated by hypoxia and metabolism of DA, also has the potential to trigger apoptosis. Normal metabolism of DA by monoamine oxidase, reaction of DA with free oxygen, and the presence of molecular iron in the microenvironment of DA neurons all lead to generation of potentially toxic levels of reactive oxygen species (39). Oxidants have been reported to directly induce apoptosis (40-42) and antioxidants can protect against apoptosis (40,43,44).

Apoptotic cell death of grafted cells during Stage 2 can also occur in response to trophic factor withdrawal. A variety of evidence indicates that many of the molecules important for DA neuron survival and growth are down-regulated or absent in the mature host striatum (45). It has been demonstrated in other neural systems that failure of a neuron to access these target-derived neurotrophic factors leads to apoptosis. In the nigrostriatal system, trophic factors derived from the striatal target may control the extent of programmed cell death of nigral DA neurons during development. Apoptotic cell death normally occurs in the substantia nigra in the form of two peaks, from E21-P6 and on P14 (46,47) and is further augmented if dopaminergic terminals or striatal target cells are lesioned (48,49). These studies illustrate that DA neurons of the substantia nigra have a period during development when they are dependent on the striatal target for protection from apoptosis.

The last stage of cell death following transplantation, Stage 3 - Established Graft, is characterized by a relatively small population of dying DA neurons. Work in our laboratory and others illustrates that after the first week following grafting (23,50,51), DA neuron survival of allografts remains relatively stable. The exception to these findings is in the case of xenografts, where a sustained immune response triggers apoptotic cell death during the weeks following, during Stage 3 (51). Given the massive cell loss occurring during Stages 1 and 2, these stages have been the target intervals for interventions leading to substantive increases in grafted DA neuron survival. The remainder of this chapter will address some of the progress that has been made to date in this field.

The majority of transplantation research today focuses on transplantation of DA neurons generated from alternative sources, not from primary mesencephalic tissue. Collection and storage of donor tissue from multiple embryos of varying gestational stages results in a grafting procedure that is difficult to standardize both in quantity and purity, resulting in the potential for variable outcome (52). In addition, the harvest of human embryonic tissue is fraught with ethical and political concerns (53,54). However, agents that generate positive results utilizing grafts of primary DA neurons should also be beneficial when applied to DA neurons derived from stem and progenitor cells. Newly grafted cells experience similar insults irregardless of their origin. Meanwhile, consistent production of DA neurons from stem cell sources that retain their phenotype after transplantation has yet to be demonstrated. Implantation of primary mesencephalic DA neurons offers researchers a reliable and well-established model to continue to investigate strategies to augment graft survival, strategies that will be applicable to alternatively derived DA neurons once their full potential has been successfully realized.

4. TREATMENT APPROACHES TO REDUCE TRIGGERS OF GRAFTED CELL DEATH

4.1. Trophic factors

Many experiments have convincingly established that exposure of intrastriatal DA grafts to a number of identified and unidentified trophic factors (by various methods of delivery) can significantly increase the survival of grafted DA neurons (table 1). However, although the increases observed have been significant, most reports indicate that typical trophic factor augmentation results in overall survival rates of approximately 10-20% (45,55-62) with two reports demonstrating increases to 30% (63,64). This doubling or tripling of DA neuron survival observed after trophic factor exposure is relatively modest when one recognizes that 70-80% of the grafted DA neurons still do not survive transplantation.

Several identified neurotrophic factors have been investigated for their potential to improve embryonic mesencephalic DA neuron survival in culture and following transplantation. Of those trophic factors studied, glial cell line-derived neurotrophic factor (GDNF), brain derived neurotrophic factor (BDNF) and neurotrophic factor-4/5 (NT-4/5) have emerged as the most promising (45). A number of unidentified mesencephalic glia-, striatal- and striatal glia-derived trophic factors also have been shown to have powerful survival and growth-promoting effects on developing DA neurons (62,65-69). The optimal factor or factors for support of embryonic DA neurons may have yet to be identified. Clearly trophic factor support alone during Stage 1 and Stage 2 has not been demonstrated to provide the degree of improvement in grafted DA neuron survival necessary to make transplantation for PD a viable therapeutic strategy. Findings from trophic factor support studies suggest that the overwhelming majority of grafted cells need nutritive assistance beyond trophic factors.

4.2. Antioxidants

Oxidative stress in the graft environment, generated by hypoxia and metabolism of DA, also has the potential to trigger apoptosis. This hypothesis has been strengthened considerably by grafting studies utilizing mice

Table 1. Increase in Grafted DA Neuron Survival

Factor	Increase in Grafted DA Neuron Survival	Reference
NTN	5-10% → 9-18%	61
GDF-5	5-10% → 9-18%	60
GDNF	5-10% → 13-30%	55, 58, 60, 64
bFGF	5-10% → 10-34%	56, 63
bFGF/GDNF/IGF-I	5-10% → 7-15%	59
Schwann	5-10% → 9-18%	111
Striatal	5-10% → 9-18%	57, 62

NTN = neurturin, GDF-5 = growth/differentiation factor-5, GDNF = glial cell line-derived neurotrophic factor, bFGF = basic fibroblast growth factor, IGF-I = insulin-like growth factor –I, Schwann = schwann cell co-grafts, striatal = striatal co-grafts

enetically engineered to overexpress Cu/Zn superoxide dismutase (SOD), an enzyme critical to the detoxification of oxygen free radicals. Survival of grafted mesencephalic tyrosine hydroxylase immunoreactive (THir, a marker for DA neurons) neurons from Cu/Zn SOD overexpressing transgenics was 4 times higher than non-transgenic littermates (70). This finding led to an initial study utilizing two different lazaroids, antioxidant compounds that inhibit lipid peroxidation (71). These antioxidants exhibited virtually identical survival promoting effects when included in the dissociation and transplantation procedure of mesencephalic cell suspension grafts to rats, an increase of 160% more THir neurons over control was observed (71).

Several subsequent studies have evaluated the effect of the lazaroid tirilazad mesylate (72,73) on grafted DA neuron survival. Tirilazad mesylate has undergone clinical safety testing and therefore has become an attractive lazaroid candidate for therapy. Tirilazad mesylate was found 1) to increase THir neuron survival in an *in oculo* grafting model (74), 2) to generate THir neuron survival rates equivalent to fresh controls when included in hibernation medium prior to implantation (72), and 3) to increase the survival of grafted human mesencephalic THir neurons by 90% when included during dissociation and transplantation (73). When tirilazad mesylate was administered to mesencephalic tissue prior to implantation as well as intravenously to patients for 3 days after surgery, patients experienced the same magnitude of improved functional performance and striatal [^{18}F]fluorodopa uptake as previous patients implanted with twice as much mesencephalic tissue (75).

4.3. Interference with anoikis

In order to standardize grafted cell number and graft volume, many investigators dissociate mesencephalic tissue into a single cell suspension prior to implantation. It has been demonstrated that the dissociation procedure itself can trigger apoptosis in mesencephalic cell suspensions, indicating that a large population of DA neurons appear committed to die prior to implantation (26). One cause of this cell death induced by dissociation may be the disruption of cell-cell interactions or cell-extracellular matrix (ECM) interactions. Detachment from the extracellular matrix, termed "anoikis", has been demonstrated to induce apoptosis in other cell types (27). In a recent study (76) we attempted to prevent anoikis-induced cell death by utilizing the extracellular matrix molecule tenascin-C and an antibody to the cell adhesion molecule L1 to specifically mimic survival signals induced by cell-matrix and cell-cell interactions. While *in vitro* both agents induced over a 100% increase in THir neurons compared to control, in contrast tenascin- and L1 Ab-treated mesencephalic cell suspension grafts did not yield an increase in THir neuron survival using our standard grafting paradigm (3µl of 100,000 cells/µl). However, under low density conditions (3µl of 3,000 cells/µl, similar to *in vitro* density), tenascin augmented grafted THir neuron survival (42% more THir neurons than control). Therefore, while the stress of detachment occurs during the dissociation process, when mesencephalic cells are returned to suspension concentrations that allow cell-cell contact it is possible that survival signals are reimplemented. These findings highlight the limitations of cell culture models as predictors for strategies to increase THir neuron survival after transplantation.

4.4. Eliminating excitotoxicity

The case for whether excitotoxicity is a significant trigger of grafted DA neuron death has yet to be made. In fact, pretreatment of mesencephalic cell suspensions with the N-methyl-D-aspartate (NMDA) receptor antagonist (+)dizocilpine hydrogen maleate (MK-801) failed to increase the survival of grafted THir neurons (77). While another study has demonstrated survival augmentation when mesencephalic grafts are pretreated with the calcium channel blockers flunarizine (78), it is unclear whether the mechanism responsible for this effect is the blockade of calcium influx or rather antioxidant or neurotrophic effects.

4.5. Enhancement of vascularization

A newly transplanted embryonic mesencephalic graft possesses little or none of its own vasculature and may not develop a patent blood supply with the host for more than a week after implantation (35-38). Therefore, newly grafted cells rely upon oxygen and other blood borne factors that diffuse from the host vasculature into the graft. This early period after grafting (1-4 days post-transplantation) corresponds with a massive wave of cell death (23,50,51). In particular, an extensive amount of cell death is frequently observed within the graft's core, the portion of the graft that, in theory, may be least accessible to diffusible factors from the host.

Advances in cancer research have led to the isolation of vascular endothelial growth factor (VEGF) (79), the primary endogenous endothelial cell mitogen involved in both vasculogenesis, or the development of embryonic vasculature (80) and angiogenesis, the growth and sprouting of adult vessels (81-83). Recently, our laboratory demonstrated that a single bolus injection of VEGF165 into the adult rat striatum significantly increases the amount of vasculature in the vicinity of the injection site in a delayed and transient manner when compared to saline controls (84). Transplantation of solid ventral

mesencephalic grafts into the VEGF165-pretreated striatum resulted in a homogeneous distribution of small blood vessels throughout the graft, a pattern that closely resembles mature adult vasculature. In contrast, grafts in the control condition contained a patchy distribution of heavily dilated vessels. Unexpectedly, however, VEGF-pretreatment failed to increase survival of THir neurons in the grafts. Delivery of VEGF via viral vector method may prove more beneficial. In an initial study, mesencephalic reaggregates transduced with a herpes simplex virus (HSV) vector to deliver the VEGF protein possessed 290% more THir neurons than reaggregates transduced with the reporter ß-galactosidase protein (85). However, it is unclear whether the reaggregate and/or viral delivery method impacted overall THir neuron survival as no freshly transplanted control condition was included in the study.

5. TREATMENT APPROACHES TO DIRECTLY INHIBIT APOPTOSIS OF GRAFTED NEURONS

The focus of the previous section addressed strategies to reduce or eliminate potential adverse triggers in the graft environment that impact grafted DA neuron survival. The following section will summarize recent approaches that target the intracellular events leading to apoptosis of grafted cells. These interventions, ideally, have the potential to reduce apoptotic cell death regardless, even when grafted cells are faced with trophic factor withdrawal, oxidant stress, hypoxia/ischemia, and disruption of cell-cell and cell-ECM interactions.

The "neurotrophic factor theory" suggests that survival of developing neurons is dependent upon the secretion of specific factors from target cells that the neurons innervate. These trophic signals appear to exert their supportive effect by suppressing an intrinsic cell "suicide" program (20), i.e. an apoptotic program that engages when such signals are absent. In cultures of sympathetic neurons, nerve growth factor (NGF) deprivation induces apoptosis, whereas NGF-exposed neurons are protected (86). At the molecular level the mechanisms of apoptosis are highly conserved. Specific genes have been identified that either induce apoptosis (CED-3 and CED-4) or suppress apoptosis (CED-9, or its mammalian homologue bcl-2) (87,88). The pattern of interactions currently envisioned proposes that CED-9/bcl-2 binds and modulates CED-4 at the mitochodrial membrane which subsequently binds and inactivates apoptotic CED-3/caspases (89-91). Transfection of neurons with bcl-2 has been demonstrated to block a variety of apoptotic triggers both *in vivo* and *in vitro* (92-95). Endogenously, bcl-2 mRNA and protein is highly expressed during CNS embryonic development and declines with aging (96-98) and bcl-2 has been demonstrated to be crucial for the prolonged survival of specific neuronal populations during early postnatal periods (99).

5.1. Caspase inhibitors

The cysteine aspartases, or caspases, are a family of proteases that have been evolutionarily conserved and comprise the essential executionary arm of the apoptotic pathway (100,101). The activity of caspases is regulated by specific molecular events initiated by external and internal signals. Caspase inhibitors are capable of selectively blocking apoptosis, provided that the specific caspases involved in carrying out the cell's execution are targeted (102).

Caspase inhibitors have been investigated for their ability to increase grafted THir neuron survival. Initial reports were extremely promising. Inclusion of Ac-YVAD-cmk (a caspase-1 inhibitor used at a pan-caspase concentration) in the cell suspension and transplant vehicle increased grafted THir neuron survival 200% over non-treated grafts (26). However, experimenters in other laboratories, including our own, have been unable to replicate these findings utilizing the same and other caspase inhibitors (103, unpublished data). These different findings may be due to varying cell densities of the graft suspension. Nevertheless, the inability of different laboratories to generate consistent augmentation of grafted THir neuron survival have led investigators to question the utility of the caspase inhibitor approach.

5.2. BCL-2 overexpression

Gene transfer of bcl-2 to mesencephalic DA neuron grafts has been attempted to protect grafted cells from their apoptotic fate. Initially, an immortalized cell line derived from rat mesencephalon was infected with a retrovirus encoding bcl-2 and then grafted (104). Two different groups have also examined the effect on graft survival of grafting mesencephalic suspensions from mice genetically engineered to over express bcl-2 (105,106). None of these three studies reported differences in cell survival between the bcl-2 overexpressing treatment groups and controls, however enhanced THir neurite extension with bcl-2 overexpression was reported (105,106). This finding is not surprising given that the bcl-2 molecule has been demonstrated to have the capacity to induce and maintain axonal growth (107). The authors concluded from their findings that cell death in grafted cells can circumvent regulation by bcl-2 (105). While this may be the case, we suggest an alternative explanation. Given that grafted cells die immediately following implantation (23-25), bcl-2 therefore must be overexpressed during this interval in order to promote graft survival. While the aforementioned studies confirmed bcl-2 overexpression, this confirmation was not conducted immediately prior to implantation. Verification of bcl-2 protein expression was conducted via Western blots from mesencephalic tissue pieces, post mortem examination of transgenic tissue or *in vitro* at 48 hours after plating (105,106). Bcl-2 overexpression was not demonstrated after tissue dissociation, a disruptive process that may temporarily interrupt constituitive protein expression and render cells quiescent. It is possible that the stress of dissociation into cell suspension downregulated bcl-2 to physiologically inactive levels during the immediate post-grafting interval.

We recently established a mesencephalic reaggregate system that allows for a time interval of 3-4 days between mesencephalic tissue dissociation and transplantation without compromising grafted DA neuron

survival and functionality (109). As monolayer cultures do not tolerate well their removal from wells after plating, the ability to transduce mesencephalic cells in an easily transplantable form is essential. We next endeavored to transduce mesencephalic reaggregates with a helper virus-free HSV amplicon that uses the 9-kb tyrosine hydroxylase (TH) promoter to drive expression of either bcl-2 or ? -galactosidase (109). However, expression of bcl-2 three days after infection was not significantly increased and consequently no effect on grafted DA neuron survival was observed (unpublished data). Therefore, although intervention with bcl-2 to promote graft survival has not yielded positive results, it is possible that key refinements in *ex vivo* transduction parameters may yet offer potential for this approach.

6. HOST FACTORS AFFECTING GRAFT SURVIVAL

Beyond treatment of the mesencephalic graft itself, there are a number of host factors that can impact the degree of grafted THir neuron survival. For example, while most transplant experiments utilize young adult animal subjects, we have demonstrated that grafts of mesencephalic cell suspension to aged rats exhibit THir neuron survival rates that are only 30% the survival rate of the same cells implanted in young, adult rats (24,110). Chronological age of the graft recipient is of particular clinical significance because in general PD is a disease of aging with the majority of affected individuals over the age of 60. The degree (111) and duration (112) of striatal denervation can also significantly influence grafted DA neuron survival. The mechanism behind the influence of age and duration of striatal denervation on graft survival likely involves changes in striatal trophic levels (112,113). Lastly, by utilizing surgical approaches that minimize acute toxic changes associated with implantation trauma, either through delaying cell injection after cannula implantation (114) or through exposing rat hosts to mild hypothermia (115), significant increases in grafted THir neuron survival can be achieved.

7. PERSPECTIVE

Systematic evaluation of strategies to increase grafted DA neuron survival is somewhat difficult due to subtle protocol differences between investigating laboratories. Even so, several therapies have emerged as the most promising. In particular, treatment strategies that aim to reduce or eliminate the triggers of grafted cell death appear to be more successful than approaches that target the intracellular apoptotic cascade. This may attest to complexity of signal transduction involved in the cell death cascade and suggest that mesencephalic cells triggered to die will initiate an apoptotic pathway to realize this goal. In particular, treatment of mesencephalic cell suspensions with isolated neurotrophic factors (GDNF, BDNF, NT 4/5) as well as glial-derived factors, antioxidant therapies and augmentation of graft vasculature have demonstrated consistent survival promoting effects. Caspase inhibition, although initially quite promising, has not been demonstrated to reliably increase grafted cell survival. Bcl-2 overexpression similarly has yet to prove beneficial, although this may be due to biologically irrelevant levels of bcl-2 present during the critical immediate post-grafting interval.

Clearly, future strategies will target a “cocktail” approach in which effective treatment agents are combined to maximize grafted DA neuron survival (73,116,117). Refinements in *ex vivo* transduction parameters will allow for efficient sustained delivery of survival promoting agents to grafted cells. Once identified, the optimal survival-enhancing treatment of grafted primary embryonic DA neurons should also benefit future transplant therapies utilizing alternatively derived DA neurons.

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