Session V- Future Directions for Research and Treatment of PNDs

Chaired by Prof. Dr. Georg F. Hoffmann

Session V focused on future interventions for PNDs, those still in development and currently being implemented in animal models, including hepatocyte and liver repopulation techniques, the use of neural stem cells, and gene therapy approaches. Most of these techniques were described using animal model systems other than PNDs.

Dr. J. Roy Chowdhury presented an overview of hepatocyte transplantation and liver repopulation for treatment of inherited diseases. In animal models, transplantation of primary hepatocytes has ameliorated several liver-based metabolic disorders, including Crigler-Najjar syndrome type 1 (CN-1; hyperbilirubinemia), analbuminemia, familial hypercholesterolemia and Wilson's disease. Hepatocyte repopulation has been used clinically in CN-1, in which repopulation of 5% of the liver mass with transplanted hepatocytes resulted in a 50% reduction of serum bilirubin. While it has become abundantly clear that adult hepatocytes have remarkable regenerative capacity, there is a need to identify a strong proliferative stimulus for transplanted hepatocytes that is not recognized by host hepatocytes.

One approach to this problem has been the use of preparative irradiation, used widely in bone marrow transplantation. Transplantation of normal hepatocytes from congenic donor rats into rats subjected to partial hepatectomy and hepatic irradiation has resulted in almost total replacement of host hepatocytes by progeny derived from transplanted non-irradiated cells. This technique, applied to the rodent CN-1 model, led to complete normalization of serum bilirubin levels. This promising methodology of hepatocyte repopulation, however, remains limited by the shortage of donor livers as source of hepatocytes. To overcome this obstacle, investigators are studying the utility of conditionally immortalized hepatocytes, which can be expanded in vitro with induction of quiescence following transplantation. Studies with immortalized hepatocytes are underway in the rodent model of CN-1.

In the second presentation, Dr. Clive Svendsen of the Waisman Center, University of Wisconsin, Madison, spoke on the topic of genetic modification of human neuronal stem cells, and their potential implications for brain repair. Stem cells represent specialized precursor cells with the capacity for indefinite expansion. By definition, pluripotent stem cells have the capacity to form most tissues, whereas multipotent stem cells (i.e., blood cells) have a more limited capacity. Totipotent stem cells have the capacity to form all tissues (i.e., fertilized egg). Totipotent stem cells form the hollow sphere known as the blastocyst. Pluripotent stem cells hold great promise in a variety of settings, including cell and tissue therapy, drug screening and other applications. However, we currently lack essential knowledge as to those factors responsible for cell specialization.

In their laboratories at the Waisman Center, Dr. Svendsen and colleagues have developed a novel method for production of large-scale quantities of human neural precursor cells. In this context, precursor cells could be stimulated to produce monolayers of both astrocytes and neurons, without production of oligodendrocytes. Dr. Svendsen showed serial rodent brain sections with clear evidence of astrocyte migration following transplantation of human neural stem cells (neurospheres). These neurospheres can be infected with wild-type vectors (i.e., tyrosine hydroxylase (TH), an enzyme critical for dopamine production), and studies have

documented long-term expression of TH activity. Loss of dopaminergic neurons is a hallmark of Parkinson's disease. However, TH production alone does not necessarily imply a functional dopaminergic neuron. An additional approach of deriving dopaminergic neurons has been to explore transfection of human neurospheres with Nurr 1, an orphan nuclear receptor which is a transcriptional activator of endogenous TH in neural progenitor cells. Dr. Svendsen showed data indicating that neurospheres could be efficiently infected with a Nurr 1 lentiviral construct.

Dr. Svendsen discussed the potential utility of overexpressed growth factors for the potential treatment of PNDs. Important growth factors in neurological disease with potential utility include nerve growth factor (NGF) in Alzheimer's disease, ciliary neurotrophic factor (CNTF) for Huntington's disease, brain derived neurotrophic factor (BDNF) and insulin-like growth factor (IGF) for amyotrophic lateral sclerosis (ALS) and stroke, and GDNF for ALS and Parkinson's disease. Dr. Svendsen posited the question as to what GDNF application might achieve in PNDs (in relation to the capacity to induce DA release)? Since GDNF and other growth factors are exceed size limitations imposed by the blood-brain barrier, direct infusion of the growth factor would be necessary, or direct infusion of stem cell grafts containing the appropriate growth factor. In closing, Dr. Svendsen noted that neural stem cells can now be produced in large numbers in the laboratory, and these cells can produce neurons with the capacity for transplantation into injured rodent brains. In the future, these neurons may have the capacity to function as "mini-pumps" for enzyme replacement within the brain of PND patients. In the final presentation, Dr. Un Jung Kang described the potential for gene therapy in catecholamine deficiency disorders, using Parkinson's disease (PD) as the model system. Dopamine (DA) is depleted in PD brain, and therapeutic trials have focused on DA repletion using L-DOPA (3,4-dihydroxyphenylalanine) and DA agonists. Production of DA requires the concerted actions of tyrosine hydroxylase (TH; converting L-tyrosine to L-DOPA) and aromatic L-amino acid decarboxylase (AADC; converting L-DOPA to DA). TH is a tetrahydrobiopterin requiring enzyme, and the production of this cofactor is dependent upon the action of GTP cyclohydrolase (GC) on GTP. Thus, three enzyme activities are critically important in the production of DA in brain.

In rodents, Parkinsonian lesions may be induced by application of 6-hydroxydopamine, which results in moderate to severe forelimb akinesia. Primary fibroblasts transfected with retroviral constructs containing TH and GC can be effectively grafted into dopamine-denervated rat striatum, and the levels of L-DOPA assessed by in vivo microdialysis. Using this system, increased and sustained amounts of L-DOPA could be obtained. However, DA production requires AADC activity as well. Retroviral co-transfection of grafts containing all three genes, TH, GC and AADC resulted in substantial in vivo DA synthesis. Unfortunately, a drawback to this approach is the capacity of DA to feedback inhibit TH activity. Thus, to avoid this feedback inhibition, the vesicular monamine transporter (VMAT) gene was introduced to the genetically modified cells in order to store DA. VMAT gene therapy led to the highest sustained levels of DA release determined in vivo. These studies have direct relevance to PNDs, many of which have significantly altered catecholamine levels. Understanding the interactions and roles of genes involved in DA synthesis and processing could help us with designing gene therapy most appropriate for the specific genetic deficits present in PN