MODEL SYSTEMS TO STUDY THE β-AMYLOID PROTEIN OF ALZHEIMER’S DISEASE

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ABSTRACT
Alzheimer’s disease (AD), a neurodegenerative disorder that severely impairs cognitive and memory function in elderly people, is partially characterized neuropathologically by extracellular deposits of β-amyloid protein. We were interested in studying how β-amyloid may be involved in aspects of AD pathogenesis. To do this, we expressed the last 100 amino acids of the amyloid precursor protein, which contains the entire β-amyloid region, in PC12 cells and in brains of transgenic mice. We found that expression of this fragment of βPP altered cytoskeletal changes in PC12 cells following nerve growth factor treatment. Using both in vitro and in vivo systems of human βPP expression, we can study the biology of βPP and test hypotheses of how it may be involved in Alzheimer’s disease.

INTRODUCTION
Alzheimer’s disease (AD), which afflicts between 2.5 and 3 million elderly people in the United States, is perhaps the most common and certainly the most widely publicized neurodegenerative disorder (1). The onset of AD, in the seventh to ninth decade of life for sporadic cases and in the fifth to seventh decade for familial forms of the disease, clearly implicates aging as a major risk factor in the development of AD. As life expectancy continues to rise with the provision of better health care, so will the incidence of Alzheimer’s disease. The need to understand, treat and prevent Alzheimer’s disease is of monumental importance as our society ages.

Clinically, Alzheimer’s patients show significant impairment of cognitive and memory functions which leave them unable to communicate and work normally. Pathologically, their brain tissue is devastated by extracellular and intracellular accumulations of insoluble proteins that most likely contribute to the neuronal loss seen in AD. Substantial advancement of AD research efforts have come following the molecular identification of these extracellular and intracellular proteins. In 1985, Glenner and Wong as well as Masters and coworkers discerned that the insoluble extracellular protein deposited around cerebral blood vessels and as plaques in AD was primarily composed of a ~40 amino acid peptide termed the β-amyloid peptide from normal and Alzheimer’s disease afflicted brain tissue (2-3).

Analysis of the β-amyloid precursor protein (βPP) reveals that it resembles a large, membrane-spanning glycoprotein. It contains a signal sequence, a cysteine rich region, an acidic domain, several putative N-glycosylation sites, a hydrophobic transmembrane region, and of course, the β-amyloid peptide (Figure 1). Through alternative splicing of the gene located on chromosome 21, several isoforms of βPP, retaining or excluding a Kunitz-like protease inhibitor domain or the β-amyloid peptide, are generated (4). Which isoform and why it is processed to yield insoluble β-amyloid peptide is currently not understood. The β-amyloid peptide, a postulated neurotoxin, may be an important mediator of AD neuropathology (5). It, along with other regions of the βPP, are consistently found in extra-cellular plaques of AD. Also, in brain tissue of old Down syndrome (trisomy of chromosome 21) individuals, neuropathological changes identical to those seen in AD are invariably observed. Additionally, numerous mutations in the βPP gene, which all cluster around the β-amyloid region, strongly suggest that βPP and β-amyloid are involved in the pathogenesis of AD (6).

Although other neuropathological changes are seen in AD, such as reduced acetylcholine levels, astrogliosis and profound cortical atrophy, identification and analysis of abundant proteins, like β-amyloid and tau, found in AD brain tissue has advanced the understanding of this disease at the cellular and molecular level. The etiology of AD is most likely multifactorial with genetic and environmental contributions culminating in the typical neuropathological scenario described earlier. However, by studying distinctive proteins believed to be important in AD pathogenesis, theories of disease initiation and progression can be tested. The amyloid precursor protein, which has certainly been the subject of intense investigation over the past 8 years, is one molecule that may enhance our knowledge of AD. This report describes approaches our lab has employed to study AD. Specifically, we have used βPP as a means of further comprehending changes seen in AD. Our work assumes βPP is important in the development of AD and that manipulation of this molecule will elucidate novel information about AD.

DISCUSSION
Strategy
We were interested in studying the role βPP or portions of βPP play in the pathogenesis of AD. We chose to use
The Models

We have expressed the human \( \beta\text{PP}_{100} \) protein in two model systems to better understand how it may be involved in AD. In the first and less complex model, PC12 cells, derived from a tumor of a rat adrenal medulla, have been stably transfected with the human \( \beta\text{PP}_{100} \) gene. PC12 cells can be easily grown in culture, and upon stimulation with nerve growth factor or basic fibroblast growth factor, they differentiate into neuron-like cells (11). Transfections were done independently with two expression vectors that utilize viral promoters, the SV40 and the JC virus early region promoters. This work is being presented in detail elsewhere and is only summarized in this paper.

In the second model, we stably introduced a human \( \beta\text{PP}_{100} \) minigene in the genome of mice. These transgenic mice are able to transmit their new human gene to their offspring, and by utilizing tissue specific expression elements, we are able to control the bodily site of transgene synthesis. Specifically, we have used the JC virus early region promoter to confer central nervous system specific expression to our transgene (12). Additionally, this promoter has tropism for glial cells within the central nervous system (13). Thus, we have two convenient laboratory systems, one in vitro and the other in vivo, to study the consequences of \( \beta\text{PP}_{100} \) protein expression.

RESULTS

We have successfully transfected PC12 cells and obtained several unique clones that harbor and express the human \( \beta\text{PP}_{100} \) protein; examination of various cell lines following nerve growth factor treatment shows an altered responsiveness in transfected cells compared to control PC12 cells (14). PC12 cells, which develop extensive neurites in response to NGF treatment, differ significantly from transfected cells that fail to develop neurites in response to NGF stimulation. Two sets of transfected cells, in which expression of \( \beta\text{PP}_{100} \) is directed by either the SV40 or the JC virus early region promoter, designated C100 and JC100, respectively, were studied. Additionally, a cell line, JC100-1M, that integrated transgene DNA, but did not express protein was used as an additional control cell line. Failure to express transgene protein in JC100-1M cells may be the result of DNA rearrangement or integration in a transcriptionally unfavorable site. These cells respond similarly to PC12 after NGF treatment.

As an example of growth factor mediated changes seen in transfected cells, we present data here on the induction of neurofilament protein in our cell system. Neurofilament (NF) proteins, the intermediary filament protein found in neurites, are induced in PC12 cells following NGF stimulation (15). We were interested in measuring the levels of NF proteins after NGF treatment because our transfected cells failed to develop extensive neurites. Using antibodies to NF-medium protein...