FINAL REPORT for Moran Foundation Project (3-93-0069)

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Project Title: " α1-antitrypsin Deficiency in a Transgenic Mouse Model"

Investigator: Richard N. Sifers, Ph.D.
Assistant Professor
Department of Pathology

PROGRESS REPORT:

Re-evaluation of proposed amino acid substitution. The logic of this proposed research was to generate a cDNA construct for human alpha1-antitrypsin (AAT) that would abrogate its protease inhibitory activity but not affect the folding of the macromolecule. Using published findings, we had originally proposed to mutate the serine (Ser) residue of the Met358-Ser359 active site to alanine (Ala). However, after discussing this strategy with several experts in the field, we chose to change that strategy. A recent publication demonstrated that the mutation of Gly349 to Pro349 within the reactive center loop peptide in the macromolecule abolished the protease inhibitory activity of AAT, and did not affect its rate of secretion from cells. Considering the misfolded protein is not secreted, we could assume that this aforementioned mutation would not result in the misfolding of the macromolecule.

Preparation of a DNA construct encoding AAT(Pro349). Using partial restriction endonuclease digestion of the human AAT gene, a 3 kb XbaI -> EcoRI fragment was excised from the cloned genomic DNA and isolated by agarose gel electrophoresis. The 3 kb DNA fragment was directionally inserted into the XbaI and EcoRI sites of pBS(-) (plasmid Bluescribe; Stratagene) to generate plasmid pPB-ATV. The recominant DNA servee as a template for site directed mutagenesis using the appropriate synthetic sense strand oligonucleotide containing the desired mutation. Incubation with dNTPs, DNA polymerase, and DNA ligase was used to complete the synthesis of the mutant DNA strand, and bacteria CJ236 (uracil +) was transformed with the ligation mixture. Transformed bacterial colonies were picked and grown in 2 x YT medium containing ampicilin. Successful mutagenesis and identification of the correct transformed bacteria was performed by isolation of double stranded and mutated pBR-ATV which was digested with XbaI + EcoRI and then subjected to hybridization with the ^{32}P-radiolabeled synthetic oligonucleotide used for mutagenesis. Direct sequencing of plasmid DNA was performed to confirm our interpretations. The mutated 3 kb XbaI -> EcoRI DNA fragment containing the Pro349 substitution will be excised from minilysate DNA of the appropriate transformed bacterial colony and re-inserted into the human AAT gene following its partial digestion with XbaI and EcoRI. Identification of the correct re-insertion will be accomplished by Southern blotting and DNA restriction mapping.

Generation of transgenic mice. A large scale preparation of the mutated gene will be obtained and the intact 14 kb human AAT gene will be excised by complete EcoRI digestion. The DNA fragment will be purified following agarose gel electrophoresis and used for injecting mouse embryos for the generation of transgenic animals.

Because of the change of our mutagenesis strategy, we have not yet begun injecting mouse embryos with DNA.

PRESENTATIONS AND PUBLICATIONS:

None at this time.