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Abstract

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Grant Number:	5K08HL002925-06
PI Name:	LINTON, MACRAE F.
PI Email:	mark.anderson@mcmail.vanderbilt.edu
PI Title:	PROFESSOR
Project Title:	APOLIPOPROTEIN B EXPRESSION STUDIES

Abstract: As an internal medicine resident, I developed an interest in lipoprotein metabolism and the pathogenesis of atherosclerotic disease. After a year of clinical of clinical training in Endocrinology and Metabolism at UCSF, I began basic research training in Dr. Stephen Young's laboratory at the Gladstone Institute. During the past three years, my research has focused on understanding several different aspects of apolipoprotein (Apo) B genetics and metabolism: 1) the effect of liver transplantation on apo B and apo E phenotype; 2) the expression of truncated apo B species in hepatoma cells; 3) the investigation of a unique allele causing hypobetalipoproteinemia, the apo B86 allele. The apo B86 allele, which contains a one nucleotide deletion (frameshift) in exon 26 of the apo B gene, results in the production of a truncated apo B species, apo B86. Remarkably, genetic and biochemical evidence from both affected family members and cell culture expression studies indicate that the apo B86 allele yields a full-length apo B100 in addition to apo B86. I have determined that the full-length protein is produced from the apo B 86 allele as a result of reading frame restoration, by a mechanism that appears to be unique in human genetics. The first specific aim of this project is to further define this mechanism for reading frame restoration. Site directed mutagenesis will be used to delineate the exact DNA sequence requirements for reading frame restoration. Mutant apo B constructs containing various DNA sequence changes in the region of the apo B86 mutation will be expressed in hepatoma cells. The apo B proteins (truncated vs. full-length) produced by the transformed cell lines will be examined by immunoblot. The mRNA from the transformed cell lines will be examined for evidence of an error in transcription that would restore the proper reading frame. Studies of the apo B86 protein suggest that apo B86 is incapable of associating with apo (a) to form lipoprotein (a) [Lp(a)]. It is widely assumed that apo B100 is linked to apo(a) by a disulfide linkage. Based on our results with apo B86, we hypothesize that one (or more of the carboxyl-terminal cysteines in apo B100 may be involved in forming a disulfide bridge with apo(a). The second specific aim is to use site directed mutagenesis and cell culture expression studies to evaluate the importance of the four carboxyl-terminal cysteines of apo B100 in its association with apo(a) to form Lp(a). Cell culture expression studies of apo B100 have obvious limitations with regard to investigating the role of apo B100 in the pathogenesis of atherosclerosis. Therefore, I plan to develop an animal model in

which to test the hypothesis that overexpression of human apo B100 is atherogenic. The third specific aim will be to overexpress human apo B100 in transgenic mice. We plan to investigate the effects of overexpression of human apo B100 in transgenic mice on lipid levels and atherogenesis. In addition, we will develop an apo B86 transgenic mouse as a model in which to study reading frame restoration in the apo B86 allele, and we propose to use transgenic mice to develop allotype specific monoclonal antibodies to human apo B100.

Thesaurus Terms:
apolipoprotein, atherosclerosis, frameshift mutation, gene expression
apolipoprotein E, blood lipid, chimeric protein, disease model, disulfide bond, nucleic acid sequence, site directed mutagenesis
laboratory mouse, monoclonal antibody, tissue /cell culture, transgenic animal, western blotting

Institution:	VANDERBILT UNIVERSITY NASHVILLE, TN 37240
Fiscal Year:	1997
Department:	ENDOCRINOLOGY
Project Start:	01-SEP-1993
Project End:	30-JUN-1998
ICD:	NATIONAL HEART, LUNG, AND BLOOD INSTITUTE
IRG:	ZHL1

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Grant Number:	5R01HL053989-03
PI Name:	LINTON, MACRAE F.
PI Email:	mark.anderson@mcmail.vanderbilt.edu
PI Title:	PROFESSOR
Project Title:	BONE MARROW TRANSPLANTION AND ATHEROSCLEROSIS

Abstract: Atherosclerosis is the major cause of morbidity and mortality in the Western World. The first recognizable lesion of atherosclerosis, the fatty streak, consists primarily of cholesteryl ester laden macrophages (foam cells) in the arterial intima. The goal of this project is to use murine bone marrow transplantation as a method to investigate the role of the macrophage in atherosclerosis and lipoprotein metabolism. The first specific aim is designed to test the hypothesis that the transfer of bone marrow cells bearing a specific genetic marker into a lethally irradiated host will result, under the appropriate atherogenic conditions, in the delivery of donor monocytes to the arterial intima and in the development of macrophage-derived foam cells of donor origin. The ROSA beta-geo 26 strain of mice which have ubiquitous expression of beta-galactosidase from the Lac Z gene will be used as bone marrow donors in these experiments, providing an easily detectable genetic marker to track the fate of donor macrophages. Once the conditions for repopulation of the host with macrophages of donor origin have been established, we will use this system to investigate the role of apolipoprotein (apo) E secretion by the macrophage in lipoprotein metabolism nd atherosclerosis. The majority of apoE in the plasma lipoproteins is of hepatic origin, and the relative contribution of extrahepatic apoE to the metabolism of the plasma lipoproteins is uncertain. ApoE is a ligand for the LDL receptor and promotes the clearance of several classes of lipoproteins from the plasma. Mice homozygous for the targeted disruption of the apoE gene develop severe hyperlipidemia and spontaneous aortic and coronary atherosclerosis. in the second specific aim, transplantation of bone marrow from mice with the normal apoE gene into apoE deficient mice will be performed to investigate the ability of extrahepatic apoE to contribute to plasma apoE levels and the clearance of plasma lipoproteins. The macrophage is known to secrete large amounts of apolipoprotein E and free cholesterol when exposed to acetylated-LDL in vitro. Foam cell formation can be viewed as an imbalance between cholesterol influx and efflux. ApoE secretion by the macrophage may facilitate cholesterol efflux from the macrophage, thus serving a protective role by preventing foam cell formation. Observations that apoE deficient mice develop spontaneous atherosclerosis, whereas transgenic mice expressing a defective apoE from the liver do not, suggest that macrophage apoE secretion may indeed play a protective role in regard to atherosclerosis susceptibility. In the third specific aim, the role of apo E secretion

by the macrophage in promoting cholesterol efflux will be tested in vitro, and bone marrow transplantation experiments will test the hypothesis that apo E secretion by the macrophage influences susceptibility to atherosclerosis in vivo. To test the hypothesis that the inability of the macrophage to secrete apoE results in an increase in the susceptibility to atherosclerosis, bone marrow from apoE deficient mice will be transplanted into C57BL/6 mice. In addition, the transplantation of bone marrow from mice with the normal apoE gene into apo E deficient mice will be tested as a means of promoting regression of atherosclerosis in apoE deficient mice.

Thesaurus Terms:
atherosclerosis, blood lipoprotein metabolism, bone marrow transplantation, macrophage apolipoprotein E, cholesterol, genetic marker, low density lipoprotein, remission /regression flow cytometry, genetic strain, immunofluorescence technique, laboratory mouse

Institution:	VANDERBILT UNIVERSITY NASHVILLE, TN 37240
Fiscal Year:	1997
Department:	MEDICINE
Project Start:	01-MAY-1995
Project End:	30-APR-2000
ICD:	NATIONAL HEART, LUNG, AND BLOOD INSTITUTE
IRG:	PTHA