

M.D. with Thesis Guidelines for Applicants

Overview

The M.D. with Thesis program acknowledges original research undertaken during the period of matriculation in the School of Medicine. It is not awarded for research done prior to acceptance to medical school and cannot be granted for work credited toward any other degree. This work may be carried out in any field appropriate to medicine where objective, critical inquiry can be made.

Governance of Program

The operation of the program will be supervised by the M.D. with Thesis Committee, appointed by the Committee on Curriculum and Education Policy and coordinated by the Assistant Dean for Student Research. The M.D. with Thesis Committee shall include representatives of the major basic science disciplines and faculty expert in clinical research and in the behavioral sciences. This committee will be the arbiter of the appropriateness of research proposals.

As soon as a student expresses an interest in participating in the program, the student will submit a proposal to the M.D. with Thesis Committee for discussion. If the student's proposal is approved, the Committee will appoint a three-member thesis committee to oversee the thesis effort. The thesis committee will include the student's research sponsor and a member of the M.D. with Thesis Committee, who will serve as liaison to the parent committee. The thesis committee will be chaired by a UCSF faculty member, usually the student's research sponsor. Following appointment of the thesis committee, the student will contact each member of the thesis committee at least once every six months during the research effort. The liaison member will maintain a formal record of contact with the student, and the student will be informed in timely fashion of any problems with respect to fulfillment of the requirements for the program. The thesis will be approved upon concurrence of the three-member thesis committee and the M.D. with Thesis parent committee.

Site of Research

Research may be performed at any academic institution or other site, which the M.D. with Thesis Committee approves. When members of a thesis committee are from other sites, they must possess academic credentials comparable to those of UCSF faculty members who would be appointed to thesis committees.

Thesis Projects

It is anticipated that thesis projects will be carried out in widely varied areas of research. To ensure a high level of scholarly endeavor, all projects must develop and/or test an original point of view and, where the work is not strictly theoretical, support it through analysis of objective experience. Specifically, the review of the work of others is excluded, as is the simple summary of experience. However, a novel analysis of data from preexisting studies could serve as the basis for a thesis. Final decisions concerning whether or not a specific project meets the program's criteria will be made by the M.D. with Thesis Committee.

EXAMPLE OF A BASIC SCIENCE PROPOSAL FOR THE MD WITH THESIS PROGRAM

TITLE: Use of Site-directed Mutagenesis to Explore the Role of ARF1 in Membrane Traffic and Organelle Structure

RESEARCH QUESTION: What effect does inactivation of the GTPase activity of the human ARF1 protein have on 1) the attachment of coatamer protein to Golgi apparatus membranes and 2) Golgi morphology?

BACKGROUND AND RELEVANT LITERATURE: Each organelle in a eukaryotic cell is a separate, membrane-bound compartment that has a specific set of biochemical activities conferred by its resident proteins. However traffic between organelles results in a continuous flow of lipids and proteins from one organelle to another. In order to maintain organelle identity, this intracellular traffic must be highly regulated. Several GTP-binding proteins, including the heterotrimeric G proteins, the rab GTPases, and a small protein called ARF, are thought to play a role in this regulation (1, 2, 3). ARF has been shown to be required for the binding of a non-clathrin coat protein complex, called coatamer, to Golgi membranes (4). The association and dissociation of coatamer is thought to be necessary for the normal structure and function of the Golgi apparatus. It has been postulated that the assembly of cytosolic coat proteins on the Golgi membrane allows this organelle to bud off coated vesicles that may then travel to another compartment and, after losing the coat, fuse with the membrane of the latter. To further our understanding of membrane trafficking, we need to know more about the regulator of coatamer binding, ARF. Like all the ras-related small GTP-binding proteins, ARF can bind either GDP or GTP and can hydrolyze GTP to GDP (5). Exposure of permeabilized cells to a non-hydrolyzable GTP analog, GTP-g-S, results in irreversible accumulation of coat on membranes, suggesting that the ARF-GTP complex facilitates binding of coatamer to membranes. Brefeldin A, a fungal metabolite, which prevents ARF from exchanging GTP for GDP, also prevents binding of coatamer to membranes (6). When Brefeldin A is added to an in vitro incubation of Golgi membranes, ARF, cytosol, and GTP, coatamer cannot assemble on the membranes, even if GTP-g-S is added later (4). These experiments and others have led to the following model of ARF function. In order to bind GTP, ARF must interact with an exchange factor, which facilitates dissociation of GDP from the nucleotide-binding site and its replacement by GTP. The ARF-GTP complex can then associate with the Golgi membrane and facilitate the binding of coatamer. The subsequent interaction of ARF with a GTPase activating protein (GAP) allows ARF to hydrolyze the bound GTP molecule to GDP. This event permits coat disassembly.

STUDY DESIGN AND METHODS: To gain further insight into the role of ARF in membrane trafficking, I will use site-directed mutagenesis to create an ARF protein with reduced GTPase activity. The altered ARF gene will be expressed in bacterial cells to permit measurement of its nucleotide-exchange and GTP-hydrolysis activities. The altered gene also will be reintroduced into eukaryotic cells, and the effect of the mutation on coatamer binding and on Golgi morphology determined. The ARF protein used in these experiments will contain

a distinctive C-terminal epitope tag so that it can be distinguished from the wild-type protein produced by the normal cellular ARF gene.

Using the well-characterized ras protein as a model, I will create a specific point mutation in the human ARF1 gene. Glutamine 61 of the ras protein is required for GTP hydrolysis. This residue is conserved in most of the more than 60 GTP-binding proteins sequenced to date. I plan to use PCR site-directed mutagenesis to convert the homologous residue of ARF, glutamine 71, to isoleucine. The presence of this mutation (ARF Q71I) will be confirmed by sequencing, and the altered gene will be subcloned into vectors appropriate for expression in either bacterial or eukaryotic cells.

The effect of the ARF Q71I mutation on nucleotide exchange will be determined using a filter-binding assay. Recombinant ARF protein will be incubated at 30 °C with a ³²P-GTP and Golgi membranes (which contain the membrane-bound exchange factor). The mixture will then be filtered, and the filters counted to determine the amount of GTP retained. Total counts will be corrected for the amount of labeled GTP that Golgi membranes bind in the absence of the ARF protein. Using the specific activity of the labeled GTP, I will calculate the amount of "enhanced exchange", that is, the amount of GTP bound due to the interaction of ARF with the exchange factor.

The rates of hydrolysis of GTP by mutant and wild-type ARF will be determined using a modification of the filter-binding assay. In this assay the amount of g-labeled GTP bound at various time points by each protein will be compared to the amount of a-labeled GTP bound. This assay reveals the rate at which previously bound GTP is converted to GDP.

The ability of mutant and wild-type ARF proteins to facilitate binding of coatamer to Golgi membranes will be assayed by incubating each recombinant ARF protein with membranes and coatamer subunits in the presence of GTP, GDP, or GTP-g-S. After incubation, the membranes will be pelleted, electrophoresed, and Western blotted using antibody against b-COP, a subunit of coatamer

The effect of the ARF Q71I mutation on Golgi apparatus morphology will be determined using immunoelectron microscopy. Using transient transfection, the mutant ARF protein will be overexpressed in COS cells. The cells will be immunostained using an antibody specific for the mutant protein's C-terminal epitope tag and antibodies for several specific compartment markers.

Originality and Significance: Previous experiments using Brefeldin A and GTP-g-S have suggested that ARF is activated by GTP binding and inactivated when the bound GTP is hydrolyzed. The present study will attempt to confirm this model. By blocking ARF function via site-directed mutagenesis rather than pharmacologically, I will be able to separate the effects of GTP hydrolysis by ARF from other potential roles of GTP in membrane trafficking. Furthermore, by examining the effect of the ARF Q71I mutation on Golgi morphology, I will be able to determine whether the amount of coatamer bound to the Golgi membranes is a determinant of organelle structure.

Preliminary Results: To date, I have succeeded in making the ARF Q71I mutation and subcloning the mutant gene into both bacterial and eukaryotic expression vectors. I also have transiently transfected COS cells with the mutated ARF gene. Preliminary electron microscopy studies suggest that the ARF Q71I protein is localized to the Golgi apparatus, but the morphology of the Golgi apparatus is not normal.

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EXAMPLE FROM EPIDEMIOLOGY FOR MD WITH THESIS PROGRAM

TITLE: Prenatal Exposure to Tobacco Smoke and Childhood Brain Tumors

HYPOTHESIS: Prenatal exposure to tobacco smoke is associated with brain tumors in children.

BACKGROUND: Tobacco smoke contains several dozen compounds that are known to be carcinogens including tobacco-specific nitrosamines (TSNA) that are N-nitroso compound (NOC) precursors found exclusively in cigarette smoke [1, 2]. In several reports, analyses of placental and umbilical cord tissue and blood of human fetuses exposed to maternal smoking have confirmed the passage of tobacco metabolites and carcinogens across the placenta [3-5]. Animal studies have demonstrated that NOC and NOC precursors are effective nervous system carcinogens in various species especially when exposure is transplacental [1, 6]. In another report, exposure of male rats to ethylnitrosourea before mating was found to increase the incidence of neurogenic tumors in offspring [7]. Thus, tobacco smoke and its metabolites may potentially have direct mutagenic effects on the fetus as well as on developing germ cells.

Two case-control studies have found positive associations between maternal smoking during pregnancy and various childhood cancers with odds ratios (OR) ranging between 1.6 and 2.9 [8, 9]. Several analyses have looked specifically at parental smoking and childhood brain tumors (CBT) with inconclusive results [8, 10-15]. Overall, most of the reports have shown neither statistically significant nor strong associations between CBT and maternal smoking before (ORs ranged between 0.4 --0.9) or during pregnancy (ORs ranged between 0.9 --1.4) [8-10, 14]. One smaller study reported an OR=5.0 (p=0.22) for continued maternal smoking during pregnancy and CBT [11]. All of these studies were considerably smaller than the study data to be analyzed for the M.D. with thesis project.

The relation between paternal smoking and CBT has been explored less thoroughly. Two studies that examined paternal smoking habits prior to pregnancy with the index child reported mixed results [10, 14]. A few analyses have shown positive associations between paternal smoking during pregnancy and CBT (ORs ranged between 1.5 -- 2.2), but these studies relied predominantly on proxy data that may have introduced inaccuracies in recall [9, 14, 15]. No dose response effect was noted in any of these studies when data were stratified by frequency of use of cigarettes.

STUDY DESIGN: The data for this project are from a large West Coast population-based, case-control study that focused on the association between N-nitroso compounds and CBT. 540 CBT patients were identified through population-based tumor registries in Los Angeles, San Francisco, and Seattle. All CBT patients under age 20 who were residents of the region covered by each registry and who were diagnosed with a benign or malignant primary tumor of the brain, cranial nerves or cranial meninges of any histologic type between 1984 and 1990 were eligible for inclusion in the study. Pathologic materials for all three geographic locations were reviewed by a UCSF neuropathologist to provide uniform histologic classification. Additional eligibility criteria included: biological mother who was English or Spanish-speaking and available for interview; subject's physician contacted prior to writing the parents; and a

telephone in the household. The 801 control children were selected using random digit dial in the three geographical areas to obtain a target control to case ratio of 2:1 in San Francisco and Seattle and 1: 1 in Los Angeles. The control subjects were frequency matched to CBT patients on gender, birth year and age at diagnosis of cases.

Personal interviews were conducted with biological mothers and personal (31%) or telephone (69%) interviews with biological fathers when they were available. Questions regarding maternal smoking before and during pregnancy with index child and passive exposure to smoke during index pregnancy were answered by the mother. Questions regarding father's smoking habits before pregnancy were answered directly by the father in 77% of interviews and by the mother in remaining interviews.

The probability of determining significantly elevated risk factors in this study depends on the number of subjects, the proportion exposed to the risk factor, and the amount the risk factor changes a subject's chance of developing the disease. Those who designed the original study calculated the probability of obtaining significant odds ratios and determined that the study has adequate power to detect factors of interest if they are truly associated with childhood brain tumors.

The data first will be analyzed by geographical region and then combined when appropriate. We will control for potential confounding factors such as age, parental age at birth of child, socioeconomic status, ethnicity, and consumption of dietary nitrites and vitamins by stratification. Logistic regression models will be used to examine factors singly and then jointly to determine whether there is confounding and/or effect modification relating to factors of interest. Analyses will focus on maternal and paternal smoking before and during pregnancy as well as mother's exposure to passive smoke from any source during pregnancy. The data also will be stratified by histologic type (the two most common CBT histologic types are astrocytoma and medulloblastoma, which may have different etiologies).

SIGNIFICANCE: Brain tumors account for about twenty percent of all incident cancer cases and deaths in children. With an incidence rate of 3/100,000 children per year, CBTs are the second most common cancer after leukemia in children under age twenty, and yet the etiology of CBT remains uncertain [16]. In epidemiologic studies conducted to date, suspected risk factors including head trauma, genetic predisposition, and exposure to ionizing radiation, have accounted only for a small proportion of incident cases. Despite recent dramatic improvements in diagnostic, surgical, and treatment techniques, mortality rates have declined only modestly in the last three decades. Currently, only about 60% of children survive five years after initial diagnosis [16], and many of those die in the subsequent five to ten years. Epidemiologic investigation into the etiology of CBT will lead to primary prevention of this disease if preventable risk factors are observed and proper education follows.

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