Research Interests

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My research interests lie in the role of proteolysis in disease. My research has encompassed many aspects of proteolysis ranging from determining the half-life of a peptide bond, to the manipulation of the immune system for the production of antibody catalysts and the uncovering of the proteases involved in angiogenesis and immune function. I favor interdisciplinary approaches to biological questions and have incorporated synthetic chemistry as well as cell biological and biochemical approaches in my research.

Below is a discussion of my previous and current research as well as future research interests. There is a clear link between the experience I gained in these projects and the development of my current scientific goals. I also describe my interest and activities in science communication.

Previous Research

I began my study of proteolysis as a graduate student, under the guidance of Dr. David Hansen in the Chemistry Department at Amherst College, by developing an efficient assay for the hydrolysis of peptide bonds involving derivitization with naphthalenedialdehyde/cyanide. With this HPLC-based assay, I determined the uncatalyzed and carboxypeptidase-catalyzed rates of hydrolysis of a peptide bond under identical, mild conditions. This allowed the calculation of the half-life of this peptide as well as the true rate enhancement and catalytic proficiency of carboxypeptidase A to be determined. The ultimate purpose of this assay was to monitor antibodies for proteolytic activity, specifically in an investigation on the effect of torsional strain on the rate of peptide-bond hydrolysis. Antibodies were elicited against the immunosuppressant FK520, a hapten that mimics a twisted peptide bond in addition to the transition state for peptide-bond hydrolysis. While none of the antibodies proved to be catalytic, they were extensively characterized by competitive ELISA and will be useful in other studies of immunosuppression.

During this time, as I was attempting to exploit the immune system to make novel catalysts, I became increasingly intrigued in the biological role of proteolysis in the immune system. I pursued this interest during my postdoctoral fellowship with Dr. Hidde Ploegh at Harvard Medical School. Proteolysis is a fundamental element of MHC Class II-restricted antigen presentation. Proteases are necessary for both the generation of antigenic peptides and the degradation of the invariant chain (Ii). Moreover, they facilitate cancer progression. For example, secreted proteases are required for angiogenesis and the invasive crossing of the basement membrane during metastasis. The ability to identify and specifically inhibit the proteases involved in these processes is useful in laboratory research as well as therapeutically.

I began my fellowship by synthesizing and characterizing a novel radiolabeled inhibitor of cathepsins, [^{125}I]-LHVS-PhOH. Using this and other active-site directed probes, I analyzed the role of cathepsin S in invariant chain degradation and trafficking in dendritic cells. I was also involved in identifying the role of cathepsin F in the same process in macrophages. The effect of the invariant chain on cathepsin L trafficking and maturation was determined using these probes and other biochemical techniques.

Beyond the study of proteases involved in antigen presentation, a collaboration with Dr. Bjorn Olsen of the Department of Cell Biology (H.M.S.) resulted in the identification of secreted Cathepsin L as the activity responsible for the release of the angiogenesis inhibitor endostatin from Collagen XVIII. Since Cathepsin L has been associated with basement membrane dissolution in tumor invasion, this finding implies that inhibitors of Cathepsin L may be most successful in preventing metastasis when combined therapeutically with anti-angiogenic molecules such as angiostatin or endostatin.

Present Research

Requisite to the success of my research has been my adeptness in many techniques and my ability to work collaboratively with other scientists on a plethora of projects. Revolving around proteolysis, my research has included biochemical analysis by HPLC, 1D and 2D SDS-PAGE, IEF, immunoprecipitation, Western Blotting, and ELISA. Moreover, it has required the synthesis and characterization of enzyme inhibitors, as well as the ability to work with animal models and cell culture systems.

I am currently beginning a project on the role of proteolysis in Systemic Lupus Erythematosus (SLE). This autoimmune disease afflicts mainly women and is influenced by hormones such as estrogen. Among the disease processes associated with SLE are altered apoptosis and antibodies autoreactive to targets such as histones, DNA, RNA, cathepsin G, and the proteasome, among others. Although SLE patients often display enhanced MHC class II expression, the role of antigen presentation in this disease is not well understood. Epitopes presented by MHC class II in murine lupus models (NZB/NZW and MRL/lpr) arise from nuclear components, the autoreactive antibodies themselves, and other targets.

The link between SLE and sex hormones such as estrogen is documented yet the mechanism of action remains unknown. Moreover, environmental estrogen mimics such as bisphenol A (BPA), which is used in the production of plastics, may act as immune modulators and affect disease progression. Estrogen has been shown to regulate the interferon-γ promotor. IFN-γ plays a central role in immunity by activating antigen presenting cells and upregulating MHC class II expression. My collaborator, Dr. Debby Walser-Kuntz (Carleton College), has observed altered IFN-γ levels in mice treated with BPA. I will be investigating the effect of hormones on the proteases involved in MHC class II antigen presentation in NZB/NZW and control mice to better understand the mechanism of hormones on SLE progression. Currently, I am directing a Carleton student in an independent research project which will initially consist of monitoring cathepsin levels in splenocytes by western blot in response to hormones (natural and environmental) and cytokines including IFN-γ. We will also investigate the apparent sex link to SLE by comparing the cathepsin levels of males versus females.

Future Research Interests

Estrogen has also been shown to alter TNF-α production in cells from SLE patients. Given the role of TNF-α as a modulator of antigen presenting cells (APCs), I would like to extend the

above comparisons to include TNF-α as well as other cytokines. Additionally, I propose to monitor the responses of professional APCs such as dendritic cells and macrophages.

Apoptosis in general is increased in SLE patients but is reduced when estrogen is present. Accordingly, I would like to investigate the effect of estrogen and environmental hormones in normal and NZB/NZW mice on caspases, the major intracellular effectors of apoptosis. Moreover, I am interested in the relationship between caspase activity and the mode of apoptosis induction. I propose an analysis that would include the induction of apoptosis by various mechanisms such as UV irradiation, staurosporine and granzymes.

Beyond the Lupus project, I am interested in investigating the trafficking and maturation of lysosomal proteases. Most of these proteases use the mannose 6-phosphate receptor for lysosomal targeting. A loss of the mannose 6-phosphate receptor causes some cathepsins to be secreted (an effect that appears to be related to the metastatic potential of cancer cells) while others are not affected. I would like to investigate alternative modes and mechanisms of lysosomal trafficking, for example, the potential role of the AP3 adaptor. AP3 knock-out mice are available for these studies or cell culture systems could be used. I envision the use of subcellular fractionation and basic biochemical (and possibly molecular biological) techniques and would welcome the opportunity to use immunofluorescence.

Interest in Science and the Media

I have a deep passion for science communication, especially through the written word. I pursued this passion at Harvard Medical School as a contributing writer to Harvard Focus magazine where I wrote news articles on cutting-edge science taking place at the Medical School. I would like to continue to author science articles for the popular press. I am currently researching for an article on the effect of ethnicity on spontaneous abortion. I am also interested in the role science plays in mainstream media and how to promote communication between scientists and the public. There are many job opportunities for students interested in both science and writing or the media.