Yale SCHOOL OF MEDICINE

YALE LIVER CENTER

Publication of The Yale Liver Center at Yale University School of Medicine Winter/Spring 2012, Vol. 4, No. 2

Inside this issue:

Yale Liver Center
The Anlyan Center for Medical Research & Education
300 Cedar Street
Room S241
New Haven, CT 06520
Telephone: 203-785-6140
Fax: 203-785-7273

During the next several months, we will update you on the progress and results for the 2011-2012 YLC pilot project recipients.

A study of the role of Macrophage Migration Inhibitory Factor (MIF) and its receptor CD74 in Autoimmune Hepatitis

David N. Assis, M.D.

Background

Autoimmune hepatitis (AIH) is a chronic inflammatory disorder of unknown etiology that is marked by circulating auto-antibodies, hypergammaglobulinemia, and liver biopsy showing plasma cell infiltration and interface hepatitis. The disease likely represents a confluence of various predisposing factors including genetic predisposition, external events (i.e. drug ingestion, infections, stress), and loss of immune tolerance (i.e. depletion/dysfunction of regulatory T cells). Over time, sustained injury results in fibrosis and cirrhosis. Standard treatment of AIH involves anti-inflammatory drugs (i.e. prednisone) and immunomodulators (i.e. azathioprine). Up to 65% of patients enter remission within 18 months, and 80% achieve remission within three years. However, only 20-30% of AIH patients will achieve sustained remission after drug withdrawal, and most will require lifelong adjustment of immunosuppression. Thus, there is a clear need to better understand and predict the pathobiology of AIH with respect to disease activity. Furthermore, there is a need to understand the genetic and environmental predisposition to AIH.

Macrophage Migration Inhibitory Factor (MIF) is an upstream pro-inflammatory cytokine which mediates a wide variety of responses to infection and stress. MIF has been implicated in the destructive process of several autoimmune diseases including rheumatoid arthritis, systemic lupus erythematosus, and scleroderma. It can assist in the activation of T cells and promotes macrophages to release pro-inflammatory cytokines that include TNF-□, IL-1, IL-6, and IL-8. *MIF* gene sequencing has revealed two polymorphisms that provide meaningful clinical information in different autoimmune or infectious disorders. The cellular receptor for MIF is CD74, a type II transmembrane protein that serves as the required cellular receptor for the MIF molecule's inflammatory cascade.

MIF has not yet been studied in the setting of autoimmune liver diseases such as AIH or PBC. Characterization of MIF and CD74 serum levels will hopefully yield important data on disease activity in these patients. Furthermore, characterization of genetic polymorphisms which increase risk for autoimmunity may have major pathophysiologic implications for the susceptibility to AIH itself.

Pilot Project Update

For the past two years, I have worked to characterize the serum and genetic profiles of AIH and PBC patient cohorts with respect to MIF and its receptor CD74. A collaboration to accomplish this has developed between the laboratories of Dr. James Boyer (Yale Liver Center) and Dr. Richard Bucala (Yale Rheumatology). The following is a summary of our principal findings to date.

- *Serum MIF and CD74 concentrations in AIH vs. PBC*

We have found a specific pattern of MIF and CD74 serum levels which may distinguish biliary liver disease (i.e. PBC) from hepatocellular injury in AIH. Specifically, while MIF levels are elevated in both conditions as compared to healthy controls, circulating CD74 levels were quite different with a lower CD74 concentration in AIH as compared to a much higher increase in PBC. We have calculated the ratio of CD74:MIF, and believe that this has phenotypic significance. This is because data generated in the lab shows that circulating CD74 can bind to and neutralize MIF, and therefore circulating CD74 may be a modulating protein of MIF cytokine activity. The disease course in AIH consists of a more pro-inflammatory phenotype compared to PBC, and we believe the CD74 to MIF ratio seen in our cohorts reflects this clinical observation.

Continued on Page 3

Director's Corner

The Yale Liver Center (YLC) is one of 16 Digestive Diseases Research Core Centers (DDRCC) supported by NIH/NIDDK. The YLC has been funded continuously for 28 years and is one of only four that focus on the liver.

Full story >

Events Calendar

Digestive Diseases Seminars Series

- Seminars
- GI Journal Club
- Liver Journal Club

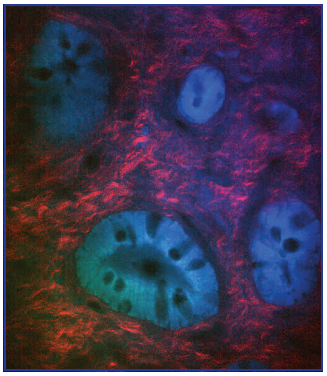
June 1 and 2 2012 NIDDK DDRCC Directors' Meeting—Rochester, Minnesota

June 16: Yale DDW Review

2011-2012 New Liver Center Members

If you are interested in becoming a member of the Yale Liver Center, please contact Kathleen Weisgable at kathleen.weisgable@yale.edu for an application.

Membership Criteria



Images of freshly excised rat common bile duct (CBDE) examined using 2-photon microscopy. Collagen fibers (red) surround ductular ephithelium seen in green near the luminal surface of the CBD. Imaged deeper into the tissue, the ductules' (blue) columnar shape is more evident as well as mucous secreting epithelial cells. Provided by Al Mennone.

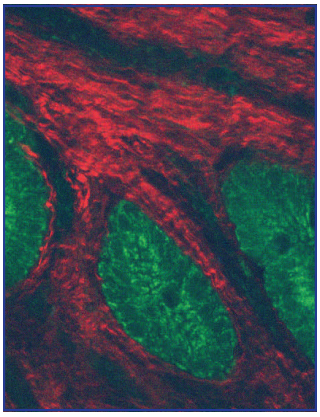
Liver Journal Club Presentation—Eugene Scott Swenson, M.D., Ph.D.

At a recent Liver Journal Club Presentation, Dr. Eugene Scott Swenson presented "Dual therapy with the nonstructural protein 5A inhibitor, Daclatasvir , and the nonstructural protein 3 protease inhibitor, Asunaprevir, in hepatitis c virus genotype 1b-infected null responders." Chayama and colleagues; Hepatology 2012;55:742-748

A short summary of this presentation follows:

This is a report of a small but very impressive pilot study in which ten non-cirrhotic Japanese patients who had previously failed standard therapy with pegylated interferon and ribavirin were treated with an all-oral, interferon-free regimen composed of a NS5A polymerase inhibitor and a NS3A polymerase inhibitor for up to 24 weeks. The study was initially designed to allow addition of standard interferon and ribavirin therapy in patients who did not achieve rapid and complete viral suppression with the oral agents. Remarkably, all ten patients responded to Daclatasvir/Asuneprevir with rapid viral suppression without virologic escape. Nine of the ten patients completed 24 weeks of the all-oral regimen, and all achieved sustained virologic response 24 weeks after completing therapy. One patient stopped treatment after only two weeks due to jaundice and diarrhea. Most impressively, even this patient who was treated for only two weeks achieved sustained virologic response. Her liver enzymes subsequently returned to normal, with no apparent sustained ill effects.

This small study provides proof-of-concept that all-oral, interferon-free antiviral regimens can provide sustained virologic response in HCV genotype 1-b-infected patients who respond poorly to interferon. Future studies will evaluate the efficacy of these agents in patients infected with other genotypes and in patients with advanced fibrosis or cirrhosis.



The Yale Liver Center is built on a tradition established by the late Gerald Klatskin, one of the country's founders of the discipline of Hepatology and a member of Yale's faculty for over 50 years.

CELLULAR AND MOLECULAR PHYSIOLOGY CORE

James L. Boyer, M.D.
Director
Shi-Ying Cai, Ph.D.
Carlo Spirli, Ph.D.
Assistant Directors

The Cellular and Molecular Physiology Core is the "work horse" of the YLC and is organized to provide technical expertise, equipment and personnel to Liver Center investigators who wish to work with **a)** Isolated cell preparations including: hepatocytes , cholangiocytes, endothelial cells, stellate cells, portal fibroblasts and hepatic lymphocytes, primarily from rat and mouse, **b) cell culture facilities** for short and long term cultures and cell lines; **c) Isolated liver perfusion preparations** for studies utilizing the whole organ; **d) altering gene expression in these cells and tissues using siRNA transfection and adenovirus infection technologies.**

The Core is divided into two essential components: 1) The Cell Isolation, Cell Culture and Organ Perfusion Component and 2) The Molecular Component. Both are housed at 300 Cedar Street on the 2nd floor of the Anlyan Center (TAC).

Equipment located at this facility includes:

- surgical instruments
- Cell isolation equipment
- Cell Culture Facilities
- Isolated Perfused Liver perfusion cabinets and equipment
- Perfusion pumps
- Blood Gas Analyzer (Radiometer)
- Quantitative PCR Machine
- Fotodyne Imaging Workstation
- BioTek Snergy 2 Multi-Detection Microplate Reader
- Matrix Anesthesia Machine
- Qiacube
- Roche light cycler 480 II system
- -80F Freezer
- USB Scientific grade camera with 1.4 megapixels and PC Image software

Several new animal models of diseases were added to the Core's resources, including models for the following diseases:

- Cystic fibrosis (CFTR KO mice)
- Alagille syndrome (Notch2 conditional KO mice and Rbpjk conditional KO mice)
- Autosomal Diminant Polycystic Kidney Disease (APKD) (Pkd1 and Pkd2 conditional KO mice)
- Autosomal Recessive Polycystic Kidney Disease (ARPKD) (Fibrocystin mutated mice)
- Biliary Atresia (migrating sea lamprey)

The availability of equipment and technical expertise for maintaining a variety of cell isolation, culture and liver perfusion models permits a large body of experimental work to be carried out that otherwise wound not be possible for individual investigators, both because of the expense of the equipment as well as the time consuming nature and technical demands of these procedures.

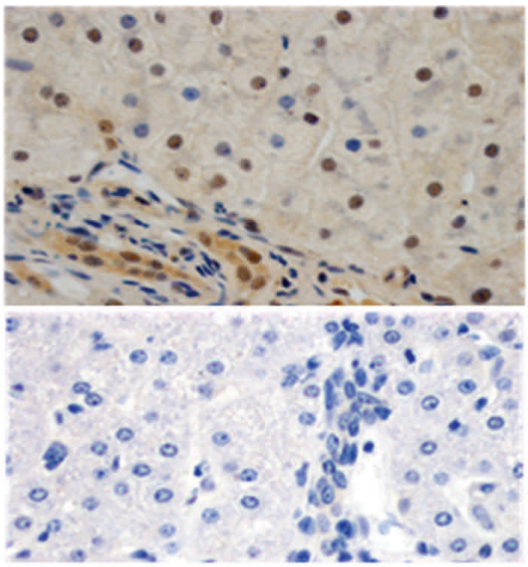


Figure. Macrophage Migration Inhibitory Factor (MIF) immunohistochemistry staining (brown) of human liver tissue from a patients with autoimmune hepatitis and from normal human control (bottom panel). The patient section displays significant increase in hepatocyte and biliary epithelial staining of MIF.

- *Functional polymorphisms in the MIF promoter region in AIH vs. PBC.*

Polymorphisms of the CATT (position -794) and also a SNP (position -173) in the *MIF* promoter have been linked with disease predisposition and also extent of disease in various inflammatory and autoimmune conditions. The $CATT_{5\text{-}8}$ is a repeat sequence in the promoter region where higher repeat numbers (i.e. 7, 8) can induce increased MIF expression. We have found that our patient cohort with AIH has a highly pro-inflammatory genetic profile with respect to the CATT repeat sequence, with increased frequency of high-expression polymorphisms. By contrast, in our PBC cohort, there was an increased frequency of low-MIF expression polymorphisms as compared to AIH and healthy controls. Therefore, the genetic profile of our patient cohorts is consistent with the clinically known inflammatory profile of these disorders.

- *Localization of CD74-positive cells via staining of human liver tissue by immunohistochemistry.*

With key assistance from the Research Histology Lab at Yale, we were able to query the specific liver cell(s) that express CD74.Through immunohistochemistry staining, we have found that sinusoidal cells ex

Press CD74 in mild autoimmune hepatitis and PBC, which is also seen in healthy control tissue. These are predominantly macrophages and possibly also with some additional staining from hepatic stellate cells. CD74 is known to be part of the MHC class II machinery, in addition to its role as transmembrane receptor for MIF, and thus the expression in the above cells is not unexpected. However, in severe AIH there was also significant hepatocellular staining, suggesting an up-regulation of CD74 in acute autoimmune disease. We interpret these findings to suggest that CD74 is intimately involved with the hepatic environment and as such may be an indicator of immune-based liver injury.

- *Characterization of the CD74 protein size and sequence in the circulation.*

Our focus over the past few months has been to precisely characterize the circulating CD74 protein, which has been recently described. It is unclear if CD74 is shed at the membrane by a sheddase protein or other enzyme, or if it is secreted in full-length (232 amino acids) from a vesicle or other intracellular mechanism. We hypothesize that CD74 is a modulator of MIF and may have regulatory mechanisms guiding its release into the serum. Our work has revealed that CD74 in the serum has a protein size consistent with a truncated protein.

We hypothesize that this protein is released from the cell membrane in response to stimulation, possibly due to known regulated intra-membrane proteolysis. Further work is needed to elucidate the mechanisms which release this cytokine receptor.

- *Serial collection of serum from patients with AIH to evaluate MIF and CD74 as biomarkers of disease activity.*

An ongoing effort is to collect serum from our patients with AIH at the Liver Clinics, to evaluate whether changes in MIF and CD74 concentrations over time correlate with disease activity, immunosuppression adjustments, and with standard liver tests. We are actively collecting serum samples from a select group of our patients, and plan to expand our collection to nearly all patients treated for AIH at our clinic. Once enough samples have been collected, we will perform detailed serum analysis and correlate changes in cytokine/receptor with standard liver tests and changes in patient immunosuppressive doses.

Significance

I believe that the results obtained add novel insight into the role of a key inflammatory cytokine and its receptor in autoimmune liver diseases. Despite the routine use of standard liver tests for monitoring of AIH patients, there is a lack of immunologic biomarkers of disease activity, and the accurate prediction of future relapses is difficult. The genetics of *MIF* polymorphisms in our US AIH cohort are point toward a pro-inflammatory genetic pattern that correlates with the nature of the disease. The serum concentrations of MIF and CD74 clearly show a distinction between biliary and hepatocellular pattern of autoimmune disease, and may have significant role in monitoring of these patients. From a mechanistic perspective, we aim to complete studies of cell secretion of CD74, which will for the first time determine the ways in which CD74 can modulate MIF action. All of these findings are crucial to an evolving understanding of the biology of MIF and CD74 in autoimmune liver diseases.

Publications and presentations resulting from pilot.

This work has been presented in poster format at the AASLD Liver Meeting in San Francisco, November 2011:

Assis DN, Grieb G, Merk M, Du X, Srivastava S, Zhang CK, McCrann C, Leng L, Zhao H, Bernhagen J, Bucala R, Boyer JL. Differential profiles of Macrophage Migration Inhibitory Factor (MIF) and its receptor CD74 in Autoimmune Hepatitis (AIH) compared to Primary Biliary Cirrhosis (PBC). Hepatology. 2011;54:S912A: Abstract 1161.

Assis DN, Srivastava S, Grieb G, Merk M, Du X, Zhang CK, McCrann C, Leng L, Zhao H, Bernhagen J, Bucala R, Boyer JL. Macrophage Migration Inhibitory Factor (MIF) and its Receptor CD74 may mediate effects of Psychological Stress in Autoimmune Hepatitis (AIH). Hepatology. 2011;54:S913A: Abstract 1164.

This work is being submitted as a manuscript for publication, and it is anticipated that it will form the basis of a K08 mentored research grant application.

Next Month:

Summary of Clinical-Translational Core

Carlo Spirli, Ph.D., Pilot Project Progress Report — Mechanisms of fibrosis in fibrocystin-deficiency associated cholangiopathies.