

MISSION STATEMENT

Samuel C. Bukantz, M.D., founded the University of South Florida College of Medicine, Department of Internal Medicine, Division of Allergy and Immunology in 1972. Richard F. Lockey, M.D. succeeded Dr. Bukantz in 1983 and is the current Director of the Division. Mrs. Joy McCann Culverhouse endowed the Division in 1997 and The Joy McCann Culverhouse Airway Disease Research Center was dedicated in February 1998. In 1998, Mabel and Ellsworth Simmons endowed the Division with a grant for education and research. The goals of the Division are: first, to provide care to patients with allergic and immunologic diseases at the University of South Florida College of Medicine, Tampa General Hospital, James A. Haley V.A. Medical Center, All Children's Hospital, and H. Lee Moffitt Cancer Center; second, to train students, residents, and fellows in the subspecialty of allergy and immunology; and third, to conduct basic and clinical research in allergy, asthma, and immunology.

Individuals interested in collaborating with members of the Division may contact Richard F. Lockey, M.D. or any faculty member at (813) 972-7631 (e-mail: rlockey@health.usf.edu).

DIVISION OF ALLERGY AND IMMUNOLOGY FACULTY AND STAFF

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BASIC RESEARCH PROJECTS

Basic research at the Joy McCann Culverhouse Airway Disease Research Center is focused on understanding the causes of asthma. Mice and rats are used as model systems to study allergic and non-allergic asthma and the effects of viral respiratory tract infections on the development and severity of asthma. Current projects include:

1. The ANP-NPRA Signaling Pathway is Involved in Asthma

Many studies have described the action of atrial natriuretic peptide, ANP, in cardiovascular disease, but its role in asthma pathology is poorly understood. Research is being performed to study ANP's effect on lung inflammation and cytokine profile in a mouse model of allergic asthma by comparing ANP receptor-deficient (NPRA KO) C57BL/6 mice to wild types (WT). Mice were sensitized and challenged with ovalbumin and airway hyperresponsiveness (Penh) was measured by whole-body plethysmography. Bronchoalveolar lavage (BAL) differential cell counts; lung histopathology and cytokine levels were also determined. Splenocyte cultures were prepared and assayed for cytokines. One group of NPRA KO mice was given an NPR-A expression plasmid intranasally to reverse the NPRA deficiency. The results of these studies show that OVA-allergic NPRA KO mice exhibit fewer eosinophils in BAL and reduced IL-4 and IL-10 in splenocyte cultures than WT. Lung sections from KO mice show less damage to mucosal epithelium and fewer infiltrating cells. Over expression of NPRA in NPRA KO mice increases the number of neutrophils and monocytes in BAL and produces higher levels of the Th2 cytokines, IL-5, IL-13 and TNF- α than in vector controls. These studies demonstrate that ANP signaling plays an important role in asthma as evidenced by reduction in lung inflammation in mice lacking the ANP receptor, and that restoration of NPRA reverses this effect.

To further examine the role of the ANP-NPRA pathway in allergic asthma, NPRA expression was silenced in allergic mice by small interfering RNA directed against the NPRA message (siNPRA). Splenocytes from siNPRA-treated mice showed reduced expression of IL-4, IL-10 and IFN- γ as well as decreased allergen-induced inflammatory lung damage and eosinophil infiltration compared to controls. These results suggest that ANP signaling via NPRA is involved in airway inflammation and that siNPRA silencing of NPRA expression may provide an effective treatment for allergies and asthma.

2. ANP has an Immunomodulatory Role in Asthma

Atrial natriuretic peptide signaling through its receptor NPRA has been reported to modulate the immune responses of dendritic cells (DCs), but the mechanism remains poorly understood. Since toll-like receptor-2 (TLR-2) is important in shaping the innate immune response, the effect of ANP signaling on TLR-2 was assessed in DCs. The results showed that exposing DCs to ANP up regulated TLR-2 expression and down regulated the adapter protein MyD88 (myeloid differentiation gene 88). Inhibition of ANP signaling by treatment of pANP-transfected DCs with the NPRA blocker isatin caused an increase in IL-10 production. Also, reduction in TLR-2 expression by RNA interference (siTLR-2) in pANP-transfected DCs up regulated IL-10, suggesting that TLR-2 is involved in NPRA signaling. Co-localization studies indicate that NPRA and TLR-2 are expressed together on the surface of HEK293 cells. These results indicate that ANP regulation of innate immunity in human DCs may involve TLR-2 signaling.

ANP appears to modulate the maturation and cytokine phenotype of DCs by altering IL-10 production, but how it does this is unclear. One possible pathway involves the suppressor of cytokine signaling-3, SOCS3, which inhibits the activity of DCs. The action of SOCS3 on ANP-activated DCs was studied by demethylation of the SOCS3 promoter. The results show that ANP down regulated SOCS3 expression compared to control, and that 5'-aza-2'dC demethylation caused SOCS3 up regulation and enhances maturation of human DCs. Also, treatment of pANP-transfected DCs with siRNA to SOCS3 up regulated IL-10 and IFN- γ . These results indicate that ANP-mediated maturation of DCs involves demethylation of the SOCS3 promoter.

3. ANP is Involved in the Genesis of Asthma

Mast cells are key initiators of allergic T helper cell type 2 (Th2) inflammation through release of various mediators, leukotrienes and cytokines. ANP has been implicated as a trigger of IgE-independent degranulation of mast cells, and based on previous observations that human mast cells express a functional ANP receptor (NPRA), we hypothesized that ANP promotes the Th2 phenotype by altering specific mast cell gene expression. To test this hypothesis, the human mast cell line HMC-1 was exposed to ANP or a scrambled peptide control. RNA was extracted and gene expression was analyzed using a microarray.

Verification of four up regulated and two down regulated genes was performed by quantitative real-time RT-PCR. ANP treatment of HMC-1 cells resulted in up regulation of 26 genes and down regulation of 17 compared to the scrambled peptide-treated cells. Real time PCR confirmed that ANP significantly down regulated IL-12 receptor β 2 expression and up regulated STAT6 expression. These observations suggest that ANP can act on mast cells in an IgE-independent manner to promote a Th2-like environment.

It was further hypothesized that ANP contained within mast cells serves as a mediator of Th2 immune responses following IgE-mediated challenge. To test this, the human mast cell line, LAD2, was studied. In LAD2 cells, IgE cross-linking induced release of ANP at 30 min, with maximal release at 2 hrs. These results show that human mast cells store and release ANP following IgE-mediated and non-immunologic challenge and that ANP may be an important mast cell mediator of allergic inflammation.

4. Prevention of Respiratory Syncytial Virus (RSV) Infection

Respiratory syncytial virus is the primary cause of severe bronchiolitis in infants and predisposes them to asthma in later life. RSV is also a serious threat to the elderly and individuals undergoing treatments that compromise the immune system. There is no available vaccine for RSV immunization and antiviral treatments are only partially effective against RSV infections. One promising new approach to specifically inhibit viral gene expression is the use of RNA interference. Small interfering RNAs (siRNA) are designed that target viral mRNAs for destruction by the cell's antiviral machinery. In this study, a plasmid expressing siRNA was constructed that blocked the expression of the non-structural protein 1 (NS1) of RSV, a protein necessary for viral replication. Human alveolar epithelial cells (A549) were transfected with either siRSV-NS1 or an unrelated siRNA as control and infected twenty-four hours later with rG RSV (recombinant RSV containing the gene encoding enhanced green fluorescence protein). The number of cells infected with rG RSV was counted by fluorescence microscopy or flow cytometry and viral protein expression in cultured cells was measured by western blotting. The expression of NS1 was undetectable after siRSV-NS1 treatment and the percentage of RSV-infected epithelial cells was significantly decreased. SiRSV-NS1 also reduced the rgRSV virus titer as determined by plaque assay compared to control. Viability assays of A549 cells revealed no cytotoxicity from siRSV-NS1 relative to controls. These results demonstrate that siRSV-NS1 is capable of significantly decreasing RSV replication in human epithelial cells and may provide potential prophylaxis and therapy of RSV infection in humans.

While RNA interference has the potential to become the RSV treatment of choice in the future, extensive human clinical trials still need to be performed to prove its safety and effectiveness. A currently available drug regimen as a promising available treatment to reduce severe asthma exacerbations caused by RSV infection is being studied. Studies are in progress to investigate the efficacy of a corticosteroid (fluticasone propionate) plus long-acting beta agonist (salmeterol) for RSV infection in a mouse model of asthma. Allergen-sensitized mice were pretreated with fluticasone, salmeterol or the two together.

Results showed a greater reduction in RSV-induced inflammation with the combination treatment. Experiments are being repeated with cultured human bronchial epithelial cells infected with RSV. These studies demonstrate the efficacy of fluticasone plus salmeterol therapy in reducing RSV infectivity and suggest that the drug combination may be useful in preventing RSV bronchiolitis and asthma exacerbation.

CLINICAL RESEARCH PROJECTS

1. Pollen and Mold Counts and Immunochemical Quantification of Outdoor Allergens

Particles, other than pollen, which transport aeroallergens, have been described. The Division, which houses the Pollen and Mold Counting Station for Tampa, has two collectors adapted to collect both pollen and pollen aeroallergens. The collectors are located on the roof of the James A. Haley V.A. Medical Center Research Building. Pollen counts are performed twice weekly, disseminated to local media once weekly, and to the Internet twice weekly.

2. Skin Aging Study

This study compared skin tests performed on both sun-exposed and non-sun-exposed areas in 120 allergic individuals in two age groups, 20-50 years of age, and 60 years and older. Tests also included nasal challenge to dust mite (*D. pter.*) and measurement of total and specific IgE to five common allergens. Findings to date indicate that: 1) older subjects have positive skin tests, 2) older men had more skin changes on their upper back than do younger and older women and younger men, 3) histamine responses were smallest on the upper back of older men, 4) specific IgE responses were positive in older and younger subjects (no statistical difference), and 5) older individuals had smaller skin tests than younger individuals. All tests have been completed and the data is being analyzed.

3. Efficacy of Using Oxymetazoline Hydrochloride Combined with Nasal Glucocorticosteroid to Treat Perennial Allergic and Non –Allergic Rhinitis in Subjects with Persistent Nasal Congestion.

This study hypothesizes that treatment with oxymetazoline, in addition to a nasal glucocorticosteroid for fourteen days, will decrease the nasal congestion persisting in subjects with allergic or non- allergic rhinitis despite maximum recommended dosages of a nasal glucocorticosteroid. It is also hypothesized that nasal glucocorticosteroid therapy will prevent the development of rhinitis medicamentosa secondary to therapy with oxymetazoline. The primary endpoint will be the change in Average Daily Nasal Congestion Scores from baseline to the end of treatment with oxymetazoline. The secondary endpoint will compare quality-of –life scores at the baseline visit to visits on Day 7, Day 14, and Day 28. Since the study began, 16 subjects have been screened and 11 subjects have been randomized. Subject recruitment is still in progress.

4. Efficacy of an Educational Program on Children at an Asthma Camp

This project evaluated the effectiveness of a children's asthma camp educational program hypothesizing that educated camp participants are more likely to correctly use their inhalers and peak flow meters at the end of camp versus at the beginning of camp. Outcome measures included: total test score of an asthma pre- and post-test (AT), peak flow usage score (PFS), inhaler usage score (IS), and spirometry. Results and conclusions included: improvement in the participants' knowledge of asthma at the end of camp, improvement in the proper use of inhalers and peak flow meters with greatest improvement in the proper use of the peak flow meter, and females having greater improvement in proper inhaler use. Improvement in asthma testing and use of the peak flow meter were the same in both genders; returning campers scored higher on the

AT, PFS, and IS pre- and post-test than did first time campers. The difference was not as broad as expected.

5. Does Addition of a Topical Antibiotic to Treat Chronic Rhinosinusitis Improve Efficacy?

Chronic rhinosinusitis is a pervasive and costly disease. Estimates reveal that the costs of treating over 20 million Americans suffering from this diagnosis exceeds \$4.3 billion per year. Primary therapy consists of oral antibiotics and nasal steroid sprays with some studies advocating nasal irrigation. Intravenous antibiotics and surgery were used for severe or recalcitrant cases. Several studies have investigated the use of topical antibiotics in rhinosinusitis, but no prospective blinded, controlled study has been done. The purpose of this prospective, randomized, double-blinded, placebo-controlled clinical study is to establish whether the addition of a topical antibiotic to a conventional regimen of oral antibiotics and topical nasal steroids results in a significant improvement in radiographic findings and quality-of-life when compared to oral antibiotics, nasal steroids, and saline placebo irrigation.

6. Role of the Natriuretic Peptide Cascade in the Genesis and Control of Asthma

The purpose of this research study is to determine indicators in the blood that may reveal a trend towards asthma and provide information on the risk of later development of allergy and asthma. Brain natriuretic peptide (BNP) and atrial natriuretic peptide (ANP) in the blood are being studied. ANP plays a role in how the lungs work and in development of the immune system and in the immune system's response to microbes and allergens. The amount of IgE in the blood will be measured, and the DNA sequence will be analyzed for specific changes that may influence a person's susceptibility to asthma.

Two groups of children/adolescents will be studied: an experimental group consisting of persons who have a physician diagnosis of allergic disease and a control group with no diagnosis of allergic disease.

7. Predicting the Diagnosis of Asthma Based on History

Despite the development of effective medications for treatment, asthma remains a significant contributor of morbidity, mortality, and financial hardship to patients with the disease. There is no single diagnostic test or symptom that defines asthma. Asthma is a syndrome consisting of a constellation of symptoms that include: wheeze, cough, shortness of breath, and chest tightness. The diagnosis of asthma takes into account history, physical examination findings, and objective measures of pulmonary function and markers of inflammation. The goal in this study is to evaluate a simplified set of questions that can be easily implemented into clinical practice that will predict the presence or absence of asthma. The primary objective is to evaluate the predictive value of a questionnaire designed to diagnose asthma in adults.

PHARMACEUTICAL SPONSORED STUDIES

Studies funded by pharmaceutical companies are conducted at the Division's Clinical Research Unit (CRU). Funds from these studies support the Division's research and clinical training program. Fourteen studies were completed in 2005, five in 2006, and eight additional studies will continue into 2007. To date, the CRU has agreements for ten new studies in 2007. The CRU is a member of the American Lung Association's Asthma Clinical Research Center network, one of 20 centers throughout the United States. The American Lung Association Clinical Research Center completed two studies in 2005, one in 2006, and two studies are in progress. The American Lung Association has nine potential protocols pending approval.

Basic and Clinical Research Support

Endowments

Joy McCann Culverhouse Endowment

Mabel and Ellsworth Simmons Endowment

Extramural Funding

American Lung Association of Florida

- Career Development Award
- Asthma Clinical Research Center Award

American Heart Association of Florida

Asthma and Allergy Foundation of Florida

Florida Biomedical Research

Genetics Institute Inc, Andover, MA

GlaxoSmithKline Medical Research Grants

Merck Medical School Grants, Merck Inc, PA

National Institute of Health, National Heart, Lung, and Blood Institute

Paterson Foundation

Veteran Affairs Merit Review Award

Pharmaceutical Sponsors

Abbott Laboratories

Altana/Byk Gulden

AstraZeneca

Aventis Pharmaceuticals

Bayer Pharmaceuticals

Baxter Healthcare

Bristol-Myers Squibb

Covance

Clintrials

Dura Pharmaceuticals

Dyax Pharmaceuticals

Forest Laboratories

Genentech

Greer Laboratories

GlaxoSmithKline

Hoffman LaRoche Pharmaceuticals

Merck and Co., Inc.

Novartis Pharmaceuticals

Ono Pharmaceuticals

Otsuka Pharmaceuticals

Pharmacia & Upjohn

Primary Immune

Schering-Plough

Sepracor

Pharming Inc.

Biotechnology Company

- ∞ A USF associated “spin-out” biotech company was formed under the direction of Shyam Mohapatra, PhD, 2004 - present.

On-Line Journal

- ∞ Shyam Mohapatra, Ph.D., Gary Hellerman PhD, and staff established an on-line journal, *Genetic Vaccines and Therapy* in 2004 - present.

PUBLICATIONS

Books or Monographs published or in press: 2003 – 2006

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Faculty and Staff Awards

Cole S: Outstanding Abstract Award; Florida Allergy Asthma and Immunology Society Annual Meeting, 2006.

Fox R: American Medical Association Physician Recognition Award, 2004-2006.

Fox R: The Best Doctors in America, 2004-2005.

Glaum M: All Children's Hospital Pediatric Clinical Research Center Pilot Grant Award (\$20,000) "Identification of inflammatory gene expression profiles in nasal polyp tissue from children with cystic fibrosis." All Children's Hospital/University of South Florida, June 2005

Ledford DK: Listed in the "America's Top Doctors" Guide, 2001 – 2006 (Chosen among 4,000 doctors nationally)

Lockey RF: Best Doctors list in May issue of *Florida Monthly Magazine*, 2005

Lockey RF: Best Doctors in America 2005-2006 database (peer selected honor), www.bestdoctors.com, 2005-2006

LockeyRF: Marquis Who's Who, 2006 edition of *Who's Who in the World*, www.marquiswhoswho.com, 2006

Lockey RF: Castle Connolly Medical Ltd. "Top Doctor 2005".

Lockey RF: Distinguished Visiting Professor, 50th Anniversary of the Faculty of Medicine of the Catholic University of Cordoba, Argentina, August 11, 2006.

Lockey RF: Honorary member, Latin-American Society of Allergy Asthma and Immunology (SLAAI), 2006.

Lockey RF: Manchester Who's Who Among Executives and Professionals, "Honors Edition", 2006/2007.

Mohapatra SS: 2005: Outstanding Biotech Achievement Award, University of South Florida-Health Science Center

Mohapatra SS: 2005: Sigma Xi 2004-05 Outstanding Faculty Researcher Award, Tampa Bay Chapter

Mohapatra SS: 2005: University of South Florida Outstanding Faculty Research Achievement Award

2005-2006 VISITING PROFESSOR GUEST LECTURERS

David P. Huston, M.D.; Cullen Chair of Immunology, Department of Medicine and Immunology; Director, Biology of Inflammation Center; Chief, Immunology, Allergy & Rheumatology, Methodist Hospital, Houston, Texas; "Autoimmunity & Urticaria", "T-32 Application", January 18-19, 2005.

Michael E. Wechsler, M.D.; Associate Physician, Pulmonary and Critical Care Medicine Asthma Research Center, Brigham, and Women's Hospital, Boston, Massachusetts; "Pharmacogenetics and Asthma Therapy: Are We There Yet?", "Pharmacogenetics" (update in asthma research); February 22-23, 2005.

Jan Nuijens, M.D.; Senior Director of Clinical Development, Pharming Technologies BV, Netherlands; "Recombinant Human C1 Inhibitor and Hereditary Angioedema", Discuss study protocol; May 3-4, 2005.

John Latall, M.D.; University of Chicago; "Allergic Bronchopulmonary Aspergillosis"; May 18, 2005.

Pravin Muniyappa, M.D.; University of Chicago; "Rhinosinusitis"; May 19, 2005.

Gerald J. Gleich, MD; Professor of Dermatology, University of Utah School of Medicine; "Serendipity Happens: Discovery of a Novel Glucocorticomimetic Agent", "Eosinophil Associated Syndromes: New Insights and New Treatments", "Eosinophil Associated Syndromes: New Insights and New Treatments", "Eosinophil Granule Major Basic Protein Homolog (MBP2): Specific Eosinophil Marker"; September 21-22, 2005.

James E. Fish, MD; Senior Medical Director, Genentech Specialty Biotherapeutics, Adjunct Professor of Medicine, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA; “IgE, Its Receptor and Role in Asthma”, “Airway Remodeling: Fact or Fiction”; October 11-12, 2005.

Dr. John Lima, PhD; Nemours Children's Clinic, Jacksonville, FL; "Pharmacogenetics of Asthma" December 13-14, 2005.

Raif S. Geha, M.D.; James L. Gamble Professor of Pediatrics, Harvard Medical and Chief of Allergy/Immunology, Rheumatology and Dermatology, Division at Children’s Boston, Massachusetts; “Mechanisms of Atopic Dermatitis”, “Immunodeficiencies Due to Defects in Isotype Switching”; February 9, 2006.

Bryan L. Martin, D.O.; Colonel, Medical Corps, U.S. Army Walter Reed Army Medical Center; “The Beta Adrenergic Controversy in Asthma”, “Skin Testing and Allergy Extract Quality Assurance”; April 11, 2006.

Larry Borish, M.D.; Professor of Medicine, University of Virginia Health System, Division of Allergy/Immunology; “Update on Chronic Sinusitis”, “Arachidonic Acid Pathway in Allergic Inflammatory Disorders”, “The Immunological Basis of Aspirin Intolerance”; May 2, 2006.

Thomas B. Casale, M.D.; Director, Clinical Research Chief, Allergy/Immunology, Creighton University, Omaha, Nebraska; “Omalizumab and Immunotherapy Combined for the Treatment of Allergic Diseases”, “New Insights into the Use of IgE Modulating Therapies”; October 10, 2006.



Left to right (back row)

John W. Sleasman, M.D.; Shyam S. Mohapatra, Ph.D.; Michael Nieder, M.D.; Dennis K. Ledford, M.D.; Monroe J. King, D.O.; Mandel R. Sher, M.D.; Richard F. Lockey, M.D.

Left to right (front row)

Matthew Morrow; Morna Dorsey, M.D., M.MSc.; Nutthapong Tangsinmankong, M.D.; Aleksandra Petrovic, M.D.; Mark C. Glaum, M.D., Ph.D.; Robert Nickeson, M.D.

Not pictured:

Samuel C. Bukantz, M.D.; Roger W. Fox, M.D.; Rama Ganguly, Ph.D., M.P.H.; Prasanna Kumar Jena, Ph.D.; Stuart M. Brooks, M.D.; Noorbibi Day, Ph.D.; Sandra G. Gompf, M.D.; Gary W. Litman, Ph.D.; Mitchel J. Seleznick, M.D.; Rosa Codina, Ph.D.; Elliot Ellis, M.D.; Mary L. Jelks, M.D.; German F. LeParc, M.D.; Brett E. Stanaland, M.D.; G. Edward Stewart II, M.D.; Hugh H. Windom, M.D.; Robert E. Windom, M.D.; Glen Whalen, Ph.D.