UNIVERSITY of NORTH TEXAS
HEALTH SCIENCE CENTER at Fort Worth
★
Education, Research, Patient Care and Service

Eighth Annual

Research Appreciation Day

March 29, 2000

Sponsored by:
Alcon Laboratories, Inc.
Graduate School of Biomedical Sciences
Office of Research and Biotechnology

RESEARCH APPRECIATION DAY 2000 SPONSORS

EVENT

Alcon Laboratories, Inc.
Graduate School of Biomedical Sciences
Office of Research and Biotechnology

AWARDS

Abbott Laboratories
Alcon Laboratories, Inc.
Fort Worth MedTech Center
Graduate School of Biomedical Sciences
Graduate Student Association
Travel Service Everywhere/Ridglea Village Travel

OTHER

Coca-Cola Bottling Company of North Texas
Promega Corporation

VENDORS

Becton Dickinson Bioscience
Brinkman Instruments
Continental Lab Products
ISC BioExpress
Nalge Nunc International
Scimetrics
Bio-Rad Laboratories
Burgoon Company
Cuevas Distribution, Inc.
Leeds Instruments, Inc.
9/5 Product Center
VWR Scientific Products

TABLE OF CONTENTS

AGENDA

7:00 - 8:00 AM	**Assemble Posters** Interdisciplinary Laboratory
8:00 - 9:30 AM	**Faculty/Non-Student Posters Session** Interdisciplinary Laboratory
9:30 - 11:30 AM	**Student/Postdoctoral Poster Competition** Interdisciplinary Laboratory
11:30 AM - 1:00 PM	**Lunch and Keynote Speaker** Luibel Auditorium
	Welcome Benjamin L. Cohen, D.O., Interim President
	Overview of RAD 2000 Activities Thomas Yorio, Ph.D., Dean Graduate School of Biomedical Sciences
	Introduction of Keynote Speaker Robert W. Gracy, Ph.D., Dean Research and Biotechnology
	"The Benefits and Difficulties of Academia Industry Collaboration - The Industry Point of View" Gerald Cagle, Ph.D., Senior Vice President for Research and Development, Alcon Laboratories, Inc.
	Presentation of the "Distinguished Alumnus Award" to Gerald Cagle, Ph.D. Neal Tate, Ph.D., Dean Toulouse School of Graduate Studies, UNT Denton
1:00 - 5:00 PM	**Student Oral Presentation Competition** Everett Hall
5:30 PM	**Award Ceremony** Everett Hall
ALL DAY	**Vendor Fair** Everett Hall Lounge/Hallway

KEYNOTE SPEAKER

Gerald Cagle, Ph.D.
Senior Vice President for Research and Development
Alcon Laboratories, Inc.

"The benefits and difficulties of academia industry collaboration -
The Industry Point of View"

Dr. Gerald Cagle's background and training is in microbiology with a career in development of ophthalmic drugs. He received his Bachelor of Science from Wayland College, his Master of Science and Doctor of Philosophy degrees from University of North Texas. After the completion of his doctoral degree in 1972, Dr. Cagle joined the faculty of the newly formed Texas College of Osteopathic Medicine, now the University of North Texas Health Science Center at Fort Worth. In 1973, he joined the faculty of The Ohio State University. He returned to Texas as a Senior Scientist with Alcon Laboratories, Inc. in 1976.

Dr. Cagle has filled a variety of positions during his career with Alcon. In his twenty-four years with the company, he has provided expertise and leadership in clinical research, international marketing, regulatory affairs, and product development. He currently serves as the Senior Vice President for Research and Development, a position he has held since 1996.

Dr. Cagle is a member of the American Society for Microbiology and is a Fellow of the American Academy of Ophthalmology. He has authored more than 25 journal articles and is a referenced contributor in five text books on microbiology and human medicine. He holds 27 patents (including pending applications). Dr. Cagle continues his scientific activities via interactions with other companies and health regulatory officials, both domestic and international.

ABBOTT LABORATORIES
RESEARCH ACHIEVEMENT AWARDS

Abbott Laboratories has been in the business of improving lives for more than a century.

With a shared commitment to the advancement of medical science, Abbott's 57,000 employees worldwide have devoted their careers to providing the highest quality in every product the company manufacturers.

This dedication to excellence has led to an outstanding record of long-term financial performance and continuous growth. Abbott consistently ranks high in the performance measures that determine the Fortune 500 and other comparisons of the world's leading corporations. For example, Abbott's 1998 worldwide sales of $12.5 billion ranks 133rd in revenues in Fortune magazine's 1999 ranking of the largest U.S.-based corporations.

Headquartered in the northern suburbs of Chicago, Abbott has manufacturing, distribution operations, and joint ventures in 44 countries. The company's products are marketed in more than 130 countries. Abbott Diagnostics Division has a facility in Irving, Texas where they manufacture diagnostic instrumentation.

Abbott maintains its standing among the world's most respected health care companies by developing innovative products at the leading edge of medical technology. This year we will reinvest more than $1 billion in research and development.

Every day, Abbott scientists are making news by discovering innovative medical technologies to improve your health. Their efforts support our overriding objective: to improve lives.

The Abbott Laboratories Research Achievement Awards are given to the top five student oral presentations as determined by a panel of judges.

See Abbott Laboratories on line at http://www.abbott.com

GRADUATE STUDENT ASSOCIATION
RESEARCH POSTER AWARDS

The Graduate Student Association (GSA) promotes the interests and opinions of the graduate student body, sponsors projects and events beneficial to students, and acts as the voice of students on matters of policy and student welfare.

GSA has co-sponsored Research Appreciation Day since its inception. This year, GSA has provided funding for travel awards ranging from $100 to $700.

The Graduate Student Association Research Poster Awards are given to the top five student poster presentations as determined by a panel of judges.

ALCON LABORATORIES
RESEARCH ACHIEVEMENT AWARD

THE ALCON GROUP

The Alcon group is the global leader in the research, development, manufacture and marketing of ophthalmic products, including surgical instruments and accessory products, intraocular lenses, prescription drugs and contact lens care solutions. The Alcon group is wholly-owned by Nestle S.A.

Founded in Fort Worth, Texas in 1947, the Alcon group now employs 10,000 individuals around the world. Total sales for 1998 exceeded $2.1 billion, with activity in more than 170 markets. One of the cornerstones of Alcon's success is the company's commitment to Research and Development. Housed at the company's headquarters in Fort Worth is the 400,000 square-foot William C. Conner Research Center, the largest and most sophisticated eye research center in the world. Over the next five years, Alcon plans to spend nearly $1 billion on eye-related research, more than any entity outside of the National Eye Institute.

The Alcon Laboratories Research Achievement Award is given to the top postdoctoral poster presentation as determined by a panel of judges.

FORT WORTH MEDTECH CENTER
INNOVATION AWARD

Fort Worth MedTech Center Innovation Award is sponsored by the Fort Worth MedTech Center, Inc., a privately funded non-profit business incubator founded in February 1998, which provides specialized and industry-specific business assistance to medical and high-technology start-up companies. This economic development effort provides a mechanism that facilitates the growth and development of emerging medical and technology companies in Fort Worth.

The mission of the Center is to attract, grow and graduate successful medical and technology companies that are financially viable and freestanding and to encourage job creation in the Fort Worth medical and technology community.

The Fort Worth MedTech Center exists to foster and assist new business ventures through the critical first years of existence. These ventures must support medical and/or technology oriented products or services which will ultimately provide economic gains, employment opportunities, and tax-base expansion in Fort Worth.

The Incubator invests time, money and expertise in emerging companies and entrepreneurs that demonstrate the potential for economic and commercial success. Medical and technology companies such as these also diversify the Fort Worth economy and make it less reliant on a single industry, while creating high-wage and high-quality jobs.

To increase the probability of success by the start-up companies, ensure a high graduation rate and sound decision making by the entrepreneurs participating in the Incubator, the Fort Worth MedTech Center provides a wide range of specialized business services that, in a pro active approach, are critical for the start-up companies.

In addition, the Fort Worth MedTech Center offers introductions and connections to a network of corporate investors, such as venture capitalists, investment and merchant bankers and private "angel" investors.

See Fort Worth MedTech Center online at http://www.medtech.org

JUDGES

The 2000 Research Appreciation Day student poster presentation judges are:

David G. Bernard, Ph.D.
Assistant Professor
University of Texas at Arlington

Warren Burggren, Ph.D.
Dean, College of Arts and Sciences
University of North Texas

Geoffrey Grant, Ph.D.
Sr. Manager for Technology
UNT Health Science Center

Warren Layne, Ph.D.
Resident Chemist/Regional Coordinator
Environmental Protection Agency

Iok Hou Pang, Ph.D.
Principal Scientist
Alcon Laboratories, Inc.

Ricardo Rodriguez, Ph.D.
Associate Professor
Texas Wesleyan University

Ken Boyce
Environmental Specialist
U.S. Environmental Protection Agency

Guy Dixon, Ph.D.
Laboratory Manager
Tarrant County Public Health Department

Jill Van Wart Hood, Ph.D.
Allied Health Coordinator
University of Texas at Arlington

Norman Miner
Research Director
MicroChem Laboratory

Manfred Reinecke, Ph.D.
Professor
Texas Christian University

The 2000 Research Appreciation Day student oral presentation judges are:

Julie Crider, Ph.D.
Senior Scientist
Alcon Laboratories, Inc.

Terry Wiernas, Ph.D.
Director of Regulatory Affairs
Alcon Laboratories, Inc.

Julia A. Nelson, M.S.
Vice President for Scientific Affairs
Summa Laboratories, Inc.

Becton Dickinson Bioscience

Representative: Neal Shea
(214) 953-1626

2600 Cole Ave., #420
Dallas, TX 75204

BD, the leader in flow cytometry for cellular analysis, serves laboratories worldwide with research and clinical applications in immunology, hematology and cell biology. The company also provides products and instruments for infectious disease diagnosis, which screen for microbial presence; grow and identify organisms; and test for antibiotic susceptibility. Products for the industrial microbiology market are used for food testing, environmental monitoring and biopharmaceutical fermentation media. Tissue culture products help advance the understanding of diseases and potential therapies. BD Labware serves the life science and drug discovery research markets. We offer innovative BD Falcon™ plasticware for tissue culture, fluid handling and high throughput screening, a unique line of BD BioCoat™ cultureware with preapplied extracellular matrix, and cell culture reagents that include ECM proteins, cytokines and media additives. BD PharMingen develops, manufactures, and markets high quality, leading edge products for immunology, apoptosis, cell biology, neurobiology, and molecular biology research. BD Immunocytometry Systems is the leader in flow cytometry, an innovative technology in the area of cellular analysis enabling health care professionals to obtain new information on a wide range of immune system diseases, such as AIDS and cancer.

Bio-Rad Laboratories

Representatives: Nikolaus Burger
Luke Dirscherl
(800) 876-3425 x1506

2000 Alfred Nobel Dr.
Hercules, CA 94547

Bio-Rad is excited to bring several great new products to the UNT vendor show. Always an industry leader in electrophoresis, Bio-Rad will display the incredible new Criterion precast gel system. The new icycler, a thermal cycler with the ability to perform quantitative real time PCR, will also be shown. A new gel documentation system, a new spectrophotometer, and samples of some of Bio-Rad's newest reagents and standards will also be available.

Brinkmann Instruments

Representative: Ed Colten
(800) 274-6562 x453

One Cantiague Rd.
Westbury, NY 11590

Brinkmann™ Instruments, Inc. stands alone as a company of people who share an ongoing mission to be the most responsive organization serving the scientific community. We offer creative solutions beyond the provision of products and support services-such as customizing products and systems to satisfy your applications needs, providing comprehensive service and maintenance contracts, and offering continuous access to technical assistance and information. That's why leading companies designate Brinkmann a "preferred vendor." Brinkmann offers a full line of products for Centrifugation, Temperature Control, Evaporation and Synthesis, Liquid Handling, Sample Preparation, and Ion Analysis. These quality products are manufactured by respected names, such as Brinkmann, Binder, Büchi, Eco Chemie, Eppendorf, KINEMATICA, Lauda, Metrohm, Retsch, Riedel-de Haën, Seward, and Tuttnauer.

VENDOR FAIR

Burgoon Company

Representatives: Larry Mitchell, Jan Wynn

2301 Strand, Suite 240
Galveston, TX 77550

(800) 287-4666

The Burgoon Company is a laboratory, medical and safety equipment and supply company based on a business philosophy of understanding the customer's needs and fulfilling them in a timely manner. The company ships worldwide and has offices in Fort Worth, Austin and Galveston. The Burgoon Company was founded in 1988 by Nita Burgoon.

Continental Lab Products

Representative: Shawn Anderson

5648 Copley Drive
San Diego, CA 92129

(888) 456-7741 x197

Continental Lab Products is a manufacturer of pipet tips, centrifuge tubes, and research quality gloves. In addition we offer pipetors reagents, thermal cyclers, and electrophoresis equipment.

Cuevas Distribution, Inc.

Representatives: Kirsten Chavera, Paul Schilly

4200 North Main St., Suite 110
Fort Worth, TX 76106

(817) 626-7110

Cuevas Distribution, Inc. is a family owned and operated business. Established in June of 1993, Cuevas provides laboratory, medical, safety supplies and equipment. We represent quality manufacturers such a Kimberly Clark, Safeskin, Barnstead/Thermolyne, Lab-line, Beckman Coulter, National Scientific, Perkin-Elmer, and many more. Cuevas puts emphasis on quality products, dependable service, and technical expertise to support the products.

ISC BioExpress

Representative: Ruth Chinn

Intermountain Scientific Corp.
420 N. Kays Dr., Kaysville, UT 84037

(800) 288-2901 x307

ISC BioExpress (Intermountain Scientific Corporation) is a small company that provides equipment and supplies to research labs, with a special focus on molecular biology labs. The company is dedicated to finding and marketing innovative products and products of the highest quality. Most ISC representatives have worked in a research lab and are familiar with how labs run and the products that are needed in them. Customer service is exceptional, with agents trained on products as well as serving the customer. ISC provides two-day express delivery at a cost of only $6.95 per order. The company strives to be a true partner in enabling labs to do the best research possible.

Leeds Instruments, Inc.

Representatives: Lisa Wright, Tracy Scully

8150 Springwood Dr. Ste 125
Irving, TX 75063

(800) 395-5998

Leeds Instruments, Inc. is the authorized distributor of Olympus microscopes in Texas. We also sell a varied line of quality products to encompass clinical, student, research and industrial applications. Our line includes microscopes, digital imaging, video equipment, image analysis, illumination, and precision measurement systems. Additionally, Leeds makes custom products to accommodate the unique needs of our customers. For over 25 years, Leeds has provided both a superior product and a support team committed to service.

VENDOR FAIR

Nalge Nunc International

Representative: Katherine Atkinson
(800) 446-2543 x4509

3365 Regent Dr.
Dallas, TX 75229

Nalge Nunc International manufactures high quality laboratory and research products. The Nalge name represents plastics from bottles and carboys to tubing and filterware. Nunc manufactures lab products for Cell Culture Molecular Biology, Cryopreservation and Immunology. For more information contact your local Sales Representative, Katherine Atkinson.

9/5 Product Center

Representatives: Bob Weatherholt
Linda Davis
Jim Barnes
(817) 255-8610

Southwest Office Systems
13960 Trinity Blvd.
Fort Worth, TX 76040

9/5 Office Product Center has over 25,000 items of office supplies and furniture. 9/5 has been in business 19 years and we are a HUB dealer. Southwest Office Systems is a Sharp copier and fax dealer. SOS also services computer systems and products.

Scimetrics

Representative: Benjamin Tepichin
(800) 231-2065 x123

1122 Stone Wall
Garland, TX 75043

Scimetrics has been serving the laboratory customer for more than twenty-five years. Independently representing a family of product lines and manufacturers that are found to be within the strongest in the industry. Our services include sales, installation, training and product service-a direct link between the laboratory and the factory. In addition, we assist in laboratory planning and product customizing. Following are only a few of the companies we represent: Alpha Innotech-Gel Doc Systems, Image Analysis; Biometra-Thermal Cyclers; Biotek-Microplate washers, and readers; Harris/Puffer Hubbard/Queue- Freezers, Refrigerators, CO2 incubators; Jouan- centrifuges; Nuaire- Bio Safety Cabinets, CO2 incubators, Solution 2000- water purification systems. There are several additional companies represented by Scimetrics both in the research and the clinical market.

VWR Scientific Products

Representatives: Blake McMonigle
Ron Yociss
Emily Heller
Billy Sharp

1001 N. Union Bower, Suite 100
Irving, TX 76561
(817) 561-0099

VWR Scientific Products is a worldwide leader in distribution of scientific equipment, supplies, chemicals and laboratory casework. VWR S/P services Life Sciences, BioTechnology, Critical Environments and Educational institutions. Our strategic partnerships with world respected manufactures enables us to service our customers with over 400,000 products from distribution centers all over the world. Key resources are our new 2000/2001 VWR S/P catalog featuring over 60,000 products and our web site (www.vwrsp.com) which features over 400,000 products. VWR S/P's strong customer service and focus is largely responsible for the corporations continued growth and success. VWR S/P's commitment to exceeding customer expectations has made us a leader in Supplying the Tools for Discovery!

TRAVEL SERVICE EVERYWHERE
RIDGLEA VILLAGE TRAVEL

TSE/Ridglea Village Travel is a long-standing supporter of the Graduate School of Biomedical Sciences and UNT Health Science Center. Their support of Research Appreciation Day 2000 includes the donation of one round-trip ticket for the first place winner of the student oral presentation competition to travel to a national scientific meeting. Please join us in thanking TSE/Ridglea Village Travel for its continued support of our activities.

TSE

TSE/Sundance
306 Main Street • Fort Worth, TX 76102
817-332-8134 Fax • Toll Free TX/USA 1-800-433-5703
(817) 332-7434

TSE/Ridglea Travel
6100 Camp Bowie Blvd. • Fort Worth, TX 76116
817-731-7130 Fax • Toll Free TX/USA 1-800-728-7507
(817) 735-1234

Two convenient locations,...managing your travel needs with expertise and care,...we're here for you,...since 1981!

Ridglea Village Travel

Coca-Cola Bottling Company of North Texas

Coca-Cola Bottling Company of North Texas has generously provided the soft drinks for our break this afternoon. Please join us in thanking Coca-Cola Bottling Company of North Texas for its support of our activities.

Promega Corporation

Promega

www•promega•com

SPECIAL SCHOOL PROGRAMS

1.	Robert L. Kaman, JD, PhD	SPECIAL SCHOOL PROGRAMS
2.	Shivang Mehta	AGONIST SENSITIVITIES OF NEURONAL NICOTINIC RECEPTORS EXPRESSED IN XENOPUS OOCYTES
3.	Priyanka Agarwal	CALCINEURIN MEDIATED DEPHOSPHORYLATION OF BAD DRIVES RAT RETINAL GANGLION CELL APOPTOSIS (A MODEL OF GLAUCOMA MEDIATED NEURAL CELL DEATH)

POSTDOCTORAL SCHOLARS ASSOCIATION (PSA)

4.	Craig Conrad	POSTDOCTORAL SCHOLARS ASSOCIATION (PSA)

RESEARCH APPRECIATION DAY 2000

First Author: *Robert L. Kaman, J.D., Ph.D.*

Department: *Graduate School of Biomedical Sciences*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

SPECIAL SCHOOL PROGRAMS; Robert L. Kaman, J.D., Ph.D., Elizabeth Davis, M.Ed., Teri Estes, J.D., Thomas Yorio, Ph.D. UNT Health Science Center - FW, Fort Worth, TX 76107.

The Office of Special School Programs administers programs whose principal goal is to increase the numbers of under represented, disadvantaged or first generation college students entering the health professions and the biomedical sciences. The programs currently in place are the Adopt-a-School Program, START, SMART, McNair, and Bridges to the Doctoral Degree.

*Adopt-a-School: This award-winning K-12 program was initiated with theFort Worth ISD in 1982 with the adoption of the Northside High School, and has expanded to include seven schools in the Northside and Dunbar High School pyramids. Health Science Center students, faculty and staff provide lectures, campus tours, mentoring, and workshops for students and school faculty. A prominent feature of this activity is the preceptorship program, in which high school students work in health science center labs and clinics two hours each day for 6 week rotations during the school year. An outgrowth of this program is the NIH-funded (currently in hiatus) Student Teacher Applied Research Training (START) Program, which extends the preceptorship experience into an eight week summer research internship.

*Summer Multicultural Advanced Research Training (SMART): This NIH-funded program, now in its second round of funding, began in 1994. Twenty college sophomores from around the country participate in a ten-week summer research internship at the health science center. Students present the findings of their research at the conclusion of the summer, and at the National Minority Research Symposium each Fall.

*Ronald E. McNair PostBacchalaureate Achievement Program: In its first year, this prestigious, Department of Education funded-program will provide year long research and mentoring experiences for up to twenty college juniors and seniors who will then apply to the graduate school at the health science center, or at others across the country.

*Bridges to the Doctoral Degree: This NIH-funded program (in hiatus) provides masters degree candidates from four partner institutions (Southern University, Jackson State University, Texas A&M University - Corpus Christi, and University of Texas - Brownsville) with scholarship support, and then facilitates entry into the Graduate School of Biomedical Sciences doctoral program. Currently, seven Bridge students are enrolled.

The outcome of the programs described here have enabled the health science center to become the leading such institution in minority enrollment in the State of Texas.

RESEARCH APPRECIATION DAY 2000

First Author: *Shivang Mehta (high school START student)*

Department: *Pharmacology*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

AGONIST SENSITIVITIES OF NEURONAL NICOTINIC RECEPTORS EXPRESSED IN XENOPUS OOCYTES. Shivang Mehta, NancyEllen C. de Fiebre and Christopher M. de Fiebre. Dept. of Pharmacology, Univ. of North Texas HSC, Fort Worth, TX 76107.

A seminal paper was published in 1991 by Luetje and Patrick (J Neurosci, 11:837-45) in which it was demonstrated that both the alpha and beta subunits contribute to the agonist sensitivity of neuronal nicotinic receptors (nAChRs). The rank ordering of potencies for four agonists at each of six nAChR subtypes was presented; however, estimates of potency were most probably erroneous due to contamination by additives to medicinal plastics. In the current study, full concentration-response analyses of four nicotinic agonists (nicotine, acetylcholine, cytisine and 1,1-dimethyl-4-phenylpiperazinium (DMPP) at seven nAChR subtypes expressed in Xenopus oocytes have been conducted. The results confirm the findings of Luetje and Patrick that both the alpha and beta subunits contribute to the agonist sensitivity of nAChRs; however, the reported extensive desensitization at high agonist concentrations was not reproduced. Further, data suggest that the "high" agonist concentrations reported by Luetje and Patrick were in fact at the lower end of agonist concentration-response curves for the various nAChR subtypes. Experiments were conducted to examine the effects of the plastics additive, Tinuvin® 770, on agonist effects. As reported by Papke et al. (J Pharmacol Exp Ther, 268:718-26, 1994), this substance is a potent, use-dependent inhibitor of neuronal nAChRs. An apparent rapid and pronounced inhibition was seen at high agonist concentrations only if drug was delivered from a syringe containing Tinuvin® 770 or from an agonist solution to which Tinuvin® 770 was directly added. These findings support the hypothesis that the rapid desensitization reported by Luetje and Patrick was due to contamination by Tinuvin® 770. These studies should assist researchers studying nAChRs in native tissues in deciphering which nAChR subtype is expressed in a given tissue by providing them with a data base of agonist potencies at known nAChR subtypes with which to compare with rank order potencies of agonists at unknown nAChR subtypes expressed in native tissues such as brain.

This project was supported by a grant from the NIAAA (AA-09585). The authors would like to acknowledge Dr. Roger Papke for helpful discussions and Dr. Jim Boulter for supplying the rat nAChR cDNAs.

RESEARCH APPRECIATION DAY 2000

First Author: *Priyanka Agarwal*

Department: *Integrative Physiology and Pathology & Anatomy*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident ☒ Summer Program Participant

CALCINEURIN MEDIATED DEPHOSPHORYLATION OF BAD DRIVES RAT RETINAL GANGLION CELL APOPTOSIS (A Model of Glaucoma Mediated Neural Cell Death) ((Priyanka Agarwal2, Hong Zeng2, Raghu R Krishnamoorthy1, Neeraj Agarwal and Stephen R Grant2)) Department of Pathology and Anatomy & Cell Biology1; Department of Integrative Physiology2, UNT Health Science Center, Fort Worth, TX 76107.

Purpose. To test our in vitro experimental model of glaucoma-mediated neural cell death, we have assesed the ability of activated calcineurin (CaN) to dephosphorylate and activate the apoptotic inducing protein, BAD. Methods. Apoptotic induction of RGC-5 cells in vitro was mediated through heterologus transfection and expression of activated form of CaN. BAD dephosphorylation and activation by CaN was assesed by immunoblot analysis. BAD mediated activation of caspase-3 was determined by enzymetic assay, and that of caspase-9 by immunoblot analysis. Apoptosis induction was assesed by "terminal deoxynucleotidyl transferase mediated fluorescinated dUTP nick end labeling" (TUNEL) and chromosomal DNA laddering. Results. The severity of apoptotic induction of RGC-5 cells was directly correlated to; (i) gene dose of heterologous CaN and (ii) degree of BAD dephosphorylation. Maximal cell apoptosis by exogenous CaN was achieved by a plasmid gene dose of 1 ug/ 200,000 cells and linear over a vector DNA range from 400 ng to 2 µg. Activation of caspase-3 and caspase-9 by dephosphorylated BAD required heterologous CaN expression. RGC-5 apoptosis in the absence of heterologous CaN remained quite low. Conclusions. Taken together, these results argue that CaN induced apoptosis of RGC_5 cells in vitro may be an excellent experimental model of retinal ganglion cell death in glaucoma. BAD mediated cell apoptosis required active CaN. Our data suggest that BAD mediated apoptosis during glaucoma may require a Ca2+–sensitive signaling cascade involving a CaN molecular mechanism.

RESEARCH APPRECIATION DAY 2000

First Author: *Craig Conrad*

Department: *Postdoctoral Scholars Association (PSA)*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☒ Postdoctoral Fellow/Resident

POSTDOCTORAL SCHOLARS ASSOCIATION (PSA)

Postdoctoral training positions were originally designed to give new Ph.D. graduates 2-3 years of practical experience. The experience gained by the post-doctoral researcher in these years would teach them to function independently in either Academic or Industrial settings. Today, many postdoctoral researchers spend 7, 8, 9 or more years trying to figure out how to work independently from their mentors. Many post-docs do not know what is expected of them in order to write papers, obtain grants, and obtain permanent employment in either Academia or Industry. For this reason, the Postdoctoral Scholars Association (PSA) was founded in 1997. The PSA is dedicated to educating post-docs on how to enhance their postdoctoral experience here at the University of North Texas Health Science Center. We believe the PSA will help post-docs strive to advance their career to the next level. The PSA provides a forum for post-docs to communicate and exchange ideas with each other. The PSA also encourages the university to follow fair and consistent policies for the management of post-graduate education.

In 1999, the PSA membership was opened to other members of the university community. Thus, graduate students, staff, and facility are invited to join PSA as associated members to introduce them to issues affecting their future post-doctoral training experience. Associate members will be invited to many of the PSA meetings each year, as well as special seminars hosted by the PSA. If you are interested in joining the PSA as an associate member, please complete the short application form, include your $5.00 membership fee, and forward the application to campus box 277.

INSTITUTE FOR CANCER RESEARCH

5.	R. H. Goldfarb, PhD	INSTITUTE FOR CANCER RESEARCH
6.	Maya Nair, PhD	DELIVERY OF ANTI-CANCER AGENTS BY LIPID/PROTEIN COMPLEXES
7.	Fu-mei Wu	PRODUCTION OF ANGIOSTATIN BY HUMAN PANCREATIC CANCER CELLS
8.	Anjuli Sinha	INVESTIGATION OF Å6: A NOVEL ANTI-ANGIOGENIC/ANTI-METASTATIC uPA-DERIVED PEPTIDE FOR ITS ADDITIVE ANTI-CANCER ACTIVITY WITH CYCLOPHOSPHAMIDE
9.	Wilson S. Chen	POTENTIAL ROLE OF ANGIOGENIC FACTORS ON NATURAL KILLER CELL ADHESION, MIGRATION AND CYTOTOXICITY
10.	Debleena Sinha	AN INVESTIGATION INTO THE DEREGULATED AKT/PKB SIGNALING PATHWAY IN OVARIAN CARCINOMA CELLS
11.	Sanghamitra Mohanty	A DEREGULATION IN PROTEIN KINASE C WAS ASSOCIATED WITH CISPLATIN RESISTANCE
12.	Porunelloor A. Mathew	GENERATION OF 2B4 GENE KNOCKOUT MICE
13.	Hilda Mendoza-Alvarez	BIOCHEMICAL CHARACTERIZATION OF POLY(ADP-RIBOSYL)ATED-P53 IN VITRO
14.	Rafael Alvarez-Gonzalez, PhD	METABOLIC CHANGES IN THE PROTEIN-POLY(ADP-RIBOSYL)ATION PATHWAY OF DIFFERENTIATING RAT GERMINAL CELLS
15.	Rafael Alvarez-Gonzalez, PhD	BACULOVIRUS-EXPRESSED RECOMBINANT RAT AND HUMAN POLY(ADP-RIBOSE) POLYMERASE: TIME COURSE OF INFECTION, PURIFICATION, AND ENZYMOLOGICAL ANALYSIS
16.	Rafael Alvarez-Gonzalez, PhD	POLY(ADP-RIBOSE) POLYMERASE AND ADP-RIBOSE POLYMERS IN THE KARYOPLASM AND THE NUCLEOLUS OF HELA CELLS IN APOPT

17.	Nils F. Confer	MOLECULAR INTERACTIONS OF POLY(ADP-RIBOSE) POLYMERASE (PARP) WITH DNA-BINDING PROTEINS DURING THE REPAIR OF DAMAGED DNA IN CULTURED CELLS
18.	Woo-Jin Chang	POLY(ADP-RIBOSYLATION) OF TRANSCRIPTION FACTOR NF-KB: EFFECTS ON DNA BINDING
19.	Miguel Angel Medina	MOLECULAR CHARACTERIZATION OF A NK CELL RECEPTOR RELATED TO 2B4 GENE
20.	Samuel S. Chuang, PhD	2B4 STIMULATION OF YT CELLS INDUCES NK CELL CYTOLYTIC FUNCTION AND INVASIVENESS
21.	Pappanaicken R. Kumaresan, PhD	GENE STRUCTURE OF THE HUMAN NK CELL RECEPTOR 2B 4
22.	Myoung H. Kim, PhD	DIFFERENTIAL EXPRESSION OF MATRIX METALLOPROTEINASES AND TISSUE INHIBITORS OF MMP S IN HUMAN NK AND T CELL LINES BY IL-2 STIMULATION
23.	Giridhar R. Akkaraju	OVEREXPRESSION OF PROTEIN KINASE C eta ATTENUATES CASPASE ACTIVATION AND TUMOR NECROSIS FACTOR-alpha INDUCED CELL DEATH
24.	Tuan Pham	FUNCTIONAL STUDY OF LECTIN-LIKE TRANSCRIPT (LLT) RECEPTOR ON HUMAN NK CELLS
25.	Min Lu	PURIFICATION AND INITIAL CHARACTERIZATION OF PROTEASOME FROM NATURAL KILLER CELLS
26.	Lori Johnson	MOLECULAR REGULATION OF INTERFERON GAMMA BY 2B4 ACTIVATION IN HUMAN NATURAL KILLER CELLS
27.	Ginelle Courchaine	INTEGRINS REGULATE NATURAL KILLER CELL PROTEOLYTIC ENZYMES IN MODULATION OF NATURAL KILLER CELL INVASION AND MIGRATION
28.	Kent S. Boles	CHARACTERIZATION OF A NOVEL LECTIN-LIKE TRANSCRIPT (LLT2) EXPRESSED ON HUMAN NK CELLS
29.	Matthew Woolard	REGULATION OF APOPTOSIS BY BCL-2 AND PROTEIN KINASE C DELTA IN HELA CELLS

RESEARCH APPRECIATION DAY 2000

First Author: *Goldfarb, RH*

Department: *Molecular Biology and Immunology, and Institute for Cancer Research, University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas 76132*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

INSTITUTE FOR CANCER RESEARCH, UNTHSC

Goldfarb, RH and Brunson, KW. Department of Molecular Biology and Immunology, and Institute for Cancer Research, University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas 76132

The University of North Texas Health Science Center's Institute for Cancer Research (ICR) was launched in January 1999 to better coordinate the research fight against life threatening forms of cancer, and to provide a focus for all aspects of cancer research in the Fort Worth region of North Texas. The ICR provides a collaborative environment for investigators working in basic science, translational research, public health and the clinic to seek a better biological basis for more effective cancer prevention, diagnosis and treatment. The Institute also acts as a focal point for interactions with biotechnology and pharmaceutical companies in its endeavor to impact on cancer. Additionally, the ICR will provide educational and research training opportunities for medical and graduate students, as well as postdoctoral and clinical fellows. In an effort to address the complexity of the cancer problem, focus groups have been formed to address: 1)Cancer Molecular Oncology, 2)Cancer Cell Biology, 3)Clinical Oncology and Clinical Investigations, 4)Cancer Preventive Medicine, Public Health, Biobehavioral Oncology and Medical Ethics and 5)Biotechnology, Technology Transfer and Public Relations. Besides about fifty talented researchers from the UNTHSC, a number of renown scientists associated with other institutions have also joined the ICR, which allows expanded interactions nationally and internationally. A distinguished panel of scientists and community leaders now serve on the Scientific and Community Advisory Council, which furnishes a broad spectrum of advice for optimal function of the ICR.

RESEARCH APPRECIATION DAY 2000

First Author: *Maya Nair, Ph.D.*

Department: *Molecular Biology and Immunology and Internal Medicine*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☒ Postdoctoral Fellow/Resident

DELIVERY OF ANTI-CANCER AGENTS BY LIPID/PROTEIN COMPLEXES

Maya H. Nair, Walter J. McConathy and Andras G. Lacko. Departments of Molecular Biology and Immunology and Internal Medicine, UNTHSC, Fort Worth, TX 76107.

A novel delivery system for hydrophobic compounds, including anticancer drugs, has been developed using recombinant high density lipoproteins (rHDL). The advantage of rHDL over liposomes and other artificial complexes is that they are smaller in size and their contents are rapidly internalized by receptors of specific cells, including tumor tissue.

Studies revealed that most tumor cells tested are nearly as efficient as ovarian granulosa cells in the incorporation of the core hydrophobic lipid, cholesteryl ester, in HDL. These findings suggest that anticancer drugs as a rHDL/drug complex may also be taken up by tumor via through an efficient, receptor mediated (SRB1) mechanism. When the anticancer drug doxorubicin (DOX) was incorporated into rHDL complexes the resultant lipoprotein was shown to be stable as they can be reisolated by either preparative ultracentrifugation or gel chromatography. In addition, the rHDL/DOX complexes have been observed to be considerably more efficient in killing HeLa cells than free DOX alone. We studied the killing potential of the drug/rHDL preparation utilizing several tumor cell lines and found killing potential. Future studies also include the chemical characterization and determination of the drug loading capacity of the rHDL complexes. Following in vitro screening, the study will assess the therapeutic effectiveness of anti-cancer drugs delivered via rHDL into tumor bearing mice.

This approach has the potential to substantially improve the delivery of anticancer drugs to tumors and thus enhance the prognosis for cancer survivors . In addition, the HDL transport vehicle may increase the specificity of drug uptake by these tumor cells and thus decrease the toxicity of chemotherapeutic agents to normal cells. Finally, the rHDL system may also be used to deliver hydrophobic radiochemicals to specific tissues to aid in the treatment and diagnosis of cancer. (Supported by the *Institute for Cancer Research*, UNTHSC).

RESEARCH APPRECIATION DAY 2000

First Author: *Fu-mei Wu*

Department: *Molecular Biology and Immunology*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☒ Staff

☐ Postdoctoral Fellow/Resident

PRODUCTION OF ANGIOSTATIN BY HUMAN PANCREATIC CANCER CELLS.

Wu, F. M., Nguyen, H., and Wu, M.C. Department of Molecular biology and Immunology and Institute for Cancer Research, University of North Texas Health Science Center, Fort Worth, TX 76107

Angiostatin is a potent inhibitor of angiogenesis and is produced by limited proteolysis of plasminogen catalyzed by plasminogen activators and other proteases.We have previously purified and characterized an urokinase-type plasminogen activator from the serum-free conditioned medium of a pancreatic carcinoma cell line MIA PaCa-2. In this study, we have investigated whether this cell cell line can produce angiostatin in the presence of exogenous plasminogen. Plasminogen has been purified from human plasma by an Affigel 10-Lys column following procedures similar to the standard Sepharose-Lys method. Serum free conditioned medium (SFCM) is prepared from cultured MIA PaCa-2 cells as described previously. Plasminogen is incubated with SFCM at 37oC under sterile condidtion. Samples are taken at different time and analyzed by SDS-PAGE for protein profile. The protein band of plasminogen disappeared after four hours incubation concomitant with the appearance of three protein bands with molecular weight of 38, 42 and 46 kda respectively. After 24 hours incubation, the reaction mixture is pooled and passed through the Affigel 10-Lys column to purify angiostatin. The purified protein fragmnets are further analyzed by SDS-PAGE. The anti-angiogenesis activity has also been assayed by its effect on endothelial cell proliferation. Our results have demonstrated that SFCM from MIA PaCa-2 cells can convert plasminogen to angiostatin-like proteins. Molecular natures of these proteins as well as the mechanism of conversion are currently under investigation.(Supported by Institute for Cancer Research, Huy Nguyen is a 1999 SMART student at UNT-HSC)

RESEARCH APPRECIATION DAY 2000

First Author: *Anjuli Sinha*

Department: *Molecular Biology & Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

INVESTIGATION OF Å6: A NOVEL ANTI-ANGIOGENIC/ANTI-METASTATIC uPA-DERIVED PEPTIDE FOR ITS ADDITIVE ANTI-CANCER ACTIVITY WITH CYCLOPHOSPHAMIDE.

A. Sinha, R.P. Kitson, Y.Xue, G. Al-Atrash, W. Chen, and R.H. Goldfarb, Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, TX. 76107 and ICR

This project is directed towards the therapy of cancer metastases using a combination therapy, which targets urokinase plasminogen activator (uPA) and its receptor (uPAR). Å6 (a non-toxic anti-angiogenic agent) is an 8-mer peptide from within the uPA sequence. It has been shown that Å6 in combination with cyclophosphamide (a standard chemotherapeutic anti-cancer agent) leads to enhanced therapy vs. either agent alone. Å6 inhibits the binding of uPA to its receptor uPAR, thus preventing plasminogen activation. Metastatic tumor cells and angiogenic endothelial cells express uPA, contributing to their ability to migrate, invade, and destroy components of the basement membrane. uPA is secreted in a single chain zymogen form (scuPA). Plasminogen activation is involved in a cascade of events, starting with the binding to scuPA to uPAR. ScuPA is activated by plasmin to form active uPA. This results in a net flux of proteolysis that can activate proteases downstream from plasmin, cause the release of growth factors from the extracellular matrix, and degrade basement membrane proteins like collagen type IV and laminin. This series of events contributes to tumor invasion by destroying the basement membrane barrier and facilitating metastasis. These studies are directed toward investigating the mechanism behind the destruction of tumors by cyclophosphamide and Å6, the interaction of cyclophosphamide with other anti-angiogenic agents (ie BB-94, angiostatin), and to identify more potent in vitro analogs of Å6 synthesized by Ångstrom Pharmaceuticals. (This work was sponsored by a grant from the Texas Advanced Technology program)

RESEARCH APPRECIATION DAY 2000

First Author: *Wilson S. Chen*

Department: *Molecular Biology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☒ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

POTENTIAL ROLE OF ANGIOGENIC FACTORS ON NATURAL KILLER CELL ADHESION, MIGRATION AND CYTOTOXICITY. Wilson S. Chen, Richard P. Kitson, Ronald H. Goldfarb, Department of Molecular Biology and Immunology, Institute for Cancer Research, University of North Texas Health Science Center, Fort Worth, Texas 76107

Angiogenesis is crucial for the growth of solid tumors and the nurturing of their metastases at distant secondary sites. This process recruits new microvessels to infiltrate these tumors in response to angiogenic factors, such as vascular endothelial cell growth factor (VEGF) and basic fibroblast growth factor (bFGF). Our laboratory has had a long standing interest in the tumor angiogenesis, cancer invasiveness, and the anti-cancer effect of natural killer (NK) cells. We have observed that interleukin-2 activated NK (A-NK) cells can accumulate within established cancer metastases followin their adoptive transfer. These A-NK cells appear to preferentially accumulate within tumor metastases which are rich in neovasculature. Furthermore, these A-NK cells not only bind to tumor cells but also to the microvascular endothelial cells. Thus, our attention has turned towards the possible involvement of angiogenic factors on NK cell functions. Initial studies will focus on the influence of angiogenic factors on the biological activities of NK cells, including: adhesion to extracellular matrices and endothelial cells; expression of degradative enzymes (e.g. urokinase type plasminogen activator and matrix metalloproteinases); modulation of migration and NK cell-mediated cytotoxicity.

RESEARCH APPRECIATION DAY 2000

First Author: *Debleena Sinha*

Department: *Molecular Biology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

AN INVESTIGATION INTO THE DEREGULATED AKT/PKB SIGNALING PATHWAY IN OVARIAN CARCINOMA CELLS. D. Sinha and A. Basu.
Department of Molecular Biology and Immunology, UNT Health Science Center, Fort Worth, TX - 76107.

Akt/Protein Kinase B is a retrovirus associated oncogene. It is a serine /threonine protein kinase and is activated via the phosphoinositide-3-OH kinase (PI3K) pathway. It is found deregulated in a variety of tumors like those of breast, ovary, prostrate and pancreas. Akt has been identified as an important component of pro-survival signaling pathways but the mechanism by which Akt prevents cell death still remains unclear.

The objective of my studies is to elucidate the Akt signaling pathway and to investigate how this deregulated signaling pathway contributes to development of ovarian carcinoma. (1) Since Akt prevents cell death, two ovarian carcinoma cell lines, 2008 and OV1063, were treated with Akt inhibitors Ly294002 and Wortmannin to see if they blocked Akt mediated cell survival. Ly294002 was more effective in killing cells than Wortmannin. (2) Ly294002 also potentiated cell death by TNF in 2008 cells. (3) To see if Ly294002 potentiated cell death via the TNF/NFkB pathway, 2008 cells were treated with LY294002 alone or in combination with cycloheximide (a protein synthesis inhibitor) in TNF treated 2008 cells. Ly294002 did not block induction and translocation of NFkB (from cytoplasm to nucleus) by TNF. (4) Ly294002 did, however, increase cleavage of caspase 3, 7, 8, and 9 which corresponded with decrease in Akt levels. Thus, inhibition of Akt causes activation of caspases which results in cleavage of Akt and abrogates its ability to promote cell survival.

RESEARCH APPRECIATION DAY 2000

First Author: *Sanghamitra Mohanty*

Department: *Microbiology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

A DEREGULATION IN PROTEIN KINASE C WAS ASSOCIATED WITH CISPLATIN RESISTANCE.

S.Mohanty, and A.Basu, Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, Texas – 76107.

Development of drug resistance is a major factor in treatment failure of cervical carcinoma. Cellular sensitivity to cisplatin is greatly influenced by protein kinase C (PKC) signal transduction pathway. PKC is a family of 11 isoforms that differ in their biochemical properties,intracellular localisation, and tissue specific expression. We have investigated whether PKC activators influence the sensitivity of cisplatin resistant HeLa (HeLa/CP) cells and characterized the expression of different PKC isoforms in both HeLa and HeLa/CP cells. Cytotoxicity (MTT) assay showed, different PKC activators like PDBu and Bryostatin I increased the sensitivity of HeLa and HeLa/cp cells to cisplatin..Increasing concentration of bryostatin I gave the biphasic effect in cytotoxicity assay in both parental as well as the resistant cells. No significant difference was observed in any of the PKC isoform expression in untreated HeLa and HeLa/CP cells. However, while PDBu and Bryo caused significant downregulation of PKC delta in HeLa cells,PDBu but not Bryo failed to do so in HeLa/CP cells.These results suggest that regulation of PKC delta is affected during development of resistance to cisplatin.

RESEARCH APPRECIATION DAY 2000

First Author: *Porunelloor A. Mathew*

Department: *MBI*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

GENERATION OF 2B4 GENE KNOCKOUT MICE

Susan E. Stepp* and Porunelloor A. Mathew
Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, TX 76107 and *Graduate program in Immunology, UT Southwestern Medical Center, Dallas, TX 75235.

The receptor 2B4 is expressed on all Natural Killer (NK) cells and a subpopulation of T cells. It is a novel member of the immunoglobulin supergene family and plays a major role in the killing of cancer cells and virally infected cells by NK cells. 2B4 associates with the signaling adaptor molecule SAP and modulates both NK and T cell functions. Defect in SAP is the molecular basis for XLP disease, an immune disorder. This implicates a role for 2B4 in maintaining normal immune functions. In both mice and human, 2B4 is the counter-receptor for CD48, which is involved in T cell activation. The proportion of 2B4+CD8+ T cells is a better predictor for disease progression in HIV-infected individuals. In order to decipher the in vivo role of 2B4 we have generated mice with a disrupted 2B4 gene by homologous recombination. Research supported by NIH grant AI 38938.

RESEARCH APPRECIATION DAY 2000

First Author: *Hilda Mendoza-Alvarez*

Department: *Molecular Biology and Immunology*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

BIOCHEMICAL CHARACTERIZATION OF POLY(ADP-RIBOSYL)ATED-P53 IN VITRO

Hilda Mendoza-Alvarez and Rafael Alvarez-Gonzalez
Department of Molecular Biology & Immunology and the Institute for Cancer Research, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107.

We have recently reported the poly(ADP-ribosyl)ation of p53 in apoptotic HeLa cells following alkylating DNA-damage with MNNG (Kumari, et al., Cancer Research, 58: 5075-5078, 1998). Now, we have proceeded to characterize the enzymology of p53-poly(ADP-ribosyl)ation using an *in vitro* reconstituted system. Our system was composed of pure recombinant p53 expressed as a $(His)_{10}$ amino-terminal fusion protein in *Escherichia coli* and pure recombinant poly(ADP-ribose) polymerase (PARP) expressed in baculovirus at various βNAD^+ concentrations. Our data indicate that the poly(ADP-ribosyl)ation of p53 is time-dependent from 0 to 120 minutes of incubation and that the addition of the tumor suppressor protein to an auto-poly(ADP-ribosyl)ation mixture results in more than a two- fold stimulation of poly(ADP-ribose) synthesis. Electrophoretic analysis of the products synthesized at low micromolar βNAD^+ concentration indicate that short oligomers predominate during the early times (0-15 min), while highly branched ADP-ribose chains are synthesized at late times of incubation (15-120 min). As expected, increasing the protein concentration of p53 from 40 nM to 1.0 μM yielded higher amounts of poly(ADP-ribosyl)ated-p53 as well. Furthermore, higher concentrations of p53 also stimulated the automodification reaction of PARP. Therefore, it appears that as the number of p53-bound ADP-ribose chains increases, the automodification reaction of PARP becomes more processive and efficient. We also observed that a deletion mutant of p53 lacking the last 30 amino acid residues at the carboxy-terminus was efficiently poly(ADP-ribosyl)ted. Since the carboxy-terminal domain of p53 allows its non-specific binding to single stranded DNA, we conclude that amino acid target sites for poly(ADP-ribosyl)ation are not located on this peptide fragment. In addition, we observed that a point mutation of p53 at residue 267 did not prevent its poly(ADP-ribosyl)ation either. Since mutant 267 of p53 does not bind its sequence specific DNA-binding site at all, our data suggest that the poly(ADP-ribosyl)ation of p53 does not depend on the formation of consensus sequence-DNA/p53 active complexes.

(This project was supported by NIH grant GM45451 to RAG.)

RESEARCH APPRECIATION DAY 2000

First Author: *Rafael Alvarez-Gonzalez, Ph.D.*

Department: *Molecular Biology and Immunology*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

METABOLIC CHANGES IN THE PROTEIN-POLY(ADP-RIBOSYL)ATION PATHWAY OF DIFFERENTIATING RAT GERMINAL CELLS

Luigia Atorino, † Rafael Alvarez-Gonzalez, ** Anna Cardone, *Iliana Lepore
* Benedetta Farina & * Piera Quesada

*Dept. of Organic & Biological Chemistry; **Dept. of Evolutive & Comparative Biology; University Federico II, 80134-Napoli,Italy and †Dept. of Molecular Biology and Immunology; University of North Texas, Health Science Center, 76107-2699 Fort Worth, TX, USA.

Endogenous levels of poly(ADP-ribose) and NAD+ have been determined in male rat germinal cells at different stages of differentiation. The levels of both metabolites decreased progressively from primary spermatocytes to secondary spermatocytes and especially in spermatids. We have also determined the size and complexity of the ADP-ribose polymers synthesized in permeabilized male rat germ cells. Polymers of increasing chain length and complexity were observed in cells incubated with increasing concentrations of radiolabeled NAD+. While short ADP-ribose polymers were observed in spermatocytes and spermatids with low NAD+ concentrations, polymers of over 20 residues in size were associated with non-histone proteins and oligomers of 20 ADP-ribose units or less were always associated with histone proteins. In addition, whereas PARP was extensively auto-poly(ADP-ribosyl)ated, by far, the H1t variant of histone H1 appeared to be the preferred ADP-ribose target in all cells examined as demonstrated after the separation of histone proteins by reverse-phase HPLC and specific radioactivity measurements. The preferential poly(ADP-ribosyl)ation of H1t, amongst all histones, was particularly evident in primary spermatocytes. Therefore, based on all metabolic parameters examined, we conclude that an active protein-poly(ADP-ribosyl)ation system is concentrated in primary spermatocytes, the germinal cells undergoing the pachytene phase of meiotic division. This project was partially supported by NIH grant GM45451 to RAG.

RESEARCH APPRECIATION DAY 2000

First Author: *Rafael Alvarez-Gonzalez, Ph.D.*

Department: *Molecular Biology and Immunology*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

BACULOVIRUS-EXPRESSED RECOMBINANT RAT AND HUMAN POLY(ADP-RIBOSE) POLYMERASE: TIME COURSE OF INFECTION, PURIFICATION, AND ENZYMOLOGICAL ANALYSIS

Sascha BENEKE*, Rafael ALVAREZ-GONZALEZ† and Alexander BÜRKLE*‡
* Abteilung Tumorvirologie, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 242, D-69120 Heidelberg, Germany and † Department of Molecular Biology and Immunology, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107-2699, USA.

DNA damage induced in higher eucaryotes by alkylating agents, oxidants or ionizing radiation leads to the synthesis of protein-conjugated poly(ADP-ribose) catalyzed by poly(ADP-ribose) polymerase-1 (PARP-1). Poly(ADP-ribose) is covalently attached to several DNA-binding proteins like histones and PARP-1 itself. Previously, the cellular poly(ADP-ribosyl)ation capacity has been shown to be positively correlated with the life span of mammalian species (Grube and Bürkle [1992] Proc. Natl. Acad. Sci. USA 89, 11759-11763). Here, we have tested the hypothesis that this correlation results from differences in kinetic parameters of the enzymatic activity of PARP-1. We therefore compared recombinant enzymes, expressed in a baculovirus system, from rat and man as two mammalian species with extremely divergent life span. In standard activity assays performed in the presence of histones as poly(ADP-ribose) acceptors both recombinant enzymes showed the expected second-order kinetics with respect to [PARP-1] (Mendoza-Alvarez and Alvarez-Gonzalez [1993] J. Biol. Chem. 268, 22575-22580) and saturation kinetics with [NAD+]. Determination of the kinetic parameters (kcat, km and kcat/km) did not reveal any differences between the two enzymes. Nevertheless, the specific activity of human PARP-1 was consistently found to be about 25% higher. In assays performed without histones, human PARP-1 displayed up to two-fold higher specific activity compared with rat PARP-1. We conclude that the correlation of cellular poly(ADP-ribosyl)ation capacity with mammalian life span is not reflected in the classical kinetic parameters, but that the subtle differences in primary structure of PARP-1 from two mammalian species of vastly different longevity control the extent of automodification. This project was partially supported by NIH grant GM45451 to RAG.

RESEARCH APPRECIATION DAY 2000

First Author: *Rafael Alvarez-Gonzalez, Ph.D.*

Department: *Molecular Biology and Immunology*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

POLY(ADP-RIBOSE) POLYMERASE AND ADP-RIBOSE POLYMERS IN THE KARYOPLASM AND THE NUCLEOLUS OF HELA CELLS IN APOPTOSIS

Rafael Alvarez-Gonzalez*&1, Herbert Springß, Marcus Müller& and Alexander Bürkle&
From: &The Division of Tumor Virology and ßthe Biomedical Structural Analysis Unit, German Cancer Research Center, Heidelberg, Germany; and &1The Department of Molecular Biology & Immunology and The Institute for Cancer Research, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX, 76107-2699, USA.

We have immunolocalized poly(ADP-ribose) polymerase (PARP), its apoptotic fragments, and poly(ADP-ribose) in specific nuclear domains of HeLa cells following exposure to 50 mM N-methyl-Ní-nitro-N-nitrosoguanidine (MNNG) by immunofluorescence and confocal microscopy. DNA oligonucleosomal fragmentation and proteolysis of PARP confirmed Apoptosis. We used monoclonal antibodies (F-I-23, C-II-10, and 10H) to specifically localize PARP, it's 29 kDa and 85 kDa fragments, and poly(ADP-ribose), respectively. As expected, while non-treated cells displayed homogeneous nucleoplasmic staining with F-I-23 and C-II-10, no immunofluorescence was observed with 10H. Instead, the intensity of 10H immunofluorescence quickly increased within 15 min of alkylating damage, and then disappeared between 15 and 180 min, suggesting that poly(ADP-ribose)glycohydrolase, the catabolic enzyme of poly(ADP-ribose), remains active during apoptosis. Interestingly, 15-120 min after MNNG, the C-II-10 and 10H immunostaining of the karyoplasm completely excluded nucleoli. Therefore, the 85-kDa fragment, which comprises the automodification and catalytic domains of PARP, quickly disappears from nucleoli with covalently bound polymers. By contrast, F-I-23 immunofluorescence remained evenly distributed over the nucleus. We conclude that the nucleolus is a highly dynamic center where some of the early biochemical changes of apoptosis take place and also strongly suggest differential roles for the proteolytic products of PARP in apoptotic execution. This project was partially supported by NIH grant GM45451 to RAG.

RESEARCH APPRECIATION DAY 2000

First Author: *Nils F. Confer*

Department: *Molecular Biology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

MOLECULAR INTERACTIONS OF POLY(ADP-RIBOSE) POLYMERASE (PARP) WITH DNA-BINDING PROTEINS DURING THE REPAIR OF DAMAGED DNA IN CULTURED CELLS. Nils F. Confer and Rafael Alvarez-Gonzalez, Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, TX 76107.

Poly(ADP-ribose) polymerase (PARP) [E.C. 2.4.2.30] is a ubiquitous protein in the nucleus of mammalian cells. This DNA-dependent enzyme catalyzes the covalent poly(ADP-ribosyl)ation of DNA-binding proteins such as histones and DNA-metabolizing enzymes. Upon covalent modification with ADP-ribose polymers, the protein acceptor detaches from DNA, presumably due to electrostatic repulsion between the negative charges on both polynucleotides. The reversibility of this pathway is insured by the efficient enzymatic removal of protein-bound polymers in a sequential manner. The catabolism of poly(ADP-ribose) is catalyzed by two enzymes, poly(ADP-ribose) glycohydrolase (PARG) and protein-(ADP-ribose) lyase (PARL), respectively. In this project, we will be examining the protein-protein interactions between PARP/PARG and polypeptides that modulate DNA-repair pathways. Amongstthe proteins of interest are: DNA polymerase □, methyl transferase(s), helicase(s), XRCC1, and ATM, to name a few. We plan to subject cultured cells to physical or chemical insults to induce either nucleotide excision repair or the base excision repair pathways. We will measure DNA repair with either a COMET assay or the TUNNEL assay. We will also measure survival rates and/or apoptotic indices depending on the dose of the DNA-damaging agent. In addition, we will also perform reciprocal co-immunoprecipitation studies with monoclonal antibodies specific for PARP/PARG and/or other proteins indicated above, followed by reciprocal immunodetection by western blotting. Finally, total intracellular levels of poly(ADP-ribose) and PARP/PARG activity will be measured to extrapolate enzyme activities with product formation and immunolocalization studies by fluorescent microscopy. This project will be supported fully by grant GM45451 from the NIH to RAG.

RESEARCH APPRECIATION DAY 2000

First Author: *Woo-Jin Chang*

Department: *Molecular Biology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

POLY(ADP-RIBOSYLATION) OF TRANSCRIPTION FACTOR NF-KB: EFFECTS ON DNA BINDING. Woo-Jin Chang and Rafael Alvarez-Gonzalez. Department of Molecular Biology & Immunology, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107.

Poly (ADP-ribose) polymerase (PARP, E.C. 2.4.2.30) is a constitutively expressed nuclear protein in mammalian cells. The enzyme comprises about 1 % of the total nuclear protein and is phylogenetically well conserved amongst higher eukaryotes. PARP is instantaneously activated upon DNA strand-break formation. When active, PARP post-transitionally modifies DNA-binding proteins including histones, topoisomerases I & II, DNA ligases, and transcription factors such as TFIIF. However, the main protein acceptor *in vitro* and *in vivo* is PARP itself. The high density of negative charges (phosphate groups) of its chemical structure and its nuclear localization make poly(ADP-ribose) a nucleic acid-like molecule. It is hypothesized that the covalent binding of these highly negatively charged polynucleotides to transcription factors inhibits their ability to bind DNA due to electrostatic repulsion. Furthermore, the short half life of ADP-ribose polymers (less than one minute *in vivo*) also suggests that this polymer is a strong candidate for the regulation of DNA-functions, such as DNA-replication, transcription and repair. In order to explore the possible role of PARP in the transcriptional control of gene expression, we have initiated experiments to test the effects of the poly(ADP-ribosylation) of PARP and transcription factor NF-kB on the DNA-binding of the latter by electrophoretic mobility shift assays (EMSA). We have observed that the ability of PARP to poly(ADP-ribosyl)ate itself as well as NF-kB is essentially dependent on the presence of nicked DNA. We have also observed that NF-kB is not a efficiently poly(ADP-ribosyl)ated under standard incubation conditions. In fact, analysis by EMSA indicates that a weakly poly(ADP-ribosyl)ated NF-kB efficiently binds to its consensus DNA sequence, suggesting that there is not enough electrostatic repulsion between consensus DNA and a small amount of protein-bound poly(ADP-ribose). We also carried out experiments with HeLa cell nuclear extracts and observed similar results. Therefore, future experiments will include the poly(ADP-ribosyl)ation of NF-kB in cultured cells as well as co-immunoprecipitation studies with monoclonal antibodies specific for PARP and NF-kB. This project should contribute to the elucidation of the exact biological role of the poly (ADP-ribosyl)ation of transcription factors in mammalian gene expression. This project was fully supported by NIH grant GM45451 to RAG.

RESEARCH APPRECIATION DAY 2000

First Author: *Miguel Angel Medina*

Department: *Biomedical Sciences*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

MOLECULAR CHARACTERIZATION OF A NK CELL RECEPTOR RELATED TO 2B4 GENE. Miguel Angel Medina, Pappanaicken R. Kumaresan, Jiabin Ann, Porunelloor A. Mathew. Univeristy of North Texas Health Science Center. Department of Biomedical Sciences. 3500 Camp Bowie Boulevard, Fort Worth, Texas 76107-2699.

2B4 is a 66 kDa monomer expressed on the surface of natural killer cells and a sub-population of T cells. 2B4 is a high-affinity counter-receptor for CD48 in mice and humans. It is a member of the immunoglobulin supergene family (IgSF), and a member of the CD2-like subgroup. Previous functional studies showed that ligation of 2B4 with monoclonal antibodies increases target cell lysis, granule exocytosis, and IFN-γ production. Mouse NK cells express two isoforms of 2B4, 2B4-L and 2B4-S. Functional studies indicate that 2B4-S may provide a positive signal, while 2B4-L appears to provide an inhibitory signal. We have obtained two 2B4 genomic clones 531 and 532 approximately 30kb in size. 531 has been fully characterized, but 532 remains to be characterized. Genomic characterization of 531 revealed that both mouse 2B4-L and 2B4-S are products of alternate splicing. The difference between the two isoforms lies in their cytoplasmic domains. Partial characterization leads us to believe that 532 is a 2B4 related gene. Partial sequencing of 532 gene revealed 87% similarity with 531 at the coding regions. Here we are reporting the preliminary data ofthe characterization of the 532 genomic clones and the expression pattern of its transcript.

RESEARCH APPRECIATION DAY 2000

First Author: *Samuel S. Chuang, Ph.D.*

Department: *Molecular Biology and Immunology*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☒ Postdoctoral Fellow/Resident

2B4 STIMULATION OF YT CELLS INDUCES NK CELL CYTOLYTIC FUNCTION AND INVASIVENESS Samuel S. Chuang, Myoung H. Kim, Lori A. Johnson, Per Albertsson, Richard P. Kitson, Ulf Nannmark, Ronald H. Goldfarb, and Porunelloor A. Mathew. Department of Molecular Biology and Immunology, and Institute for Cancer Research, University of North Texas Health Science Center, Fort Worth, TX 76107; University of Göteborg, Göteborg, Sweden.

2B4 is a surface molecule found on all human NK cells, a subset of CD8+ T cells, monocytes and basophils. It was originally identified on mouse NK cells and the subset of T cells that mediate non-MHC-restricted killing. Recently we have cloned the human homologue of 2B4 (h2B4) [Boles et al., Molecular characterization of a novel human natural killer cell receptor homologous to mouse 2B4, Tissue Antigens 54 (1999) 27-34] and found h2B4 to also mediate non-MHC-restricted cytotoxicity. In this study, we examine h2B4 in regulating various functions of NK cells using a human NK cell line YT, with mAb C1.7, an antibody that specifically recognizes h2B4. Ligation of surface 2B4 with mAb C1.7, increases YT's ability to destroy tumor cells. In the presence of mAb C1.7, the production of IFN-g by YT cells is greatly enhanced. Engagement of surface 2B4 by mAb C1.7 down regulates the expression of h2B4 at the cell surface as well as the expression of h2B4 mRNA. Also, signaling through h2B4 causes the increased expression of matrix metalloproteinase-2, a member of the matrix degrading proteinase family. Thus in addition to modulating cytolytic function and cytokine production of NK cells, activation through surface 2B4 may play a role in up regulating the machinery for degradation of extracellular matrices to promote invasion of the tumor by NK cells.

RESEARCH APPRECIATION DAY 2000

First Author: *Pappanaicken R. Kumaresan, Ph.D.*

Department: *Department of Molecular Biology and Immunology*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☒ Postdoctoral Fellow/Resident

GENE STRUCTURE OF THE HUMAN NK CELL RECEPTOR 2B4.

P.R.Kumaresan and P.A.Mathew. Department of Molecular Biology and Immunology and Institute for Cancer Research,University of North Texas Health Science Center, Fort Worth,Texas 76107.

The receptor 2B4 is a cell surface glycoprotein of the immunoglobulin superfamily expressed on all NK cells and the subset of T cells that mediate non-MHC-restricted killing. 2B4 is the high-affinity counter-receptor for CD48 in both mice and humans. Interaction of 2B4 with monoclonal antibodies or CD48 increase target cell lysis and production of IFN-gamma. 2B4 is a member of the CD2 subgroup of the immunoglobulin superfamily which includes CD48, LFA-3, CD84, LY9 and SLAM. In mice 2B4 is expressed in two isoforms that differ solely in their cytoplasmic regions and function as inhibitory and stimulatory receptors. In human 2B4 functions as an activating receptor on NK cells and as an activation marker for CD8+ T cells. Here we report the genomic cloning and gene structure of the human 2B4 gene. Human 2B4 is about 30 kb long and contains at least nine exons, the first exon corresponds to the 5' untranslated and leader sequence, the second exon codes for the V domain, the third exon codes for the C2 domain, and the fourth codes for the transmembrane region. The cytoplasmic and 3' untranslated regions are derived from exons 5, 6 and part of exon 7. Overall, the genomic organization of human 2B4 is similar to that of other members of the immunoglobulin superfamily. This study was supported by NIH grant PO1 AI 38938.

RESEARCH APPRECIATION DAY 2000

First Author: *Myoung H. Kim, Ph.D.*

Department: *Mol. Bio. and Imm.*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☒ Postdoctoral Fellow/Resident

DIFFERENTIAL EXPRESSION OF MATRIX METALLOPROTEINASES AND TISSUE INHIBITORS OF MMPS IN HUMAN NK AND T CELL LINES BY IL-2 STIMULATION

1M.H. Kim, 2,3P. Albertsson, 1R.P. Kitson, 2U. Nannmark, and 1R.H. Goldfarb Dept. of 1Molecular Biology and Immunology, UNT Health Science Center and Institute for Cancer Research, Fort Worth, TX 76107-2699; and 2Depts of Anatomy and Cell Biology and 3Oncology, U. of Göteborg, Göteborg, Sweden

We have previously reported that IL-2 activated NK (A-NK) cells produce an array of matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) including membrane type-MMPs (MT-MMPs). These A-NK cell MMPs have been shown to play an important role in migration of these cells through a model basement membrane, and possibly in tumor infiltration. T lymphocytes also produce MMP-9 constitutively, but not MMP-2 which is induced by IL-2.

Here, we report that IL-2 stimulation of YT and Jurkat cells, human NK and T-cell lines respectively, causes the differential expression of MMPs and TIMPs. Moreover, we demonstrate for the first time MMP-8 expression in human NK and T-cell lines. IL-2 stimulation of YT cells induced the expression of MMP-8 (neutrophil collagenase), while down-regulating TIMP-1. However, Jurkat cells constitutively express MMP-8, TIMP-1 and TIMP-2 independent of IL-2 stimulation. Most significantly, RT-PCR analysis of IL-2-stimulated YT cells revealed the expression of mRNA for MMP-12 (macrophage metalloelastase), but not in unstimulated YT cells nor in Jurkat cells. MMP-12 has been shown to degrade plasminogen to angiostatin, an anti-angiogenic molecule.

These findings suggest that the IL-2 stimulation of YT cells can coordinately up-regulate the expression of MMP-8 and MMP-12, while down-regulating the expression of TIMP-1. Moreover, this effect may be mediated by a cell-type specific signaling pathway, since IL-2 has no effect on the expression of MMP-8 and TIMPs in Jurkat cells.

RESEARCH APPRECIATION DAY 2000

First Author: *Giridhar R. Akkaraju*

Department: *Molecular Biology and Immunology*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☒ Postdoctoral Fellow/Resident

OVEREXPRESSION OF PROTEIN KINASE C eta ATTENUATES CASPASE ACTIVATION AND TUMOR NECROSIS FACTOR-alpha INDUCED CELL DEATH.

Giridhar R. Akkaraju and Alakananda Basu, Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth TX 76107

Tumor necrosis factor-alpha is a cytokine that can trigger cell death by binding to its receptor. TNF receptor (TNFR I) forms a homotrimer upon activation by TNF-alpha and in turn activates cell death enzymes, the caspases. Caspases are cysteine proteases that cleave after specific aspartate residues within essential cellular proteins, leading to the physical changes seen during apoptosis. Protein Kinase C has been shown to be involved in TNF-alpha-induced apoptotic cell death. For instance, the catalytic fragment of PKCeta has been shown to trigger apoptosis in some cell types. Upregulation of PKCeta has been correlated with a decrease in cellular sensitivity to TNF-alpha. In this study, we investigate the role played by PKCeta in TNF-alpha-induced cell death. Cell lines overexpressing PKCeta were created by transfection with an expression vector encoding the gene. These cells showed a decreased sensitivity to TNF-alpha-induced cell death. PKCeta overexpression coincided with a decrease in the activation of caspase-8, -7 and -9. Control cells transfected with just the parental vector, on the other hand, showed a time dependent activation of caspase-8 and -7. Cells exposed to TNF-alpha also show a time dependent induction of PKCeta. These results suggest that PKCeta blocks caspase activation and TNF-alpha-induced cell death in MCF-7 cells.

RESEARCH APPRECIATION DAY 2000

First Author: *Tuan Pham*

Department: *Molecular Biology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

FUNCTIONAL STUDY OF LECTIN-LIKE TRANSCRIPT (LLT) RECEPTOR ON HUMAN NK CELLS. Hoang-Tuan K. Pham, Kent S. Boles, Samuel S. Chuang, P.A. Mathew. University of North Texas Health Science Center, Department of Molecular Biology and Immunology, Fort Worth, TX 76107-2699.

Natural killer (NK) cells constitute a population of lymphocytes that spontaneously kill neoplastic and virally infected cells and mediate allograft rejection via the interaction of inhibitory and activating surface receptors with target ligands. A group of NK cell receptors belongs to the C-type lectin superfamily and localizes to the NK complex on chromosomes 6 and 12 in mouse and human, respectively. The NK gene complex encodes type II receptors including the families of NKR-P1, Ly-49, and NKG-2 receptors. We identified and cloned the cDNA of a human lectin-like transcript 1 (LLT1) receptor expressed on NK, T, and B cells. The LLT1 gene localizes to the NK gene complex. The LLT1 cDNA encodes a predicted protein of 191 amino acid residues. The predicted protein contains a transmembrane domain near the N-terminus and an extracellular domain of 131 amino acid residues homologous to C-type lectins. Our goal is to characterize LLT1 function. We PCR-amplified the LLT1 cDNA with a set of primers designed to introduce a C-terminal histag epitope. Subsequently, it was subcloned into a mammalian expression vector pCI-neo and transfected into a murine T-cell lymphoma cell line (BW). By antibiotic resistance selection we had selected several positive clones, and by Western analysis, we have confirmed the expression of LLT1 protein in these clones. We are in the process of immunizing AKR/J strain mice with LLT1 protein for anti-LLT1 monoclonal antibody production. We have also subcloned LLT1 cDNA into a bacterial expression vector pQE-60 for protein expression in bacteria. We are now confirming LLT1 protein production in bacteria that will be used to immunize rabbits for polyclonal antibody production. Generated antibodies will allow us to study the functional role of LLT1 receptors in human NK cells and to decipher the molecular mechanism of LLT1 receptor signaling.

RESEARCH APPRECIATION DAY 2000

First Author: *Min Lu*

Department: *Molecular Biology & Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

PURIFICATION AND INITIAL CHARACTERIZATION OF PROTEASOME FROM NATURAL KILLER CELLS Min Lu, Richard P. Kitson, Ph.D. and Ronald H. Goldfarb, Ph.D. Department of Molecular Biology and Immunology & Institute for Cancer Research, UNT Health Science Center, Fort Worth, TX 76107

The proteasome is a key enzyme in the degradation of proteins in all cells including eukaryotic cells, and has been implicated in numerous cellular processes such as cell-cycle control and cellular differentiation. We have previously reported that the chymotrypsin-like activity of the rat natural killer cell proteasome may play a role in natural killer (NK) cell-mediated cytotoxicity. In addition, we also noted that there were some differences in biochemical properties of proteasome between rat NK cells and rat hepatic cells. Here, we applied isopycnic sucrose gradient fractionation of postnuclear supernatants, molecular sieve chromatography, and heparin-Sepharose chromatography to partially purify the proteasome from the rat NK leukemic cell line CRNK-16 cells. The purification achieved to date is 299 fold. Polyacrylamide gel electrophoresis of the final proteasome preparation gave an apparent single protein band under nondenaturing conditions as assessed by Coomassie Blue staining and await further analysis by silver staining. The substrate specificity of proteasomes indicated that they contain at least three types of activity, namely, chymotrypsin-like, trypsin-like, and postglutamyl cleaving activities. Four peptide aldehydes achieved an 80% inhibition rate for chymotrypsin-like activity of proteasomes from NK cells and were ranked the most useful inhibitors among 18 inhibitors tested. Although proteasomes from rat NK cells and rat hepatic cells share a common preference on most substrates, there were still some significant differences in specificity for some substrates, most of which are chymotryptic substrates. Differences of inhibition rates of some agents on the proteasome activities between rat NK cells and rat hepatic cells were also observed. Moreover, CEP1612, a single synthetic selective chymotryptic proteasome inhibitor, had differential effects on two chymotryptic substrates in the studies with the Jurkat cell line. Therefore, these distinguished properties of proteasomes from NK cells implied that proteasomes in NK cells may have a unique structure and contain more than one site for chymotryptic cleaving activity. (This study is supported by a grant from Robert A. Welch Foundation.)

RESEARCH APPRECIATION DAY 2000

First Author: *Lori Johnson*

Department: *Molecular Biology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

MOLECULAR REGULATION OF INTERFERON GAMMA BY 2B4 ACTIVATION IN HUMAN NATURAL KILLER CELLS. Lori A. Johnson, Ronald H. Goldfarb and Porunelloor A. Mathew. University of North Texas Health Science Center at Fort Worth Texas. Department of Molecular Biology and Immunology. Fort Worth, Texas, 76107-2699.

Interferon gamma (IFNγ) is a cytokine shown to stimulate signaling cascades resulting in activation of gene transcription. This activity is important in anti-viral, anti-proliferative, and immunomodulatory processes. For instance, in antigen presenting cells, IFNγ has been shown to upregulate expression of major histocompatibility (MHC) Class II molecules and antagonize viral replication. IFNγ can also assist in the activation of macrophages, promote T cell differentiation, and promote expression of certain immunoglobulin (Ig) isotypes in B cells. The overall role of IFNγ in immune response is critical and it is believed to have potential in treating clinical diseases. We previously identified a natural killer and T cell surface molecule, 2B4. 2B4 is involved in non-MHC-restricted lysis of virus-infected cells and some tumor cells. The interaction of murine 2B4 with anti-2B4 monoclonal antibody has resulted in increased IFNγ secretion and granule exocytosis in γδ epidermal T cells. The aim of the current study is to completely decipher the molecular regulation of IFNγ by 2B4 activation in human natural killer cells. Proposed studies include the determination of IFNγ secretion, message stability, transcription level, and promoter analysis. ELISA analysis has revealed that IFNγ secretion is greatly enhanced in human natural killer cells following 2B4 activation, whereas IL-2 stimulation results in only a moderate increase in IFNγ secretion and stimulation with an isotype control antibody does not augment IFNγ secretion. We have cloned the human IFNγ promoter by PCR from genomic DNA. Several deletion mutations of the IFNγ promoter were made. These deletion constructs are being subcloned into a reporter vector that will allow us to study its activity. Additionally, mRNA stability studies are currently in progress. Results from this study will allow us to better understand the importance of 2B4 in natural killer cell activation.

RESEARCH APPRECIATION DAY 2000

First Author: *Ginelle Courchaine*

Department: *Molecular Biology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

INTEGRINS REGULATE NATURAL KILLER CELL PROTEOLYTIC ENZYMES IN MODULATION OF NATURAL KILLER CELL INVASION AND MIGRATION.

Ginelle Courchaine, Richard P. Kitson Ph.D., and Ronald H. Goldfarb Ph.D.
Department of Molecular Biology and Immunology, Institute for Cancer Research, University of North Texas Health Science Center, Fort Worth, TX, 76107.

Lymphocyte cell adhesion to the extracellular matrix is mediated by integrins. Integrins are a family of transmembrane receptors consisting of noncovalently associated alpha and beta subunits. Each heterodimer complex forms the extracellular ligand-binding region for various components of the extracellular matrix. Integrins regulate aspects of lymphocyte invasion and migration by mediating adherence to extracellular matrix components, such as laminin, fibronectin, collagen, and vitronectin. Furthermore, integrins play an important role as signaling receptors that contribute to cell migration. Previous work in tumor cells and certain leukocytes has shown that integrins are capable of localizing proteolytic activities at the cell surface by direct interaction between the integrins and the extracellular matrix-degrading proteases and/or their receptors, such as matrix metalloproteinases (MMPs) and the urokinase plasinogen activator receptor (uPAR). We therefore hypothesize that integrins are capable of directly interacting with proteases on the surface of interleukin-2 (IL-2) activated natural killer (A-NK) cells to facilitate A-NK cell invasion, migration, and cytotoxicity. Our studies will contribute to our understanding of how integrins play a role in A-NK cell accumulation within established cancer metastases and provide potential and novel targets for enhancing therapy of established cancer metastases.

RESEARCH APPRECIATION DAY 2000

First Author: *Kent S. Boles*

Department: *MBI*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

CHARACTERIZATION OF A NOVEL LECTIN-LIKE TRANSCRIPT (LLT2) EXPRESSED ON HUMAN NK CELLS. Kent S. Boles and Porunelloor A. Mathew. Department of Molecular Biology and Immunology & Institute for Cancer Research, University of North Texas Health Science Center, Fort Worth, TX.

Natural killer (NK) cells are lymphocytes that spontaneously detect and kill cancerous and virally infected cells through receptors that transduce either activating or inhibiting signals. Many NK cell receptors belong to the C-type lectin superfamily and include CD69, Ly-49, and the NKG2/CD94 heterodimer. We have previously described a lectin-like transcript (LLT1) expressed on human NK cells which mapped to the NK gene complex on chromosome 12. Here we report the molecular characterization of a second human lectin-like transcript (LLT2). The cDNA encodes a predicted protein of 242 amino acid residues with a transmembrane domain near the N-terminus and an extracellular domain of 199 amino acid residues with homology to the carbohydrate recognition domain of C-type lectins. The predicted protein of LLT2 shows 46, 49, and 51% similarity to NKG2-D, CD94, and LLT1, respectively. The predicted protein does not contain intracellular ITIM motifs suggesting that LLT2 may be involved in mediating activation signals. Supported by NIH grant AI38938.

RESEARCH APPRECIATION DAY 2000

First Author: *Matthew Woolard*

Department: *Molecular Biology and Immunology*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☒ Staff

☐ Postdoctoral Fellow/Resident

REGULATION OF APOPTOSIS BY BCL-2 AND PROTEIN KINASE C DELTA IN HELA CELLS. Matthew D. Woolard, and Alakananda Basu. Department of Molecular Biology and Immunology. UNTHSC at Fort Worth, Fort Worth, TX. 76107.

An inbalance between cell proliferation and cell death leads to cancer. Anti-cancer agents not only block cell proliferation but also induce cell death by apoptosis. The main components of this apoptosis pathway are a family of cysteine proteases called caspases. With initiator caspases (caspase-8 and -9) first being activated, they then activate executioner caspases (caspase-3, -7, and -2). Once these proteases are activated they are irreversible committed to cell death. There are several proteins that regulate the activation of caspases. The anti-apoptotic protein Bcl-2 is a mitochondrial membrane bound protein, that either blocks the release of cytochrome C from the mitochondria inner matrix, or binds up Apaf-1, a protein that is integral in the activation of caspase-9, or both of these functions. Protein Kinase C Delta (PKC delta) is a cytosolic protein, that has been shown to be a substrate of caspase-3 and also regulates the activation of caspases. In the present study we have investigated the regulation of cisplatin (CP) and Tumor Necrosis Factor (TNF) mediated apoptosis by Bcl-2 and PKC delta. While rottlerin, a PKC delta inhibitor, blocked cisplatin induced activation of caspase-9, -8, -3, and -7, it potentiated the activation of these caspases during TNF mediated apoptosis. Overexpression of Bcl-2 in HeLa cells blokced cisplatin mediated activation of caspase-9, -8, -3, and -7, but failed to block TNF mediated activation of caspase-3 and -8. These results suggest that Bcl-2 and rottlerin regulate the TNF and cisplatin mediated apoptosis via distinct mechanisms.

CARDIOVASCULAR RESEARCH INSTITUTE

30.	Peter B. Raven, PhD	CARDIOVASCULAR RESEARCH INSTITUTE
31.	Hong Zeng	THE LABORATORY OF CARDIAC AND VASCULAR MOLECULAR GENETICS
32.	Tom Valencia	TETRACYCLINE-INDUCIBLE CAM KINASE II SILENCES HYPERTROPHY-SENSITIVE GENE EXPRESSION
33.	Janelle Hardisty	ROLE OF TRANSCRIPTION FACTOR YY1 IN REGULATING GENES OF CARDIAC HYPERTROPHY
34.	Rebecca A. Deaton	INCREASED WORKLOAD CAUSES TRANSCRIPTIONAL UP-REGULATION OF CARDIAC HYPERTROPHY-SENSITIVE GENES IN THE RAT NEONATE CARDIOMYOCYTE
35.	G. S. Boswell	DEVELOPMENT OF THE HUMAN ARTERIAL EQUIVALENT. PART 1. ISOLATION AND PROPAGATION OF THE CELLULAR COMPONENTS
36.	Xiaoming Bian	LIFE SAVING IMPROVEMENT NI SYSTEMIC HEMODYNAMIC S WITH ENHANCED INTRA-AORTIC ASSIST DEVICE: EVALUATION IN CANINES WITH SEVERE, ACUTE LEFT VENTRICULAR FAILURE
37.	Paul Fadel, M.S.	ARTERIAL BAROREFLEX CONTROL OF SYMPATHETIC NERVE ACTIVITY DURING ACUTE HYPOTENSION
38.	Shigehiko Ogoh	INCREASES IN INTRAMUSCULAR PRESSURE RAISE ARTERIAL BLOOD PRESSURE DURING DYNAMIC EXERCISE
39.	M.A. Williams	CORONARY VASCULAR RESISTANCE DURING EXERCISE IN DOGS WITH RENOVASCULAR HYPERTENSION (RVH)
40.	Srinath Setty	ADENOSINE MODULATES MYOCARDIAL OXYGEN CONSUMPTION (MVO2) OF RIGHT VENTRICLE (RV) IN DOGS WITH INHIBITED SYNTHESIS OF ENDOTHELIUM-DERIVED NITRIC OXIDE (NO)

41. Jie Sun — PYRUVATE RESTORES FUNCTION OF HYDROGEN PEROXIDE-INJURED MYOCARDIUM

42. Eric B. Pearlman — MET ENKEPHALIN ARG PHE (MERF) AND METABOLISM OF MERF ACROSS THE CANINE HEART VASCULAR BED

43. Martin Farias III — SUPER DALDA ENHANCES ADRENERGIC RESPONSES IN THE DOG

RESEARCH APPRECIATION DAY 2000

First Author: *Peter B. Raven*

Department: *Cardiovascular Research Institute*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

WELCOME TO THE CRI

Peter B. Raven, Ph.D., Director, Cardiovascular Research Institute
UNT Health Science Center at Fort Worth
3500 Camp Bowie Blvd., Fort Worth, Texas 76107

Medical consumers and patients are removed from the realm of research being conducted in universities and hospitals all over the world. Sometimes a specific scientific breakthrough is reported and you may wonder how that relates to you. At the Cardiovascular Research Institute we want to educate the medical consumer with the information necessary to make sound medical decisions. We also want to educate the patient to understand not only how research can be applied to produce a safer medical treatment, but to bring definitions to the medical terminology the patient hears but often doesn't quite understand.

While the Cardiovascular Research Institute is continually researching the many diseases that make up the genre known as heart disease, such as myocardial infarction, hypertension, and congestive heart failure, we are also looking at ways to improve the overall patient tolerance of medications and developing devices to insure a better recovery for patients of cardiac surgery.

MISSION STATEMENT: The Cardiovascular Research Institute was established in 1995 as a Center for Excellence of the UNT Health Science Center at Fort Worth. It is a multidisciplinary program designed to promote basic and clinical research, education, clinical practice and community outreach programs in the prevention, diagnosis, treatment and rehabilitation of cardiovascular disease of human beings of all ages.

RESEARCH APPRECIATION DAY 2000

First Author: *HONG ZENG*

Department: *CARDIOVASCULAR RESEARCH INSTITUTE*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☒ Staff

☐ Postdoctoral Fellow/Resident

THE LABORATORY OF CARDIAC AND VASCULAR MOLECULAR GENETICS.
Hong Zeng and Stephen R. Grant. Cardiovascular Research Institute, University of North Texas Health Science Center, Fort Worth, TX 76107.

The laboratory of cardiac and vascular molecular genetics is housed in the Cardiac Research Institute of UNT Health Science Center. The research interest for this lab is mainly focused on nuclear regulatory mechanisms involved in the control of cardiac hypertrophy-sensitive contractile protein gene expression and events which lead to its downstream disease state. This laboratory is also interested in exploring nuclear mechanisms controlling different signals for smooth muscle myocytes hypertrophy growth versus hyperplastic expansion in vascular smooth muscle system. The research approach for this lab is utilizing three rodent systems for gene activity assessment, the neonate and post-differentiated primary cardiomyocytes, the arterial smooth myocytes, and the murine transgenic hearts. This lab has explored mechanisms of cardiac gene induction and/or gene repression under conditions of cardiovascular hypertrophic growth, dilated cardiomyopathy, work overload-related stress, and vascular hypertention. These research interests have lead to establishment of a international recognized calcineurin-driven nuclear signal transduction pathway for cardiac hypertrophy and identification of the following mechanisms, three other calcium-sensitive nuclear signaling pathways, two new models for enzymatic cross-regulation of cardiac gene expression, and silencing of myocyte contractile protein gene expression by direct phosphorylation and attenuation of three enzymes identified as mediators of hypertrophic gene induction. In collaboration with Dr. Eric Olsen, UT Southwestern Medical School, this lab has established three transgenic models for cardiac hypertrophy and heart failure. These transgenic models and related primary cardiomyocyte research in this lab demonstrate direct relevance to the molecular mechanisms of chronic hypertrophy, heart failure, and sudden death. This lab so far has 4 pending international patterns and has attracted Myogen Inc., a 40-million-dollar biotechnology company, to sponsor these research. The ultimate goal for this laboratory is to develop a new generation of cardiac drugs which will prevent enlarged heart formation in the aging American population and hopefully clinical intervention for prevention of heart failure.

RESEARCH APPRECIATION DAY 2000

First Author: *Tom Valencia*

Department: *Integrative Physiology*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☒ Staff

☐ Postdoctoral Fellow/Resident

TETRACYCLINE-INDUCIBLE CAM KINASE II SILENCES HYPERTROPHY-SENSITIVE GENE EXPRESION Tom Valencia, Don Roberts, Hong Zeng and Stephen R. Grant UNTHSC, Cardiovascular Research Institute, Fort Worth, TX 76107

Recent work from this laboratory both in rodent primary neonate cardiomyocytes and in ventricular tissue of transgenic mouse models of induced hypertrophy has identified two calcium/calmodulin-dependent nuclear signaling cascades. The first involves the phosphatase, calcineurin (CaN). The second is the CaM kinase kinase nuclear signaling cascade which involves CaM kinase I and CaM kinase IV. Each of these signaling cascades strongly up-regulate transcription of hypertrophy-sensitive genes in the rodent ventricular cardiomyocyte. We have documented that over-expression of a heterologous active cytoplasmic form of CaM kinase II silenced transcriptional induction of hypertrophy-sensitive genes. The purpose of this study was to generate an inducible CaM kinase II expression system in order to correlate its expression with the silencing of hypertrophic-sensitive reporters. A truncated form of CaM KII, CaM KII (1-290) was subcloned downstream and proximal to an inducible promoter under transcriptional control (induction) of the tetracycline-regulated transcription factor, tet-TransActivator (tTA). When this system was co-expressed with viral-driven over expression plasmids either alone or in combination harboring active forms of CaN, CaM KI or CaM KIV, induced CaM KII expression silenced CaN, CaM kinase I, or CaM kinase IV driven reporter activity 16.5, 19.3, and 13.8 fold below their maximal values, respectively. Myocyte exposure to doxycycline (DOX) blocked tTA driven CaM KII expression and restored CaN and CaM KI or CaN and CaM KIV driven reporter activation. This study demonstrates, for the first time, that active cytoplasmic CaM KII silences calcium-sensitive nuclear signaling cascades for transcription up-regulation of cardiomyocyte hypertrophy.

RESEARCH APPRECIATION DAY 2000

First Author: *Janelle Hardisty*

Department: *Integrative Physiology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

ROLE OF TRANSCRIPTION FACTOR YY1 IN REGULATING GENES OF CARDIAC HYPERTROPHY

Janelle Hardisty, Tom Valencia, and Stephen R. Grant, Ph.D.
Cardiovascular Research Institute, University of North Texas Health Science Center, Fort Worth, TX 76107

Chronic stimulation of cardiac hypertrophy can lead to heart failure in humans. Models exist for up-regulation of gene transcription, but very little work has been done on pathways which down-regulate gene transcription. The transcription factor, Yin Yang 1(YY1), has been shown to regulate transcription of many genes, both positively and negatively. This work deals with the effects of YY1on cardiac hypertrophy-sensitive genes. We subcloned YY1 downstream and proximal to a minimal viral promoter under the strict transcriptional control of the tetracycline-regulated TransActivator (tTA). This new tTA-inducible YY1 is expressed only in the presence of tTA and can be effectively shut off by the addition of doxycycline (DOX), thus halting any YY1-mediated effect. This study looks at the effects of YY1 on three hypertrophy-sensitive reporters: atrial natriuretic factor (ANF), skeletal □-actin (SkA), and cardiac □-actin (CaA). All three of these promoters have shown repression in basal expression in the presence of tTA-YY1. Moreover, tTA-YY1 is capable of silencing hypertrophy-sensitive reporters in the presence of calcineurin (CaN), a Ca2+/calmodulin-dependent phosphatase that has been shown to up-regulate hypertrophy-sensitive reporters. In cardiomyocytes, it appears that YY1's ability to repress transcription of hypertrophiy-sensitive genes is independent of a YY1 DNA binding motif. This suggests that YY1 is capable of affecting transcription through a mechanism other than direct DNA interaction.

RESEARCH APPRECIATION DAY 2000

First Author: *Rebecca A. Deaton*

Department: *Biomedical Science*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

INCREASED WORKLOAD CAUSES TRANSCRIPTIONAL UP-REGULATION OF CARDIAC HYPERTROPHY-SENSITIVE GENES IN THE RAT NEONATE CARDIOMYOCYTE . Rebecca A. Deaton and Stephen R. Grant PhD. Laboratory of Cardiac and Vascular Molecular Genetics, Cardiac Research Institute, University of North Texas Health Science Center at Fort Worth, 3500 Camp Bowie Boulevard, Fort Worth, Texas 76017

Increased workload on the heart through training has been shown to induce cardiac hypertrophy. This abberant form of growth is hallmarked by up-regulation of several genes, including Beta Myosin Heavy Chain, Skeletal Alpha Actin, Cardiac Alpha Actin and Atrial Natriuretic Factor. This laboratory has previously shown that the expression of these genes is regulated by Ca2+/CaM-dependent protein kinases, however, the complete signaling pathway through which these enzymes accomplish this is largely unknown. The molecular mechanisms of how calcium mediated signaling can induce and maintain cardiac hypertrophy is of great importance in understanding the remodeling of the myocardium resulting from exercise training or heart disease in the elderly. In particular, we are interested in mechanisms that can block or inhibit this signaling in order to reduce hypertrophy. Understanding these mechanisms will help in the treatment of debilitative cardiac hypertrophy, which is often associated with chronic heart failure in the aged person as well as show us how to maintain helpful hypertrophy associated with exercise training.

Work-overload can be simulated in rat neonate cardiomyocytes by subjecting them to electrical stimulated contraction. We show here that prolonged stimulation causes up-regulation of several cardiac hypertrophy-sensitive genes including Skeletal Alpha Actin and Cardiac Alpha Actin. We also demonstrate that transient transfection of constitutively active CaMK II alpha can silence the increased expression of these genes seen after stimulation. We propose a pathway in which modulation of this gene expression depends upon a calcium-sensitive CREB/ATF (cAMP response element binding protein/ activating transcription factor) family of transcription factors. We hypothesize that these transcription factors can be activated or inactivated by Ca2+/CaM dependent enzymes in order to modulate gene expression due to the presence or absence of hypertrophic stimulii.

RESEARCH APPRECIATION DAY 2000

First Author: **G. S. Boswell**

Department: **Molecular Biology and Immunology/Cardiovascular Research institute**

GSBS Student SPH Student PA Student

TCOM Student Faculty X Staff

Postdoctoral Fellow/Resident

Development of the Human Arterial Equivalent. Part I. Isolation and Propagation of the Cellular Components G. S. Boswell and S.D. Dimitrijevich, Departments of Molecular Biology and Immunology and Cardiovascular Research institute, University of North Texas Health Science Center, Fort Worth, TX 76107

Vascular tissue damage due to cardiovascular diseases (e.g. hypertension, atherosclerosis), is a major cause of death in the industrial world. Although replacement of dysfunctional tissue is limited by the supply of suitable pressure vessels, grafts engineered from human cellular components, are becoming a viable alternative. We have recently developed several hetero-poly-cellular Human Tissue Equivalents (HTE), based on a three dimensional non-contracted collagen type I matrix. These are populated with appropriate human mesenchymal cells, and support a fully differentiated epithelium and where appropriate a functioning endothelium.

The goal of this project is to establish a Human Arterial Tissue Equivalent using human vascular smooth muscle cells, endothelial cells and fibroblasts, and type I collagen. In order to accomplish this task there must be a ready source of normal human vascular cells, primarily the endothelial cells and the smooth muscle cells. Based on our previous experiences with the skin and eye tissue we have developed methodology for obtaining homogeneous cultures of normal human endothelial and smooth muscle cells from umbilical artery. In vitro senescence is a common disadvantage of normal human cells. Although we have developed media which delay this process in UASMC and HUAEC a more appropriate solution is to immortalize these cells. Recently catalytic sub unit of human telomerase (hTERT) has been cloned. Its over expression in normal human fibroblasts and retinal pigment epithelial cells has been shown to lead to a greatly extended in vitro life span. Using retroviral infection technology we have successfully transfected UASMC and HUAEC cells with hTERT and have shown that transfectants express the appropriate markers typical of the wild type cells. These cells can now be used to study the parameters involved in the construction of the pressure vessels.

RESEARCH APPRECIATION DAY 2000

First Author: *Xiaoming Bian*

Department: *Integrative Physiology*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

Life Saving Improvement in Systemic Hemodynamics with Enhanced Intra-Aortic Assist Device: Evaluation in Canines with Severe, Acute Left Ventricular Failure

Xiaoming Bian, H. Fred Downey

Objective: For presently available intra-aortic balloon (IAB) pumps (IABP) to be effective, the mean aortic pressure must be usually greater than 50 mmHg. Thus, these devices cannot sustain systemic hemodynamics if the left heart is severely injured. We recently developed an enhanced IAB Assist Device (EIAB, patent pending). The EIAB concept was evaluated in open chest dogs with acute LV failure. **Methods**: In five dogs (20-23 kg) anesthetized with pentobarbital and fentanyl, a 12 cc IAB was inserted to the descending aorta through the femoral artery. An additional, external chamber containing a 30 cc IAB was connected to the arterial circulation through a 4.4mm catheter inserted into the left subclavian artery. This simulated the EIAB's integrated system with its external chamber in series with an internal IAB. Inflation and deflation of one (IABP) or two balloons (EIABP) was accomplished simultaneously by a IABP control unit synchronized with the ECG. LV failure was created by: two 15-min LAD occlusions, separated by 10-min reperfusion, and 3-min ventricular fibrillation. **Results**:

	HR (beats/min)	SP (mmHg)	AP (mmHg)	MPF (mmHg)	CBF (ml/min/g)	CABF (ml/min)
UT	$132 \pm 6^{\dagger}$	$47 \pm 4^{\dagger}$	32 ± 3 (DP)	34 ± 3	0.50 ± 0.03	42.7 ± 14.1
IABP	128 ± 7	36 ± 5	42 ± 5	27 ± 2	0.57 ± 0.05	43.5 ± 14.9
EIABP	120 ± 4*	41 ± 5	87 ± 3*	36 ± 2*	1.08 ± 0.10*	62.5 ± 10.1*

UT, untreated control; HR, Heart rate; SP, Systolic aortic pressure; AP, Augmented pressure; DP, Diastolic aortic pressure; MPF, Mean femoral artery pressure (distal to the IAB); CBF, Coronary blood flow; CABF, Left carotid artery blood flow. *$P < 0.05$ vs Control and IABP; $^{\dagger}P < 0.05$ vs IABP.

After 20 min of EIABP, LV function had greatly improved, so that mean carotid artery pressure was 87 ± 6 mmHg and mean femoral artery pressure was 77 ± 6 mmHg.

Conclusion: Under conditions where a presently available IABP could not improve systemic hemodynamics, EIAB strikingly increased systemic pressures and flows. EIAB is a life saving device for the patient with severe acute LV failure.

RESEARCH APPRECIATION DAY 2000

First Author: *Paul Fadel, M.S.*

Department: *Integrative Physiology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

ARTERIAL BAROREFLEX CONTROL OF SYMPATHETIC NERVE ACTIVITY DURING ACUTE HYPOTENSION. P.J. Fadel, M. Stromstad, J. Hansen, M. Sander, K.M. Gallagher, K. Horn, N.H.Secher and P.B Raven. CRI-UNTHSC, Ft. Worth, TX, 76107; Copenhagen Muscle Research Center (CMRC), Denmark.

The purpose of this investigation was to examine arterial baroreflex, carotid sinus and aortic arch, control of muscle sympathetic nerve activity (MSNA) during abrupt decreases in mean arterial pressure (MAP). Acute hypotension was induced non-pharmacologically in nine healthy subjects, aged 25.1 ± 1.1 (mean ± SE), by releasing a unilateral arterial thigh cuff (300 Torr) following 9 minutes of ischemia. To assess the relative influence of the aortic baroreflex (ABR) and carotid baroreflex (CBR) cuff release was performed under two conditions: control (ABR and CBR deactivation) and neck suction (ABR deactivation alone). The neck suction (NS) was applied during the initial 14 seconds after cuff release to maintain carotid sinus pressure and limit the contribution of the carotid baroreflex to the sudden decrease in MAP. Muscle SNA (peroneal microneurography), MAP, heart rate and central venous pressure were measured throughout the cuff release periods. Mean arterial pressure decreased 14.4 ± 2.0 mmHg during control (ABR and CBR deactivation) compared to 19.4 ± 2.1 mmHg during cuff release with neck suction ($p<0.05$). Furthermore, the MSNA response was significantly attenuated by the application of NS (increased 185.3 ± 50.4 (NS) vs. 244.7 ± 55.6 (control) total activity units $p<0.05$). Central venous pressure was unchanged during both conditions. These data suggest that 1) carotid baroreflex deactivation is necessary for a full normal increase in sympathetic nerve activity during acute hypotension and 2) the aortic baroreflex, when deactivated alone, is able to produce a sizeable sympathetic nervous response.

(Support-CMRC/NIH Grant /#HL45547)

RESEARCH APPRECIATION DAY 2000

First Author: *Shigehiko Ogoh*

Department: *Integrative Physiology*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☒ Postdoctoral Fellow/Resident

INCREASES IN INTRAMUSCULAR PRESSURE RAISE ARTERIAL BLOOD PRESSURE DURING DYNAMIC EXERCISE

S. Ogoh, K.M. Gallagher, P.J. Fadel, S.A. Smith, K.H. Norton, R.G. Querry, A. Olivencia-Yurvati and P.B. Raven

Department of Integrative Physiology and the Cardiovascular Research Institute, University of North Texas Health Science Center, Fort Worth, Texas 76107

The purpose of this investigation was to assess the role of intramuscular pressure sensitive mechanoreceptors and chemical sensitive metaboreceptors in affecting the blood pressure response to dynamic exercise. Subjects performed incremental (20W/min) leg cycle exercise to maximum under four conditions: 1)no intervention (control), 2)thigh cuff occlusion of +90mmHg (Cuff occlusion), 3)lower body positive pressure (LBPP) of 45 Torr and 4)a combination of thigh cuff occlusion and LBPP (Combination). During each exercise test, we measured arterial blood pressure (ABP), Heart rate (HR), Central venous pressure (CVP), intramuscular pressure (IMP), rating of perceived exertion (RPE), electromyographic activity (EMG) of vastus medialis and lateralis muscles, oxygen uptake (VO2) and cardiac output (Qc). Mean arterial pressure (MAP) was not significantly increased when metabolites were trapped in the active skeletal muscle by Cuff occlusion. However, significant elevations in IMP by mechanical effect of LBPP resulted in significant increases in MAP at rest and throughout exercise in LBPP and the Combination condition ($P<0.05$). In addition, these changes in MAP do not appear to be influenced by central command since HR, EMG, VO2 and RPE of whole body (RPEbody) were not significantly altered by application of the four conditions. These findings suggest that the mechanoreflex is the primary exercise pressor mediator of arterial blood pressure during submaximal dynamic exercise.

RESEARCH APPRECIATION DAY 2000

First Author: *M.A. Williams*

Department: *Integrative Physiology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

CORONARY VASCULAR RESISTANCE DURING EXERCISE IN DOGS WITH RENOVASCULAR HYPERTENSION (RVH)

M.A. Williams, B.L Graves, G.P. Kline and P.A. Gwirtz. University of North Texas Health Science Center, Fort Worth, Tx. 76107

Hypertensive patients have a limited tolerance to exercise. This study examined whether coronary vascular resistance is greater during exercise stress and limits coronary blood flow delivery. Six Healty adult dogs were chronically instrumented to measure aortic pressure (AoP), cardiac output (CO), heart rate (HR), circumflex blood flow (CBF) and resistance (CVR) at rest (R) and during a progressive submaximal exercise test (EX). Dogs were studied before (normotensive, NT) and after three weeks of RVH. Data for R and EX at 4 mph, 16% incline are shown in the table below (*$p<0.05$ vs. respective NT value, repeated-measures ANOVA):

	mean AoP (mmHg)	CO (L/m)	HR (bpm)	CBF (mmHg)	CVR (mmHg/ml/m)
NT R	100+/-6	3.96+/-1.5	99+/-6	72+/-13	1.67+/-0.2
EX	123+/-6	12.3+/-1.7	237+/-10	128+/-14	1.01+/-0.1
RVH R	124+/-6*	3.63+/-0.4	112+/-17	55+/-8	2.45+/-0.3*
EX	147+/-11*	12.47+/-0.8	206+/-12*	17+/-16*	1.49+/-0.2*

These data indicate RVH results in a greater CVR at all levels of submaximal exercise. Preliminary studies suggest that the greater CVR may be due, in part, to a greater coronary alpha-1 constrictor tone combined with impaired endothelial nitric oxide-mediated vasodilation.

(Sponsor: Patricia A. Gwirtz, Ph.D.)

RESEARCH APPRECIATION DAY 2000

First Author: *Srinath Setty*

Department: *Integrative Physiology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

ADENOSINE MODULATES MY0CARDIAL OXYGEN CONSUMPTION (MVO_2) OF RIGHT VENTRICLE (RV) IN DOGS WITH INHIBITED SYNTHESIS OF ENDOTHELIUM-DERIVED NITRIC OXIDE (NO). S. Setty, X. Bian, and H. F. Downey. Dept. Integ. Physiol., Univ. N. Tex. Hlth. Sci. Ctr., Fort Worth, TX 76107.

Endothelium-derived nitric oxide (NO) augments right coronary blood flow (RCBF) at rest, but it is unclear how NO synthesis inhibition affects MVO_2. In addition, some investigators have shown that adenosine receptor blockade increases oxygen demand. It appears that adenosine and nitric oxide may synergistically affect MVO_2. The present study was conducted to evaluate the possible role of adenosine on RCBF and RV MVO_2 in the presence of NOS blockade. In open chest dogs (n=6) RCBF and RV MVO_2 were measured at control and following infusion of L-NAME (NO synthase inhibitor N^w-nitro-L-arginine methyl ester) both in the absence and presence of adenosine receptor agonist 8 SPT (8 sulpho phenyl theophylline) in the right coronary artery. L-NAME alone reduced both RCBF and MVO_2. Addition of 8 SPT along with L-NAME attenuated the decrease in MVO_2 without change in RCBF compared to infusion of L-NAME alone. Reduction in MVO_2 should not be interpreted as a direct effect of NO, as RCBF was also reduced. We conclude that in the canine RV when NO synthase is inhibited, concomitant adenosine blockade may further increase MVO_2.

	Control	L-NAME	L-NAME+8SPT
RCBF	0.61±0.01	0.50±0.03*	0.51±0.03*
RV MVO_2	4.62±0.18	4.24±0.17*	4.45±0.18*ξ

Values are mean±SEM. * $p<0.05$ vs Control, ξ $p<0.05$ vs L-NAME alone. (Supported by NIH Grant HL35027.)

ABSTRACT #40

RESEARCH APPRECIATION DAY 2000

First Author: *Jie Sun*

Department: *Integrative Physiology*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☒ Staff

☐ Postdoctoral Fellow/Resident

PYRUVATE RESTORES FUNCTION OF HYDROGEN PEROXIDE-INJURED MYOCARDIUM. Jie Sun, Robert T. Mallet. Dept. Integrative Physiology, Univ. North Texas Health Science Center, Fort Worth, TX 76107.

Pyruvate, a metabolic fuel and potent antioxidant in heart muscle, could protect myocardium from oxyradical injury. The aims of this study were twofold: 1. To determine whether pyruvate could reverse H_2O_2-induced cardiac contractile dysfunction and oxidative stress if provided 30 min after H_2O_2 exposure; 2. To compare pyruvate's effects with those of lactate, which is catabolized by the same metabolic pathways but which lacks pyruvate's antioxidant properties. Isolated working guinea-pig hearts, perfused with 10 mM glucose-fortified Krebs-Henseleit buffer, were challenged with 100 μM H_2O_2 for 10 min, followed by 90 min H_2O_2-free perfusion. Cardiac power ($mJ \cdot min^{-1} \cdot g^{-1}$), glutathione redox state (myocardial content ratio of reduced glutathione (GSH)/glutathione disulfide (GSSG)), and contents ($nmol \cdot g \cdot dry^{-1}$) of gluththiolated protein (GSSP) and malondiakldehyde (MDA), an index of lipid peroxidation, were measured in H_2O_2-free time controls (TC), in untreated H2O2-challenged hearts (No Tx), and in H_2O_2-challenged hearts treated with 5 mM pyruvate (PYR) or 5 mM lactate (LAC) at 30-90 min post- H_2O_2. Table: means ± /SE, n=6; *P< 0.05 *vs* TC; †P < 0.05 *vs.* No Tx.

Group	Power	GSH/GSSG	{GSSP}	{MDA}
TC	124±8	47±5	191±38	64±5
No Tx	33±8*	30±2*	108±27	96±4*
PYR	119±15†	44±3†	169±22	89±8*
LAC	31±2*	35±5	163±31	90±6*

H_2O_2 decreased power 73%, partially oxidized the glutathione system, and increased myocardial lipid peroxides. Pyruvate restored contractile function and the antioxidant GSH/GSSG ratio, but lactate was ineffective. Neither pyruvate nor lactate ameliorated H_2O_2 induced myocardial lipid peroxidation. **Conclusions:** H_2O_2 depleted the endogenous glutathione antioxidant reserve and increased lipid peroxidation in guinea-pig myocardium, but did not cause glutathiolation of protein sulfhydryl moieties. Pyruvate restored contractile performance of H_2O_2-injured myocardium without reserving lipid peroxidtion. Lactate was an ineffective antioxidant and did not improve contractile function following H_2O_2 exposure. Supported by grants from NIH (HL50441) and MY-TECH, INC.

RESEARCH APPRECIATION DAY 2000

First Author: *Eric B. Pearlman*

Department: *Integrative Physiology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

MET ENKEPHALIN ARG PHE (MERF) AND METABOLISM OF MERF ACROSS THE CANINE HEART VASCULAR BED. E. PEARLMAN, M. LOFTUS, C. BEENY, B. A. BARRON. University of North Texas Health Science Center, Dept. of Integrative Physiology, Fort Worth, TX 76107.

MERF has been shown to be co-stored with catecholamines in vesicles. The catecholamines appear to decrease the degradation rate of 3H-MERF in vitro. Mongrel dogs were anesthetized and instrumented to record heart rate, arterial pressure, coronary blood flow, dP/dt, left ventricular pressure, infuse 3H-MERF, and obtain blood samples across the heart. Blood samples were taken at 1, 2, 3, 30, 60, and 75 min after start and at ½, 1, and 3 min after stopping 3H-MERF infusion. Chromatography separated intact from degraded 3H-MERF. Steady state concentration of 3H-MERF was measured after 30 min of infusion. Three experimental groups were used; control, propranolol plus isoproterenol and propranolol only. Blockade was necessary to prevent changes in coronary blood flow. Propranolol (0.2 mg/kg) was administered IV at 50 min. Confirmation of b blockade was with a 5 ug bolus of isoproterenol. 3 ug/min isoproterenol or 0.5 ml/min normal saline was infused starting at 72 min until the end of sample collection. The 3H-MERF venous - arterial (V-A) difference at 75 min was -1572 ± 672 cpm, showing that the MERF is either being degraded in the plasma or taken up by the heart. The V-A difference at 75 min was also used to calculate the effect of the infusions on the degradation or uptake of the 3H-MERF; this value was unchanged by any treatment. Heart rate was significantly lower for the propranolol only group compared to control. Blood pressure and coronary flow were unchanged. In conclusion, b adrenergic blockade does not affect MERF across the heart; however, more catecholamines need to be investigated.

RESEARCH APPRECIATION DAY 2000

First Author: *Martin Farias III*

Department: *Integrative Physiology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

SUPER DALDA ENHANCES ADRENERGIC RESPONSES IN THE DOG. Martin Farias, Keith Jackson, Darice Yoshishige, Hazel Szeto, and James L. Caffrey. University of North Texas Health Science Center, Fort Worth, TX 76107 and Cornell University School of Medicine, New York, New York.

Szeto et. al reported that systemic infusion of the highly selective mu agonist, super DALDA increased blood pressure when administered in sheep. The current studies were conducted to test the hypothesis that super DALDA increases the cardiovascular response to adrenergic activation. Several methods were employed to activate adrenergic systems responsible for the control of heart rate. These methods included the infusion of norepinephrine into the sinoatrial (SA) node by microdialysis and both the direct (electrical stimulation) and reflex activation (bilateral carotid occlusion) of the sympathetic input to the heart. Each challenge was conducted in the presence and absence of super DALDA. Blood pressure and heart rate were monitored throughout. Blood pressure was significantly higher during carotid occlusion performed in the presence of super DALDA. However, heart rate was not different. In contrast, heart rate was significantly higher when super DALDA was combined with exogenous norepinephrine, administered into the SA node via microdialysis. There was no apparent effect of super DALDA on heart rate or blood pressure during direct sympathetic nerve stimulation. A potential effect of sympathetic nerve stimulation on heart rate may have been obscured due to the variability in this response. The results suggest that super DALDA may enhance both the pressor and heart rate responses to norepinephrine in the canine model. The underlying mechanism remains to be elucidated.

CELLULAR SCIENCE

44.	Jami R. Kern	TELOMERASE ACTIVITY DOES NOT ALTER FIBROBLAST BEHAVIOR IN A THREE-DIMENSIONAL IN VITRO MODEL
45.	Eve Ettinger	PROLIFERATION OF BETA CELLS AND THEIR AGGREGATION INTO NEO-ISLETS
46.	Karen R. Murray	INVESTIGATION OF THE 121-136 DOMAIN, A PUTATIVE LIPOPROTEIN BINDING SITE OF LECITHIN: CHOLESTEROL ACYLTRANSFERASE
47.	Anson Pierce	OXIDATION/REDUCTION ENZYME ACTIVITIES IN MOUSE PERITONEAL MACROPHAGES ISOLATED AFTER WHOLE-ANIMAL HYPERBARIC OXYGEN TREATMENT
48.	Vinay Kumar Parameswara	PHOSPHOPROTEIN PHOSPHATASE 2 A IS LOCALIZED TO INSULIN SECRETORY GRANULES IN PANCREATIC BETA CELLS
49.	Harshika Bhatt	GLUCOSE ACTIVATES CAM KINASE II VIA THE KATP CHANNEL-INDEPENDENT PATHWAY OF INSULIN SECRETION
50.	Christina A. Malakowsky	A REPRODUCIBLE, QUANTITATABLE, LINEAR STANDARD FOR CHEMILUMINESCENT BLOTS
51.	Dmitriy S. Ushakov	STEADY-STATE FLUORESCENCE POLARIZATION OF RHODAMINE ATTACHED TO MYOSIN REGULATORY LIGHT CHAIN IN SKELETAL MUSCLE FIBERS

RESEARCH APPRECIATION DAY 2000

First Author: *Jami R. Kern*

Department: *Molecular Biology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

TELOMERASE ACTIVITY DOES NOT ALTER FIBROBLAST BEHAVIOR IN A THREE-DIMENSIONAL IN VITRO MODEL. JR Kern and SD Dimitrijevich, Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, TX. 76107.

In tissue repair, fibroblasts (fbs) are the principle cell recruited to the wound bed to restore and remodel granulation tissue into a scar. We have developed a non-contracting, three-dimensional model composed of a collagen type I matrix populated with normal human dermal fbs. This is an ideal model for studying the process of wound contraction since tissue contraction is independent of cell number (within the range of 100,000 - 500,000 cells/ml collagen) and of collagen concentration (3 - 5 mg/ml collagen). It has been shown in culture that the proliferative capacity of fbs (number of population doublings) declines with donor age. This in vitro senescence has been linked to telomere shortening. Recently the catalytic subunit of human telomerase (hTERT) has been cloned and expressed in several normal human cell types. In human dermal fbs this has led to, if not immortalization, a cell line with greatly extended in vitro lifespan. Since cancer cells invariably have upregulated telomerase activity, there is a concern that in vivo upregulation of telomerase in normal cells could lead to hyperplasia. We are therefore studying the behavior of telomerase transfected fbs (hTERT fbs) in the tissue context using our three-dimensional tissue equivalent (TE). In this model we compare the proliferation, migration, contractile properties and matrix biosynthesis of hTERT fbs with normal dermal fbs. In the TE, hTERT fbs should not be not hyperproliferative and should be able to synthesize ECM (collagen, GAGs), migrate, and contract the matrix. We have shown that the hTERT fbs do not hyperproliferate in the TE and can contract it. In order to study cell migration and matrix remodeling as well as interaction of TEs as grafts with the recipient's tissue we have labeled the fbs with green fluorescent protein (GFP). Stable expression of GFP in hTERT fbs was achieved using a lentiviral vector (VSV-G pseudotyped HIV-1) with about 28% efficiency. Since the vector did not contain an antibiotic resistance gene, the GFP expressing cells were sorted using Becton Dickinson Cell Sorter to isolate a pure population expressing GFP. As a component of the TE, hTERT-GFP fbs extend the utility of our three-dimensional model and make possible real time studies of cell-matrix interactions using scanning confocal microscopy. In the future we hope to show that our model populated with hTERT transfected cells does not undergo neoplastic transformation when exposed to carcinogens and thus will be a feasible source of transplantable engineered tissue.

RESEARCH APPRECIATION DAY 2000

First Author: *Eve Ettinger*

Department: *Molecular Biology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

PROLIFERATION OF BETA CELLS AND THEIR AGGREGATION INTO NEO-ISLETS. ES Ettinger, RA Easom and SD Dimitrijevich, Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, TX. 76107

Diabetes mellitus is a leading cause of death in the United States, afflicting over 16 million people. Some consequences of diabetes include blindness, end-stage renal failure and cardiovascular disease. Type 1, insulin-dependent diabetes mellitus (IDDM) patients, as well as some non-insulin dependent diabetes mellitus (NIDDM) patients require constant treatment with exogenous insulin to regulate their blood glucose level. A therapeutic approach being studied clinically is implantation of pancreatic beta cells of human or porcine origin. An even more relevant approach would consider using islet implantation because the islet is the optimal glycemic control unit. The purpose of this study is to identify the progenitor of the beta cells and study the process of cytodifferentiation into functional islets. The focus of this study is the in vitro maintenance of viable islets and their expansion. We have shown that murine, porcine and human islets can be sustained in a three-dimensional culture for more than 6 weeks and that they secrete insulin in response to elevated glucose. We have also shown that murine, porcine and human islets can be a source of endocrine epithelial cells. Immunohistochemical studies demonstrated that these cells were composed of insulin, glucagon and cells producing both insulin and glucagon. When placed in a specific microenvironment, these endocrine epithelial cells spontaneously aggregate into neo-islets. These results suggest the feasibility of propagation and differentiation of functional islet cells in vitro and assembly of these into neo-islets. Future studies will address functional maturation and appropriate glucose responsiveness in the neo-islets. It is expected that this approach will provide a source of good quality islets suitable for transplantation, thereby eliminating the need for exogenously administered insulin to diabetic patients.

RESEARCH APPRECIATION DAY 2000

First Author: *Karen R. Murray*

Department: *Molecular Biology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

INVESTIGATION OF THE 121-136 DOMAIN, A PUTATIVE LIPOPROTEIN BINDING SITE OF LECITHIN: CHOLESTEROL ACYLTRANSFERASE

Karen R. Murray* , Maya P. Nair* , P. Haydn Pritchard#, and Andras G. Lacko*
*Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, Texas and #Department of Pathology, University of British Columbia, Vancouver, Canada

The purpoase of these studies was to investigate the reactivity of the enzyme lecithin:cholesterol acyltranserase (LCAT) with the site directed and general polyclonal antibodies to probe the site of a putative substrate binding domain (residues 121-136). The two polyclonal antibodies, one against human plasma LCAT and the other against purified recombinant LCAT, and one site specific antibody, directed against the 121-136 region of LCAT were employed. All antibodies reacted with a recombinant form of purified LCAT secreted by baby hamster kidney (BHK) cells. However, only the polyclonal antibodies were able to recognize the enzyme when it was first adsorbed to a hydrophobic surface in a solid phase immunoassay, or when bound to HDL in a sink immunoassay. In addition, three mutant forms of LCAT, representing alterations in the 121-136 region were tested for immunoreactivity with the same panel of antibodies as described for the wildtype above. These studies suggest that the 121-136 region of LCAT indeed represents a region with high affinity for hydrophobic surfaces that could function as a lipoprotein substrate binding domain.

RESEARCH APPRECIATION DAY 2000

First Author: *Anson Pierce*

Department: *Molecular Biology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

OXIDATION/REDUCTION ENZYME ACTIVITIES IN MOUSE PERITONEAL MACROPHAGES ISOLATED AFTER WHOLE-ANIMAL HYPERBARIC OXYGEN TREATMENT. Anson Pierce and Ladislav Dory, The University of North Texas Health Science Center, Dept. Molecular Biology & Immunology, Fort Worth, TX 76107

It has been shown that hyperbaric oxygen (HBO) treatment results in the protection from and regression of atherosclerosis in the rabbit model of atherosclerosis. The mechanisms of these effects of HBO treatment are unknown. The purpose of this study is to reproduce these observations in an apolipoprotein E K.O. (ApoE-/-) mouse model for atherosclerosis and to examine the activities as well as levels of expression of oxidation/reduction enzymes in peritoneal macrophages (MPM's) isolated from the mice after HBO treatment. Oxidation of low-density lipoproteins (LDL) is thought to be a major contributing factor to atherosclerotic lesion formation. Macrophages may play a major role in the oxidation of LDL through the production of superoxide anions by the NADPH oxidase complex. We plan to test the ability of the NADPH oxidase complex in MPM's to produce superoxides after HBO treatment of ApoE-/- mice. Superoxide anion production by MPM's will be measured as the superoxide dismutase-inhibitable reduction of acetyl ferricytochrome C by the microtiter plate technique. Other enzymes such as paraoxonase I (PON-1) may be responsible for the reduction of LDL into a less atherogenic form on the LDL particle itself. Expression levels of these enzymes before and after HBO treatment are of interest as well, and will be measured using ribonuclease protection assay or Northern Blotting techniques. It has already been shown that PCR cycle-dependent differences in expression of 9 enzymes can be detected by RT-PCR in this lab, after treatment of MPM's with 25-hydroxycholesterol. Activities and levels of expression of these oxidizing and reducing enzymes in MPM's and plasma after HBO treatment of whole mice would further characterize the protective effect of HBO treatment in atherogenesis.(This work will be supported by NIH funding to LD).

RESEARCH APPRECIATION DAY 2000

First Author: *Vinay Kuma r Parameswara*

Department: *Molecular Biology and Iimmunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

PHOSPHOPROTEIN PHOSPHATASE 2 A IS LOCALIZED TO INSULIN SECRETORY GRANULES IN PANCREATIC BETA CELLS. Vinay K. Parameswara and Richard A. Easom, Department of Molecular Biology and Immunology, UNTHSC, 3500 Camp Bowie, Fort Worth, Texas- 76107

Type II Diabetes is characterized in part by an inability of the pancreatic beta cells to secrete insulin in response to glucose. How glucose induces insulin exocytosis is not fully understood, but there is good evidence that it is dependent on reversible protein phosphorylation. A number of kinases including calcium/calmodulin dependent protein kinase II (CaMKII), are implicated in this process. In contrast, less is known about the potential involvement of phosphoprotein phosphatases. In the current study, protein phosphatase expression in the beta cells has been charectarized by microcystin affinity chromatography and immunoblot analyses. These studies reveal the expression of a Serine-Threonine phosphoprotein phosphatase, phosphoprotein phosphatase 2A (PP2A) in the beta cell. PP2A has a wide range of cellular functions including cellular metabolism, transcription and translation, ion transport, development, cell growth and differentiation. Further analysis has revealed the expression of all three PP2A subunits A (65 Kda), B (subtype delta 55Kda) and the catalytic (37Kda) subunit. PP2A was also localized to the insulin secretory granule (ISG). These studies have characterized the presence of PP2A in the pancreatic beta cells. The localization of PP2A to ISG implies the involvement of the phosphatase in insulin exocytosis possibly via the regulation of kinases associated with the granules or via the dephosphorylation of regulatory phophoproteins required for granule recruitment and exocytosis.

RESEARCH APPRECIATION DAY 2000

First Author: *Harshika Bhatt*

Department: *Molecular Biology & Immunology*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☒ Postdoctoral Fellow/Resident

GLUCOSE ACTIVATES CAM KINASE II VIA THE KATP CHANNEL-INDEPENDENT PATHWAY OF INSULIN SECRETION. Harshika Bhatt, Amanda Lam, and Richard A. Easom, UNTHSC, Fort Worth, Texas, 76107.

D-Glucose is the primary physiological stimulator of insulin secretion from the pancreatic beta-cell and achieves this via two primary pathways. In the best understood pathway, glucose metabolism elevates cytosolic calcium via the sequential closing of a KATP channel, cell depolarization and activation of L-type calcium channels. In the second pathway, glucose enhances insulin secretion under conditions in which the KATP channel is clamped open, but remains dependent on the elevation of basal calcium levels and glucose metabolism. The cellular mechanisms mediating this pathway of insulin secretion are not understood. In the present study, we have demonstrated that glucose induces the activation of a calcium/calmodulin-dependent protein kinase, CaM Kinase II, in the presence of diazoxide, to clamp the KATP channel in an open state, and 30 mM KCl, to artificially depolarize the beta-cell and elevate calcium. This effect correlated closely with insulin secretion and was totally dependent on glucose metabolism. The mitochondrial poison, sodium azide, reversibly inhibited both insulin secretion and CaM Kinase II activation indicating that both parameters may be sensitive to the energetic state of the beta-cell. Indeed, the extent of activation of CaM kinase II by calcium in permeabilized cells was dependent on the ratio ATP:ADP. These data are significant in that they identifiy CaM Kinase II as an important mediator of KATP channel-independent insulin secretion but also that CaM Kinase II activation is sensitive to ATP:ADP ratio of the beta-cell. Since we have previously established that CaM Kinase II is important for calcium-induced insulin secretion via mechanisms involving the KATP-channel, these data support a central role for this kinase in the coordination of physiological pathways of glucose-induced insulin secretion. (Supported by the National Institutes of Health, DK 47925).

RESEARCH APPRECIATION DAY 2000

First Author: *Christina A. Malakowsky*

Department: *Molecular Biology and Immunology*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☒ Staff

☐ Postdoctoral Fellow/Resident

A REPRODUCIBLE, QUANTITATABLE, LINEAR STANDARD FOR CHEMILUMINESCENT BLOTS. Christina Malakowsky, Robert W. Gracy and Craig C. Conrad. Molecular Aging Unit, Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, Texas, 76107.

Sensitive chemiluminescent probes offer analytical analysis of DNA, RNA, and proteins. The common method used for detection and visualization involves blotting of samples from a gel matrix onto a support matrix (e.g. nitrocellulose, nylon). Visualization of the signal involves chemicals that emit light. Because the emission of light is continuous, the exposure time of the blot to the detection medium (e.g., film or digital camera) is a critical variable in the final results. Thus, detection media exposed to chemiluminescent blots will yield darker exposures after longer periods of exposure. Although the ratio of samples on a single blot will remain constant, comparison of separate blots done on different days requires a chemiluminescent standard. This standard uses substrates specific for the chemiluminescent probes to calculate a correction factor and to eliminate differences in light emission that occurs between the different blots, allowing the blots to be compared. Such a standard has been developed for Western blots of 2-D PAGE and is discussed. Such standards can also be used for Northern and Southern blots.

RESEARCH APPRECIATION DAY 2000

First Author: *Dmitriy S. Ushakov*

Department: *Molecular Biology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

STEADY-STATE FLUORESCENCE POLARIZATION OF RHODAMINE ATTACHED TO MYOSIN REGULATORY LIGHT CHAIN IN SKELETAL MUSCLE FIBERS

D.S. Ushakov, J. Borejdo
University of North Texas Health Science Center, Fort Worth, Texas, 76107

The hydrolysis of ATP by myosin induces a change in the conformation of its light chain-binding domain. However, the significance of this change for the motion of actin relative to myosin is not clear. We used a steady-state fluorescence polarization approach in order to study the orientation of rhodamine attached to a single cysteine of myosin regulatory light chain (RLC). Chicken fast skeletal muscle RLC cDNA containing a single cysteine 73 (provided by S. Lowey, Univ. Vermont) was expressed in E.coli BL21(DE3)pLysS cells. The Cys73 was labeled with 5'-iodo-acetamido-tetramethyl-rhodamine and exchanged into skeletal muscle fibers. The fluorescence polarization was measured in the direction perpendicular ($P_\perp$) and parallel ($P_\parallel$) to the fiber axis. The values of $P_\perp$ and $P_\parallel$ in relaxed fibers were 0.301 and 0.281, respectively. They were only a little different in rigor (0.316 and 0.275). The values of $P_\perp$ and $P_\parallel$ are in agreement with literature (Sabido-David et al., J Mol Biol, 279, 387, 1998). The difference between the perpendicular and parallel polarizations (δP) is a measure of the degree of order of the fluorophore. The fact that this difference was small, both in relaxation and in rigor, suggest that cys73 is mobile. Relatively low δP values agree well with the structural data (Rayment et. al, Science, 261, 50, 1993) on myosin head showing that the residue 73 of RLC is located in the flexible loop of EF-hand II and exposed to the solvent. The slight increase in δP in rigor in comparison to relaxed and active muscle fibers can be explained by the formation of a tight complex between actin and myosin in the absence of ATP.
Acknowledgment: we acknowledge assistance and discussions with Dr. Tony Romeo. Supported by NIH #AR40095.

FAMILY MEDICINE

52.	S. T. Coleridge, D.O.	ESTABLISHING A RESEARCH CULTURE IN FAMILY MEDICINE
53.	Linda E. Reed, MEd, PA	MASTER OF PHYSICIAN ASSISTANT STUDIES DEGREE
54.	Carol Stehly, M.S. Med	PROSPECTIVE ON PHYSICIAN ASSISTANT PRACTICUM IN RURAL COMMUNITIES
55.	Arushi Sinha, MA	BORDER HEALTH: A 5-YEAR RETROSPECTIVE IN PREDOCTORAL CULTURAL SENSITIVITY TRAINING
56.	Henri Migala, MA, MPH	NORTH CENTRAL TEXAS DIVISION, EAST TEXAS AREA HEALTH EDUCATION CENTER (AHEC)
57.	Barbara D. Adams, MSA	RURAL TRACK SITE UTILIZATION AND STUDENT PARTICIPATION, 1996 TO PRESENT

RESEARCH APPRECIATION DAY 2000

First Author: *ST Coleridge, DO Chaiman of Family Medicine*

Department: *Family Medicine*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

ESTABLISHING A RESEARCH CULTURE IN FAMILY MEDICINE.

ST Coleridge, DO Chairman Family Medicine, Peggy Smith-Barbaro, Ph.D.
Divisision of Education and Research
Family Medicine Department
Univerity of North Texas Health Science Center - Fort Worth
Fort Worth, TX 76107

Building and maintaining a successful research program in Family Medicine starts with creating a research culture designed to identify the benefits of medical reseach to faculty, their patients and the academic community. In May 1999, a project was initiated with the objective of developing a research culture in the family medicine department utilizing several techniques. Educational tools employed included the following: (1) establishing a Division of Education and Research (DEAR) manual designed to provide information to faculty and staff about writing grant proposals, developing scholarly articles and presentations, and (2) presenting lectures to residents to encourage scholastic activity and research. Motivational tools utilized to help establish a research culture included the following: (1) development of a monthly newletter highlighting individual faculty member's contributions to scholastic activity, (2) use of a Scholarly Activity Report (SAR) which summarized the status of grants, pending grants, education contracts, publications / presentations and family practice residents' papers, and (3) establishment of a bulletin board in a highly visable location within the Department of Family Medicine highlighting the extramural and educational funding, current grants, research news and newly published articles written by faculty members. Individual faculty members research interests were identified and leveraged against potential funding sources. Over the seven months since project initiation there have been over seventy (70) requests for research / scholastic activity assistance made by family medicine faculty members. The interest generated by these innovative strategies to research culture development suggests that these approaches can provide a cornerstone for the development of a successful research program in Family Medicine.

(Funded by HRSA -Grant for Establishment of Department of Family Medicine)

RESEARCH APPRECIATION DAY 2000

First Author: *Reed, Linda E., MEd, PA*

Department: *Physician Assistant Studies (Family Medicine)*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

MASTER OF PHYSICIAN ASSISTANT STUDIES DEGREE

Linda E. Reed, MEd, PA
Henry R. Lemke, MMS, PA
J. Warren Anderson, EdD

Division of Physician Assistant Studies
University of North Texas Health Science Center at Fort Worth
Fort Worth, Texas 76107

The Master of Physician Assistant Studies Program at the University of North Texas Health Science Center at Fort Worth is designed to expand the competencies of physician assistants to meet the needs of a changing health care environment, while maintaining a strong focus on the delivery of primary care to underserved Texans. To meet this goal, the PA Studies Program examined current and projected health care needs reported by the State of Texas and the United States in the literature. The program also examined the educational trends and challenges of physician assistant curricula nationwide. As a result the Physician Assistant Studies program redesigned and expanded its curriculum to include two tracks of study in addition to the clinical competencies required of all physician assistant students. The first two masters' tracks designed through this project are: Rural/Underserved Primary Care and Medical Education.

Students accepted into the new Masters of Physician Assistant Studies (MPAS) Program may choose either area of focus in their degree plan. All students in the MPAS program will gain increased knowledge and skills in biostatistics, epidemiology, and clinical research methods. Students choosing the Rural/Underserved Primary Care track will learn how to provide primary care with limited resources, understand cultural differences of underserved patient populations, and become familiar with the principles of office management, including health care financing. Those students who choose the medical education track will learn basic educational theories and teaching methods, improve their skills in the organization of instruction, and understand testing and evaluation methods.

The Masters of Physician Assistant Studies Program is scheduled to begin in the fall semester of 2000.

RESEARCH APPRECIATION DAY 2000

First Author: *Carol Stehly, M.S. MEd*

Department: *Family Medicine, Physician Assistant Studies*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

PROSPECTIVE ON PHYSICIAN ASSISTANT PRACTICUM IN RURAL COMMUNITIES

Carol Stehly, MS,MEd
Physician Assistant Studies Program
University of North Texas Health Science Center-Fort Worth
Fort Worth, TX 76107

There is a growing need for qualified, highly trained and culturally sensitive Physician Assistants to practice in medically underserved rural communities. To address this need, the Physician Asistant Studies Division of the Department of Medicine at the University of North Texas Health Science Center at Fort Worth developed a rural practicum to encourage PA students to choose rural or medically unserderved areas as their professional destination. The purpose of this presentation is to describe the rural practicum and summarize student experiences for the Class of 1999.

Eleven students placed randomly at sites throughout Texas completed the required four-week practicum living and training in a rural or medically underserved community. Practicum components included community selective assignments, community assessments and clinical expereinces. Each student completed required activities for the practicum that included mandatory readings, visits to three community service agencies and written evaluation of the agency visits, community assessment and clinical expereinces.

A review of student evaluations reveal unique and memoprable expereinces and suggest that the rural practicum increased student awareness of healthcare concerns, practices and services.

(Health Resources and Service Administration Physician Assistant Training Grant)

RESEARCH APPRECIATION DAY 2000

First Author: *Arushi Sinha, MA*

Department: *Family Medicine*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

BORDER HEALTH: A 5-YEAR RETROSPECTIVE IN PREDOCTORAL CULTURAL SENSITIVITY TRAINING

Arushi Sinha, MA, Barbara D. Adams, MSA, Henri Migala, MA, MPH and Carol Stehly, M.Ed.

University of North Texas Health Science Center at Fort Worth, Fort Worth Texas, 76107

The Department of Family Medicine has for the past five years organized trips to border health regions in order to increase students' awareness about the general health needs of the region as well as the specific needs of the Hispanic-American community of the area. Pre-Doctoral students spend one week in a US-Mexico border region exploring health needs and existing health infrastructure. Have our efforts in this area made a difference? This study evaluates five years of student data, student surveys (pre- and post-), students' reactions, and students' papers. Initial evaluation suggests that student sensitivity and awareness about the particular needs of this population has increased.

(This project was jointly supported by the Department of Family Medicine, Health Resources Services Administration (HRSA) Predoctoral Training Grant, the Lower Rio Grande Valley Area Health Education Center of the South Texas Area Health Education Center (LRGV AHEC), and The Health Education Training Centers Alliance of Texas (HETCAT) operated with the Texas Tech-El Paso Area Health Education Centers (AHEC)).

RESEARCH APPRECIATION DAY 2000

First Author: *Henri Migala, MA, MPH*

Department: *Family Medicine*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

NORTH CENTRAL TEXAS DIVISION,
EAST TEXAS AREA HEALTH EDUCATION CENTER (AHEC)
Henri F. Migala, MPH, MA; Gloria Overstreet, BBA
Univeristy of North Texas Health Science Center at Fort Worth, TX 76107
website address: etxahec.org

The Area Health Education Center, AHEC, is a multi-institutional, multi-disciplinary, community-based program that seeks to address the primary health care workforce needs of medically underserved populations and communities through the following four programmatic areas:
1. Health Careers Promotion (among local schools and students)
2. Community-Based Clinical Education (for all health professions)
3. Practice Entry and Support (for all health professions)
4. Community Health Initiatives (including community leadership and health systems support)

East Texas AHEC Mission:
To improve the health of the population, especially the underserved, by creating partnerships among community and academic organizations. The AHEC links health care resources to build and strengthen community-based education programs.

East Texas AHEC Vision:
That all people (in the service region) achieve optimal health through access to quality health care provided by well-trained health professionals.

North Central Texas Division statistics:
33 Counties
4.9 million people
28,494 square miles
Served by 3 Local AHEC Centers:

East Texas AHEC statistics (including the North Central Texas Division)
111 Counties
12.3 million people (About two-thirds of the state's 19 million people)
Would be the 5th largest State by population (behind California, Texas, New York and Florida)
93,204 square miles (Larger that 39 States)
Served by 8 Local AHEC Centers

RESEARCH APPRECIATION DAY 2000

First Author: *Barbara D. Adams, MSA*

Department: *Family Medicine*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

RURAL TRACK SITE UTILIZATION AND STUDENT PARTICIPATION, 1996 TO PRESENT

Barbara D. Adams, MSA; John R. Bowling, DO; Shirley King, AAS; Samuel T. Coleridge, DO

Since its inception in October 1996, the Rural Family Medicine Track has provided medical students with the opportunity to complete their family medicine training in a rural setting. The purpose of this research was to ascertain site utilization and student participation patterns during the first four years that the Track has been implemented. Students who elect to participate in the Rural Track are matched to a community with a community-based private practice faculty member who serves as preceptor. During the predoctoral years, students return several times to spend a total of 18 weeks in the community and clinical training site. Tracking data were analyzed for site activity and student participation for each component of the Track. Analysis indicates that 24 training sites have been utilized with the number of students precepted ranging from 1 to 11 per site. Student participation levels have varied from class to class; however, a total of 108 students have participated in some aspect of the Track program. Fifty-nine students have enrolled for full Rural Track participation, and an additional 49 have completed their third year family medicine clerkship at Rural Track sites. Though it is too early to assess long term outcomes, results suggest that participation is increasing as students become familiarized with the opportunity available through the Rural Family Medicine Track program. (Project support by Health Resources and Service Administration Predoctoral Training in Family Medicine Grant)

INFECTIOUS DISEASE

58.	B. A. Atkinson	A PILOT STUDY TO DEFINE THE DEGREE OF LOSS OF BON E MASS IN HIV-INFECTED INDIVIDUALS
59.	Harlan P. Jones	TH2 CELL RESPONSES PREDOMINATE IN LUNGS AFTER INTRANASAL IMMUNIZATION W ITH ANTIGEN ALONE, BUT NOT AFTER USING THE MUCOSAL ADJUVANT, CHOLERA TOXIN
60.	James R. Saunders	DIFFERENCES IN IFN-γ__PRODUCTION ARE ASSOCIATED WITH SEVERITY OF MYCOPLASMA RESPIRATORY DISEASE IN C3H AND DBA/2N MICE
61.	Bangdong Wei	POST-TRANSCRIPTIONAL REGULATION OF BACTERIAL MOTILITY AND FLHDC EXPRESSI ON BY THE RNA-BINDING PROTEIN CSRA
62.	Thomas Weilbacher	IDENTIFICATION OF A NOVEL REGULATORY RNA INVOLVED IN GLYCOGEN BIO SYNTHESIS IN ESCHERICHIA COLI
63.	Seshagirirao Gudapaty	EFFECT OF *csrA,* AND *rpoS* ON LEVELS OF CsrB AND CsrA IN *ESCHERICHIA coli*

RESEARCH APPRECIATION DAY 2000

First Author: *Atkinson, B.A.*

Department: *Internal Medicine*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

A PILOT STUDY TO DEFINE THE DEGREE OF LOSS OF BONE MASS IN HIV-INFECTED INDIVIDUALS

B.A. Atkinson, D.O., F.X. Blais, D.O., and B. R. Rubin, D.O.
Department of Medicine, University of North Texas Health Scienc Center, Fort Worth, Texas 76107

Twenty-nine individuals infected with the human immunodeficiency virus (HIV) were assessed for their degree of bone loss via Dual eneryg x-ray absorptiometry (DXA scan, a non invasive method that measeures bone mass and risk of fracture. All patients were receiving antiretroviral therayp but most scans were performed prior to current therapeutic guidelines of three or more drugs as standard of care. There were 12 females and 17 males, average age was 33 years amd CD4 T-lymphocyte counts were used as markers of the degree of immunosuppression. Nineteen patients had CD4 counts of <200 cell/mm3, criteria for the diagonsis of AIDS.

Scans were abnormal in 18 patients. Criteria for abnormal DXA scans is the WHO classification. CD4 T-lymphocyte counts correlated indirectly with risk of fracture. Three of more antiretroviral agents (highly active antiretroviral therapy (HARRT), the current standard of care may prevent osteoporosis in HIV infected individuals. Future studies should address this issue, as well as the role of drugs used to treat osteoporosis in patients with high risk of fracture.

RESEARCH APPRECIATION DAY 2000

First Author: *Harlan P. Jones*

Department: *Molecular Biology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

TH2 CELL RESPONSES PREDOMINATE IN LUNGS AFTER INTRANASAL IMMUNIZATION WITH ANTIGEN ALONE, BUT NOT AFTER USING THE MUCOSAL ADJUVANT, CHOLERA TOXIN. H.P. Jones, L. M. Hodge, and J. W. Simecka, Dept. of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, TX. 76107

We evaluated T helper (Th) cell populations in lungs and their response to intranasal (i.n.) immunization. The major T cell population in lungs of BALB/c mice was CD4+ Th cells as determined by FACS. Lung and splenic lymphocytes from mice were stimulated in vitro with anti-CD3 antibody (Ab), and supernatants were analyzed for Th1 (IFN-gamma) and Th2 (IL-4 and IL-5) cytokines using ELISA. Lung lymphocytes produced primarily Th2 (IL-4 and IL-5) cytokines in contrast to stimulated splenic lymphocytes which secreted high levels of IFN-gamma. Thus, resident lung T cells are primarily Th2-like, suggesting i.n. immunization will preferentially stimulate a Th2 cell response in lungs. To examine this possibility, mice were i.n. immunized with influenza vaccine antigen (Ag). There was little change in lung lymphocyte numbers, including CD4+ T cells, as compared to control mice given PBS. Total RNA was isolated from lungs of mice immunized as above, and we examined Th cell cytokine mRNA expression using RT-PCR. Th2 cytokine mRNA (IL-4, IL-5) levels were increased in mice immunized with Ag alone. Whereas increases in Th1 cytokine (IL-2 and IFN-gamma) mRNA expression were not detected. These results are consistent with the cytokine production of resident T cell population after in vitro anti-CD3 Ab stimulation. The mucosal adjuvant, cholera toxin (CT), is commonly used to investigate immune responses after i.n. immunization. In contrast to mice given Ag alone, there was a 4-fold increase in lung lymphocyte numbers after i.n. immunization using CT. Furthermore, FACS suggests that there is a preferential increase in CD4+ Th cells. As above, we examined Th1 and Th2 cytokine mRNA expression by RT-PCR and RNAse protection assay. As expected, Th2 cytokine (IL-4,IL-5,IL-6,IL-10, IL-13) mRNA levels were higher using CT. Importantly, there were large increases in Th1 (IL-2,IFN-gamma) cytokine mRNA levels in the lungs of mice given CT. Ag-specific lung lymphocyte responses were determined using in vitro stimulation with Ag and measuring cytokines in culture supernatants. Consistent with mRNA expression, IL-4 was secreted by lung lymphocytes after immunization with Ag alone. Using CT during immunization however not only enhanced IL-4 levels in culture supernatants, but also stimulated high levels of IFN-gamma . This is consistent with in vivo generation of Th1 cell responses. In support, we demonstrated Ag-specific delayed type hypersensitivity responses in mice i.n. immunized using CT. Furthermore, mRNA expression of the chemotactic cytokines, MIP-1 alpha , and MIP-1 beta , was increased in lungs after i.n. immunization using CT. Thus, Th cells in lungs are predominantly Th2-like. However, recruitment of lymphocytes into lungs in response to an adjuvant, or infectious agents, may alter developing immune responses in the lung.

RESEARCH APPRECIATION DAY 2000

First Author: *James R. Saunders*

Department: *Molecular Biology and Immunology.*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

DIFFERENCES IN IFN-γ PRODUCTION ARE ASSOCIATED WITH SEVERITY OF MYCOPLASMA RESPIRATORY DISEASE IN C3H AND DBA/2N MICE. James R. Saunders, Harlan P. Jones, Jerry W. Simecka, Dept. of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, Texas 76107.

Mycoplasma is a major cause of morbidity and mortality, not only in animals but in humans as well. Despite the significance of this genus of microorganism, the mechanisms of protective immunity remain poorly understood. Murine respiratory mycoplasmosis due to *Mycoplasma pulmonis* is an excellent animal model of human respiratory mycoplasmal disease with similar etiology to human disease. Despite similar etiologies, not all strains of mice respond identically to infection with *M. pulmonis*. It has been demonstrated in other studies that the clinical course of disease in C3H/HeN and DBA/2NCr mice differ significantly with DBA/2NCr mice being highly susceptible to dying even though the number of organisms in the lungs of both strains are basically identical. Interestingly, the lung lesions are more severe in C3H/HeN mice. The infiltration of mononuclear cells (e.g. lymphocytes, macrophages) is a significant component of the disease in C3H/HeN mice, but not in DBA/2N mice. The histopathologic differences suggest that the differences in disease are linked to some form of impairment in the immune or inflammatory responses generated in response to mycoplasma infection in DBA/2N mice. To begin to examine this possibility, mice from both strains were inoculated intranasally with 10^4 CFU of *M. pulmonis* UAB CT and evaluated at 14 days post infection. Lung tissue was then examined for cytokines by RT-PCR. RT-PCR results indicated that the major difference between the two strains was a lower level of IFN-γ in the DBA/2NCr mice, whereas there was no apparent difference in IL-4 mRNA levels. These results support our hypothesis that the differences of disease between these mouse strains are linked to some form of immune system dysfunction. Further studies will determine if cytokines, other than IFN-γ, are associated with the difference in disease in these two mouse strains. Furthermore, we will evaluate the generation of antibody responses after infection to begin determining if differences in lymphocyte activation exist. In addition, we will look for the presence of *M. pulmonis* in tissues other than the lungs. This will indicate whether impaired inflammatory or immune responses in DBA/2N mice allow systemic dissemination of mycoplasma, leading to death. Overall, the results from these studies will lead to a better understanding of anti-mycoplasmal host defenses, allowing the development of novel vaccine strategies against mycoplasma infections.

RESEARCH APPRECIATION DAY 2000

First Author: *Bangdong Wei*

Department: *Molecular Biology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

POST-TRANSCRIPTIONAL REGULATION OF BACTERIAL MOTILITY AND FLHDC EXPRESSION BY THE RNA-BINDING PROTEIN CSRA B. Wei and T. Romeo. Department of Molecular Biology and Immunology, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107-2699

Escherichia coli can respond to their environmental conditions and decide to move toward some substances and away from others using flagellar propulsion. Motility plays an important role in several respects of bacterial pathogenesis, especially in colonization of host tissues. In order to examine the effects of a global regulator, the RNA-binding protein CsrA, on motility and biosynthesis of flagella in *E. coli*, and to determine the molecular mechanism by which CsrA regulates the expression of flagella, we first compared the growth of *csrA* wild-type and mutant strains on semisolid tryptone agar plates and in liquid medium. Our results showed that *csrA* is required for motility. Negative staining electron microscopy revealed that *csrA::kanR* cells lack flagella. The master regulatory operon for flagella biosynthesis, *flhDC*, was found to be expressed 3- to 5-fold higher in *csrA* wild-type versus mutant strains, using an *flhDC::lacZ* translational fusion. In vitro S-30 coupled transcription and translation assay further demonstrated that purified recombinant CsrA protein directly stimulated the expression of *flhDC*. In addition, *flhDC* mRNA in *csrA::kanR* mutant cells was found to be significantly decreased by reverse-transcription polymerase chain reaction (RT-PCR) and by primer extension analysis. This study reveals that *csrA* is essential for bacterial motility under these conditions and is first demonstration of the mechanism of positive regulation by CsrA

RESEARCH APPRECIATION DAY 2000

First Author: *Thomas Weilbacher*

Department: *Microbiology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

IDENTIFICATION OF A NOVEL REGULATORY RNA INVOLVED IN GLYCOGEN BIOSYNTHESIS IN ESCHERICHIA COLI. Thomas Weilbacher, and Tony Romeo. Department of Molecular Biology and Immunology , University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107-2699

As a bacterial culture undergoes the transition from exponential to stationary phase, a series of metabolic alterations occur which prepare the cell for survival under suboptimal conditions. One such alteration is the commencement of glycogen biosynthesis. Regulation of glycogen synthesis is accomplished via several global regulatory factors, including a novel kind of system, Csr. Csr utilizes an RNA-binding protein, CsrA, as an effector to alter mRNA stability, and is itself antagonized by a structural RNA molecule CsrB, which complexes with ~18 CsrA subunits. In attempt to clone additional genes responsible for glycogen biosynthesis (glg), a low copy plasmid containing a genomic insert from the Escherichia coli chromosome was isolated, which stimulated glycogen biosynthesis, but did not encode any known glg gene. Deletion analysis of this clone revealed a deduced functional region that did not contain an open reading frame, but did possess a putative Rho-independent transcriptional terminator sequence. This lead us to hypothesize that a non-translated regulatory RNA was responsible for the observed effects on glycogen synthesis. Northern hybridization analysis revealed that a small RNA is transcribed from this region, and that the levels of this RNA molecule are significantly decreased in a csrA mutant. This further suggested that the RNA might be interacting with the Csr system of glycogen regulation. Further sequence analysis of the RNA gene revealed the presence of seven sites which bear homology with the proposed CsrA-binding sites of CsrB. These observations strengthen our hypothesis that this region of the E. coli chromosome encodes a regulatory RNA, which is responsible for the observed stimulatory effect on glycogen biosynthesis, and that this RNA may be working through the Csr system by a mechanism similar to that of CsrB.

RESEARCH APPRECIATION DAY 2000

First Author: *SESHAGIRIRAO GUDAPATY*

Department: Molecularbioloy & Immunology

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☒ Postdoctoral Fellow/Resident

EFFECT OF *csrA, csrB*, AND *rpoS* ON LEVELS OF CsrB AND CsrA IN *ESCHERICHIA coli*

Seshagirirao Gudapaty and Tony Romeo, UNTHSC, Ft.Worth.TX 76107.USA

The global regulatory system known as Csr (Carbon storage regulator) of *Escherichia coli* represses biological functions that are induced upon entry into the stationary phase of growth, including glycogen synthesis and turnover, gluconeogenesis and biofilm formation, while it activates glycolysis and motility.Csr includes an RNA binding protein, CsrA, a potent modulator of specific mRNA stability.CsrA also binds to a non-coding RNA molecule, CsrB, to form a large ribonucleoprotein complex, which antagonizes CsrA activity.We have monitored *csrA* and *csrB* expression through growth curves of E. *coli* K-12 strain MG1655 and mutants defective in *csrA,csrB ,rpoS or both csrA and rpoS. The csrB mutant* was constructed by allelic replacement with chloramphenicol marker. Northern and Western blots for CsrA mRNA, CsrB RNA and CsrA protein, respectively were prepared using chemilluminscent probes and signals were quantitated by phosphoimage analysis. Intracellular *CsrB* levels were significantly decreased by ~20 fold at all stages of growth in *csrA* and *csrA rpoS* mutants No significant change was seen in *rpoS* mutant. *CsrA* message levels were similar in all strains up to mid-exponential phase of growth but thereafter a decrease of~4 fold was noted in the *csrB* and bot*h rpoS* mutants. CsrA protein levels accumulated as the cultures approached the stationary phase of growth: no significant differences were observed between any of the strains. We conclude that CsrA is needed to maintain steady state levels of CsrB RNA, and that *rpoS* affects CsrA mRNA levels without altering levels of CsrA protein. This suggests that a compensatory mechanism exist for post-transcriptional control of CsrA levels.(Supported by NIH Grant # 1R01GM59969-01)

MUSIC AND MEDICINE

64.	Kris Chesky, PhD	THE TEXAS CENTER FOR MUSIC & MEDICINE
65.	Kris Chesky, PhD	EXCEEDING THE STANDARD: SOUND EXPOSURE LEVELS I N A VARIETY OF COLLEGE MUSIC ENSEMBLES
66.	Kris Chesky, PhD	FORCES AGAINST THE RIGHT THUMB DURING CLARINET PERFORMANCE

RESEARCH APPRECIATION DAY 2000

First Author: *Kris Chesky, Ph.D.*

Department: *School of Public Health*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

THE TEXAS CENTER FOR MUSIC & MEDICINE Kris Chesky, Ph.D., School of Public Health; Bernard Rubin, D.O., Department of Medicine; Miriam Henoch, Ph.D., Department of Speech and Hearing Sciences; John Hipple, Ph.D., Testing and Counseling Center.

The talent, energy, and hard work of musicians and other performers give us the pleasures and deeper experiences, and the diversions we need to enrich our lives.

Like athletes, musicians require health, strength and stamina to perform in today's competitive music world and to meet their own high artistic expectations. Training for a career in music is difficult and even the most successful performers may be subject to severe and, at times, crippling stresses and strains. In many cases the ailments and disabilities of musicians arise from the specific uses of the body required to perform, such as the overuse disorder of an instrumentalist's hand, or they may be related to performing itself, such as the common but nonetheless debilitating anxiety of stage fright. For these reasons, a group of professionals at the University of North Texas and the University of North Texas Health Science Center have developed the Texas Center for Music & Medicine.

Considered as the pioneering arts medicine group in the Southwest, it is dedicated to the study, prevention, diagnosis, and treatment of illness that may arise in the course of a musician's career. At the Texas Center for Music & Medicine we lead the search for better musician health, education and performance practices. Our research and service team seeks answers together, sharing the challenge, excitement, and joy of our work. Unique as one of the world's premier and largest musical training grounds, together with a vibrant Health Science Center, the University of North Texas is committed to finding and delivering state of the art knowledge and services to the community of musicians.

A vital link between the biomedical sciences and musicians, the Texas Center for Music & Medicine capitalizes on the proximity of educational venues and patient care services adjacent to research laboratories and interdisciplinary facilities. Interaction among researchers fosters fresh concepts that may enhance music and become new topics in education and promote breakthroughs in clinical application. Many of the pedagogical and clinical techniques that impact music education still await discovery.

At the Texas Center for Music & Medicine, the current and future teams of researchers will interact to make these discoveries. Through the Texas Center for Music & Medicine, students and professional musicians will benefit from research that examines musicians' hearing loss, musculoskeletal and sensory dysfunction associated with over use and repetitive strain injury of tendinitis and focal dystonia, stage fright and performance anxiety, occupational health and safety, and other work-related physical and psychosocial stresses.

RESEARCH APPRECIATION DAY 2000

First Author: *Kris Chesky, Ph.D.*

Department: *School of Public Health*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

EXCEEDING THE STANDARD: SOUND EXPOSURE LEVELS IN A VARIETY OF COLLEGE MUSIC ENSEMBLES; Kris Chesky, Ph.D., School of Public Health and Research and Education Director, Texas Center for Music & Medicine; Miriam A. Henoch, Ph.D.Associate Professor Department of Speech and Hearing Sciences, University of North Texas, Denton, Texas 76203, Bernard Rubin, D.O. , Medical Director, Texas Center for Music & Medicine, 76107

Little is known about sound pressure levels within various musical ensembles, the inter-ensemble variability, or the variability of loudness levels produced between performance times. The ensemble types included those that have not been the focus of extensive research, i.e. jazz bands, symphonic/concert bands, percussion ensembles, and wind symphony. Throughout the 1998/1999 academic year, loudness levels experienced by individual musicians in a variety of musical ensembles at the University of North Texas College of Music were collected.

A personal dosimeter, attached to the selected musicians, measured and stored the sound pressure levels generated during the entire performances or rehearsal period. The collected measures included time-weighted average and daily noise dose. The data derived from this research was compared to OSHA (1983) criteria to determine which musicians were at risk for hearing loss according to the standards in place for industrial workers.

(This project was sponsored, in part, through a research grant from the National Academy of Recording Arts and Sciences and the University of North Texas Office of Sponsored Projects.)

RESEARCH APPRECIATION DAY 2000

First Author: *Kris Chesky, Ph.D.*

Department: *School of Public Health/Texas Center for Music & Medicine*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

FORCES AGAINST THE RIGHT THUMB DURING CLARINET PERFORMANCE

Kris Chesky, Ph.D., George Kondraske, Ph.D., Bernard Rubin, D.O. Texas Center for Music & Medicine, School of Public Health, University of North Texas Health Science Center, Fort Worth, Texas

Musicians playing clarinet may be at risk for occupationally related injuries. In our previous study, 37% of clarinetists reported some level of pain in their right wrist that ranged from episodic discomfort while playing the instrument to persistent pain with loss of control and use of the hand. Pain may be a potential precursor of more severe disease and indicates potential Cumulative Trauma Disorders (CTD's) such as carpal tunnel syndrome and wrist tendinitis. Epidemiologic evidence links force, repetition, and posture as risk factors. The forces at the right thumb during clarinet performance are probably produced by a combination of flexors and abductors of the carpometacarpal (CMC) joint in combination with abductors of the metacarpophalangeal (MCP) joint. In relation to the clarinet a composite force is generated from both a radial force, produced by mostly flexors of CMC and MCP, and an axial force produced mostly by abductors of the CMC. The purpose of this study was to determine the level of forces produced at the right thumb of clarinetists and to assess whether an elastic neck strap alters that force.

A new Buffet clarinet was fitted with a special two-axis sensor designed to measure thumb forces at the interface of the right thumb to the clarinet. This sensor measured axial thumb force along the longitudinal axis of the clarinet as well as radial force along the radial axis of the clarinet, orthogonal to the axial axis. Force measures were taken from nine clarinetists and used to determine forces associated with individual notes and fingerings, and the influence of an elastic neck strap. Continuous sound level measures determined the initiation, timing, and cessation of all within-subjects performance events. We found that forces increased and force angles decreased as the number of keys pressed elevated. Linear trend line equations for force ($y = 0.5355x + 9.4055$; $R2 = 0.8331$) and angle ($y = -3.1782x + 70.615$; $R2 = 0.8722$) demonstrated strong linear relationships to the number of pressed keys. Five pair-wise comparisons demonstrated that the elastic neck strap significantly increased the radial forces and significantly decreased the axial forces, composite forces and force angles. Overall, results suggested that the elastic neck strap could reduce the risk for CTD's by significantly reducing the composite forces against the right thumb during clarinet performance without hindering performance technique.

(supported through grants from the National Academy of Recording Arts & Sciences and the UNTHSC Office of Research and Biotechnology)

NORTH TEXAS EYE RESEARCH INSTITUTE

67.	James E. Turner, PhD	NORTH TEXAS EYE RESEARCH INSTITUTE (NTERI)
68.	James E. Turner, PhD	REGULATION OF OCULAR FETUIN IN RETINAL DEVELOPMENT AND DISEASE
69.	Tammy Hancock Nelson	OCULAR FETUIN PROTECTS RETINAL CELLS FROM APOPTOSIS
70.	Harold J. Sheedlo, PhD	ALTERED EXPRESSION OF NERVE GROWTH FACTOR, TrkA AND p75NTR PROTEIN AND mRNA IN DYSTROPHIC RETINAS
71.	Rajnee Agarwal	THE EXPRESSION OF BONE MORPHOGENETIC PROTEIN (BMP) AND BMP RECEPTOR mRNA BY HUMAN TRABECULAR MESHWORK AND OPTIC NERVE HEAD CELLS AND TISSUES
72.	Debra Shade	INTERLEUKIN-1α INDUCES HUMAN TRABECULAR MESHWORK CELL STROMELYSIN-1 PRODUCTION VIA ACTIVATOR PROTEIN-1
73.	Xiaochun Liu	PROTEIN EXPRESSION OF THE CILIARY NEUROTROPHIC FACTOR (CNTF) RECEPTOR COMPLEX BY HUMAN TRABECULAR MESHWORK (HTM) CELLS, LAMINA CRIBROSA (LC) CELLS AND OPTIC NERVE HEAD (ONH) ASTROCYTES
74.	Wendi Lambert	MAP KINASE ACTIVATION AND CELL PROLIFERATION OF HUMAN CULTURED LAMINA CRIBROSA CELLS FOLLOWING EXOGENOUS NERVE GROWTH FACTOR TREATMENT
75.	Santosh Narayan	REGULATION OF ENDOTHELIN-1 AND ENDOTHELIN CONVERTING ENZYME-1 EXPRESSION BY TUMOR NECROSIS FACTOR-alpha (TNF-alpha) AND CARBACHOL IN HUMAN NON-PIGMENTED EPITHELIAL (HNPE) CELLS
76.	Xinyu Zhang	ENDOTHELIN-1 INDUCES NITRIC OXIDE SYNTHASE IN tHNPE CELLS
77.	Raghu Krishnamoorthy, PhD	REGULATION OF Na,K-ATPase mRNA LEVELS BY ENDOTHELIN-1 IN TRANSFORMED HUMAN CILIARY NONPIGMENTED EPITHELIAL (t-HNPE) CELLS

78. Kelly Vopat — MECHANISM OF ISCHEMIA INDUCED APOPTOSIS OF CULTURED RAT RETINAL GANGLION CELLS

79. Bhalchandra J. Kudchodkar — PLASMA LIPID PEROXIDES AND PARAOXONASE -1 ACTIVITY AS MODULATORS OF OCULAR TISSUE CHOLESTEROL AND LIPID PEROXIDE ACCUMULATION; EFFECT OF HYPERBARIC OXYGEN TREATMENT

RESEARCH APPRECIATION DAY 2000

First Author: *James E. Turner*

Department: *North Texas Eye Research Institute*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

The North Texas Eye Research Institute (NTERI) was established in 1992 as one of the Centers of Excellence at the University of North Texas Health Science Center at Fort Worth. The purpose of the NTERI is to serve as an academic and research focus for basic and clinical science activities within the vision community of Fort Worth and the surrounding areas. Consequently, over 20 faculty are involved in visual science research and have appointments to the NTERI. These faculty are located in three basic science departments (Anatomy and Cell Biology, Pharmacology, and Molecular Biology and Immunology) at the UNT Health Science Center, at Alcon Research Laboratories, and within the ophthalmology community of Fort Worth.

Within NTERI, there are a number of groups of faculty interested in retina research, ocular diabetes, autoimmune diseases of the eye, optic nerve regeneration, glaucoma, corneal wound healing and aging. Specific areas of research interests within the various groups include: neovascularization, trophic factors, wound healing, aging, cell death, retina transplantation, glaucoma and diabetic complications. Broad technical areas of expertise are found through faculty interests within the NTERI to include: morphology, cell biology, biochemistry, molecular biology, pharmacology, electrophysiology and clinical expertise. All of the areas of expertise are supported by modern, sophisticated equipment and a skilled technical staff.

RESEARCH APPRECIATION DAY 2000

First Author: *James E. Turner*

Department: *Pathology and Anatomy*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

REGULATION OF OCULAR FETUIN IN RETINAL DEVELOPMENT AND DISEASE. J.E.Turner, T.H. Nelson, H.J. Sheedlo, M.H. Chaitin, N. Agarwal, J.W. Petry, W.A. Lambert, R.S. Roque. Dept. of Pathology and Anatomy, University of North Texas Health Science Center, Fort Worth, TX 76107.

Purpose. Recently we reported the presence of fetuin, a cysteine protease inhibitor and TGF-beta antagonist , and its message in ocular tissues, including the RPE, retina and corneal and ciliary body epithelia. In addition, fetuin has been reported to be present in high levels in the developing CNS in many species. In this study, fetuin expression was analyzed under developmental and disease conditions. Methods. Embryonic, postnatal and adult eye tissues were analyzed from Royal College of Surgeons (RCS) dystrophic rats, retinal degeneration (rd) mice and adult humans. Results. Early postnatal rat retinas showed fetuin immunolabeling in the neuroblast layer and later in ganglion cell bodies and outer plexiform layer. Western blots showed a peak of retinal fetuin protein at 2-6 postnatal days with a significant decrease at later time periods. Late surviving photoreceptor cells in RCS rat retinas were densely immunolabeled for fetuin, particularly in the retinal periphery. Western blot analysis confirmed these findings and indicated significant upregulation of fetuin at 2 months after birth, a time of maximum photoreceptor cell degeneration, with a graded decrease thereafter to barely detectable levels by 6 months. In contrast, normal retinas during these time periods demonstrated minimal to barely detectable levels of fetuin. Immunocytichemical analysis also revealed a similar upregulation followed by a decrease of fetuin in the RCS rat corneal and ciliary body epithelium. In contrast, immunocytochemistry did not reveal increased fetuin in rd/rd mouse eyes, prior to or after photoreceptor degeneration. Adult human retinas were immunolabeled for fetuin, particularly in the inner segments of cone photoreceptor cells. Conclusions. Fetuin is known to be a TGF- beta and bone morphogenetic protein antagonist and thus may figure in ocular development and diseases. Our results add to this hypothesis since we observed an upregulation of fetuin during these two events. Further studies are in order to determine the actual role of ocular fetuin in eye tissues.

RESEARCH APPRECIATION DAY 2000

First Author: *Tammy Hancock Nelson*

Department: *Pathology and Anatomy*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☒ Staff

☐ Postdoctoral Fellow/Resident

OCULAR FETUIN PROTECTS RETINAL CELLS FROM APOPTOSIS. T.H. Nelson, H.J. Sheedlo, J.W. Petry, W.A. Lambert, J.E.Turner,. Dept. of Pathology and Anatomy, University of North Texas Health Science Center, Fort Worth, TX 76107.

Purpose. Fetuin, a cysteine protease inhibitor and TGF-beta antagonist was reported for the first time in eye tissues, including RPE, retina, corneal and ciliary body epithelium. TGF-beta and other members of this superfamily, the BMP (bone morphogenetic proteins) family have been shown to regulate cell proliferation, differentiation, development and apoptosis. Fetuin's ability to bind TGF-beta and BMPs may enable it to protect retinal cells from apoptosis, resulting in increased proliferation and survival. Because of this, we have investigated fetuin's ability to protect retinal cells from apoptosis. Methods. Apoptosis was induced in transformed mouse retinal photoreceptor cells (661W) and assessed with ethidium bromide/acridine orange flourescence. Cells were grown in the presence or absence of fetuin prior to the apoptosis induction. Apoptotic-stained cells counts and tetrazolium cell proliferation assays were used to determine fetuin protection. Fetuin intravitreal injections were done into postnatal rd mouse eyes prior to the onset of degeneration and examined at 21 days. Results. Fetuin pre-treatment for 24 hours prior to the induction of apoptosis protected 661w cells from dying. A significant decrease in the number of stained apoptotic cells was seen with fetuin compared to controls. In addition, a cell proliferation assay showed the presence of significantly more cells 24 hours after induction in cultures pre-treated with fetuin compared to controls, confirming that fetuin caused an increase in cell survival. Injections of fetuin in rd mouse eyes prior to the onset of degeneration increased the number of surviving retinal cells. An increase in total retina thickness, especially in the inner retina, along with an increase in the number of photoreceptor cells was seen in fetuin-injected eyes. Conclusions. Ocular fetuin can protect retinal cells from induced apoptosis in vitro as well as protecting some cells from apoptotic cell death in the rd mouse retina. Retinal degeneration via apoptosis is present in many retinal pathologies therefore fetuin may have therapeutic potential in protecting dying retinal cells.

RESEARCH APPRECIATION DAY 2000

First Author: *Harold J. Sheedlo, Ph.D.*

Department: *Pathology and Anatomy*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

ALTERED EXPRESSION OF NERVE GROWTH FACTOR, TrkA AND p75NTR PROTEIN AND mRNA IN DYSTROPHIC RETINAS. H.J. Sheedlo, B. Srinivasan, A.M. Brun-Zinkernagel, L.X. Oakford, R.J. Wordinger, J.E. Aschenbrenner, J.E. Turner, R.S. Roque. Department of Pathology and Anatomy, University of North Texas Health Science Center, Fort Worth, TX 76107.

It is known that photoreceptor death in retinas of Royal College of Surgeons (RCS) dystrophic rats is via apoptosis. However, the initiating factor in this photoreceptor cell loss remains unknown. The disease process could involve the defective retinal pigment epithelial (RPE) cells, microglial cells and/or Müller cells. Nerve growth factor (NGF) and its low-affinity p75 receptor (p75NTR) controls death of some neurons in the central nervous system. In this study, we investigated the role of NGF and NGF receptors, TrkA and p75NTR, in photoreceptor death in retinas of RCS dystrophic rats. For this study, expression of NGF, TrkA and p75NTR protein and mRNA in retinas of RCS dystrophic rats were compared to retinas of normal rats using immunocytochemistry and semi-quantitative RT-PCR. In this investigation, in normal adult retinas, NGF was localized to RPE and ganglion cells, p75NTR immunolabeling was observed in photoreceptors, and TtrkA immunoreactivity was restricted to the inner retina. In early degenerating RCS dystrophic retinas, dense immunoreactivity for NGF was localized to Müller cell bodies and processes, which was not observed in normal retinas. Immunostaining for p75NTR in photoreceptor cells was denser in 1-month-old RCS retinas compared to normal retinas. TrkA immunolabeling in RCS retinas was restricted to the inner retina, which remains relatively intact throughout the life of RCS dystrophic rats. As shown by PCR, mRNA levels for NGF, TrkA and p75NTR were increased in 1-month-old RCS retinas when compared to normal adult rat retinas. In conclusion, increased levels of NGF and p75NTR, but not TrkA, in photoreceptors during retinal degeneration in RCS dystrophic retinas suggest that NGF is directly involved in photoreceptor death during retinal degeneration. The mechanism of NGF-induced cell death during this degeneration may be mediated by a p75NTR-cell death cascade. The source of the NGF may include RPE, Müller and/or microglial cells. (EY 10766)

RESEARCH APPRECIATION DAY 2000

First Author: *Rajnee Agarwal*

Department: *Pathology and Anatomy*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☒ Staff

☐ Postdoctoral Fellow/Resident

THE EXPRESSION OF BONE MORPHOGENETIC PROTEIN (BMP) AND BMP RECEPTOR mRNA BY HUMAN TRABECULAR MESHWORK AND OPTIC NERVE HEAD CELLS AND TISSUES ((R. Agarwal1, M. Talati1, S. Fern1, E. Clayton1, W. Lambert1, A. Clark,1,2,3 and R. Wordinger1,2)) Department of Anatomy and Cell Biology1 and North Texas Eye Research Institute2, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX.; Glaucoma Research, Alcon Research Ltd3., Fort Worth, TX.

Purpose: Bone morphogenetic proteins (BMP) constitute the largest subfamily of growth factors within the TGFbeta superfamily. BMPs were originally shown to induce bone and cartilage formation but are now considered to be multifunctional cytokines having a wide range of effects on numerous cell types. BMPs signal via a receptor complex consisting of BMPR-I and BMPR-II. We have previously reported that superfamily members TGFbeta and TGFbetaR {Agarwal et al., IOVS 38(4) S563; Lambert et al., IOVS 38(4) S162} and GDNF and GDNFR {Wordinger et al., IOVS 40(4) S504); Liu et al., IOVS 40(4) S 673)} are expressed by both human trabecular meshwork (HTM) and optic nerve head (ONH) cells. The purpose of this study was to demonstrate mRNA expression of BMPs and BMPRs in cultured HTM and ONH cells and tissues. Methods: The expression of mRNA for BMP-2, BMP-4, BMP-5, BMP-7, BMPR-IA, BMPR-IB, and BMPR-II was examined by total cellular and tissue RNA isolation, RT-PCR, and agarose gel electrophoresis using well characterized, early passage HTM cells, lamina cribrosa cells (LC) and ONH astrocytes isolated from neonatal and adult donors. Results: Using RT-PCR we detected mRNA's for BMPR-IA, BMPR-IB, and BMPR-II in all HTM, LC, and ONH astrocyte cell lines and tissues. There were no apparent differences in expression due to age of the donor. mRNA for BMP-2 and BMP-4 was expressed by all HTM and ONH cell lines and tissues but BMP-5 and BMP-7 was variably expressed. Conclusions: These studies are the first to report that HTM, LC, and ONH astrocytes cells and tissues express mRNA for BMPs and BMPRs. These results further demonstrate that members of the TGFgbeta superfamily are potential modulators of cellular function within the human trabecular meshwork and the ONH. CR: C2, E. Support: National Glaucoma Program of the American Health Assistance Foundation, Rockville, MD., The Glaucoma Foundation, New York, NY., and Alcon Research Ltd., Fort Worth TX

RESEARCH APPRECIATION DAY 2000

First Author: *Debra Shade*

Department: *Pharmacology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

INTERLEUKIN-1α INDUCES HUMAN TRABECULAR MESHWORK CELL STROMELYSIN-1 PRODUCTION VIA ACTIVATOR PROTEIN-1. D.L. Shade, A.F. Clark, I.-H. Pang. Alcon Research Ltd., Fort Worth, TX 76134 and University of North Texas Health Science Center, Fort Worth, TX 76107.

Purpose: Accumulation of extracellular matrix (ECM) in the trabecular meshwork (TM) region of the human eye is believed to increase aqueous outflow resistance, and, hence, elevate intraocular pressure. Interleukin-1α (IL-1α) is a highly efficacious stimulator of TM production of stromelysin-1 (MMP-3), an enzyme which degrades ECM. Thus, we determined the intracellular signaling mechanisms by which IL-1α induces human TM (HTM) MMP-3 production. **Methods:** HTM cells were cultured in 24 well plates in the presence and absence of IL-1α and/or specific inhibitors of various intracellular signaling pathways. Secreted proMMP-3 levels were then quantified in aliquots of culture supernatant by a commercial ELISA (The Binding Site); neutral red uptake was used to determine viability of the cell monolayers. **Results:** Basal HTM pro-MMP-3 levels were was 0.122 ± 0.005 ng/well/24 h (mean ± SEM, n = 158). IL-1α (5 ng/mL) increased its level dramatically: 1844 ± 90% compared to control (n = 30; $p < 0.001$). This stimulative effect of IL-1α was blocked by Gö6976 [protein kinase Cμ (PKCμ) inhibitor, 200 nM], PD98059 [MEK inhibitor, 100 μM], SB202190 [p38 inhibitor, 100 nM] and SR11302 [activator protein-1 (AP-1) sequester, 100 nM], but not significantly affected by inhibitors of casein kinase II, nuclear factor κB (NFκB), phospholipase A_2 (PLA_2), phospholipase D, cycloxygenases, lipoxygenases, orsphingomyelinase. All compounds were tested at concentrations that did not compromise cell viability. **Conclusions:** A variety of intracellular signaling pathways are known to transduce the effects of IL-1α in other tissues: NFκB-mediated protein expression, PLA_2-activated lipid transmitter production, and AP-1 associated transcription, for example. Our data indicate that AP-1 and its associated upstream enzymatic cascades (i.e. PKCμ, MEK, and p38) are the predominant means by which IL-1α stimulates in vitro HTM production of stromelysin-1. Therefore, we predict that AP-1 "activators" will upregulate MMP-3 production in vivo and improve aqueous outflow. (Supported by Alcon Research, Ltd.)

RESEARCH APPRECIATION DAY 2000

First Author: *Xiaochun Liu*

Department: *Pathology and Anatomy*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

PROTEIN EXPRESSION OF THE CILIARY NEUROTROPHIC FACTOR (CNTF) RECEPTOR COMPLEX BY HUMAN TRABECULAR MESHWORK (HTM) CELLS, LAMINA CRIBROSA (LC) CELLS AND OPTIC NERVE HEAD (ONH) ASTROCYTES. ((X. Liu, (1) M. Talati,(1) W. Lambert,(1) R. Agarwal,(1) and A. F. Clark,(1,2) and R. Wordinger,(1))) North Texas Eye Research Institute and Department of Anatomy and Cell Biology, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX.(1); Glaucoma Research, Alcon Research Ltd., Fort Worth, TX.(2)

Purpose: Ciliary neurotrophic factor (CNTF) is a polypeptide growth factor that provides trophic support and protection for neurons and glial cells. However, neurotrophin and neurotrophic growth factors are expressed and appear to function in many non-neural cell populations. For example, we have previously demonstrated that human trabecular meshwork (HTM) cells express neurotrophins (NGF, BDNF, NT-3, NT-4 {Wordinger et al., 1998; IOVS; 39[4], S436}). Previous studies have demonstrated that mRNA for CNTF and its receptor complex (CNTF-R, LIFR, and gp130) was expressed in HTM cells {Wordinger et al., 1999; IOVS; 40[4], S504} as well as lamina cribrosa (LC) cells and optic nerve head ONH astrocytes {Liu et al., 1999; IOVS; 40[4], S673}. The purpose of this study was to demonstrate protein expression for CNTF-R, LIFR, and gp130 in HTM cells, LC cells and ONH astrocytes. Methods: A combination of immunolocalization and Western blot analysis for the CNTF receptor complex was performed using HTM cells, LC cells and ONH astrocytes from normal donors of various ages. Results: Both immunolocalization and Western blot analysis detected protein expression of CNTF-R, LIFR, and gp 130 by HTM cells, LC cells, and ONH astrocytes. Conclusions: To our knowledge, this is the first report that proteins of the CNTF receptor signaling complex are expressed by cultured HTM cells, LC cells and ONH astrocytes. This raises the distinct possibility that paracrine/autocrine signaling via CNTF and the CNTFR complex exists within the human trabecular meshwork and optic nerve head. CR: C2, E; (Support: National Glaucoma Program of the American Health Assistance Foundation, Rockville, MD., The Glaucoma Foundation, New York, NY., and Alcon Research Ltd., Fort Worth, TX.)

RESEARCH APPRECIATION DAY 2000

First Author: *Wendi Lambert*

Department: *Pathology and Anatomy*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

MAP KINASE ACTIVATION AND CELL PROLIFERATION OF HUMAN CULTURED LAMINA CRIBROSA CELLS FOLLOWING EXOGENOUS NERVE GROWTH FACTOR TREATMENT. Wendi Lambert (1), Rajnee Agarwal (1), Abbot F. Clark (1,2,3) and Robert J.Wordinger (1, 2). Department of Anatomy and Cell Biology (1) and North Texas Eye Research Institute (2), University of North Texas Health Science Center at Fort Worth, TX ; Glaucoma Research, Alcon Research Ltd., Fort Worth, TX (3).

Purpose: We have previously shown that lamina cribrosa (LC) cells isolated from the human optic nerve head (ONH) express and secrete the neurotrophins (e.g. nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3)). We have also demonstrated that LC cells express both high affinity neurotrophin receptors (trk A, trk B and trk C) specific for the neurotrophins, and truncated trk receptor isoforms (trk B.T and trk C.T) {Lambert et al., 1999; IOVS, 40(4): S673 and Lambert et al., 1998; IOVS, 39(4): S260}. In response to NGF bining, trk A receptors are activated via autophosphorylation, and within certain cell types activate the mitogen-activated protein (MAP) kinase pathway. The purpose of this study was to (a) demonstrate MAP kinase activation and (b) LC cell proliferation following exogenous administration of NGF. Methods: Lamina cribrosa cells were isolated from the ONH and were exposed to NGF (50 ng/ml) in serumless media for 5 minutes (MAP kinase activation) or 14 days (cell proliferation). Cellular proteins were collected to be used in Western blot analysis for activated MAP kinase and phosphotyrosine (Promega). Cell proliferation was determined using a Coulter counter. Results: Phosphotyrosine and activated MAP kinase were detected in LC cells treated with exogenous NGF. In LC cells treated with serumless media alone, phosphotyrosine and activated MAP kinase were also detected, but to a lesser degree. Exogenous NGF treatment stimulated LC cell proliferation (100% of control). Conclusions: These results demonstrate that exogenous NGF leads to tyrosine and MAP kinase phosphorylation within human LC cells. In addition, exogenous NGF stimulates LC cell proliferation. This suggests that human LC cells possess functional trk A receptors that may participate in both exogenous and endogenous NGF signaling. These results raise the possibility of neurotrophin signaling within the human ONH via trk receptor activation. (Support: The Glaucoma Foundation and Alcon Research Limited.)

RESEARCH APPRECIATION DAY 2000

First Author: *Santosh Narayan*

Department: *Pharamcology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

REGULATION OF ENDOTHELIN-1 AND ENDOTHELIN CONVERTING ENZYME-1 EXPRESSION BY TUMOR NECROSIS FACTOR-alpha (TNF-alpha) AND CARBACHOL IN HUMAN NON-PIGMENTED EPITHELIAL (HNPE) CELLS.

Santosh Narayan, Ganesh Prasanna, Raghu Krishnamoorthy, Christina Hulet and Thomas Yorio.

Department of Pharmacology, University of North Texas Health Science Center at Fort Worth, TX-76107

Purpose. Endothelin-1 (ET-1) is a potent vasoactive peptide produced from precursor big ET-1 by the action of endothelin converting enzyme (ECE-1). Previous studies suggest that ET-1 may be involved in aqueous humor dynamics and regulation of intraocular pressure (IOP). Cholinergic agents such as carbachol (CCH) and pilocarpine, also regulate IOP by increasing aqueous humor outflow via actions on the ciliary muscle. We have previously shown enhanced ET-1 synthesis in HNPE cells following cytokine (TNF-alpha) treatment. The present study determines if ET-1 synthesis and release as well as ECE-1 expression are also under the regulation of cholinergic treatments in HNPE cells. Methods. HNPE cells were grown in 6-well plates and were treated (at least in triplicate) with serum-free culture media containing either carbachol (1, 10 and 100 micro M) or TNF-alpha (10 nM; positive control) for 24 hours. Immunoreactive ET-1 (ir-ET-1) released from these cells into the culture media was quantitated by radioimmunoassay (RIA). Expression of preproendothelin-1 (ppET-1) and ECE-1 was determined using RT-PCR. Results. TNF-alpha significantly increased ir-ET-1 levels in HNPE cells (8 +/- 0.1 pg/ml/well, n=3) compared to untreated controls (4 +/- 0.3 pg/ml/well, n=4). Carbachol dose-dependently increased ir-ET-1 in HNPE cells (CCH 1micro M: 7 +/- 0.1 pg/ml/well, n=3; CCH 10 micro M: 9 +/- 0.5 pg/ml/well, n=3; CCH 100 micro M: 11 +/- 0.6 pg/ml/well, n=3). TNF-alpha caused an up-regulation in mRNA expression for ppET-1 and ECE-1. Conclusions. Regulation of ECE-1 activity and expression in HNPE cells may be important relative to the concentrations of ET-1 in aqueous humor and its actions in enhancing outflow.

(Supported by NEI/ NIH EY 11979)

RESEARCH APPRECIATION DAY 2000

First Author: *Xinyu Zhang*

Department: *Pharmacology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

ENDOTHELIN-1 INDUCES NITRIC OXIDE SYNTHASE IN tHNPE CELLS

Xinyu Zhang, Ganesh Prasanna, Thomas Yorio?
Department of University of North Texas Health Science Center at Fort Worth, TX-76107

Purpose: Nitric Oxide (NO) is an important signaling molecule that mediates a variety of essential physiological process including neurotransmission, vasodilatation and host cell defenses. Most recently increases in NO concentrations have been linked to damage to retinal ganglion cells in Glaucoma. Endothelin-1(ET-1), a potent vasoconstrictive peptide, has been shown to be elevated in Glaucomatous eyes. Our laboratory has been shown that ET-1 can increase iNOS in tHNPE cells. Methods: The final products of NO released in culture media are nitrite (NO2) and nitrate (NO3). Hence the best index of total NO producted is the sum of both [NO2] and [NO3]. Using a colorimetric assay (with nitric oxide synthase assay kit), NO synthase activity was examined inhuman non-pigmented ciliary epithelial (HNPE) cells treated with ET-1 (1, 10, and 100 nM) or cytokine cocktail (TNF-?, IL-1? , IFN- ? and LPS) as a positive control for 24 hrs in serum-free culture media. Results: An increase in NO production was observed in cells treated with ET-1 or cytokine cocktail, but not in control. ET-1 100nM had the greatest effect .PD142893, an ETA/B receptor antagonist, blocked the effedts of ET-1. Conclusion: ET-1 effectively stimulate the production of NO in tHNPE cells through an ET receptor event. Whether such increases in NO are detrimental to HNPE cells has yet to be determined.
(Supported by NEI/NIH EY 11979)

RESEARCH APPRECIATION DAY 2000

First Author: *RAGHU KRISHNAMOORTHY PH.D.*

Department: *BIOMEDICAL SCIENCES*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☒ Postdoctoral Fellow/Resident

REGULATION OF Na,K-ATPase mRNA LEVELS BY ENDOTHELIN-1 IN TRANSFORMED HUMAN CILIARY NONPIGMENTED EPITHELIAL (t-HNPE) CELLS ((R. R. Krishnamoorthy, G. Prasanna, C. Hulet, and T. Yorio)) Department of Pharmacology, University of North Texas Health Science Center, Fort Worth, TX 76107.

Purpose: Endothelin-1 (ET-1), a potent vasoconstrictive agent is found to be elevated in aqueous humor of patients with glaucoma, compared to control subjects. Intravitreal injection of ET-1 has been shown to reduce intraocular pressure (IOP) by possibly enhancing outflow mechanisms via the trabecular meshwork or uveoscleral route and also by reducing aqueous humor formation. Previous studies by our group showed that ET-1 (1 to 100 nM) decreases the activity of sodium potassium ATPase (Na,K-ATPase), a key enzyme involved in ion transport and aqueous humor production, in t-HNPE cells. This decrease in Na,K-ATPase activity could have a retarding influence on the rate of aqueous humor production and IOP. In the present study, we sought to determine if ET-1 also alters mRNA levels of Na,K-ATPase subunit genes. Methods: t-HNPE cells were treated acutely (15 min) with ET-1 (1, 10 and 100 nM) and total RNA from these cells were isolated using the Trizol reagent. Northern blot analysis of Na,K-ATPase mRNA levels was carried out using cDNA for the alpha subunit of the enzyme. Results: Treatment of t-HNPE cells with ET-1 caused a dose-dependent increase in mRNA levels of the alpha subunit of Na,K-ATPase. Beta-actin expression was not appreciably altered between the control and experimental groups. Conclusions: In addition to attenuating Na,K-ATPase activity in t-HNPE cells, ET-1 also increases the expression of the catalytically active alpha subunit of the enzyme. The observed increase in mRNA of the alpha subunit could occur either by transcriptional regulatory mechanisms or by altering template stability of the message and could consequently have a significant effect on aqueous humor formation.

RESEARCH APPRECIATION DAY 2000

First Author: *Kelly Vopat*

Department: *Pathology and Anatomy*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

MECHANSIM OF ISCHEMIA INDUCED APOPTOSIS OF CULTURED RAT RETINAL GANGLION CELLS. Kelly Vopat, Ross S. Fuller, Neeraj Agarwal, Department of Pathology and Anatomy, University of North Texas Health Science Center, Fort Worth, TX 76107.

Purpose: To test the hypothesis that the retinal ganglion cells (RGCs) in glaucoma are exposed to ischemic conditions leading to apoptosis of RGCs. Therefore, the purpose of this study was to explore the mechanisms of apoptosis of RGCs using an in vitro model of RGCs.

Methods: RGC-5 cells were exposed to ischemic conditions for six hours. We included six groups of various conditions with zero glucose and no serum in growth medium with nitrogen gas replacing carbon dioxide and oxygen, representing the ischemic conditions along with a control group of RGC-5 cells. A TUNEL assay was used to determine the apoptosis of RGC-5 cells on exposure to ischemic conditions. Using RT-PCR analysis, we quantitated the mRNA levels of both apoptotic marker genes including Bcl-2 and Bax, and stress proteins including c-fos, hsp70, and heme oxygenase (HO). Furthermore, we also quantitated the levels of various neurotrophins under the ischemic conditions.

Results: The TUNEL assay showed apoptosis occurring in the ischemically stressed RGC-5 cells as compared with the control RGC-5 cells in growth medium containing glucose and serum under oxygen and carbon dioxide. The Bcl-2/Bax mRNA ratio decreased in the RGC-5 cells exposed to ischemic conditions as shown by RT-PCR analysis. The mRNA levels of various neurotrophins including BDNF, GDNF, and NGF were elevated in the ischemically stressed cells. The stress proteins (c-fos, hsp70, and HO) levels were also elevated in the ischemic cells.

Conclusion: With the above studies, we conclude that the retinal ganglion cells undergo apoptosis in ischemic conditions via a decrease in Bcl-2/Bax ratio. These results further suggest that the RGCs try to overcome the ischemic stress by up regulation of various neurotrophins along with stress protein mRNA levels.

RESEARCH APPRECIATION DAY 2000

First Author: *Bhalchandra J. Kudchodkar*

Department: *Molecular Biology & Immunology*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

PLASMA LIPID PEROXIDES AND PARAOXONASE -1 ACTIVITY AS MODULATORS OF OCULAR TISSUE CHOLESTEROL AND LIPID PEROXIDE ACCUMULATION; EFFECT OF HYPERBARIC OXYGEN TREATMENT.

Bhalchandra J. Kudchodkar, Andras Lacko and Ladislav Dory, Department of Molecular Biology & Immunology, UNT HSC at Fort Worth, Fort Worth, TX 76107.

The deposition of oxidized lipids in the lens and cornea (ocular tissue) plays an important role in the developmet of cataracts. We have previously shown that administration of cholesterol-rich diet to rabbits leads to a profound increase in plasma oxidized lipid concentration. This increase is accompanied by a nearly 50% decrease in plasma paraoxonase-1 (PON-1) activity, an high density lipoprotein (HDL)- associated enzyme thought to be responsible, at least in part, for the hydrolysis of oxidized lipids. Significantly, exposure of these animals to hyperbaric oxygen (HBO) reversed the extent of lipid oxidation and PON-1 inactivation. The marked increase in plasma cholesterol and oxidized lipids concentrations of the cholesterol-fed animals was also reflected in the ocular tissues. Cholesteryl ester content increased 14-fold, while oxidized lipids increased over 4-fold (0.1±0.07 vs. 1.4±0.7 mg/g tissue and 1.7±0.4 vs. 7.2±2.6 nmol/g, respectively). Treatment with HBO dramatically decreased both, ocular tissue cholesterol (0.35±0.7 mg/g tisue) and lipid peroxides (2.5±1.5 nmol/g). These changes correlated inversely with plasma and eye aqueous humor PON-1. In contrast to the general circulation, where the vast majority of lipids is carried in very low and low density lipoproteins (LDL) , the aqueous humor contains only HDL . Our data therefore suggest that HDL and not LDL may regulate the extent of lipid and lipid peroxide deposition in ocular tissue and paraoxonase-1 plays an important protective role.(Supported in part by Bank One and NIH grants to LD).

NEUROSCIENCE AND AGING
GERIATRICS EDUCATION AND RESEARCH INSTITUTE

80.	Thomas J. Fairchild, PhD	GERIATRICS EDUCATION AND RESEARCH INSTITUTE
81.	Chau N. Pham, DO	PROFILE OF THE TARRANT COUNTY VIETNAMESE OLDER ADULTS: NEEDS ASSESSMENT AND ACCESS TO HEALTH CARE SERVICES
82.	John M. Talent	ALZHEIMER'S DISEASE INCREASES THE SUSCEPTIBILITY OF PROTEINS TO OXIDIZED PROTEIN ISOFORMS AS DIAGNOSTIC BIOMARKERS IN ALZHEIMER'S DISEASE: TOTAL OXIDIZED PROTEIN LEVELS IN HUMAN SERA
83.	John M. Talent	OXIDIZED PROTEIN ISOFORMS AS DIAGNOSTIC BIOMARKERS IN ALZHEIMER'S DISEASE: TOTAL OXIDIZED PROTEIN LEVELS IN HUMAN SERA
84.	Martine Pastorcic	CONTROL OF TRANSCRIPTION OF THE PRESENILIN 1 GENE BY ETS TRANSCRIPTION FACTORS
85.	Pamela L. Marshall	ALZHEIMER'S DISEASE INCREASES THE SUSCEPTIBILITY OF PROTEINS TO OXIDATIVE DAMAGE
86.	Joungil Choi	VARIOUS REACTIVE OXYGEN SPECIES (ROS) CAUSE DIFFERENTIAL EXPRESSION OF PROTEINS IN NEURONAL CELL LINES
87.	Michael L. Moeller	DEVELOPMENT OF A NOVEL SYSTEM FOR THE INVESTIGATION OF FACTORS CONTROLLING THE GROWTH AND DIFFERENTIATION OF DIFFERENT CELL TYPES FROM NEUROSPHERES
88.	Shelley McDonald	A NOVEL NITRONE IMPROVES MEMORY IN OLD MICE
89.	Jay Mishalanie	THE EFFECTS OF AGING AND CALORIC RESTRICTION ON AUDITORY AND SHOCK STARTLE REACTIONS IN MICE
90.	Christopher M. de Fiebre, PhD	NEUROPROTECTIVE PROPERTIES OF NICOTINIC AGONISTS AGAINST ETHANOL-INDUCED NEUROTOXICITY

91.	Robert Luedtke, PhD	CHARACTERIZATION OF ^{125} I-IABN, A RADIOLIGAND SELECTIVE FOR D2-LIKE DOPAMINE RECEPTORS
92.	Paromita Das	PHARMACOLOGY OF 5-HT3 RECEPTORS
93.	Susan Laufer/Edward L. Orr	ACCUMULATION OF CEREBRAL CORTICAL HISTAMINE AND EVAN'S BLUE AFTER MILD-TO-MODERATE HEAD TRAUMA IN THE MOUSE

RESEARCH APPRECIATION DAY 2000

First Author: *Thomas J. Fairchild, Ph.D.*

Department: *Geriatrics Education and Research Institute*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

GERIATRICS EDUCATION AND RESEARCH INSTITUTE
Thomas J. Fairchild, Ph.D., University of North Texas Health Science Center; Fort Worth, Texas 76107

Purpose: To create an "introduction" to the Geriatrics Education and Research Institute for viewers and to create a "lead" poster presentation preceding other posters/abstracts on aging supported or endorsed by GERI.

Methodology: The mission of GERI, a general focus on aging demonstrating a national focus and GERI's focus, and a "food for thought" section constitute the totality of the poster display.

Conclusions: GERI is an inter-disciplinary and multi-disciplinary Institute that focuses on the phenomenon of aging. Its Executive Council reflects most general areas surrounding the study of aging.

RESEARCH APPRECIATION DAY 2000

First Author: *Chau N. Pham, DO*

Department: *Geriatric Fellowship Program*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☒ Postdoctoral Fellow/Resident

PROFILE OF THE TARRANT COUNTY VIETNAMESE OLDER ADULTS: NEEDS ASSESSMENT AND ACCESS TO HEALTH CARE SERVICES. Chau N. Pham, D.O., Anthony Nguyen, D.O., Thuc-Nguyen Tran, D.O., Janice Knebl, D.O. University of North Texas Health Science Center, Ft. Worth, Texas, 76107.

Purpose: Most Vietnamese living in the United States today entered the country as immigrants. They face health care challenges: there is less community/neighborhood support and less support from children to care for parents than traditional Vietnamese are accustomed to. Cultural attitudes for older adults continue to dictate how health care is accessed. There are no current studies focusing on attitudes of older Vietnamese adults toward health care services and its usage. This qualitative study provides baseline information on 1) awareness of community services, 2) attitudes and barriers toward health care access, and 3) perceived basic needs.

Methods: Vietnamese age 50 years and older were invited to a general session where demographic information was gathered and focus groups were identified. To facilitate data collection, focus groups were gender specific. Data were gathered via scribes' notes and taped sessions.

Results: There were a total of 28 participants, 18 females and 10 males. Ninety percent came to the U.S. in the 90's (63% male and 53% female). Most (94% female and 90% male) live with someone i.e. children or relatives. Therefore the participants are Medicare and Medicaid ineligible.

Conclusion: Language and transportation continue to be barriers to health care. Our recommendations include 1) providing literatures on community services and eligibility requirements in Vietnamese, 2) creating a list of local Vietnamese physicians, 3) creating a list of Vietnamese students who may provide translation services, 4) disseminating health care information through Vietnamese radio stations and newspapers and local health fairs, 5) encouraging churches to organize scheduled transportation and the use of MITS, 6) providing a bilingual social worker with expertise in Medicaid and Medicare, and 7) a pilot Senior Center for older Vietnamese older adults funded by the Area Agency on Aging.

(Funded by the Area Agency on Aging of Tarrant County)

RESEARCH APPRECIATION DAY 2000

First Author: *John M. Talent*

Department: *Molecular Biology and Immunology*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☒ Staff

☐ Postdoctoral Fellow/Resident

ALZHEIMER'S DISEASE INCREASES THE SUSCEPTIBILITY OF PROTEINS TO OXIDIZED PROTEIN ISOFORMS AS DIAGNOSTIC BIOMARKERS IN ALZHEIMER'S DISEASE: TOTAL OXIDIZED PROTEIN LEVELS IN HUMAN SERA John M. Talent, Craig C. Conrad, Christina A. Malakowsky, and R.W. Gracy. UNTHSC, Fort Worth, Texas 76107. The oxidation of proteins has been shown to be a causative factor in loss of cognitive abilities in Alzheimer's Disease (AD). a neurodegenerative affliction among the elderly. With the aging of the human population, AD is expected to reach epidemic levels. The initial stages of AD begin long before clinical symptoms are apparent. Unfortunately, postmortem observation of brain tissue is the only reliable method to date for the 100% confirmation of AD. Therefore, predictive diagnostic biomarkers for AD are needed. Although genetic biomarkers can be used to predict familial AD (a subset comprising less that 3% of those likely to develop the disease), the genetic biomarkers are of little use for monitoring the development, progression or prevention of AD. For such purposes, oxidized protein isoforms would offer the best potential diagnostic tool. Since the reactive oxygen species (ROS) may also damage cells that make up the blood brain barrier, the oxidative damage of AD may not be restricted to proteins only in the brain. Thus, it is likely that specific oxidized proteins may be found in the blood or cerebral spinal fluid (CSF) of persons susceptible to or suffering from AD. Utilizing high performance liquid chromatography (HPLC), our laboratory has found evidence of such oxidized protein biomarkers in the blood and is currently isolating, identifying and characterizing these proteins. A preliminary aspect, reported here, is determination by HPLC of the total levels of oxidized proteins in sera of AD patients and their relatives compared to non-AD controls. Based on data reported here, AD subjects have significantly higher levels of oxidized proteins, confirming that proteins in AD patients are more susceptible to oxidation.

RESEARCH APPRECIATION DAY 2000

First Author: *John M. Talent*

Department: *Molecular Biology & Immunology*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☒ Staff

☐ Postdoctoral Fellow/Resident

OXIDIZED PROTEIN ISOFORMS AS DIAGNOSTIC BIOMARKERS IN ALZHEIMER'S DISEASE: TOTAL OXIDIZED PROTEIN LEVELS IN HUMAN SERA. John M. Talent, Craig C. Conrad, Christina a. Malakowsky, and R. W. Gracy. Molecular Aging Unit, Department of Molecular Biology and Immunology, UNTHSC, Fort Worth, Texas 76107.

The oxidation of proteins has been shown to be a causative factor in loss of cognitive abilities in Alzheimer's Disease (AD), a neurodegenerative affliction among the elderly. with the aging of the human population, AD is expected to reach epidemic levels. The initial stages of AD begin long before clinical synptoms are apparent. Unfortunately, postmortem observation of brain tissue is the onlyreliable method to date for the 100% confirmation of AD. Therefore, predictive diagnostic biomarkers for AD are needed. Although genetic biomarkers can be used to predict familial AD (a subset comprising less than 3% of those likely to develop the disease), the genetic biomarkers are of little use for monitoring the development, progression, or prevention of AD. For such purposes oxidized protein isoforms would offer the best potential diagnostic tool. since the reactive oxygen species (ROS) may also damage cells that make up the blood brain barrier, the oxidative damage of AD may not be restricted to proteins only in the brain. Thus, it is likely that specific oxidized proteins may be found in the blood or cerebral spinal fluid (CSF) of persons susceptible to or suffering from AD. Utilizing 1-D and 2-D polyacrylamide gel electrophoresis (PAGE) and high performance liquid chromatography (HPLC), our laboratory has found evidence of such oxidized biomarkers in the blood and is currently isolating, identifying, and characterizing these proteins. A preliminary aspect, reported here, is determination by HPLC of the levels of oxidized proteins in sera of AD patients and their relatives compared to non-AD controls. Based on early data, it is expected that AD subjects will have significantly higher levels of oxidized proteins, confirming that proteins in AD patients are more susceptible to oxidation. (This research was supported by grants from the Robert A. Welch Foundation (BK0502) and the Alzheimer's Association (IIRG-98-037)).

RESEARCH APPRECIATION DAY 2000

First Author: *Martine Pastorcic*

Department: *Pharmacology*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☒ Postdoctoral Fellow/Resident

CONTROL OF TRANSCRIPTION OF THE PRESENILIN 1 GENE BY ETS TRANSCRIPTION FACTORS. Martine Pastorcic and Hriday K. Das. University of North Texas Health Science Center. Department of Pharmacology. Fort Worth, Texas 76107

We have previously identified a crucial DNA element controlling 90% of the expression of the human presenilin 1 gene (PS1) between position -22 and -6 upstream from the transcription start site. This region contains an Ets transcription factor binding motif, and a 2 base pair alteration within the core sequence (GGAA) of the Ets consensus also reduced transcription by over 90%. We now show that cotransfection of a PS1 promoter-CAT reporter including the (-22 to -6) promoter fragment with Ets1 and Ets2 expression vectors increases PS1 transcription by 4 to 5 fold in human neuroblastoma SK-N-SH cells and hepatoma HepG2 cells. This correlates with electrophoretic mobility shift assays (EMSAs) including in vitro translated proteins which show that Ets transcription factors recognize the -20 PS1 element, and that binding is abolished by mutating the Ets motif. Previous reports have indicated that the cellular mRNA encoding presenilin 1 is down regulated by the p53 protein. We show that the same promoter area (-22, -6) contains a response element(s) for the regulation by p53. Cotransfection of PS1 promoter-CAT constructs with a p53 expression vector results in a drastic reduction (over 10 fold) of PS1 transcription and inhibits transactivation by Ets1 and Ets2. Thus the p53 protein appears to downregulate the transcription of the PS1 gene by interfering with its transactivation by Ets transcription factors.

RESEARCH APPRECIATION DAY 2000

First Author: *Pamela L. Marshall*

Department: *Molecular Biology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

ALZHEIMER'S DISEASE INCREASES THE SUSCEPTIBILITY OF PROTEINS TO OXIDATIVE DAMAGE. Pamela L. Marshall, Christina Malakowsky, Monica Mendez, John Talent, Joungil Choi, Robert W. Gracy and Craig C. Conrad. Molecular Aging Unit, Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, Texas, 76107.

Reactive oxygen species (ROS) are generated by a variety of sources from the environment (e.g., photo-oxidations & emissions) and normal cellular functions (e.g., mitochondrial metabolism & neutrophil activation). ROS include free radicals such as superoxide and hydroxyl radicals, nonradical oxygen species (hydrogen peroxide and peroxynitrite) and reactive lipids and carbohydrates (e.g., ketoaldehydes, hydroxynonenal). The accumulation of oxidized proteins appears to play a causative role in many age-related diseases including Alzheimer's Disease (AD).

These studies are designed to identify the proteins that are most susceptible to ROS damage and to use these as potential biomarkers for the early diagnosis of AD The effects of a variety of potential oxidants have been examined. Cell survival studies and 1-D and 2-D blot analysis show that (a) fibroblasts from AD individuals are more susceptible to oxidative damage by hydrogen peroxide and Fenton Reaction than age matched controls, (b) with increased oxidative stress, there is an increase in AD fibroblast death, (c)sera proteins from AD individuals are more susceptible to oxidative damage than age matched controls, (d) oxidative protein modification is not random. Mechanistic insight into oxidative damage may allow intervention or prevention of cellular oxidative damage in AD.

RESEARCH APPRECIATION DAY 2000

First Author: *Joungil Choi*

Department: *Molecular Biology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

VARIOUS REACTIVE OXYGEN SPECIES (ROS) CAUSE DIFFERENTIAL EXPRESSION OF PROTEINS IN NEURONAL CELL LINES. Joungil Choi, Pamela L. Marshall, Christina Malakowsky, Robert W. Gracy and Craig C. Conrad. Molecular Aging Unit, Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, Texas, 76107.

Reactive oxygen species (ROS) are generated inside the cell by a variety of sources that range from normal cellular metabolism to environment pollutants. The nervous system is especially sensitive to ROS due to its high metabolic rate and unique lipid composition. Pathological lesions (e.g., amyloid beta peptide (Ab)) found in the brains of Alzheimer's Disease (AD) patients can produce ROS, which is consistent with the observed increased oxidative damage to brain proteins.

These studies are designed to investigate neuronal cell survival and identify newly synthesized proteins produced with in vitro conditions that increase ROS (e.g., beta-amyloid). The survival of neuronal HT-22 cell showed that exposure of cells to beta-amyloid (200uM) and hyperbaric stress gives a significant reduction (>50%) of cell survival after 2-hours compared to both beta-amyloid alone and hyperbaric oxygen alone. Two-Dimensional gel analysis showed that several patterns of newly synthesized proteins occur under the similar ROS stress condition. This study identifies the newly synthesized proteins that are induced upon treatment of stress

RESEARCH APPRECIATION DAY 2000

First Author: *Michael L. Moeller*

Department: *Molecular Biology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

DEVELOPMENT OF A NOVEL SYSTEM FOR THE INVESTIGATION OF FACTORS CONTROLLING THE GROWTH AND DIFFERENTIATION OF DIFFERENT CELL TYPES FROM NEUROSPHERES Michael L. Moeller and S. Dan Dimitrijevich, Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, TX 76107

Cell clusters derived from the neuroectoderm of the human fetal neural tube might be potential sources of stem and progenitor cells for multiple classes of neurons, glial cells, and melanocytes. Although these neurospheres may be expanded in suspension, adoption of distinct phenotypes and neurite extension appears to be observed only following attachment or implantation. We have designed a novel in vitro experimental system to investigate the commitment status of various cell types contained within human-derived neurospheres. By embedding and culturing neurospheres in a three dimensional collagen I matrix, the neuroepithelial cells are able to proliferate and differentiate in an in vivo like fashion, including three dimensional growth of neurite extensions. This model will also facilitate future studies, designed to understand regulatory effect of growth factors on differentiation of neuroepithelial cells.

In vitro environments with varying growth factor profiles gives rise to various states of neuroepithelial proliferation or differentiation showing populations of isolated neurons, highly reticulate neural networks, and extensive monolayers sheets of contiguous, undifferentiated neuroepithelial cells. Specifically, stimulation with hFGF and hEGF has been observed to induce the developments of neuroepithelial sheets, retaining cells in apparently undifferentiated states. The neuroepithelial cells have been shown to be highly proliferative, rapidly covering plastic growth surfaces. From these neuroepithelial sheets, new spheroids eventually emerge from localized aggregates of highly compacted cells which connect to the parent sheets via unique multicellular connecting bridges. As we have observed with endocrine epithelial cells, the presence of PMA and lower concentrations of hFGF and hEGF result in the rapid differentiation of the cell sheets into reticulate networks of highly-connected cells with distinct neuronal or glial appearance. This method of proliferation of neuroepithelium followed by differentiation to the neuronal phenotypes has been evaluated in a tissue equivalent model populated with human dermal fibroblasts in addition to the neurospheres. This essentially sets the stage for multicellular heterologous interactions found in vivo. Preliminary observations indicate that this strategy will be useful in the development of fully-innervated tissue equivalents.

Following the above discussed observations, investigations have been initiated to define soluble growth factors which will specify neurite extension, target axonal extension, and drive differentiation of the stem cells to development of sensory neurons, motor neurons, glial cells, and melanocytes. Changes in cell surface adhesion proteins, including cell/cell attachment proteins such as NCAM and L1 and cell/matrix attachment proteins such as laminin specific integrins, will also be assessed at various stages of growth and differentiation using this model system. It is hoped that the resulting increase in understanding of these events will facilitate the development of innervated tissue models.

RESEARCH APPRECIATION DAY 2000

First Author: *Shelley McDonald*

Department: *Pharmacology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

A NOVEL NITRONE IMPROVES MEMORY IN OLD MICE. S. R. McDonald, J. Kelleher-Andersson, B. K. Koller, and M. J. Forster. Department of Pharmacology, UNTHSC, Fort Worth, TX 76107 and Centaur Pharmaceuticals, Sunnyvale, CA 94086.

Recent studies suggest that inhibition of NFκB activity by nitrones may account for their ability to improve age-related cognitive impairments in aged animal models. NFκB appears to play a pivotal role in regulating oxidative stress and immune function during aging, thus nitrone compounds with greater efficacy for suppression of NFκB may have a greater potential to ameliorate cognitive deficits associated with aging in C57BL/6 mice. In this study, we tested a novel nitrone, one with a greater potency for regulating NFκB in cultured hepatocytes or neuronal cells than the well-characterized nitrone, phenylbutylnitrone (PBN) to determine if the ability to regulate NFκB *in vitro* was associated with functional efficacy. Old (23.5 months) and young (4.5 months) C57BL/6 mice received daily oral treatment with the novel nitrone or the vehicle for a period of up to 29 weeks. Following 2 weeks of treatment, the mice began testing on a discriminated avoidance, recent memory task. Previous studies using this task had indicated that aged C57BL/6 mice showed slower acquisition of memory performance, as well as faster time-dependent decay of recently acquired memory (Forster and Lal, *Behav. Pharmacol.* 3: 337-349,1992). The old mice receiving 0.1 or 10 mg/kg/day of the novel nitrone showed more rapid learning of the memory task when compared with the old vehicle-treated mice. The rate of learning by the old mice treated with the novel nitrone was comparable to that of the young, vehicle-treated mice. During the retention phase, when mice were tested for memory under conditions of high demand (after a delay of 90 minutes), performance was more accurate in old mice treated with 0.1 or 10 mg/kg of the novel nitrone than in the old vehicle controls. The effects of the novel nitrone on cognitive performance were generally of greater magnitude when compared with the effects of PBN in a comparable study. The results indicate that the novel nitrone may be more efficacious than PBN in the treatment of memory dysfunction associated with normal aging or degeneration, and may delay the progress of memory decline associated with those conditions. Furthermore, these studies suggest that the efficacy of nitrone compounds may relate to their ability to regulate NFκB, in addition to free radical trapping. (Centaur Pharmaceuticals)

RESEARCH APPRECIATION DAY 2000

First Author: *Jay Mishalanie*

Department: *Pharmacology*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☒ Staff

☐ Postdoctoral Fellow/Resident

THE EFFECTS OF AGING AND CALORIC RESTRICTION ON AUDITORY AND SHOCK STARTLE REACTIONS IN MICE. J.F. Mishalanie, C. Beeny, R. Reynolds, P. Morris, H. Lal and M.J. Forster. Department of Pharmacology, University of North Texas Health Science Center. Fort Worth, TX 76107.

This study was part of a larger investigation of the usefulness of non-invasive tests of brain function in assessing biological aging by determining their sensitivity to experimental interventions which increase longevity. Accordingly, in a longitudinal study, beginning at 6 months of age C57BL/6 and B6D2F$_1$ mice were assessed at 6 month intervals across their lifespan, for their response (the maximum amplitude of response (peak response) and the time to peak response (reaction time)) to different intensities of both auditory and shock stimuli. For each strain, separate groups were fed under either *ad libitum* conditions, or from 4 months of age, under conditions of 40% restriction of caloric intake. With increasing age, the response to both auditory and shock stimuli changed: regardless of strain, peak response to auditory stimuli gradually decreased, despite a relatively stable reaction time; and in response to shock stimuli, although peak response remained stable, reaction time gradually increased. With caloric restriction, regardless of strain, there was a marginal increase in the peak response and a decrease in the reaction time to auditory stimuli; and for shock stimuli, caloric restriction substantially increased peak response, without influencing reaction time. The two dietary groups also exhibited differences in longevity. Both the level of mortality and the rate of mortality were greater in the *ad libitum* group, underscoring the improved longevity associated with the caloric restriction condition. Taken together, these results suggest that measures of auditory and shock startle reactions are possible indicators of biological aging and may provide a useful additional method for assessing potential anti-aging interventions. Supported by NIA grant AG07695.

RESEARCH APPRECIATION DAY 2000

First Author: *Christopher M. de Fiebre, Ph.D.*

Department: *Pharmacology*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

NEUROPROTECTIVE PROPERTIES OF NICOTINIC AGONISTS AGAINST ETHANOL-INDUCED NEUROTOXICITY. Christopher M. de Fiebre and NancyEllen C. de Fiebre. Dept. of Pharmacology, Univ. of North Texas HSC, Fort Worth, TX 76107.

Although alcohol and nicotine are often used together, very little is understood about the pharmacological interactions between these agents or the biological mechanisms by which this most common form of polydrug abuse arises. In an ongoing series of studies utilizing both in vivo and in vitro model systems, we have been examining potential interactions between ethanol and nicotinic agonists in regulating neuronal viability. In the in vitro studies, hippocampal and or septal tissue was removed from E-18 to E-21 Long Evans hooded rat fetuses. Cells were dissociated and were cultured for 7 days in plates coated with polyornithine. Cells were treated with 1 nM cytosine beta-D-arabino-furanoside (Ara-C) on Day 2 to inhibit glial cell proliferation. Approximately 67% of the media was changed every 3-4 days. Subsequently the cells were exposed to ethanol and/or nicotinic agents for 4-5 days . Neuronal viability was assessed using a cellular proliferation assay, MTT. Preliminary results suggest that the alpha7-selective nicotinic agonist, DMXB, protects against ethanol-induced neurotoxicity in a concentration dependent fashion. In in vitro studies, male Long-Evans rats were treated for 24 weeks with either a Sustacal-based, EtOH-containing or isocaloric, sucrose-containing liquid diet as the sole source of nutrition and were given twice daily injections (i.p.) of saline (SAL), nicotine (NIC), mecamylamine (MEC), combined NIC/MEC or DMXB. After 3 weeks of withdrawal from all drugs, rats were tested for learning ability using two different paradigms. One task assessed spatial learning/memory (Morris Water Task) while the other assessed associative learning (active avoidance). In both task, nicotinic agonists appeared to attenuate ethanol-induced deficits in learning and memory. Actual neuronal counts are currently being conducted to determine if this effect of nicotinic agonists on learning is due to a neuroprotective action. However, even in the absence of these counts, these in vitro and in vivo data taken together strongly suggest that nicotinic agonists can protect against ethanol-induced neurotoxicity. Further, this may suggest why alcoholics are almost invariably smokers.

This project was supported by a grant from the NIAAA (AA-11597).

ABSTRACT #90

First Author: *Robert Luedtke, Ph.D.*

Department: *Pharmacology*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

CHARACTERIZATION OF ^{125}I-IABN, A RADIOLIGAND SELECTIVE FOR D2-LIKE DOPAMINE RECEPTORS.

R.R. Luedtke[1]*, R. Freeman[1], M. Martin[1], V.A. Boundy[2], Y. Huang, and R.H. Mach[3].

[1]Department of Pharmacology, University of North Texas Health Science Center. Fort Worth, TX; [2]Ergo Science Corporation, Charlestown, MA; [3]Departments of Radiology and Physiology and Pharmacology, Wake Forest School of Medicine, Winston-Salem, NC.

^{125}I-IABN, was developed as a high affinity radioligand selective for D2-like (D2, D3 and D4) dopamine receptors. ^{125}I-IABN binds with picomolar affinity and nonselectively to rat D2 and D3 dopamine receptors expressed in Sf9 cells (Kd = 50 pM and 40 pM, respectively). ^{125}I-IABN binds with lower affinity to human D4.4 dopamine receptors expressed in HEK 293 cells (Kd = 610 pM). Dissociation constants (Kd) calculated from kinetic experiments are in agreement with Kd values obtained from equilibrium binding studies. Scatchard plots of the binding of ^{125}I-IABN with rat caudate homogenates exhibit low nonspecific binding and are linear, suggesting that the ligand is binding primarily to D2 dopamine receptors. Quantitative autoradiographic studies using rat brain slices indicate that ^{125}I-IABN selectively labels the striatum and the olfactory tubercle area. ^{125}I-IABN inhibits PGE1-dependent stimulation of D2 or D4.4 receptors expressed in HEK cells in a dose dependent manner. Therefore, ^{125}I-IABN appears to be a high affinity, selective antagonist at D2-like dopamine receptors. *Supported by the Scottish Rite Schizophrenia Program and NIDA (DA 09142 and DA 09147).*

RESEARCH APPRECIATION DAY 2000

First Author: *Paromita Das*

Department: *Pharmacology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

PHARMACOLOGY OF 5-HT3 RECEPTORS. Paromita Das and Glenn. H. Dillon
Dept. of Pharmacology, University of North Texas Health Science Center, Ft.Worth, TX 76107.

The focus of our lab has been studies on ligand gated ion channels like GABAA and glycine receptors using patch clamp techniques. Recently, we have started studies on another member of the superfamily of ligand gated ion channels, namely the 5-HT3 receptor. The 5-HT3 receptor, in common with other members of this superfamily, is a pentameric assembly of subunits. Each subunit is predicted to contain four hydrophobic transmembrane domains, M1-M4. In the neurons, 5-HT3 receptors mediate fast synaptic transmission and activation of the receptor leads to opening of a cation selective channel. Our initial attempt has been to characterize the pharmacology of this receptor using whole cell recording techniques.

HEK 293 cells transiently transfected with murine WT 5-HT3A receptors were used for whole cell recordings. WT 5-HT3A receptors mediated rapidly activating currents in response to different concentrations of 5-HT. Concentration-effect relationships for 5-HT revealed an EC50 of 1.2+/- 0.12 □M and a Hill co-efficient of 2.3+/- 0.43.

A previous report has shown that L-type calcium channel blockers like verapamil, diltiazem and dihydropyridines affect the rate of decay of 5-HT evoked currents without any change in current amplitude. Our initial experiments with verapamil have also demonstrated that currents evoked by application of 30 □M 5-HT (in presence of verapamil) were decayed rapidly without significantly affecting the peak amplitude.

Our studies have demonstrated that heterologously expressed 5-HT3 receptors are useful for pharmacological characterization of the receptors. Currently we are attempting to create a stable cell line expressing 5-HT3 receptors. Future studies will focus on the structure and function relationship of the channel itself and regulation by various ligands.

(Supported by NIH ES 07904)

RESEARCH APPRECIATION DAY 2000

Presenter: *Susan Laufer*

First Author: *Edward L. Orr*

Department: *Pathology and Anatomy*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

ACCUMULATION OF CEREBRAL CORTICAL HISTAMINE AND EVAN'S BLUE AFTER MILD-TO-MODERATE HEAD TRAUMA IN THE MOUSE Edward L. Orr* and Susan Laufer#, *Department of Pathology and Anatomy, UNTHSC, Fort Worth, TX and #Department of Biological Sciences, UNT, Denton, TX.

As part of our ongoing program to develop and validate an animal model of mild-to-moderate head injury, we have tested whether one can quantify the changes in cerebral cortical histamine and extent of disruption of the blood-brain or cerebrospinal fluid-blood barriers which occurs after head injury. SJL/J mice were anesthetized by inhalation of methoxyflurane. To quantify breakdown of the BBB, mice were injected intravenously via the lateral tail vein with 0.1 ml of 2% Evan's blue in saline. Their calvaria were then exposed by a midline incision through the skin of the head and 2 parallel grooves were made in the left dorsal parietal bone using a Dremel tool. Care was taken to not cut into the dura mater. At various times after injury, the chest cavity was opened and, after removing 0.1 ml of blood from the heart of the mice injected with Evan's blue, mice were perfused through the left ventricle with 30 ml saline then decapitated. The calvarium was removed, and plugs of cerebral cortex subjacent to the injured skull were removed and frozen. Similar plugs of cerebral cortex from the opposite uninjured side were also removed and frozen. The cerebral cortical plugs were subsequently homogenized in water (histamine) or 50% trichloroacetic acid (Evan's blue) and centrifuged. Samples of supernatant were assayed for histamine using a radioenzymatic assay or Evan's blue using a fluorescence method. The results show that the cerebral cortex underlying the site of injury contained significantly morehistamine at 5, 10 and 20 min after injury and significantly more Evan's blue 20 min after injury than were found in the opposite cerebral cortex. Thus, scoring of the dorsal calvarium without direct injury to subjacent tissues resulted in quantifiable accumulation of histamine and Evan's blue in the underlying cerebral cortex. We suggest that the accumulation of Evan's blue may be due to an histamine-induced increase in the permeability of the cortical cerebral vasculature and/or overlying arachnoidal barrier cells in response to injury to the skull.

PHYSICAL MEDICINE INSTITUTE

94.	Jeff Siu	PHYSICAL MEDICINE INSTITUTE AT THE UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER
95.	Lisa Butler	A MODEL FOR OSTEOPATHIC MANIPULATIVE MEDICINE CURRICULUM REVISION
96.	Jeff Siu	COMPLEMENTARY AND ALTERNATIVE MEDICINE CLINICAL RESEARCH CURRICULUM DEVELOPMENT WITH EMPHASIS ON OSTEOPATHIC MANIPULATIVE MEDICINE
97.	Lynn F. Johnson, MIS	OSTEOPATHIC LITERATURE DATABASE PROJECT
98.	David Russo/John Licciardone	A RANDOMIZED CONTROLLED TRIAL OF OSTEOPATHIC MANIPULATIVE TREATMENT IN PATIENTS WITH CHRONIC LOW BACK PAIN
99.	Jeff Siu/Jerry L. Dickey	A RANDOMIZED CONTROLLED TRIAL OF OSTEOPATHIC MANIPULATIVE TREATMENT (OMT) VERSUS PLACEBO IN PATIENTS UNDERGOING CORONARY ARTERY BYPASS GRAFT (CABG) SURGERY
100.	John Licciardone, D.O.	A RANDOMIZED CONTROLLED TRIAL OF OSTEOPATHIC MANIPULATION FOLLOWING KNEE OR HIP ARTHROPLASTY
101.	Jon Swift Jr.	THE EFFECT OF PARAVERTEBRAL MANIPULATION ON THE SYMPATHETIC NERVOUS SYSTEM
102.	Jeff Siu/Scott T. Stoll	DERMATOMAL SOMATOSENSORY EVOKED POTENTIALS: EVALUATION OF MANIPULATIVE MEDICINE IN THE TREATMENT OF CERVICAL AND LUMBAR RADICULAR SYMPTOMS

RESEARCH APPRECIATION DAY 2000

First Author: *Jeff Siu*

Department: *Department of Osteopathic Manipulative Medicine, Physical Medicine Institute*

☒ GSBS Student ☒ SPH Student ☐ PA Student

☒ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

PHYSICAL MEDICINE INSTITUTE AT THE UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER. Scott T. Stoll, D.O., Ph.D., Jeffrey Siu, B.A.S.

The goal of the Physical Medicine Institute is to promote basic and clinical research, education, clinical practice and community outreach programs, in the prevention, diagnosis, treatment and rehabilitation of neuromusculoskeletal disease of human beings of all ages.

The strategic plan of the Physical Medicine Institute is as follows:

1. To foster high quality basic science research projects to investigate the mechanisms of action of Manipulative Medicine/Osteopathic Manipulative Treatment in treatment of neuromusculoskeletal disease with the goal of publication in nationally peer reviewed journals.
2. To foster high quality clinical outcome research projects to investigate efficacy of Manipulative Medicine/Osteopathic Manipulative Treatment in the prevention and treatment of neuromusculoskeletal disease with the goal of publication in nationally recognized peer review journals.
3. Develop a broad, universally accessible literature database pertaining to Manipulative Medicine/Osteopathic Manipulative Treatment in the prevention and treatment of neuromusculoskeletal disease necessary to support research, education and clinical services.
4. To provide education to appropriate students, physicians, researchers, community leaders and to the community as to the state of the art of clinical management of neuromusculoskeletal disorders.
5. Provide state of the art clinical services to people affected by neuromusculoskeletal disorders emphasizing cost-effectiveness and clinical efficacy.
6. Facilitate the development of an international, interdisciplinary taxonomy of manual medicine techniques. This is a necessary foundation for future interdisciplinary discussion and eventual consensus on certification requirements for clinical practice as well as for interpretation of research publications by separate professional disciplines.
7. Develop funding support, facilities and administration independent of the Department of Osteopathic Manipulative Medicine.

RESEARCH APPRECIATION DAY 2000

First Author: *Lisa Butler*

Department: *OMM*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☒ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

A MODEL FOR OSTEOPATHIC MANIPULATIVE MEDICINE CURRICULUM REVISION

Lisa Butler, B.A., Scott Stoll, D.O., Ph.D., Jerry Dickey, D.O., Russell Gamber, D.O., Anthony Wright, D.O., Gloria Wright, D.O., Sankar Pemmaraju, D.O., Shane Maxwell, Dave Tanner, D.O.
Department of Osteopathic Manipulative Medicine, University of North Texas Health Science Center,
Fort Worth, TX 76107-2699

Though the number of osteopathic medical school graduates is rising, the number of D.O. physicians using OMT in the clinical setting is dramatically decreasing. Surveys show that a leading reason for this decline is insufficient training in a clinically integrated setting. This presents the challenge to osteopathic educators to integrate osteopathic principles and practices (OP&P) into student's clinical decision making skills so that they will include OP&P in their diagnosis and treatment for all aspects of medicine. The OMM department is developing a seven year curriculum plan to integrate OP&P into the recently revised general medical curriculum at the Texas College of Osteopathic Medicine.

RESEARCH APPRECIATION DAY 2000

First Author: *Jeff Siu*

Department: *Department of Osteopathic Manipulative Medicine, Physical Medicine Institute*

☒ GSBS Student ☒ SPH Student ☐ PA Student

☒ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

COMPLEMENTARY AND ALTERNATIVE MEDICINE CLINICAL RESEARCH CURRICULUM DEVELOPMENT WITH EMPHASIS ON OSTEOPATHIC MANIPULATIVE MEDICINE

Jeff Siu, B.A.S., Scott T. Stoll, D.O., Ph.D., John C. Licciardone, D.O., M.S., M.B.A.
Department of Osteopathic Manipulative Medicine, Physical Medicine Institute, University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas 76107-2699

The Physical Medicine Institute in conjunction with the Department of Osteopathic Manipulative Medicine (OMM) formalized a Predoctoral Research Fellowship in OMM at the University of North Texas Health Science Center in 1998 as an extension of a Predoctoral Teaching Fellowship in OMM founded 1985. This program was designed to develop successful researchers in complementary & alternative medicine (CAM), specifically OMM. It collaborates with the School of Public Health, Graduate School of Biomedical Sciences, & various departments of Clinical Medicine; & is coordinating efforts to offer predoctoral fellows the combined D.O./M.P.H. degree through enrollment in a variety of research & CAM related courses. We propose to enhance the administrative, curricular, mentoring, & funding structures of the current research program. Development is targeted at: (1) curriculum expansion with courses on hypothesis building; biostatistics; epidemiology; clinical trial design; research methods; responsible conduct; ethical & regulatory issues in research, (2) development of new CAM-focused courses; (3) program extension to attract a wider audience including faculty, pre- and post- doctoral fellows, and allied health professionals; (4) establishment of annual CAM research conferences; (5) development of Continuing Medical Education courses in CAM; (6) formalization of various degree tracks; & (7) use of computer technologies for curricular advancement. Research fellows will acquire the skills necessary to successfully develop basic science & clinical research projects, attain funding, implement studies & publish quality research in CAM with the opportunity to advance toward various degrees in combination with or addition to their Doctor of Medicine degree. Fellows, at present, are competitively selected from a diverse multicultural pool of osteopathic medical students from the UNTHSC. This evolving predoctoral fellowship program has a successful track record of graduating accomplished clinical researchers, educators & administrators in OMM. This expanded & improved program will continue to develop future leaders and researchers capable of successful and competitive clinical & basic science research in CAM.

(Submitted to NIH/NCCAM February 1, 2000 in response to PAS-00-024; allowable cost is $300,000 for 5 years)

RESEARCH APPRECIATION DAY 2000

First Author: *Lynn F. Johnson, MIS*

Department: *Gibson D. Lewis Library*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

OSTEOPATHIC LITERATURE DATABASE PROJECT

Lynn F. Johnson MIS; Ann Brooks MLS, MBA; Craig Elam MLS; Dohn Martin, MSLS;
Gibson D. Lewis Health Science Library,
University of North Texas Health Science Center, Fort Worth, TX 76107

Lawrence Onsager ML, MA; Robert Sanders MA, MEd
A. T. Still Memorial Library,
Kirksville College of Osteopathic Medicine, Kirksville, MO 63501

The need for information on osteopathic medicine and osteopathic manipulative treatment has increased greatly in recent years, both in the United States and worldwide. Current access to the 100-year old osteopathic literature is extremely limited, incomplete, and scattered among several databases with inadequate subject control. To solve this problem, the American Osteopathic Association (AOA) joined the American Association of Colleges of Osteopathic Medicine (AACOM) to sponsor the development of the world's first comprehensive index to the osteopathic literature. Similar to MEDLINE for biomedical information, the index will allow the osteopathic profession global access to its own unique literature. Work began in September 1997 when a five-year $385,000 contract was awarded to the Gibson D. Lewis Library at the Univeristy of North Texas Health Science Center at Fort Worth, Texas (UNTHSC) and the A.T. Still Memorial Library at the Kirksville College of Osteopathic Medicine (KCOM), Kirksville, Missouri. UNTHSC provides project administration, database development, technical assistance, and quality control. UNTHSC is also responsible for indexing the current (post-1950) literature, while KCOM, as the founding school for osteopathic medicine, is responsible for indexing its unique historical (pre-1950) materials. Using Cuadra STAR database software, the index will be accessible via the Web as soon as a sufficient quantity of materials is indexed. The index consists of citations and abstracts of articles, book chapters, audiovisual, and electronic resources from around the world, covering all aspects of osteopathic medicine, osteopathic manipulation, and relevant manual medicine topics. Subject access is provided through keywords, NLM Medical Subject Headings (MeSH), and a structured thesaurus comprised of local headings derived from the AACOM "Glossary of Osteopathic Terminology", osteopathic physicians, and other appropriate resources. This thesaurus will be a major product of the project. By helping standardize the osteopathic nomenclature, scholarly communication will be facilitated and unity within the profession will be promoted.
(Sponsored by a grant from the American Association of Colleges of Osteopathic Medicine (AACOM) and the American Osteopathic Association (AOA).)

RESEARCH APPRECIATION DAY 2000

Presenter: *David Russo*

First Author: *John Licciardone, D.O., M.S., M.B.A.*

Department: *School of Public Health*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

A RANDOMIZED CONTROLLED TRIAL OF OSTEOPATHIC MANIPULATIVE TREATMENT IN PATIENTS WITH CHRONIC LOW BACK PAIN

John C. Licciardone, D.O., M.S., M.B.A., Scott T. Stoll, D.O., Ph.D., Russel G. Gamber, D.O., Jeff Siu, B.A.S., David Russo, B.A., William Winn, B.S.
Department of Osteopathic Manipulative Medicine, Physical Medicine Institute, University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas 76107-2699

The primary purpose of the proposed research is to determine the efficacy of osteopathic manipulative treatment (OMT) for chronic low back pain. We will study subjects in an ambulatory clinic setting and measure health status and clinical outcomes over a one-year follow-up period. We anticipate being able to demonstrate the efficacy of OMT in this adult population by fulfilling the following objectives:

1. Planning and implementing a scientifically rigorous research design -the randomized, controlled trial-to minimize sources of experimental bias.

2. Recruiting sufficiently large numbers of experimental and control subjects to ensure reasonable study power and to minimize the probability of type II errors in hypothesis testing.

3. Providing OMT to the experimental group according to a research protocol that allows individualized treatment and use of a variety of osteopathic manipulative techniques.

4. Providing either light touch therapy that is designed to simulate OMT or no intervention to the control groups .

5. Completely and accurately collecting health status measures and clinical outcomes data.

6. Analyzing the research data in a statistically-appropriate manner to test the null hypothesis and to minimize the probability of type I errors in hypothesis testing.

(Received $35,000 Extramural Funding from American Osteopathic Association, 1999-2000)

RESEARCH APPRECIATION DAY 2000

Presenter: *Jeff Siu*

First Author: *Jerry L. Dickey*

Department: *Department of Osteopathic Manipulative Medicine, Physical Medicine Institute*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

A RANDOMIZED CONTROLLED TRIAL OF OSTEOPATHIC MANIPULATIVE TREATMENT (OMT) VERSUS PLACEBO IN PATIENTS UNDERGOING CORONARY ARTERY BYPASS GRAFT (CABG) SURGERY

Jerry L. Dickey, D.O., F.A.A.O., Martin S. Weiss, D.O., Albert H. O-Yurvati, D.O., F.A.C.O.S., F.I.C.S., William Wallace, D.O., F.A.C.O.S., F.C.C.P., Scott Stoll, D.O.,Ph.D., Scott Feree, D.O., David Russo, B.A., Jeff Siu, B.A.S., William Winn, B.S.
Department of Osteopathic Manipulative Medicine, Physical Medicine Institute, University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas 76107-2699

This study is the first to measure the effect of OMT on post-CABG patients in a systematic way. Based upon previously identifed prognosticators of post-CABG outcomes, objective and quantifiable patient data will be collected including 1) length of hospital stay, 2) length of mechanical ventilation, 3) occurrence of post-operative arrythimias, 4) dosage and frequency of pain medication, 5) dosage and frequency of anti-arrhythmic medication, 6) amount of chest-tube drainage, 7) diet advancement, 8) ambulatory distances, 9) and occurrence of wound, lung, peripheral vasculature, and device complications. It is expected that the OMT group will be superior to the placebo group in at least one outcome domain. Such a finding would help establish the efficacy of OMT as an adjunctive therapy to improve overall CABG outcomes. It is possible that OMT exerts no effect relative to placebo on any of these CABG outcome variables. This would suggest, contrary to current literature, that OMT is not an effective modality for improving outcome in patients undergoing this procedure.

(Received $8,000 Intramural Funding from Office of Research and Biotechnology, 2000)

RESEARCH APPRECIATION DAY 2000

First Author: *John Licciardone, D.O.*

Department: *School of Public Health*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

A RANDOMIZED CONTROLLED TRIAL OF OSTEOPATHIC MANIPULATION FOLLOWING KNEE OR HIP ARTHROPLASTY.

J.C. Licciardone, D.O.*, S.T. Stoll, D.O.†, K.M. Herron, M.P.H.‡, R.G. Gamber, D.O.†, J. Swift, M.A.†, W. Winn, B.S.†

*School of Public Health and †Department of Manipulative Medicine, University of North Texas Health Science Center, Fort Worth, TX. ‡Health Resources and Services Administration, Dallas, TX.

CONTEXT: Previous studies suggest that osteopathic manipulation may reduce pain, improve ambulation, and increase rehabilitation efficiency in patients undergoing knee or hip arthroplasty.

OBJECTIVE: We performed a randomized controlled trial to further assess the efficacy of osteopathic manipulation in acute inpatient rehabilitation patients having chronic knee or hip osteoarthritis or a hip fracture.

METHODS: Patients received either osteopathic or sham manipulation, according to study guidelines on frequency, duration, and technique, in addition to standard rehabilitation unit care. Primary outcome measures included changes in Functional Independence Measure (FIM) scores and in daily analgesic use during the rehabilitation unit stay; length-of-stay; rehabilitation efficiency, defined as the FIM total score change per rehabilitation unit day; and changes in the Medical Outcomes Study Short Form– 36 (SF-36) scores from rehabilitation unit admission to four weeks following discharge.

RESULTS: Randomization appeared successful as baseline characteristics of the 30 osteopathic- and 30 sham-manipulation patients were comparable, and any differences could be attributed to chance alone. Preliminary results failed to demonstrate the efficacy of osteopathic manipulation in any of the 19 primary outcomes. Osteopathic manipulation was associated with greater length-of-stay (15.0 vs. 8.3 days, P=.004) and reduced rehabilitation efficiency (2.1 vs. 3.4 FIM total score points per day, P<.001) in patients with knee osteoarthritis.

CONCLUSIONS: The osteopathic manipulation protocol used does not appear to be efficacious in this hospital rehabilitation population. Further research is needed to determine if an artificial knee implant diminishes responsiveness to osteopathic manipulation.

(Supported in part by the American Osteopathic Association [Grant no. 98-11-464], the Osteopathic Health Foundation, and the Carl Everett Charitable Lead Trust Fund.)

RESEARCH APPRECIATION DAY 2000

First Author: *Jon Swift Jr.*

Department: *Manipulative Medicine*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☒ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

THE EFFECT OF PARAVERTEBRAL MANIPULATION ON THE SYMPATHETIC NERVOUS SYSTEM Jon Swift Jr., M.A., Shane Maxwell, B.S., Jerry Dickey, D.O., Michael Smith, Ph.D. UNT Health Science Center, Ft. Worth TX, 76107

Introduction: Osteopathic manipulative treatment (OMT) has been reported to affect the sympathetic nervous system in patients. Paravertebral manipulation (a type of OMT) is believed to increase (excitatory technique) or decrease (inhibitory technique) sympathetic nervous activity (SNA) depending on its application. This study involved both excitatory and inhibitory techniques as well as a "light touch" placebo technique. *Hypothesis:* Paravertebral manipulation will significantly increase or decrease sympathetic nevous system activity in human subjects. *Methods:* Paravertebral manipulation was performed on subjects by predoctoral fellows in manipulative medicine under the guidance of an osteopathic physician. Healthy human subjects (n=9) were instrumented with various direct and indirect measures of SNA, including: heart rate, blood pressure, efferent sympathetic nerve activity (directly by microneurography of the peroneal nerve), and peripheral mixed plasma catecholamines (not shown). Subjects were allowed to rest quietly for twenty minutes to obtain baseline data. Each subject received three 5 minute treatments in random order: excitatory (EX), inhibitory (IN) and placebo (PL). Each treatment was followed by a 20 min washout period, the last 5 min of which became the baseline for subsequent treatments. All measures of SNA were recorded for specified time periods at the end of baseline: after 30 seconds of treatment, 4 min of treatment and 5 min post- treamtent. For each treatment, baseline SNA measures were compared to measures at the end of treatment using paired t-tests. Treatment effects (change from baseline to end of treatment) were compared to each other using an ANOVA. *Results:* Heart rate decreased slightly during EX (-2.4±0.9 bpm, p=0.03) and IN (-2.1±0.9 bpm, p=0.05), but was unchanged during PL (-1.0±0.7 bpm, p=0.20). No differences among treatments were observed (p=0.46). Mean blood pressure was not significantly different from baseline for any treatment and no differences between treatments were observed (p>0.75). Efferent sympathetic nerve activity data were not significantly changed from baseline (p=0.50) and no significant differences between treatments were found (p=0.36). *Summary:* Overall, paravertebral manipulation, (including excitatory, inhibitory and placebo techniques) did not significantly affect SNA in healthy human subjects. Although a slight decrease in heart rate was noted, it is believed to be clinically insignificant. Future studies will focus on patient populations in which the underlying pathology is associated with elevated SNA.

RESEARCH APPRECIATION DAY 2000

Presenter: *Jeff Siu*

First Author: *Scott T. Stoll*

Department: *Department of Osteopathic Manipulative Medicine, Physical Medicine Institute*

☒ GSBS Student ☒ SPH Student ☐ PA Student

☒ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

DERMATOMAL SOMATOSENSORY EVOKED POTENTIALS: EVALUATION OF MANIPULATIVE MEDICINE IN THE TREATMENT OF CERVICAL AND LUMBAR RADICULAR SYMPTOMS.

S.T. Stoll, D.O., Ph.D., J.L. Caffrey, Ph.D., T.J. Wright, R.T., Jeff Siu, B.A.S.
University of North Texas Health Science Center, Departments of Integrative Physiology and Osteopathic Manipulative Medicine. Fort Worth, TX, 76107

Dermatomal Somatosensory Evoked Potentials (DSEPs) have been successfully employed to monitor spinal cord function during spinal surgery and to document successful spinal root decompression. We are using DSEP to determine whether segmental, cervical or lumbar spinal manipulation in patients with radicular symptoms actually leads to decompression of spinal nerve roots and therein increases spinal nerve root conduction velocity and decreases DSEP latency. DSEP evaluation of a total of 41 healthy control subjects before and after spinal manipulation demonstrates that DSEP is a reliable and reproducible measure of spinal nerve root conduction velocity in this population in our laboratory. Clear DSEP waveforms were not obtainable from a population of 25 individuals with chronic (>6 months) radicular pain. These subjects with chronic pain generated electrodiagnostic data with increased electrical noise and decreased DSEP waveform amplitude. We feel that the ability to measure and improve waveforms may depend on the duration of the dysfunction. This study currently focuses on the recruitment and testing of patients with acute (less than 6 weeks) cervical or lumbar radicular symptoms. These patients will receive bilateral, multi-level DSEP evaluations before and after a thirty-minute waiting period (no treatment) and then again after spinal manipulation in order to show the consistency of DSEP evaluation without treatment and any changes in segmental spinal nerve root conduction velocity after treatment with spinal manipulation.

(Received $8,000 Office of Research and Biotechnology Intramural Faculty Grant, 1999-2000; and received

PUBLIC HEALTH

103.	Sue Gena Lurie, PhD	HOMELESSNESS: ASSESSMENT AND SERVICES
104.	Daryhl Johnson	AN EPIDEMIOLOGICAL ANALYSIS OF FOREIGN-BORN PATIENTS WITH *MYCOBACTERIUM TUBERCULOSIS* INFECTION IN TARRANT COUNTY, TEXAS FROM 1980 TO 1999
105.	S/D Mark Gamber	PRESERVING THE HEALTH OF POSTMENOPAUSAL MEXICA N AMERICAN WOMEN

RESEARCH APPRECIATION DAY 2000

First Author: *Sue Gena Lurie, Ph.D.*

Department: *School of Public Health*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☒ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

HOMELESSNESS: ASSESSMENT AND SERVICES - Sue Gena Lurie, Ph.D. School of Public Health. University of North Texas Health Science Center. Fort Worth,Tx.76107

Hypothesis: Assessment of local demographic characteristics of homelessness and service needs of homeless persons is a function of urban planning and social policy change. Methods: Participation in the Homeless Task Force over a six-month period and in the Tarrant County Homeless Survey were employed to integrate information on: assessment of homelessness, service needs, agency networks and city planning goals, using longitudinal and cross-sectional qualitative observation and interview methods. Results and conclusions: Study findings support hypothesis. Homelessness and service assessment are linked to urban planning goals for managing numbers, concentrating shelters and decentralizing employment and support services. Social policy changes impact both housing and support services.
(Fort Worth Homeless Task Force and Tarrant County Homeless Coalition - unfunded.)

RESEARCH APPRECIATION DAY 2000

First Author: *Daryhl Johnson*

Department: *School of Public Health & Dept. of Internal Medicine*

☐ GSBS Student ☒ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

AN EPIDEMIOLOGICAL ANALYSIS OF FOREIGN-BORN PATIENTS WITH *MYCOBACTERIUM TUBERCULOSIS* INFECTION IN TARRANT COUNTY, TEXAS FROM 1980 TO 1999. Authors: Daryhl Johnson, Francis Blais, DO, Antonio Rene, PhD, Walter McConathy, PhD, Stephen Weis,DO. School of Public Health & Dept. of Internal Medicine, Univ. Of North Texas Health Science Center, Ft. Worth, Tx 76107

I. Purpose of the Research: Since the numbers of foreign born *Mycobacterium tuberculosis* (MTB) cases are increasing as a consequence of increased immigration from high prevalence countries, this analysis retrospectively reviews MTB cases in Tarrant County from 1980 to 1999. The specific aims of the study are: 1) identify countries of origin of foreign born patients immigrating to the US with MTB; 2)develop epedemiologic profiles of foreign born persons in Tarrant County who are at high risk for MTB to facilitate selective screening of these populations. II Research Design and Methodolgy: *Mycobacterium tuberculosis* (MTB) cases reported to the Tarrant County Health Dept. between 1980 and 1999 were included in the analyis. All adults were considered a verified MTB case if they had a positive culture for MTB. Children were included if they met all the following criteria: positive tuberculin skin test, contact with a person with active TB, and abnormal chest X-ray. Foreign born cases were investigated further with respect to their gender, age time in US before diagnosis and relapse status. The SPSS statistical package was used in the analysis. III. Results & Discussion: During the study period, a total of 1478 confirmed cases were reported to the Tarrant County Health Department. Persons born in the USA accounted for 74% of the cases; foreign born persons accounted for 25% of the cases. A total of 63% of the MTB cases were among persons aged 18-44. Of the foreign born persons for whom a date of arrival was available, 187 (53%) had been in the US less than 5 years before being diagnosed with MTB. Persons from Mexico and Vietnam accounted for 68% of the foreign borm MTB cases. Foreign born cases (391, 86%) were intial cases, suggesting that tuberculosis in these patients resulted from reactivation of latent infection acquired prior to their arrival in Tarrant County. The migration of persons already infected with MTB into Tarrant County appears to be responsible for most foreign born tuberculosis cases in the county. New arrivals in the US who have the highest rates of tuberculosis are more likely to live with other foreign born persons in their own ethnic communities. There is an urgent need for these patients to be identified and treated before progressing to active tuberculosis and transmitting tuberculosis further in the community. (Source of Support: Sch of Pub Hlth & Dept. of Int. Med)

RESEARCH APPRECIATION DAY 2000

First Author: *S/D Mark Gamber*

Department: *Medical Student*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☒ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

PRESERVING THE HEALTH OF POSTMENOPAUSAL MEXICAN AMERICAN WOMEN, M. Gamber, C Whiting, DO, K Godwin PhD. University of North Texas Health Science Center, Fort Worth, TX 76107.

Purpose: This qualitative study evaluates attitudes and knowledge of post menopausal, Mexican American women towards osteoporosis and hormone replacement therapy and compares them to previously recorded data on Anglo and African American women from other surveys. The results will be used to refine questions and ideas appropriate for a focus group study with Mexican American women concerning osteoporosis and hormone replacement therapy. This cohort of women is the fastest growing in the nation. The risk for osteoporosis increases with a decrease in the calcium intake in the daily diet. The in-migration dietary patterns of Mexican-American women indicate that there is a reduction of calcium as they become more assimilated into the American culture.

Methods: Participants were either members of the Northside Community Center or patients at the Northside Clinic of the University of North Texas Health Science Center. Surveys were delivered in either English or Spanish and filled out by the participant. Inclusion criteria specify for female Mexican Americans of at least 40 years of age.

Results: Study participants not taking hormone replacement therapy exhibited a lower knowledge base concerning predisposing factors to osteoporosis. Only 50% (control 60%) of participants believed a decrease in dietary calcium predisposes to osteoporosis. Mexican American women were more likely to believe in causal associations which thus far have not been linked to osteoporosis. These include race, vitamin C deficiency, and being overweight. Finally, study participants more frequently visit the doctor for the emotional symptoms of menopause, whereas previous studies indicate participants see physicians more often for physical symptoms.

Conclusion: The previous notion that Mexican American women are at a decreased risk for osteoporosis may not be true. The results of this pilot indicate that Mexican American women have different attitudes and knowledge base towards osteoporosis and hormone replacement therapy. Subsequent focus groups research will attempt to better understand causality.

STUDENT ORAL PRESENTATIONS

(1:00 PM)	Bhooma Srinivasan	RETINAL MICROGLIA-DERIVED NERVE GROWTH FACTOR PROMOTES PHOTORECEPTOR CELL DEATH VIA p75NTR
(1:12 PM)	Mohammed Dibas	DEFINING THE CONVULSIVE SITE IN GABAA RECEPTORS
(1:24 PM)	Stephen L Wasmund	SELECTIVE CARDIAC PARASYMPATHETIC DENERVATION FOLLOWING RADIOFREQUENCY ABLATION IN THE POSTEROSEPTAL REGION
(1:36 PM)	L. Don Roberts	CA+2/CALMODULIN-DEPENDENT PROTEIN KINASE-IIa NEGATIVELY REGULATES THE INDUCTION OF HYPERTROPHIC RESPONSE INIATED BY Ca+2 DEPENDENT PROTEIN KINASES AND PHOSPHATASES
(1:48 PM)	Keith Jackson	AGONISTS/ANTAGONISTS PROFILES INDICATE DELTA OPIOID RECEPTOR CONTROL OF HEART RATE IN THE SINOATRIAL NODE
(2:00 PM)	Bradley Hart	CANINE RIGHT VENTRICULAR OXYGEN SUPPLY/DEMAND BALANCE DURING GRADED EXERCISE
(2:12 PM)	Robert Carter III, M.S.	TIDAL LUNG VOLUME AND CARDIOVASCULAR RESPONSES TO APNEA
(2:24 PM)	Peter Gargalovic	CAVEOLIN-1 AND CAVEOLIN-2 EXPRESSION IN MOUSE MACROPHAGES AND REGULATION BY CHOLESTEROL METABOLISM
(2:36 PM)	Kevin Formes	VAGAL BLOCKADE INDUCES BLOOD PRESSURE INSTABILITY (PATHOPHYSIOLOGICAL IMPLICATIONS)
(2:48 PM)	Lisa Hodge	INTRANASAL IMMUNIZATION IS MORE EFFECTIVE THAN SYSTEMIC IMMUNIZATION IN PRIMING MUCOSAL ANTIBODY RESPONSES IN THE UPPER RESPIRATORY TRACT
(3:12 PM)	Debra White	BACTERIAL BIOFILM FORMATION AND DISPERSAL UNDER THE INFLUENCE OF THE GLOBAL REGULATOR

(3:24 PM)	Michelle L. Wright	ISOLATION AND PRELIMINARY CHARACTERIZATION OF A PUTATIVE DETERMINANT OF MANGANESE SUPEROXZIDE DISMUTASE (*sod)* FROM *Staphylococcus aureus*
(3:36 PM)	Katie Overheim	THE GLOBAL REGULATORS (*AGR* AND *SAR*) OF VIRULENCE FACTORS PROMOTE SEVERE STAPHYLOCOCCAL PNEUMONIA IN A MOUSE MODEL
(3:48 PM)	P. John Kamthong	ATTENUATION OF MACROPHAGE COLONY-STIMULATING FACTOR EXPRESSION BY CYCLIC ADENOSINE MONOPHOSPHATE: NOT THROUGH INHIBITION OF IκB KINASE
(4:00 PM)	Van Huynh	POLYMORPHISM IN THE 2B4 GENE OF INBRED MOUSE STRAINS
(4:12 PM)	Kent S. Boles	IDENTIFICATION OF A 2B4 VARIANT AND RELATED RECEPTOR ON HUMAN NK CELLS
(4:24 PM)	Gheath Al-Atrash	ROLE OF UROKINASE PLASMINOGEN ACTIVATOR AND ITS RECEPTOR IN EXTRACELLULAR MATRIX DEGRADATION BY NATURAL KILLER CELLS
(4:36 PM)	Michael C. Lawrence	CALCIUM AND CYCLIC AMP UPREGULATE INSULIN GENE TRANSCRIPTION BY A CALCINEURIN/NFAT PATHWAY IN PANCREATIC B-CELLS
(4:48 PM)	Paramjit Kaur Gill	DETERMINATION OF THE INTRACELLULAR LEVELS OF CYCLIC ADP-RIBOSE IN CULTURED HUMAN CELLS USING A NEW HIGHLY SENSITIVE FLUORESCENT HPLC METHOD

RESEARCH APPRECIATION DAY 2000

First Author: *Bhooma Srinivasan*

Department: *Pathology and Anatomy*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

RETINAL MICROGLIA-DERIVED NERVE GROWTH FACTOR PROMOTES PHOTORECEPTOR CELL DEATH VIA p75NTR. ((B. Srinivasan, H.J. Sheedlo, S. Taylor, and R.S. Roque)) Department of Pathology and Anatomy, University of North Texas Health Science Center, Fort Worth, TX 76107.

In animals with retinal degeneration, the early stages of photoreceptor cell death are accompanied by activation and migration of glial cells, and increased expression of nerve growth factor (NGF) in glial cells and of the low affinity NGF receptor, p75NTR, in photoreceptor cells. Previous findings that microglial cells may release soluble factors that promote apoptosis of cultured photoreceptor cells led us to investigate the role of NGF in photoreceptor degeneration. RT-PCR was used to determine the expression of NGF receptors, trkA and p75NTR, in freshly isolated normal rat photoreceptor cells and in a mouse photoreceptor cell line (661w). 661w cells were also treated with microglial cell conditioned medium (MGCM) or NGF 0-100 ng/ml in basal medium (DMEM with 0.01% bovine serum albumin) and assayed for cell death/ survival. The effects of NGF were also neutralized using anti-NGF IgG. p75NTR, but not trkA, was expressed in normal photoreceptor cells and in 661w cells. Treatment of 661w cells with MGCM or NGF 50-100 ng/ml for 48 hrs. induced morphological changes and cell death as compared to cells incubated in basal medium alone. MGCM and NGF-treated cultures exhibited fewer number of cells, most of which were non-adherent and appeared rounded and refractile. Treated cells labeled with ethidium homodimer as compared with untreated cells, which stained with calcein-AM. The cytotoxic effects of MGCM were inhibited using anti-NGF polyclonal antibody. The induction of cell death in cultured photoreceptor cells by MGCM and NGF and the inhibition of MGCM-induced cell death by anti-NGF IgG support our hypothesis that NGF may be involved in photoreceptor cell loss during retinal degeneration. Moreover, the high expression of p75NTR, but not trkA, in the degenerating photoreceptor cells suggest that the NGF-induced cell death may be mediated by p75NTR-cell death cascade. (Supported by NIH EY10766 and the University of North Texas Health Science Center at Fort Worth Intramural Research Program)

1:00 PM

RESEARCH APPRECIATION DAY 2000

First Author: *Dibas, Mohammed*

Department: *PHARMACOLOGY*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

DEFINING THE CONVULSIVE SITE IN GABAA RECEPTORS.

M.I.Dibas* and G.H.Dillon Department of pharmacology, University of North Texas HSC at Fort Worth, Fort Worth, TX 76107

Picrotoxin (PTX) and related drugs like pentylenetetrazole (PTZ), insecticides (dieldren), and U93631 are convulsant drugs that inhibit GABA mediated Cl- current. Yet, the exact location of the convulsive site is not known. Recent studies have shown that mutation in TMII of rat beta2 subunit abolished picrotoxin effect on rat a1b2(T246F)g2. These results supports the contention that picrotoxin-binding domain might be within the channel lumen that is lined by TMII. Using site-directed mutagenesis, and patch clamp technique, we tested the hypothesis that the inhibitory effect of other convulsants presumably to act at lumen of the channel, would be affected in rat GABAA receptor a3b2(T246F)g2. We found that the inhibitory effect of PTZ , TBPS, and picrotoxin has been abolished. Furthermore, U93631 inhibited the GABA current with decreased affinity. Preliminary data from our lab indicate that the inhibtory effect of insecticide dieldren has been greatly affected by the mutation. These results suggest that the insecticidal action is mediated through the picrotoxin site, and also provide an insight about the molecular interaction between the insecticide molecule and the amino acids comprising the binding site. In addition, recent studies have indicated that a novel compound, a-IMGBL showed antagonistic effect on the picrotoxin site. Our lab studies showed that a-IMGBL protected the wild type receptor from interaction with picrotoxin. This compound might be a potential candidate to expolre the convulsive site in other ion channel. (Supported by NIH ES07904).

RESEARCH APPRECIATION DAY 2000

First Author: *Stephen L Wasmund*

Department: *Integrative Physiology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

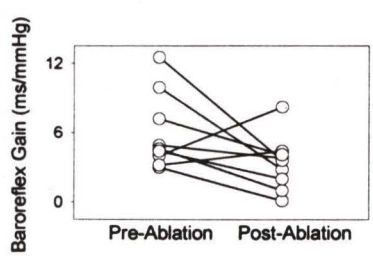
☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

SELECTIVE CARDIAC PARASYMPATHETIC DENERVATION FOLLOWING RADIOFREQUENCY ABLATION IN THE POSTEROSEPTAL REGION.

SL Wasmund, ML Smith, †RL Page, †J Zagrodzky, †C Sheehan, †K Ramaswamy, †L Nelson, †JA Joglar, ‡MH Hamdan, Dept of Integrative Physiology, University of North Texas Health Science Center, Ft Worth, TX, †Division of Cardiology, UT-Southwestern Medical Center, Dallas, TX, ‡VA Medical Center, Dallas, TX.

Sinus tachycardia had been reported following radiofrequency (RF) ablation in the posteroseptal (PS) region in patients with atrioventricular nodal reentrant tachycardia (AVNRT) or PS accessory pathways. The underlying mechanism is thought to be either an increase in sympathetic activity or a decrease in parasympathetic activity. We hypothesize that RF ablation in the posteroseptal region may result in selective parasympathetic denervation of the sinus node. METHODS: Nine patients with the diagnosis of AVNRT (n=5) or PS pathways (n=4) were studied. Coronary sinus norepinephrine levels (cardiac sympathetic nerve activity) and baroreflex heart rate gain (index of cardiac parasympathetic control) were measured before and after RF ablation. Baroreflex gain was measured using the bolus phenylephrine (PE) technique. RESULTS: Baroreflex gain decreased in 6 patients and increased in 2 as shown in the figure ($p=0.07$). There was no significant change in coronary sinus norepinephrine levels (1.7 ± 0.4 vs 1.6 ± 0.3 pmol/ml, $p=0.45$). CONCLUSION: In most patients, RF ablation in the PS region appears to result in selective parasympathetic denervation of the sinus node. This is probably secondary to direct damage to parasympathetic ganglia of postganglionic fibers that converge over the posterior intrapericardial space.



RESEARCH APPRECIATION DAY 2000

First Author: *L. Don Roberts*

Department: *CRI*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

CA+2/CALMODULIN-DEPENDENT PROTEIN KINASE-IIa NEGATIVELY REGULATES THE INDUCTION OF HYPERTROPHIC RESPONSE INIATED BY Ca+2 DEPENDENT PROTEIN KINASES AND PHOSPHATASES. L. Don Roberts, Tomas Valencia, Hong Zeng, Stephen Grant, Cardiovascular Research Institute, University of North Texas Health Science Center, Fort Worth, Texas.

Chronic stimulation of mechanisms promoting cardiac hypertrophy can lead to heart failure in humans. Molecular mechanism(s) which drive this adaptive growth process are currently not well understood. It has been shown that adult cardiomyocytes express an embryonic genetic program during cardiac hypertrophy, and continuous up-regulation of cardiac hypertrophy-sensitive gene program results in growth of the heart and subsequently progression toward congestive heart failure. Recent studies have documented a functional role for calcium signaling in initiating the hypertrophic response. Findings from our laboratory suggest that Ca2+/calmodulin-dependent kinases and phosphatase are principle in the regulation of classical cardiac hypertrophic sensitive genes (e.g. atrial natriuretic factor, skeletal-a-actin) during hypertrophy. Our laboratory has observed both CaM kinase-IV (CaMK-IV) and Calcineurin (CaN) effectively up-regulating hypertrophic-sensitive genes 6-12 fold over control. In contrast, the presence of CaM kinase-II (CaMK-II) silences any effect induced by CaN and/or CaMK-IV. This study utilize a doxycycline (Dox) inducible CaMK-II expression system that allows the turning on/off of expression of CaMK-II this study illustrates, in the presence over-expressed CaN and/or CaMK-IV, that during the period when CaMK-II is actively transcribed a dominate silencing of the induced hypertrophic response is evident. Furthermore, when CaMK-II transcription is silenced, with the addition of Dox, the hypertrophic response is restored. This, in conjunction, with in vitro findings of CaMK-II capacity to conduct phosphorylation events on both CaN and CaMK-IV and the fashion by which each enzyme is activated during different Ca2+ management profiles gives a plausible mechanism of both positive and negative regulation of hypertrophic response systems contained within the Ca2+/calmodulin-dependent enzymatic cascades. This further supports the idea that induction of hypertrophic response systems in cardiac tissue is a result of calcium of mismanagement.

RESEARCH APPRECIATION DAY 2000

First Author: *Keith Jackson*

Department: *Integrative Physiology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

AGONISTS/ANTAGONISTS PROFILES INDICATE DELTA OPIOID RECEPTOR CONTROL OF HEART RATE IN THE SINOATRIAL NODE. Keith Jackson, M. Farias, A. Goode, and J.L. Caffrey. Department of Integrative Physiology, University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas, 76107-2699.

Met-enkephalin-arg-phe (MEAP) is an endogenous opiate derived from the C-terminal sequence of proenkephalin. This heptapeptide is abundant in the myocardium and has significant vagolytic activity when infused systemically. The practical significance of the vagus is illustrated by the fact that patients, who regain vagal control of heart rate soon after suffering heart attacks, are 4-5 times more likely to have survived. These studies were conducted to determine which opioid receptor was responsible for the observed vagolytic effect of MEAP. Microdialysis probes were placed in the sinoatrial (SA) node of mongrel dogs and perfused at 5 µl/minute. Increasing doses of MEAP were included in the nodal perfusate and approximately two-thirds of the vagal bradycardia was inhibited with a maximal effect at 0.3nmoles/µl and a half-maximal response near 0.1nmoles/µl. When deltorphin II (a delta opioid receptor agonist) was infused into the SA node more than 95% of the vagal bradycardia was eliminated at 0.3nmoles/µl with the half-maximal response near 0.1nmoles/µl indicating that deltorphin II was more efficacious than MEAP. The maximal deltorphin II and MEAP effects were both similarly reversed by the paired infusion of increasing doses of the delta opiate receptor antagonist, naltrindole. There were no significant effects on vagal function observed when similar doses of mu (endomorphin-1, super DALDA) and kappa (U50, 488, Dynorphin) receptor agonists were infused into the SA node. Nor-binaltorphimine (kappa antagonist) was also unable to block the vagolytic effects of MEAP. These data suggest that the vagal effects of exogenous MEAP involve the activation of delta opiate receptors within the SA node. (Local Funds)

RESEARCH APPRECIATION DAY 2000

First Author: *Bradley Hart*

Department: *Integrative Physiology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

CANINE RIGHT VENTRICULAR OXYGEN SUPPLY/DEMAND BALANCE DURING GRADED EXERCISE. B.J. Hart, X. Bian, P.A. Gwirtz, and H.F. Downey. Dept. Integ. Physiol., Univ. N. Tex. Hlth. Sci. Ctr., Fort Worth, TX 76107.

To date, there are no data describing right ventricular (RV) O_2 extraction and consumption in an exercising animal model. This investigation was conducted to delineate mechanisms regulating RV O_2 supply/demand balance during moderate exercise. **Methods:** Four dogs were instrumented with a mammary artery catheter, a right coronary (RC) venous catheter, and a RC flow transducer. Hemodynamic data were recorded and blood samples were taken for blood gas analyses with the dog resting quietly on the treadmill and during 3 min exercise at 4 mph (Exercise 1) and 4 mph + 4% incline (Exercise 2). **Results:** See Table. At rest, RV O_2 extraction was only 49%. Exercise produced expected increases in heart rate (HR) and RV myocardial O_2 consumption (MVO_2). The large increase in O_2 demand was met by increased O_2 extraction, as RC blood flow (RCBF) was not significantly increased. Although RC venous PO_2 fell significantly from 29.7±0.8 to 19.7±0.8 mmHg, RC resistance was unchanged. **Conclusions:** RV has a large O_2 extraction reserve at rest. During moderate exercise, the O_2 extraction reserve is mobilized before the RC flow reserve. Under these conditions, RC resistance is unaffected by reduced RC venous PO_2.

	HR (beats/min)	AoP (mmHg)	RCBF (ml/min/g)	O_2 Extraction (%)	MVO_2 (ml/min/100g)
est	103±2	117±5	0.52±0.03	49±1	4.8±0.4
xercise 1	165±4*	121±3	0.56±0.05	71±3*	6.8±0.9*
xercise 2	175±8*	125±4	0.56±0.06	75±4*	7.6±1.0*

Values are mean±SEM. AoP: peak aortic pressure. *Significantly different from respective values at rest, $p<0.05$. (Supported by NIH Grant HL35027.)

2:00 PM

RESEARCH APPRECIATION DAY 2000

First Author: *Robert Carter III, M.S.*

Department: *Integrative Physiology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

TIDAL LUNG VOLUME AND CARDIOVASCULAR RESPONSES TO APNEA.

R. Carter III, D.E. Watenpaugh, S.L. Wasmund, N.K. Muenter, W.L. Wasmund, M.L. Smith. Integrative Physiology, UNT Health Science Center, Fort Worth, Texas, USA.

Obstructive sleep apneas occur at end expiration and lead to hypoxemia and hypercapnia, which in turn increase sympathetic nerve activity (SNA) and arterial pressure.We hypothesized that low lung volume during apnea also contributes to the sympathoexcitation. Nine healthy subjects and 7 sleep apneic patients held their breath at end tidal expiration (functional residual capacity) and end tidal inspiration. They breathed 12% O2 plus 3% CO2 for 1 min before apnea. SNA (microneurography), beat-to-beat arterial pressure, arterial oxygen saturation (SaO2), and end tidal PCO2 were measured. SNA and arterial pressure were compared between expiratory and inspiratory apnea at times during which SaO2 and PCO2 values were comparable. SNA increased to 731 ± 145% of baseline levels during expiratory apnea, and to 751 ± 150% of baseline levels during inspiratory apnea in healthy subjects (mean ± SE; NSD). SNA increased to 612 ± 126% of baseline levels during expiratory apnea, and to 711 ± 139% of baseline levels during inspiratory apneas in patients (NSD). Mean arterial pressure (MAP) in patients increased similarly during expiratory and inspiratory apneas 13 ± 4 mm Hg and 18 ± 5 mm Hg, respectively. In healthy subjects, there were also no significant differences in MAP elevation between expiratory (12 ± 5 mm Hg) and inspiratory apneas (17 ± 5 mm Hg). Greater lung volume at end-inspiration does not play a direct role in apnea-induced sympathoexcitation when SaO2 is equivalent. Differences in the SNA response between inspiratory and expiratory apneas are due only to the effect on rate of oxygen desaturation.

RESEARCH APPRECIATION DAY 2000

First Author: *Peter Gargalovic*

Department: *Molecular Biology/Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

CAVEOLIN-1 AND CAVEOLIN-2 EXPRESSION IN MOUSE MACROPHAGES AND REGULATION BY CHOLESTEROL METABOLISM.

Peter Gargalovic and Ladislav Dory, UNTHSC, Dept. Molecular Biology & Immunology, Fort Worth, TX 76107

Caveolins, integral membrane proteins forming cholesterol- and phospholipid- rich caveolae in the plasma membrane are believed to participate in cholesterol trafficking within the cell. Although macrophages may beone of the most active cells in cholesterol trafficking during the development of atherosclerosis, caveolin (cav) expression in these cells has not been reported. In the present studies we characterize cav-1 and cav-2 expression in primary mouse macrophages as well as J774 cells and demonstrate the regulation of their expression by cellular cholesterol.

Western blotting, RT-PCR and ribonuclease protection assay (RPA) were used to demonstrate, for the first time, the expression of the □ and ß forms of cav-1 and cav-2 in mouse macrophages. Relative to 3T3 cells, the cav-1 / cav-2 ratio in macrophages is significantly lower (approx. 1:20 vs. ~1:1), an observation that may explain the surprising lack of cellular co-localization. Immunocytochemical analysesclearly establish that most of cav-1 is located on the cell membrane, while cav-2 is found almost exclusively in the Golgi / ER. The relatively low level of cav-1 expression may be responsible for the lack of cav-2 transport to cell membrane. In contrast to primary macropha-ges, J774 macrophages express only cav-2.

The expression of cav-1 and cav-2 is regulated by cellular sterol content and flux. Manipulation of the cellular cholesterol pool by acLDL, oxysterols or statins significantly affect the expression of cav-1 and, to a lesser extent, that of cav-2. HDL- mediated cholesterol efflux results in an over 100% increase in cav-1 expression. Our data provide strong evidence that the regulation of caveolin expression in macrophages is complex and depends on the cellular levels of cholesterol and the rates of cholesterol biosynthesis and efflux. (Suuported by a grant from NIH to LD).

RESEARCH APPRECIATION DAY 2000

First Author: *Kevin Formes*

Department: *Integrative Physiology*

☐ GSBS Student ☐ SPH Student ☐ PA Student

☒ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

VAGAL BLOCKADE INDUCES BLOOD PRESSURE INSTABILITY (PATHOPHYSIOLOGICAL IMPLICATIONS)

K.J. Formes, D.W. Wray, M.S. Weiss, A.H. Yurvati, and X. Shi
Department of Integrative Physiology and Cardiovascular Research Institute, UNTHSC, Fort Worth, TX 76107

The aim of this study was to test the hypothesis that a diminished baroreflex gain is responsible for a blood pressure (BP) instability. Gain was determined from the slope of changes in heart rate (HR) to systolic BP (SBP, measured by an intra-radial arterial catheter or a radial arterial tonography) during hypotensive and hypertensive stimuli, elicited by cuff deflation following three minute bilateral thigh occlusion > 200 mmHg and after phenylephrine injection (1ug/kg), respectively, before (B) and after vagal blockade using atropine (A) or glycopyrrolate (G) in nine healthy young adults. Systolic and diastolic BP (DBP) were not significantly affected by A or G despite an increased HR (see table). Gain was significantly reduced and BP variability was substantially augmented in terms of the magnitude (dP) or rate (dP/t) of changes in SBP following A or G. We concluded that vagal blockade diminished the buffering effect of HR response and caused a BP instability. A vagal dysfunction associated with the normal aging process may be the mechanism responsible for some of the hemodynamic related clinical symptoms occurring in the elderly population.

	HR bpm	SBP mmHg	DBP mmHg	HYPOTENSIVE			HYPERTENSIVE		
				Gain PI/mmHg	dP mmHg	dP/*t* mmHg	Gain PI/mmHg	dP mmHg	dP/*t* mmHg
B	59±3	123±3	66±2	10.6±1.9	-9±1	-2.3±.2	17.3±3.9	+14±2	0.9±.2
A	103±6	130±3	72±3	0.9±0.1	-15±1	-3.2±.3	1.3±0.6	+26±2	1.9±.2
G	110±4	129±5	71±4	0.6±0.1	-14±2	-3.0±.4	1.4±0.7	+27±3	2.2±.5

(Supported by NIA AG14219, NIH HL45547, UNTHSC Faculty Research Grants)

RESEARCH APPRECIATION DAY 2000

First Author: *Lisa Hodge*

Department: *Molecular Biology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

INTRANASAL IMMUNIZATION IS MORE EFFECTIVE THAN SYSTEMIC IMMUNIZATION IN PRIMING MUCOSAL ANTIBODY RESPONSES IN THE UPPER RESPIRATORY TRACT

Lisa Hodge and Jerry Simecka

Department of Microbiology and Immunology, University of North Texas Health Science Center, Fort Worth, Texas, 76107

Mucosal immunity, particularly IgA antibody, is the first line of defense for the upper respiratory tract and should be considered during the development of any respiratory vaccine. In our studies we found vaccination via the intranasal route is more effective than systemic immunization in priming respiratory antibody responses. Mice were immunized with whole Philippines Influenza hemagglutinin (HA) antigen in combination with cholera toxin (CT) either intranasally (IN) or intraperitoneally (IP). Fourteen days following primary immunization, serum antibody responses were detected by ELISA. Mice that received intranasal immunizations produced the highest level of serum, nasal wash and fecal IgA. Serum IgG and IgM levels were high in both intranasal and intraperitoneally immunized mice. These antibodies are important in preventing subsequent lower respiratory tract infection. To further asses antibody responses, anti-HA antibody-forming cells among lungs, spleens, nasal passages, upper respiratory nodes and lower respiratory nodes were isolated fourteen days following primary immunization. In concurrence with serum antibody production, IgA antibody forming cells in these tissues were highest in mice that had received intranasal immunizations, while IgG and IgM antibody forming cells were similar between IN and IP immunized groups. Therefore, we conclude that intranasal immunization induces a higher IgA response than intraperitoneal immunization, as well as producing antibodies that protect the lower respiratory tract (American Lung Association of Texas).

RESEARCH APPRECIATION DAY 2000

First Author: *Debra White*

Department: *MolecularBiology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

BACTERIAL BIOFILM FORMATION AND DISPERSAL UNDER THE INFLUENCE OF THE GLOBAL REGULATOR *csrA*. Debra L. White, Lawrence Oakford, Jerry Simecka, Mark E. Hart, and Tony Romeo. Department of Molecular Biology and Immunology , University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107-2699

Carbon Storage Regulator A (*csrA*) is a global regulator of carbon metabolism and of genes expressed in early stationary phase of bacterial growth. A mutation in the *csrA* gene of *Escherichia coli* K-12 alters a variety of surface properties including mucoidy, hydrophobicity, and adherence to abiotic surfaces, i.e. biofilm formation. Biofilm provides an environment that protects the bacterium against harsh conditions, including attack by the human immune system and certain antimicrobials. *E coli* biofilms have been proposed to be involved in human diseases such as biliary tract infection, bacterial prostatitis, and urinary catheter cystitis. Regulation of *E. coli* biofilm formation and development is poorly understood, while dispersal of pelagic cells from biofilm is essentially unexplored in any bacterium. Several extracellular components, including type I pili, curli fimbriae, flagella, and colanic acid have been previously proposed to play roles in *E. coli* biofilm formation. We now report that a *csrA* mutant, unlike the parent strain, forms a significant biofilm in the absence each known adherence factor. Microscopic analyses indicated that biofilm of the *csrA* mutant was structurally similar to that of the isogenic parental strain. However, quantitative staining of surface-bound cells using crystal violet showed that biofilm formed at a highly accelerated rate in the *csrA* mutant. We constructed a strain of *E. coli* harboring an inducible *csrA* gene. Biofilm was readily formed by this strain in the absence of *csrA* expression. Furthermore, this biofilm was specifically dispersed following *csrA* induction. Overexpression of *the E. coli csrA* gene in several clinical isolates, including *Citrobacter* and *E. coli* strains isolated from urinary catheter-biofilms, decreased their abilities form biofilms in vitro. The *csrA* gene represents the first example of a repressor of bacterial biofilm formation, and our data provide the first evidence for a genetic signaling pathway for biofilm dispersal. (National Science Foundation MCB9726197)

RESEARCH APPRECIATION DAY 2000

First Author: *Michelle L. Wright*

Department: *Molecular Biology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

ISOLATION AND PRELIMINARY CHARACTERIZATION OF A PUTATIVE DETERMINANT OF MANGANESE SUPEROXZIDE DISMUTASE (*sod)* FROM *Staphylococcus aureus*

Michelle L. Wright and Mark E. Hart. University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas, 76107-2699

Staphylococcus aureus invasion of the skin's underlying tissues is confronted by professional phagocytes. The ability of these phagocytes (polymorphonuclear leokocytes and macrophages) relies in part on the production of toxic reactive oxygen intermediates (ROI) such as superoxide, hydrogen peroxide, and hydroxyl radicals. To counteract the toxic effects of these ROIs, bacteria have evolved enzymes that ultimately convert these radicals to oxygen and water. Superoxide dismutase (SOD), which converts superoxide to hydrogen peroxide and oxygen, is an important enzyme to bacteria that undergo aerobic respiration and, in some bacteria, is critical in preventing killing by ROIs within professional phagocytes. *S. aureus*, a gram positive coccus, is commonly found on the skin and mucous membranes of humans. Invasion of subcutaneous tissues often leads to localized abscess formation that can lead to more life-threatening diseases such as osteomyelitis, endocarditis, and meningitis. The role staphylococcal SOD plays in the disease process is currently unknown. Therefore, we hypothesize that the staphylococcal SOD may play a role in evading killing by professional phagocytes. A BLAST search of the *S. aureus* genomic sequence database with a partial staphylococcal *sod* sequence revealed a 787bp contiguous region containing a full-length gene with a putative promoter and Shine Delgarno sequence. This region was amplified by PCR and used to rescue an *E.coli sodA, sodB* mutant grown on minimal media under aerobic conditions. Amino acid sequence comparisons indicate that the gene encodes a manganese SOD (MnSOD). Activity gels of whole cell lysates from *S. aureus* demonstrated three closely migrating low molecular weight bands that are resistant to hydrogen peroxide, a characteristic of MnSODs. Whole cell lysates of the rescued *E. coli* strain showed a band of activity that migrated between the upper and middle bands of the three activities observed in *S. aureus* whole cell lysates. Northern analysis of total RNA revealed a single transcript of the expected size. Aeration appears to have a decreasing effect on message level at 12 hours of growth, a phenomenon also observed with SOD activity gels. Efforts are underway to generate a *S. aureus sod* mutant to determine the role SOD plays in survival inside professional phagocytes. (National Institutes of Health)

RESEARCH APPRECIATION DAY 2000

First Author: *Katie Overheim*

Department: Microbiology and Immunology

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

THE GLOBAL REGULATORS (*AGR* AND *SAR*) OF VIRULENCE FACTORS PROMOTE SEVERE STAPHYLOCOCCAL PNEUMONIA IN A MOUSE MODEL.

Katie A. Overheim, Leslie Tabor, Jerry W. Simecka and Mark E. Hart, University of North Texas Health Science Center, Fort Worth, Texas 76107-2699

Staphylococcus aureus is a gram-positive bacterium and a leading cause of nosocomial infections. Among the nosocomial infections caused by *S. aureus*, pneumonia represents one of the more life-threatening diseases, particularly in the elderly population. Although *S. aureus* produces numerous toxins that contribute to various diseases, little is known about the involvement of these toxins in causingpneumonia. We hypothesize that toxins contribute to the severity of staphylococcal pneumonia, and that approaches that block toxin production or their effects will have a tremendous therapeutic effect. To begin to examine the role of toxin production on pneumonia, we took advantage of the fact that production of many toxins is regulated by at least two genetic loci, the accessory gene regulator (*agr*) and the staphylococcal accessory regulator (*sar*). Mutations in either or both of these regulators result in reduced levels of expression for many of the toxins with a concomitant reduction in virulence in several animal models of disease. To investigate the roles of these regulators in staphylococcal pneumonia, Balb/c mice were intranasally inoculated with *S. aureus* RN6390 or its isogenic mutants RN6911 (*agr)* and ALC488 (*sar).* Greater than 90% of the mice given 10^8 colony forming units of the parent strain of *S. aureus* died within 2 days after infection. In contrast, about 50% of mice similarly infected with *sar* mutant survived, whereas all mice infected with *agr* mutant survived. The difference in virulence was not due to number of bacteria surviving in the lung. Also, bacterial dissemination to extrapulmonary sites was not the cause for lethality of the organism. These studies demonstrate that the severity of staphylococcal pneumonia in a mouse model is increased with a functional *agr* and *sar* regulatory system, and supports a role for toxin production in the pathogenesis of a life-threatening lung disease.

RESEARCH APPRECIATION DAY 2000

First Author: *P. John Kamthong*

Department: *Molecular Biology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

ATTENUATION OF MACROPHAGE COLONY-STIMULATING FACTOR EXPRESSION BY CYCLIC ADENOSINE MONOPHOSPHATE: NOT THROUGH INHIBITION OF IκB KINASE P. John Kamthong and Ming-chi Wu
Department of Molecular Biology & Immunology, University of North Texas HSC, Fort Worth, Texas 76107.

The receptor-mediated mechanism of Macrophage colony-stimulating factor (M-CSF) has been thoroughly investigated. However, only limited information is available on the signal transduction of M-CSF expression. We have observed that IL-1α can induce human M-CSF expression and this inducibility is attributed to the NF-κB activation. Treatment of human pancreatic cancer cell MIA PaCa-2 with forskolin or cAMP attenuated the NF-κB activation as well as M-CSF expression. In this study, we further investigate the mechanism of cAMP attenuation. MIA PaCa-2 cells were incubated with forskolin or dibutyryl-cAMP and then stimulated with IL-1 for 1 hour. Cell lysates were immunoprecipitated by anti-IκB kinase (IKKβ) antibody and assayed the immune complex for kinase activity using IκBα as substrate. The total cellular protein levels of IKK were measured by respective western blot. The results showed that the level of IKK protein remained constant in the presence of either cAMP and/or IL-1, while IKK kinase activity is greatly stimulated by IL-1. Nonetheless, this activity is not affected by forskolin. These results suggest that cAMP had no effect at all on IKK activity that induced by IL-1 treatment. We then proceed to examine the IκB level in cAMP treated cells by western blot analysis. IκB level decreased markedly in IL-1 treated cells comparing to the untreated. By contrast, cells treated with cAMP or forskolin possessed significantly higher IκB level. The results have shown clearly that cAMP indeed increase the IκB level even in the presence of IL-1. We conclude that the attenuation by cAMP in the IL-1 stimulated M-CSF expression is not by the inhibition of IKK, but rather by inducing the higher expression of IκB which prevents the NF-κB release from the cytosolic NF-κB/IκB complex and attenuates M-CSF expression.

RESEARCH APPRECIATION DAY 2000

First Author: *Van Huynh*

Department: *Molecular Biology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

POLYMORPHISM IN THE 2B4 GENE OF INBRED MOUSE STRAINS. Van T. Huynh, Pappanaicken R. Kumaresan, Porunelloor A. Mathew. University of North Texas Health Science Center, Department of Molecular Biology and Immunology. 3500 Camp Bowie Boulevard, Fort Worth, Texas. 76107-2699.

Natural killer cells are lymphocytes that play a role against cancer and viral infections. 2B4 gene is a membrane glycoprotein expressed on all natural killer cells and is implicated in the killing of cancer cells. It is a counter-receptor for CD48 and a member of the CD2 subgroup of the immunoglobulin superfamily. In human, 2B4 is also expressed on T cells. It has been reported that 2B4 expression on T cells can be used as a marker in AIDS disease progression. This indicates that the 2B4 molecule plays an important role in the regulation of immune cell function. Earlier studies have shown that anti-2B4 mAb specifically recognizes the epitopes expressed on NK cells derived from mice strains C57BL/6 and C59/J. However, our lab has shown, by northern analysis, that 2B4 transcripts are expressed in all mouse strains. Failure of anti-2B4 mAb to recognize 2B4 in different mouse strains may be due to the polymorphism of the 2B4 gene. The molecular characterization of 2B4 from mice strains BALB/c, 129/Svj and A.CA show that the polymorphic residues in 2B4 are located in the immunoglobulin V-domain. Results from the polymorphism studies indicate that the nucleotide sequence of A.CA-2B4 clone is highly homologous to C57BL/6-2B4, whereas the nucleotide sequence of 129/Svj-2B4 clone is highly homologous to BALB/c-2B4. In addition, the peptide homology between the strains showed that C57BL/6 and A.CA have 99% similarity and BALB/c and 129/Svj have 100% similarity. Future studies will be to localize the binding sites important in 2B4/CD48 interactions through site-directed mutagenesis. This may lead to developing new strategies for immunotherapy of cancer using NK cells.

4:00 PM

RESEARCH APPRECIATION DAY 2000

First Author: *Kent S. Boles*

Department: *MBI*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

IDENTIFICATION OF A 2B4 VARIANT AND RELATED RECEPTOR ON HUMAN NK CELLS. Kent S. Boles and Porunelloor A. Mathew. Department of Molecular Biology and Immunology & Institute for Cancer Research, University of North Texas Health Science Center, Fort Worth, TX.

Natural killer (NK) cells spontaneously detect and kill cancerous and virally infected cells through receptors that transduce either activating or inhibiting signals. The majority of well studied NK receptors are involved in inhibitory signaling. However, we have previously described an activating receptor, 2B4, expressed on NK cells and a subset of T cells that mediate non-MHC restricted killing. Anti-2B4 monoclonal antibodies directed against NK cells and a NK cell line enhanced their destruction of tumor cells. Other related receptors including SLAM and CD48 are implicated in disease. Elevated levels of soluble CD48 are observed in patients with leukemia or autoimmunity. Genetic defects in an adaptor molecule associated with SLAM (SAP) cause XLP (X-linked lymphoproliferative disorder). 2B4 signaling is thought to occur via four tyrosine motifs (TxYxxI/V) in the cytoplasmic domain. Evidence suggests that both SAP and SHP-2 bind these tyrosine motifs. In the mouse, alternative splicing leads to a shorter cytoplasmic tail (2B4S) with the elimination of the two C-terminal tyrosine motifs. Similar variants have been identified in both CD84 and SLAM. It has been suggested that the longer (2B4L) and the 2B4S variants may have opposing functions. Here we report the identification of a splice variant in the cytoplasmic domain of the human 2B4 receptor. There is a twenty amino acid residue deletion encompassing the second tyrosine motif distal to the membrane. Elimination of the tyrosine motif could lead to differential binding of adaptor molecules and subsequent alteration of effector function. Furthermore, we have identified a new NK cell receptor related to 2B4. Its cytoplasmic domain contains four tyrosine motifs similar to 2B4, possibly transducing similar signals. Characterization and modulation of these receptors may lead to advances in the immunotherapy of cancer.

RESEARCH APPRECIATION DAY 2000

First Author: *Gheath Al-Atrash*

Department: *Molecular Biology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☒ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

ROLE OF UROKINASE PLASMINOGEN ACTIVATOR AND ITS RECEPTOR IN EXTRACELLULAR MATRIX DEGRADATION BY NATURAL KILLER CELLS

Gheath Al-Atrash,1,2 Richard P. Kitson,1,2 Yaming Xue1,2 Myoung H. Kim,1,2 Andrew. P. Mazar,3 and Ronald H. Goldfarb. 1,2 1Dept. of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, Texas, 76107, USA. 2Institute for Cancer Research, University of North Texas Health Science Center, Ft Worth, Texas, 76107, USA. 3Ångstrom Pharmaceuticals Inc., San Diego, California, 92121, USA

The uPA system has been correlated with cellular migration and invasiveness in a variety of tumor cells and cells of the immune system including macrophages, neutrophils, and T-lymphocytes. We provide evidence for the production by Natural Killer (NK) cells of urokinase plasminogen activator (uPA), and its receptor (uPAR), at both the molecular and protein level. NK cell uPA has a Mr slightly lower (48-50kD) than that characteristic for uPA of other cell types (52-55kD). NK cell uPA appears enzymatically active on casein/plasminogen zymography and chromogenic substrate assays, and is recognized by monoclonal antibodies to human uPA. uPAR was detected using Western blots and fluorescence microscopy using BODIPY labeled uPA. Regulation of both uPA and uPAR mRNA by IL-2 and by binding of cells to extracellular matrix components was examined. While no significant changes in uPA mRNA levels were found, a dramatic increase in uPAR mRNA following stimulation of NK cells with IL-2 was observed. uPA dependent in vitro invasion by NK cells was observed using Matrigel invasion assays. Since plasmin is documented to cleave basement membrane laminin, the uPA system in NK cells appears to play an important role in extracellular matrix degradation. The uPA system may therefore contribute, at least in part, to our previous electron microscopic observations revealing basement membrane degradation in vivo by A-NK cells, and their subsequent accumulation into tumor metastases following their adoptive transfer.

RESEARCH APPRECIATION DAY 2000

First Author: *Michael C. Lawrence*

Department: *Molecular Biology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

CALCIUM AND CYCLIC AMP UPREGULATE INSULIN GENE TRANSCRIPTION BY A CALCINEURIN/NFAT PATHWAY IN PANCREATIC B-CELLS.

Michael C. Lawrence and Richard A. Easom. UNT Health Science Center., Fort Worth, TX 76107.

Diabetes is a chronic disease with no known cure and is the seventh leading cause of death in the United States (ADA, 1998). The main underlying cause of this disease is the loss of pancreatic b-cells or the impairment of b-cell function. Glucose induces both insulin secretion and transcription in pancreatic b-cells. However, the molecular mechanisms involving increased insulin production are unclear. Therefore, we sought to determine physiological components that increase insulin transcription in pancreatic b-cells. It is known that glucose stimulates secretion by driving cell depolarization and increased intracellular calcium, and it was hypothesized that these events lead to the upregulation of insulin gene transcription as well. We transfected INS-1 cells with either the wild-type insulin promoter-reporter or an NFAT-driven reporter, which both showed increased reporter activity when stimulated by glucose, K+, or forskolin. Reporter activity also increased when these reporters were co-transfected with the calcium/calmodulin-dependent phosphatase, calcineurin. In all cases, the calcineurin-selective immunosuppressant FK506 blocked the increase in transcription. Insulin gene promoter-reporters containing NFAT binding-site mutations resulted in reduced activity as well. These results show that calcium and cAMP can upregulate insulin gene transcription, and demonstrate that calcineurin and NFAT play a pivotal role in producing this response. These findings may not only provide insight to formulating new treatments for diabetes, but also lead to the development of new immunosuppressant drugs, which do not induce diabetes during clinical use. (Supported by The Advanced Research Program of the Texas Higher Education Coordinating Board)

RESEARCH APPRECIATION DAY 2000

First Author: *Paramjit Kaur Gill*

Department: *Molecular Biology and Immunology*

☒ GSBS Student ☐ SPH Student ☐ PA Student

☐ TCOM Student ☐ Faculty ☐ Staff

☐ Postdoctoral Fellow/Resident

DETERMINATION OF THE INTRACELLULAR LEVELS OF CYCLIC ADP-RIBOSE IN CULTURED HUMAN CELLS USING A NEW HIGHLY SENSITIVE FLUORESCENT HPLC METHOD Paramjit Kaur Gill and Rafael Alvarez-Gonzalez
Department of Molecular Biology and Immunology, University of North Texas Health Science Center at Fort Worth, TX 76107-2699.

Cyclic ADP-ribose (cADPR) is a second messenger that mobilizes intracellular stores of calcium in higher eucaryotic cells. The intracellular concentration of cADPR has previously been estimated to be in the femto- to nanomolar range. Therefore, there is a need for a highly sensitive assay to measure the levels of this nucleotide in just a few million cells. Here, we have developed a highly sensitive, specific, and reproducible fluorescent HPLC method to determine the intracellular concentration of cADPR in cultured cells. The procedure involves extraction of the total nucleotide pool in 20% (W/V) TCA followed by the purification of soluble molecules containing two or more riboses by boronate affinity chromatography. Purified nucleotides are then digested with phosphodiesterase to degrade all non-cyclic molecules, leaving cADPR intact. Contaminating products of phosphohydrolysis are then eliminated by a second boronate step and the pure preparation of cADPR obtained is converted to monomeric ADP-ribose with NADase (isolated from *Bungarus fasciatus)*. After a third boronate purification, ADP-ribose is chemically derivatized to the etheno-adenine fluorescent form with chloroacetaldehyde at 60 °C, and the εADP-Ribose formed is quantified by fluorescent-HPLC on a Partisil 10-SAX column. The specificity of our method was monitored by determining the yield at every step of the protocol with [^{32}P]cADPR. Radiolabeled cADPR was synthesized from [^{32}P]β-NAD+ and pure ADP-ribosyl cyclase from *Aplysia californica.* [^{32}P]cADPR was subsequently purified by HPLC on a Partisil 10-SAX and a C-18 reverse phase column placed in tandem. While the recovery of a known amount of cADPR through each boronate step of the 4-day protocol was approximately 90%, the overall recovery throughout the procedure was between 30-40%. As expected, our mock incubations (negative controls) in the absence of phosphodiesterase or NADase treatment, as well as chloroacetaldehyde, yielded no εADP-ribose peak. Furthermore, spiking of a cell extract with commercially available cADPR resulted in the formation of a bigger fluorescent peak. Finally, our method indicated an intracellular concentration of cADPR in HeLa cells of 980 pmol of cADPR/10^8 cells. Considering that HeLa cells have a larger cytoplasm compared to blood cells, our results agree well with those reported by Da Silva et al. who observed that the intracellular concentration of cADPR was 198 pmol/10^8 Jurkat cells, using a less sensitive chromatographic assay.

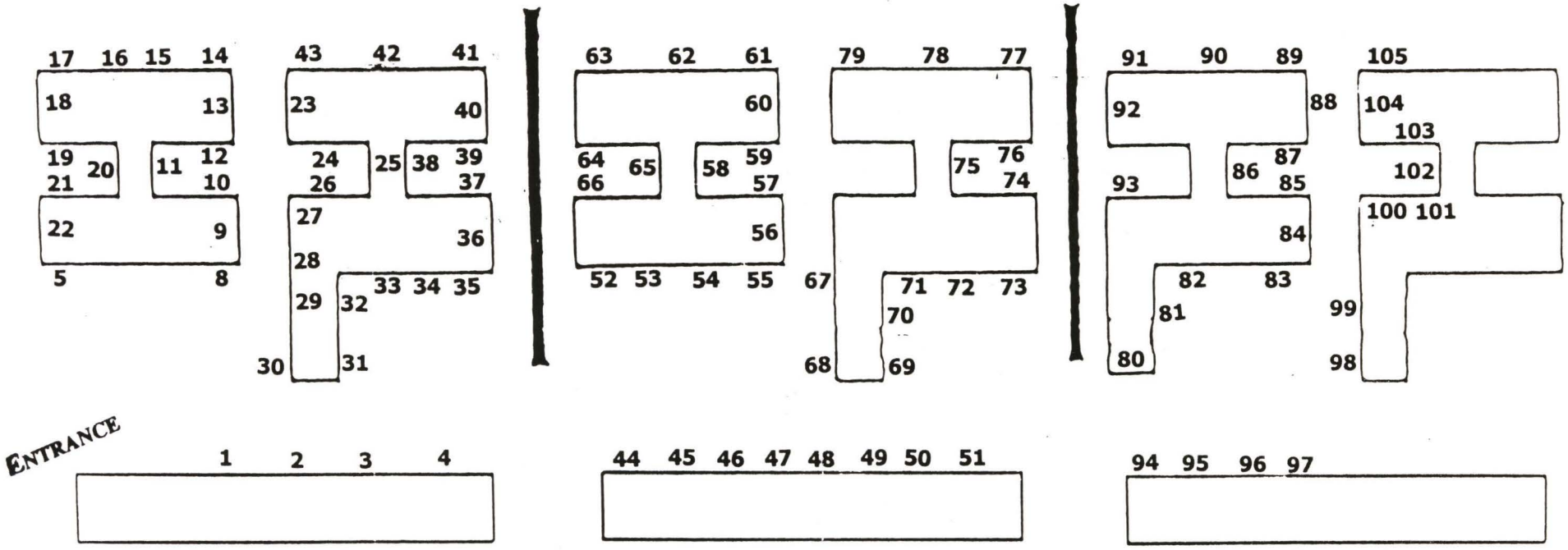
FIRST AUTHOR/PRESENTER INDEX

DEPARTMENT/UNIT SUBMISSIONS

	# Submitted
Biomedical Sciences	4
Family Medicine	5
Geriatrics Education Research Institute	2
Integrative Physiology	16
Cardiovascular Research Institute	3
Internal Medicine	1
Library	1
Molecular Biology and Immunology	56
Osteopathic Manipulative Medicine	6
PA Program	2
Pathology and Anatomy	10
Pharmacology	11
Public Health	8
TOTAL NUMBER OF ABSTRACTS SUBMITTED:	**124**

POSTER MAP

Research Appreciation Day 2000



1 - 3	Special School Programs	44 - 51	Cellular Science	80 - 93	Neuroscience and Aging
4	Postdoctoral Scholars Association	52 - 57	Family Medicine	94 - 102	Physical Medicine Institute
5 - 29	Institute for Cancer Research	58 - 63	Infectious Disease	103 - 105	Public Health
30 - 43	Cardiovascular Research Institute	64 - 66	Music and Medicine		
		67 - 79	North Texas Eye Research Institute		