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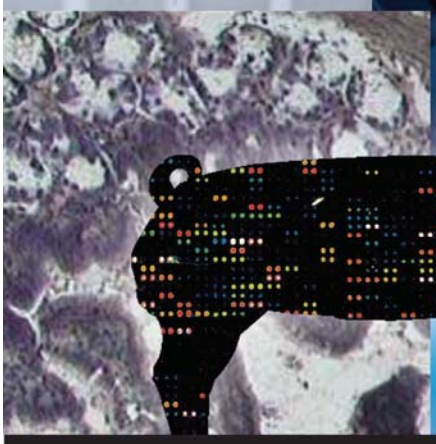
SWINE

IN BIOMEDICAL RESEARCH CONFERENCE

Building Blocks of Animal Models: Genomics, Bioengineering, Transgenesis and Cloning



JANUARY 27-29, 2005
FAIRMONT HOTEL, CHICAGO, IL

HOSTED BY THE UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

Organizing Committee:

Lawrence B. Schook, Ph.D. (Chair)
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National Institute of Agrobiological Sciences, Sukuba, Japan

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Sharon Donovan, Food Science & Human Nutrition
H. Rex Gaskins, Animal Sciences
Russell Jamison, Materials Science & Engineering
Lawrence Schook, Animal Sciences
Kelley Tappenden, Food Science & Human Nutrition
Michael Tumbleson, Agricultural Engineering (honorary chair)
Matthew Wheeler, Animal Sciences
Federico Zuckermann, Veterinary Pathobiology

Poster Session Moderators:

Bioengineering, Russ Jamison, UIUC
Immunology & Infectious Diseases, Federico Zuckermann, UIUC
Transplantation (allo & xeno), Doug Smith, Baylor & Mark Rutherford, U.Minn.
Nutrition (Obesity and Diabetes), Sharon Donovan, UIUC
Genomics and Cloning, Jon Beever, UIUC & Max Rothschild, ISU
Cardiovascular, Rex Gaskins, UIUC
Physiology, Jack Odle, NCSU
Cancer, Craig Beattie, UNR
Clinical Models, Steve Niemi, Harvard

Sponsored by:

Institute for Genomic Biology, College of Agricultural, Consumer, and Environmental Services, College of Liberal Arts & Sciences, College of Veterinary Medicine, and Office of the Vice-Chancellor of Research at the University of Illinois at Urbana-Champaign

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Hosted by:

The Institute for Genomic Biology, University of Illinois at Urbana-Champaign

Special Acknowledgement:

The organizers wish to express their appreciation to Helen Neef (Institute for Genomic Biology), Scott Miller (UIUC Conferences), and Shannon Tomlinson (UIUC)

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Swine in Biomedial Research
Historical Perspective and Conference Objectives

The Swine in Biomedical Research Conference Organizing Committee welcomes you to this third meeting focused on the use of the pig in biomedical research. We wish to recognize both the financial support and encouragement of our sponsors to host this conference.

This conference addresses the biomedical community needs to develop relevant animal models. The last conference was hosted by the Mike Tumbelson and Larry Schook at the University of Maryland University College, College Park, MD, October 22-25, 1995 (proceedings published by Plenum Press, *Advances in Swine in Biomedical Research*, ed. by M.E. Tumbleson and L.B. Schook, 1996). Since that time, the pig has been identified through the NIH White paper process as a high priority species for sequencing and the USDA has initiated targeted structural and functional genomics efforts for understanding the diverse pig phenotypes. The emergence of genetic information and the tools to target manipulations combined with the ability to clone pigs provides a new and highly relevant animal model. The growing relevance is further demonstrated by a recent CRISP search (1999-2003) that shows that NIH sponsored research (over 20 institute and centers) supported > 2,500 separate grants using the pig. Thus, a broad foundation utilizing the pig in biomedical research already exists from which to build future programs.

Goals: The conference goals are to identify areas of study or methodologies that will enhance the utility of pigs as biomedical models. In particular, the conference will focus on identifying appropriate human diseases where traditional rodent models have not proven relevant, where the historical use of swine in this regard may be enhanced, or where no useful models exist today and for which swine may be considered. The conference is organized to identify resource needs and areas in which new approaches or methodologies are required. The conference is structured to stimulate interactions between researchers working within swine and human genomics. To date, these are separate research communities and the conference will provide a forum for introductions and to show case the utility of the pig as an invaluable model. The increasing relevance of existing pig models for human diseases and the emerging ability to capture genomic information to create novel models has stimulated the organization of this conference. The conference has been organized to permit active discussion and to identify the needs and opportunities for continued exploitation of this animal model.

Building Blocks of Animal Models: Genomics, Transgenesis and Cloning

Swine in Biomedical Research Conference
Fairmont Hotel, Chicago, IL
January 27 - 29, 2005

Program

Thursday, January 27, 2005

2:00 - 9:00 pm ..... Arrivals and Registration (International Ballroom)
4:00 - 7:00 pm ..... Poster Setup (International Ballroom)
7:00 - 9:00 pm ..... Comparative Genomics: A Driver for Better Models of Human Diseases
Discussion Leaders: Lawrence Schook (University of Illinois), Hiroshi Yasue (National Institute of Agrobiological Sciences)
7:00 pm ..... *Welcome*, **Harris Lewin**, Director of the Institute for Genomic Biology, University of Illinois
7:10 pm ..... Introduction: *Defining the Promise of the Pig Model*, **Lawrence Schook**
7:30 pm ..... *Historical Perspective Biology of the Pig and Relevance to Medicine*, **Leif Andersson**, Uppsala University, Sweden
8:30 - 10:00 pm ..... Reception (Moulin Rouge) and Review of Posters (International Ballroom)

Friday, January 28, 2005

7:30 - 8:30 am ..... Breakfast and Poster Reviews (International Ballroom)
8:30 am-12:15 pm ..... Building Blocks of Models: Bioengineering, Genomics, Transgenesis and Cloning
Discussion Leaders: Merete Fredholm (Den Kgl. Veterinærog Landbohøjskole), Randall Prather (University of Missouri)
8:30 am ..... *The Pig Genome Sequencing Project*, **Jane Rogers**, Sanger Institute
9:15 am ..... *Perspectives on Porcine Transgenesis and Cloning*, **Randall Prather**
10:00 am ..... Break
10:30 am ..... *Bioinformatics for Phenotypes of Human Diseases*, **Janan Eppig**, Jackson Laboratory
11:00 am–12:15 pm .. Selected Talk(s) from Abstracts. Discussion Lead by Session Moderators
11:00 am ..... *The Sino-Danish Pig Genome Project and Genetically Manipulated Pig Models,* **Lars Bolund**, Aarhus University,
11:15 am ..... *Pig Genome Resources at NCBI,* **Melissa Landrum**, National Center for Biotechnology Information
11:30 am ..... *Mouse Models of Human Cancers Consortium*, **Cheryl Marks**, National Cancer Institute
11:45 am ..... Insulin-like Growth Factor-I and Piglet Intestinal Development in Biomedical Models and Transgenics, **Sharon Donovan**, University of Illinois
12:00 noon ..... *Ossabaw Miniature Swine as a Novel Metabolic Syndrome and Subsequent Cardiovascular Disease,* **Emily Mokelke**, Indiana University
12:15 - 2:00 pm ..... Lunch (Moulin Rouge) and Review of Posters (International Ballroom)
2:00 - 6:00 pm ..... Clinical Implications: Lesson from Successful Models
Discussion Leaders: Thalachallour Mohanakumar (Washington University), Steven Niemi (Massachusetts General Hospital)
2:00 pm ..... *Value of Pigs in Transplantation Models*, **David Sachs**, Harvard Medical School
2:45 pm ..... *Diabetes and Porcine Islet Cells*, **Thalachallour Mohanakumar**
3:15 pm ..... *Porcine Diabetes Models,* **Michael Sturek**, Indiana University School of Medicine
3:30 pm ..... Break

4:00 pm ..... *A Genetically Malleable Porcine Model of Human Cancer*, **Christopher Counter**, Duke University

4:45 pm ..... *Porcine Eye Models*, **Michael Young**, Harvard Medical School

5:30 - 6:00 pm ..... Selected Talk(s) from Abstracts. Discussion Lead by Session Moderators

5:30 pm ..... *Transcriptional Profiling of Stress-Response in Cultured Porcine Islets,* **Scott Fahrenkrug**, University of Minnesota

5:45 pm ..... *Use of the MeLiM Swine Model to Search for Novel Loci of Hereditary Cutaneous Melanoma,* **Claudine Geffrotin**, National Institute for Agricultural Research

Saturday, January 29, 2005

7:30 am - 8:30 am ..... Continental Breakfast and Poster Reviews (International Ballroom)

8:30 am - 12:00 noon ..... Needs and Opportunities for Creating New Models
Discussion Leaders: Christopher Counter (Duke University), Walter Simson (Infigen, Inc.)

8:30 am ..... *Creating Porcine Genomics Platforms for Biomedical Applications*, **Lawrence Schook**

9:00 am ..... *Development of Resources,* **Lela Riley,** Director, National Swine Resource & Research Center, University of Missouri

9:45 am ..... Break

10:15 am ..... *Pigs in Bioengineering*, **Scott Hollister**, University of Michigan

11:00 am ..... *Porcine Viral Models of Human Diseases*, **Linda Saif**, Ohio State University

11:45 am-12:30 pm ... Selected Talk(s) from Abstracts. Discussion Lead by Session Moderators

11:45 am ..... *Use of Experimental Porcine Models to Provide Biomarkers of Human Nutrient and Disease Interactions,* **Harry Dawson**, ARS-USDA

12:00 noon ..... *The Genetics of Skin Wound Healing and Scarring: A Porcine Model,* **Corrie Gallant-Behm**, University of Calgary

12:15 pm ..... *Osteoblastic Differentiation of Porcine Derived Mesenchymal Stem Cells on 3D Scaffolds for Bone Tissue, Engineering.* **Aylin Sendemir-Urkmez**, University of Illinois

12:30 – 1:00 pm ..... Closing Discussions by Session Moderators

1:00 pm ..... Lunch (provided)

Departures

Speakers and Program Members (alphabetical)

Leif Andersson, Ph.D.

Leif Andersson is guest professor in Functional Genomics at Uppsala University and professor in Animal Genetics at the Swedish University of Agricultural Sciences in Uppsala, Sweden. The work in his group focuses on unraveling the molecular basis for phenotypic diversity in domestic animals, from coat color to metabolic traits. He is particularly interested in domestication as an evolutionary model and in using domestic animals for biomedical research. He and his group have generated highly informative intercrosses between the wild boar and domestic pigs as well as between the red junglefowl and domestic chicken. The intercrosses have been used for detection of Quantitative Trait Loci (QTLs). Andersson is an elected member of the Royal Swedish Academy of Sciences, the Royal Swedish Academy of Forestry and Agriculture and the Royal Physiographic Society in Lund.

Christopher M Counter, Ph.D.

Chris received his B.Sc. and Ph.D. in biology and molecular virology respectively at McMaster University. His postdoctoral training was in the laboratory of Robert Weinberg at MIT where he focused on cell immortality. He currently is an Assistant Professor in the Departments of Pharmacology and Cancer Biology, Radiation Oncology at Duke University Medical Center. He is the recipient of the MIT/MERCK postdoctoral fellowship, was appointed a Whitehead Scholar and a Kimmel Foundation Scholar. He also has received the Leukemia and Lymphoma Society Scholars Award. His research focuses on the mechanisms of neoplastic transformation and the molecular basis of telomerase function and regulation. His laboratory currently is exploring the use of the pig in cancer models and to dissect the complex human neuromuscular disease ataxia telectangesia as well as neurofibromatosis type I.

Sharon M. Donovan, Ph.D., R.D.

Sharon M. Donovan received her B.S. (1983) and Ph.D. (1988) in Nutrition from the University of California, Davis. After completing a post-doctoral fellowship in Pediatric Endocrinology at Stanford University School of Medicine (1988-1991), Dr. Donovan accepted a faculty position at the University of Illinois, Urbana as an Assistant Professor in 1991. She was promoted to Associate Professor in 1997 and Professor in 2001. In 2003, Dr. Donovan was named the first recipient of the Melissa N. Noel Endowed Chair in Nutrition and Health at the University of Illinois. Since 1999, Dr. Donovan has also served as Director of the Division of Nutritional Sciences, the interdisciplinary nutrition graduate program at the University of Illinois. Dr. Donovan's research focuses in the areas of neonatal nutrition, with an emphasis on optimization of neonatal intestinal development. Her work compares the biological effects of human milk and infant formulas on intestinal function in healthy neonatal piglets and in various models of intestinal disease. She has published over 70 peer-reviewed publications, review articles and conference proceedings. She is the recipient of a number of awards in recognition of her research contributions including the International Life Sciences Institute (ILSI) Future Leader in Nutrition award (1992), the Young Investigator Award from the International Society for Research in Human Milk and Lactation (1995), and the Mead Johnson Award from the American Society for Nutritional Sciences. On the University of Illinois campus, she received the College of ACES Faculty Award for Excellence in Research in 1997 and was named University Scholar from 1998-2001.

Janan T. Eppig, Ph.D.

Janan T. Eppig received her B.S. in Mathematics at the University of Washington and her Ph.D. in Genetics at the University of Maine. Her former work included studies on the genetics of ovarian teratomas and histiocytic sarcomas. She developed an early version of linkage analysis programs for automating map construction for mouse and was part of the program project that developed the Encyclopedia of the Mouse Genome software, the precursor to the Mouse Genome Database. She currently is a Senior Staff Scientist at The Jackson Laboratory in Bar Harbor, ME, USA and the Director of the Mouse Genome Database (MGD; http://www.informatics.jax.org). She is also PI for the Mouse Tumor Biology Database and co-PI of the Gene Expression Database. She is chair of the International Committee on Standardized Nomenclature for Mice, an appointed member of the Rat Genome and Nomenclature Committee, and recently completed a term on the NIH Genome Study Section. She is on the Advisory Board of a number of model organism database projects and interna-

tional experimental systems consortia. She is a founding member of the Gene Ontology Consortium (GO). Dr. Eppig's research interests include comparative genomics, genome organization, model systems for human disease, bioinformatics, and the development of database resources, including semantic standards for annotation and data sharing.

Erik J. Forsberg, Ph.D.

Erik J. Forsberg graduated magna cum laude from Kalamazoo College in 1979 with a B.A. in biology. He obtained his Ph.D. in 1983 at the University of Chicago in the Department of Pharmacological and Physiological Sciences. His dissertation focused on the control of hormone secretion from intestinal endocrine cells, work that led to a student research prize from the American Gastroenterological Association. Dr. Forsberg then worked as a Staff Fellow and Senior Staff Fellow in the Laboratory of Cell Biology and Genetics at the National Institutes of Health from 1983-89 where he studied signal transduction mechanisms in adrenal endocrine cells and vascular endothelial cells. In 1989 Dr. Forsberg was appointed as an Assistant Professor in the Department of Physiology at the University of Wisconsin Medical School where he studied the electrophysiological and biochemical events that control adrenalin secretion and vascular tone. In August of 1997, Dr. Forsberg joined Infigen as a scientist in the cloning laboratory and was promoted to Director of Cell Biology and Transgenesis in 1999. Dr. Forsberg was appointed as Infigen's Vice President of Development in 2001 and was responsible for production cloning, intellectual property management, and Quality Assurance. Erik also managed the relationships with Infigen's major partners including Pharming N.V. and Immerge BioTherapeutics, Inc. Recently, Dr. Forsberg accepted appointments at Minitube of America as the Director of Nuclear Transfer Technology and in the Department of Surgery at the University of Wisconsin as a Senior Scientist.

Merete Fredholm, Ph.D.

Merete Fredholm is a Professor of Animal Genetics in the Department of Basic Animal and Veterinary Sciences, The Royal Veterinary and Agricultural University (RVAU) in Copenhagen, Denmark. She received her D.V.M. degree in 1982, a Ph.D. in Animal Genetics in 1986 and a Doctorate of Veterinary Science in 1996, all from RVAU. Her Ph.D. thesis was titled "Immunogenetic investigations of the major histocompatibility complex in Danish Swine Breeds" and her doctoral thesis was titled "A molecular approach to investigation of basic mammalian genetics". Dr. Fredholm's current research interests are directed towards gene mapping and genome analysis in farm animals with emphasis on host pathogen interaction, disease genetics and comparative genomics. Furthermore, she is takes part in the international efforts towards sequencing the swine genome. She has published 65 publications in international, peer reviewed journals.

Scott J. Hollister, Ph.D.

Dr. Hollister is currently an Associate Professor of Biomedical Engineering, Surgery, and Mechanical Engineering at the University of Michigan, where he directs the Scaffold Tissue Engineering Group. He received his Ph.D. in Bioengineering from the University of Michigan in 1991. His research interests include bone and cartilage tissue engineering, computational design and fabrication of tissue engineering scaffolds for a wide range of tissue applications. He has received the New Investigator Award from the Orthopaedic Research Society in 1992, an award for Outstanding Research in Biomedical Engineering from the Whitaker Foundation in 1994, and the Henry Russell award for Teaching and Research from the University of Michigan in 2003.

Russ Jamison, Ph.D.

Russ Jamison is Professor of Bioengineering and Materials Science and Engineering at the University of Illinois at Urbana-Champaign. He is an affiliate of the Beckman Institute for Advanced Science and Technology and a core faculty member of the Institute for Genomic Biology. Professor Jamison joined the faculty in 1998. Previously he was Senior Vice President for Research and Development for Smith & Nephew Orthopedics, a developer and manufacturer of orthopedic implants and instruments. Before that Dr. Jamison was Associate Professor of Mechanical Engineering at the U.S. Naval Academy where he was director of the Composite Materials Laboratory. Professor Jamison received the Ph.D. degree from Virginia Tech in materials engineering science. He was senior visiting

research fellow at the University of Bath, England. His current research is in the area of tissue engineering approaches to hard and soft tissue replacement.

Harris A. Lewin, Ph.D.

Harris Lewin is Professor of Immunogenetics, with a primary appointment in the Department of Animal Sciences. Lewin was the founding Director of the W. M. Keck Center for Comparative and Functional Genomics, a nationally recognized facility that conducts genome research on microbes, plants and animals. He is currently Director of the new Institute for Genomic Biology at the University of Illinois. Professor Lewin's research interests are in the area of mammalian comparative and functional genomics, with particular emphasis on host genes affecting the progression of bovine leukemia virus infection. His research has resulted in the development of high-density comparative maps for the cattle and human genomes, and novel software for *in silico* gene mapping using the human genome as a template. In addition, his group produced the first large-scale cDNA microarray for functional genomics of ruminants. Lewin holds the Gutgsell Endowed Chair and is the recipient of several departmental, college, university and external awards and recognitions. He is Associate Editor of the journal *Animal Biotechnology* and serves on the Editorial Board of *Physiological Genomics.* Lewin chairs the Scientific Advisory Board for GenoMar AS, a Norwegian aquaculture and biotechnology company, and is on the Science Advisory Board of Pyxis Genomics, a company he founded. In 2004, he was elected as a Fellow of the American Association for the Advancement of Science.

Thalachallour Mohanakumar, Ph.D.

T. Mohanakumar, Ph.D. is the Jacqueline G. and William Maritz Professor of Surgery at Washington University School of Medicine in St. Louis, Missouri. He is a Professor of Surgery, Pathology and Immunology, and the Director of the Clinical Histocompatibility and Immunogenetics Laboratory at Barnes-Jewish Hospital, Washington University School of Medicine. Dr. Mohanakumar is also the Director of the Islet Isolation Facility at Washington University School of Medicine. Dr. Mohanakumar, received his Ph.D. in Microbiology and Immunology in 1974 from Duke University. He is currently funded for his research in Transplantation and Tumor Immunology by the NIH, Juvenile Diabetes Foundation, Susan G. Komen Foundation, and American Liver Foundation. Dr. Mohanakumar has been a member of several NIH Peer review groups for research funding and was the Chairman of the Transplantation Biology and Immunology Subcommittee. He is a member of the International Xenotransplantation Society, International Society Heart Lung Transplantation, Transplantation Society, American Society of Transplantation, American Society of Histocompatibility and Immunogenetics, and the American Society for Cancer Research. He is also a consultant for review groups of the Juvenile Diabetes Foundation and the US Department of Defense for Breast Cancer Research. He is currently serving as a member on the Editorial Board of scientific journals including Transplantation, Journal of Immunology, Human Immunology, and Transplantation Immunology. He was awarded the 2001 Fujisawa Basic Science Award by the American Society of Transplantation and a 2003 Distinguished Scientist Award from the American Society of Histocompatibility and Immunogenetics. He has trained several Ph.D. students, and post-doctoral fellows during his 30 year career, and is very proud of the fact that many of them are successful scientists/chairs in the field of Histocompatibility and Immunogenetics and Transplantation.

Steven M. Niemi, D.V.M.

Dr. Niemi has over 28 years experience in biomedical research and biotechnology, including executive positions in start-ups engaged in drug development, gene therapy, and genomics. He earned an A.B. in Biology from Harvard College, a D.V.M. from Washington State University, and was a Postdoctoral Fellow in the Division of Comparative Medicine at the Massachusetts Institute of Technology where he received a Public Health Service National Research Service Award. He later completed the Program for Management Development at the Harvard Business School.

Dr. Niemi is a Diplomate and past Director of the American College of Laboratory Animal Medicine. He has also served on the boards of the Biotechnology Industry Organization Food and Agriculture Governing Body, Illinois Biotechnology Industry Organization, National Association for Biomedical Research, Massachusetts Biotechnology Council, Massachusetts Society for Medical Research, and Scientists Center for Animal Welfare. Dr. Niemi has written or co-authored over 30 scientific publications and abstracts, and has served on numerous government-industry committees and task forces addressing medical product development and laboratory animal welfare.

Randall S. Prather, Ph.D.

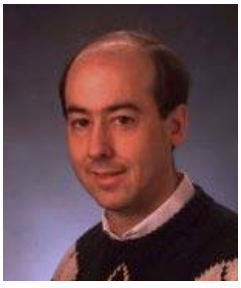
Since 1982 Dr. Prather's research has focused on the early mammalian embryo. He earned his B.S. and M.S. from Kansas State University, and Ph.D. and Postdoc from the University of Wisconsin-Madison. He is best known for his work on cattle and pig cloning by nuclear transfer. While at Wisconsin he cloned some of the first cattle by nuclear transfer. His group at the University of Missouri has created miniature pigs that have the alpha 1,3 galactosyltransferase gene knocked out, thus paving the way for xenotransplantation. In addition to his transgenic pig research, he and his collaborators have funding to conduct two projects that will identify new genes in the reproductive tissues of pigs and cattle. An understanding of the pattern of gene expression in these tissues will help to reduce the 30% loss of pregnancies that occurs in mammals. Other projects in his laboratory continue to describe the cellular and molecular program of events that occurs during development of the preimplantation pig embryo.

Lela K. Riley, Ph.D.

Dr. Lela K. Riley is Professor of Veterinary Pathobiology and Director of the Research Animal Diagnostic Laboratory (RADIL) at the University of Missouri. Her research interests include molecular mechanisms of infectious agents, emerging pathogens, development of novel diagnostic techniques, mammalian genetics and animal models of human health and disease. Along with her colleagues, Dr. Riley has established three NIH-funded Animal Resource and Research Centers: the Mutant Mouse Resource and Research Center (MMRRC), the Rat Resource and Research Center (RRRC), and the National Swine Resource and Research Center (NSRRC). All three centers serve as repositories and distribution centers for animal models. In addition, research conducted in the centers is designed to improve animal models and facilitate research with animal models.

Max F Rothschild, Ph.D.

Dr. Rothschild was born in Highland Park, Michigan in 1952. He received his B.S. in animal science and with a background in genetics at the University of California, Davis in 1974 and his M.S. at the University of Wisconsin in animal science in 1975. In 1978 he obtained his Ph.D. in animal breeding with minors in statistics and genetics from Cornell University. From 1978-1980 he was an assistant professor at the University of Maryland. In 1980 he joined the Department of Animal Science at the Iowa State University where he was eventually promoted to the highest rank of C.F. Curtiss Distinguished Professor of Agriculture in 1999. Recent research has been directed towards identifying genes controlling traits of economic importance in the pig. More recently he was named co-director of the Center for Integrated Animal Genomics. Since 1993 Rothschild has served as the USDA Pig Genome Mapping Coordinator. He is a member of many national and international societies. He has presented numerous invited papers in over 30 countries and has over 220 referred publications, 450 other publications and 5 patents. His awards include AAAS fellow, USDA Group Honor Award, ASAS award in Animal Breeding and Genetics, two R&D100 awards and was named Iowa Inventor of the year in 2002.



Mark S. Rutherford, Ph.D.

Mark Rutherford is an Associate Professor of Immunology, with a primary appointment in the Department of Veterinary and Biomedical Sciences, University of Minnesota. Rutherford is currently the Director of Graduate Studies for the Comparative and Molecular Biosciences graduate program. He is a member of the Mucosal and Vaccine Research Center, the Animal Biotechnology Center, and the Cancer Center. Dr. Rutherford's laboratory was responsible for cloning the porcine ß2-integrin molecules, CD18 and CD11b. Dr. Rutherford's current research interests are in the area of functional genomics of the host response to intracellular infection. He uses a primary alveolar macrophage culture system to examine changes in expression patterns subsequent to infection by the Porcine Reproductive and Respiratory Syndrome Virus. In addition, Dr. Rutherford's group has cloned many porcine expressed sequence tags and placed them on the porcine chromosomes by radiation hybrid mapping. Dr. Rutherford is also on the editorial board of the journal *Animal Biotechnology.*

David H. Sachs, M.D.

Dr. David H. Sachs is Professor of Surgery and Immunology at Harvard Medical School and Director of the Transplantation Biology Research Center at the Massachusetts General Hospital. He has worked at the interface between basic research and clinical applications in transplantation for more than 20 years, and has been active in the Transplantation Society throughout this time. His research interests include transplantation tolerance, xenotransplantation and immunogenetics of the MHC. Dr. Sachs was the recipient of the Public Health Service Commendation Medal in 1979 and of the Meritorious Service Award in 1984. He was a Councilor of the Transplantation Society from 1988-1994 and Vice-President from 1996-1998. From 1992-1996, Dr. Sachs was a member of the Immunobiology Study Section at the National Institutes of Health, and has served on the Immunology Executive Committee at Harvard Medical School since 1991. Dr. Sachs was elected to the Institute of Medicine of the National Academy of Sciences in 1996. He serves as Chairman of the Scientific Advisory Board of Immerge BioTherapeutics, Inc. and serves on the Scientific Advisory Board of the Lombard Odier Immunology Fund. In 1998 Dr. Sachs received the Jean Borel Award in Transplantation and the ASTP/Novartis Established Investigator Award. In 2001 Dr. Sachs was presented the Award for Distinguished Contributions to Health Research by The Medical Foundation and the Mary Jane Kugel Award by the Juvenile Diabetes Research Foundation.

Lawrence B. Schook, Ph.D.

Larry Schook received his B.A. (biology) from Albion College and his M.S. (microbiology) and Ph.D. (microbiology and immunology) from Wayne State School of Medicine. He performed postdoctoral training at the Institute for Clinical Immunology, Berne, Switzerland and at the University of Michigan. His research has focused on genetic resistance to disease and in the development of the pig as a model for biomedical research. Dr. Schook is the founding editor of Animal Biotechnology and was the Director of the Food Animal Biotechnology Center and Associated Dean for Research at the College of Veterinary Medicine at U. Minnesota. During that tenure he chaired the AAVMC Research Deans and Directors Committee. Currently at the U. Illinois, he is a Theme Leader in the Institute for Genomic Biology, his laboratory has developed human-pig comparative radiation hybrid map and characterized the swine MHC. Dr Schook has been recognized as a University Scholar, a Fellow at the National Center for Supercomputer Applications and is the recipient of the Pfizer Animal Health Award. He currently serves as the Co-Chair, International Swine Sequencing Consortium and Chairs the Steering Committee of the Alliance for Animal Genome Research. He has active collaborations for development of clinical models and most recently his laboratory has focused on technology platforms for using DNA sequence information to target genetic manipulations. Dr. Schook also serves on the Scientific Advisory Board for Pyxis Genomics an animal genomics company and on the boards of the Illinois Biotechnology Organization, the Food and Agriculture Governing Board of BIO and the ABIC Foundation.

Walter A. Simson III

Walter Simson is experienced as both a CEO and CFO in successful life sciences companies. As CEO of Infigen, Inc., a pioneer of nuclear transfer ("cloning") technology, he refocused the operation on opportunities in biopharmaceutical production and xenotransplantation research. This involved selling a division and substantial internal changes. The company is now focused on cloned animal models. The first application of this strategy is the knockout of the LDL receptor in miniature swine, with the cardiovascular device industry as the target market. Other novel models with applications in cardiovascular health, diabetes and oncology are also underway. He was formerly CFO of MJ Research, a DNA instruments company in Waltham, Mass., and CEO of Research Biochemicals, Inc. ("RBI") of Natick, Mass. Walter started his career at the Chase Manhattan Bank, where he worked in investment banking, syndications and workouts. He holds a B.A. from Columbia University and an M.B.A. from New York University. He has lectured in turnarounds and venture capital at both Columbia and NYU graduate Business Schools. In addition, he currently serves on the Board of Cambria Biosciences (Boston, Mass.) and Protein Genetics, Inc. (Madison, WI) as well as a number of community and cultural organizations. He lives with his family in Madison, Wisconsin.

Michael S. Sturek, Ph.D.

Dr. Sturek received his B.A. from Augustana College and his M.S. from Purdue University. Studies during his Ph.D. training at the University of Iowa encompassed rodent models of exercise and cardiovascular disease and comparative studies of ion transport in the vasculature. In postdoctoral training at the University of Chicago he compared ion channels in the coronary circulation between bovine and porcine species. On faculty at the University

of Missouri his studies on the coronary circulation in swine were mainly on effects of chronic exercise, hyperlipidemia, and diabetes. Studies using a wide variety of clinically relevant methods, such as angiography, intravascular ultrasound, coronary flow velocity, echocardiography, coronary angioplasty and stent placement, and in vivo glucose regulation enabled comparison to human clinical studies. Sturek established that chemically-induced swine models having gross diabetes (hyperglycemia) and dyslipidemia show numerous cardiovascular complications similar to humans, including accelerated atherosclerosis, retinopathy, autonomic neuropathy, and cardiomyopathy. Nearly 3 years ago Sturek conducted an expedition to Ossabaw Island, Georgia to obtain the endangered Ossabaw pig. The "thrifty genotype" of the obese Ossabaw gives rise to naturally occurring "pre-diabetes" ("metabolic syndrome"). Selective breeding efforts are underway to optimize the line. Sturek recently joined the Indiana University School of Medicine as Chair of Cellular & Integrative Physiology. In the Comparative Medicine Program between Indiana and Purdue University Sturek is expanding the Ossabaw colony for studies of the constellation of factors underlying the metabolic syndrome. These studies have been funded by the National Institutes of Health and American Diabetes Association.

Kelly Tappenden, Ph.D.

Dr. Kelly Tappenden received her Ph.D. in Nutrition and Metabolism at the University of Alberta and clinical training, as a Registered Dietitian, at the Misericordia Hospital in Edmonton, Alberta. After completing a post-doctoral fellowship in Gastrointestinal Physiology at the University of Texas - Houston Medical School, she joined the University of Illinois at Urbana-Champaign as an Assistant Professor of Nutrition in 1997. In 2003, Dr. Tappenden was promoted to Associate Professor. The long-range goal of Dr. Tappenden's research program is to understand the regulation of small intestinal function by various nutrients and gut-specific peptides. She has received national and international recognition for exceptional innovation regarding the hypotheses that she tests and the diversity of experimental models that she uses. Dr. Tappenden's skill with porcine biomedical models has established her as a resource for scientists at the University of Illinois and other institutions. Further, Dr. Tappenden has served as an active member of the Institutional Animal Care and Use Committee since 2000. Peer recognition for her expertise has led investigators from institutions as far as Australia and Denmark to travel to the University of Illinois to learn surgical skills employed in Dr. Tappenden's laboratory. Dr. Tappenden also has traveled internationally to teach other scientists regarding the intricacies of some of the porcine models used in her laboratory.

Hiroshi Yasue, Ph.D.

Dr. Hiroshi Yasue received his Ph.D. from the Osaka University (Biological Chemistry, Faculty of Science) in Osaka, Japan in 1977. He started his career as a research staff in the department of viral oncology in Aichi Cancer Center. In 1986 he moved to the National Institute of Animal Industry. Since then, his research activities have been focused on animal genomics, especially on swine genome analysis. He developed his research in the INRA Laboratory at Jouy-en-Josas (France) from 1988 to 1989 and also in the University of Minnesota (USA) in 1999. Currently he serves as Research Leader at the Genome Research Department in the National Institute of Agrobiological Sciences and focuses on comparative and functional genomics. He is also appointed as a collaborative research staff in the Human Genome Research Group of Riken Yokohama Institute. He is on the Editorial Board of Animal Biotechnology. Dr. Yasue has been a member of ISAG since 1994 and serves currently as a member of the domestic animal genome sequencing committee in ISAG. He also serves as a member of ISAG Executive Committee from 2004.

Michael J. Young, Ph.D.

Michael Young received his B.S. degree in behavioral neuroscience from the University of Pittsburgh in 1989. He then received his Ph.D. in anatomy/neuroscience from the University of Cambridge in 1995. His thesis work involved the study of intracerebral retinal transplantation, and how transplant- and –derived information is integrated in the central nervous system. A postdoctoral fellowship at the Institute of Ophthalmology, University College, London in 1995 was followed by a postdoctoral fellowship at the Massachusetts Institute of Technology, Department of Brain and Cognitive Sciences. In 1998, Dr. Young joined the Schepens Eye Research Institute. He was promoted to Assistant Scientist in 2000, and Assistant Professor in the Department of Ophthalmology, Harvard Medical School in 2001. He was named the director of the Minda de Gunzburg Research Center for the Retinal Transplantation in 2002. His research involves the use of stem cells derived from the central nervous system, and neuroretinal transplantation in animal models of retinal degeneration.

Abstracts

Bioengineering (BE)

BE1.

Comparison of Osteogenic Potential between Porcine and Human Bone Marrow Derived Stem Cells, In Vitro

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In recent years, the pig has increasingly become the subject of biomedical research, particularly in the area of bone tissue engineering. Due to similar bone turnover rates, bone size, and nutritional habits between pigs and humans, the pig is a promising animal model for humans for bone regeneration by mesenchymal stem cells (MSCs). However, there is a lack of knowledge on the differences between the osteogenic differentiation characteristics of porcine MSCs and human MSCs. To verify the suitability of the pig to serve as an animal model for human bone healing, an in vitro comparison will be used to assess the osteoblastic differentiation potential and expression differences between human and porcine MSCs.

Stem cells from the bone marrow of humans and pigs were obtained. The potential for osteoblastic differentiation of the stem cells is determined in vitro by differentiating human MSCs and porcine MSCs into osteoblasts on 2D polystyrene tissue culture plates with osteogenic media for four weeks. The results of the osteoblastic activity of the cultured cells are presented according to morphology, proliferation rate, and expression of osteoblastic phenotypic markers. The presence of osteoblastic phenotypic markers are determined by alkaline phosphatase, calcified matrix, collagen, osteocalcin and osteonectin through chemical assays, histological staining and reverse-transcriptase polymerized chain reaction. This work will demonstrate if the pig is a suitable animal model for studying the role of MSCs in bone regeneration in humans.

BE2.

Osteoblastic Differentiation of Porcine Derived Mesenchymal Stem Cells on 3D Scaffolds for Bone Tissue Engineering

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Mesenchymal stem cells (MSC,s) are attractive for use in tissue engineering constructs since they are believed to be part of natural healing mechanism of adult tissues. Among other lineages, the osteoblastic differentiation potential of MSC,s is particularly well-documented. The majority of the MSC research is, however, conducted in rodents, which differ from humans substantially in terms of size, skeletal mechanics, bone turnover rate and healing properties. In our laboratories, we have developed a porcine mandibular model that better represents human bone for tissue engineering research. Porcine bone marrow derived MSC,s have been shown to differentiate into osteoblastic phenotype in vitro on 2D, but little is known about their differentiation on 3D scaffolds. For tissue engineering applications, it is essential that cell viability and differentiation on 3D constructs before and after implantation be maintained.

We have successfully cultured bone marrow derived porcine MSC,s and shown their osteoblastic differentiation on chitosan/biphasic calcium phosphate porous scaffolds for up to four weeks. The cells produce collagenous extracellular matrix on the surface and within the bulk of the scaffolds. The alkaline phosphatase production was detected at two weeks, and peaked at three weeks showing a similar pattern with the rat and mice MSC,s and confirming early stages of osteoblastic differentiation. The evaluation of the production of other osteoblastic marker genes is in progress. The expression of these markers will be

compared to those of the same batch of cells on 2D culture conditions evaluating the effects of 3D structure on cell differentiation.

BE3.

Refinement of Techniques for Implantation of Biomaterials

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The implantation of biomaterials such as catheters, cannulas and interventional devices (stents) are some of the most common surgical procedures performed in swine in research. Swine have species-specific rquirements for implantation of devices in order to prevent postoperative complications. Behavioral and caging characteristics need to be taken into account. Because swine tend to rub the area of implantation against their caging, implanted devices need to be located on the dorsum and the cage environment needs to be free of structures that could result in self-injury. Non inflammatory suture materials and subcuticular suture patterns need to be utilized for incisional closure. Proper design of devices and material selection are key issues. The rapid growth rate of swine needs to be considered during the design phase in order to prevent dislodgement of catheters and cannulas over time. Strict aseptic technique, inclusing the use of iodine impregnated adhesive drapes, can be utilized to reduce postoperative complications. Antibiotics should not be necessary to prevent infection if proper procedures for implantation, maintenance and access of implanted devices are followed. Complication rates of >10% should not be encountered in our experience if these procedures are followed.

Cardiovascular (CV)

CV1.

Coronary Vascular Effects Of Cyclooxygenase-2 Inhibition in Hypercholesterolemic Swine

M. Alloosh[1], E. A. Mokelke[1], N. J. Dietz[1], J. M. Sturek[1] and M. Sturek[1,2]
Medical Pharmacology & Physiology[1] and Internal Medicine[2], School of Medicine, University of Missouri, Columbia, Missouri, USA 65212

Hypercholesterolemic swine were treated with the selective cyclooxygenase-2 (COX-2) inhibitor SC-236 to clarify the role of chronic COX-2 inhibition in coronary disease and K+ current regulation in smooth muscle. Control (n=7), high fat/cholesterol fed (hypercholesterolemic, H, n=7), and hypercholesterolemic treated with SC-236 (SC-236, 1 mg/kg twice weekly, n=6) were maintained 20 weeks. In vivo intravascular ultrasound assessed conduit vasoreactivity and atheroma and Doppler blood flow velocity assessed microvascular reactivity. SC-236 treated pigs had increased blood pressure and greater constriction to prostaglandin F2a (PGF, 8 mg/kg) vs. H, while SC-236 dilated less to bradykinin vs. control and H ($p<0.05$). Atheroma in SC-236 was greater only than control, while H did not differ from control or SC-236. The increase in bradykinin-induced (4 ng/kg) blood flow after PGF-induced constriction was less in SC-236 vs. control. In vitro relaxation to bradykinin (10-11 to 10-6 M) was impaired in SC-236 vs. control and H. Steady state and spontaneous transient outward K+ currents were decreased in conduit and microvascular smooth muscle, respectively, in SC-236 vs. control and H. We conclude that in hypercholesterolemic swine COX-2 regulates blood pressure and protects coronary artery. Support: NIH RR13223, HL62552, HL10474, Pfizer.

Fig 1. Impact on CD3+ Lymphocytes

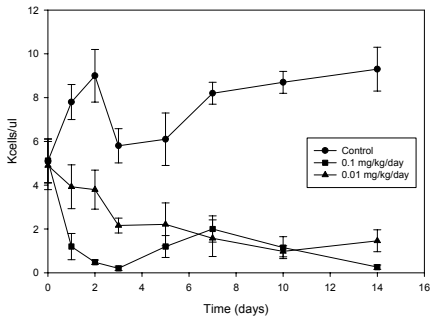
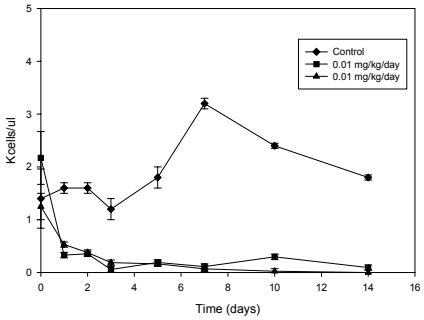


Fig 2. Impact on CD21+ Lymphocytes



CV2.

Adaptive Coronary Vessel Remodeling Following Surgical Coronary Artery-to-Left Ventricle Stent (VSTENT˙) Implantation

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Background: Recently, a left anterior descending (LAD) artery-to-left ventricle (LV) conduit has been developed as a novel approach to myocardial revascularization. Initial feasibility experiments have demonstrated that this conduit provides forward flow to the distal LAD during proximal LAD occlusion and preserves regional myocardial function. In the present experiments, we evaluate the chronic physiological reaction in the absence of proximal LAD occlusion following conduit placement in the mid-LAD.

Methods and Results: Eight pigs (40-60 kg) were implanted with an ePTFE-covered, balloonexpandable stent (VSTENT˙) through the posterior wall of the mid-LAD to LV via a left thoracotomy. Coronary angiography was performed immediately post-implant to confirm VSTENT˙ patency. The maximal luminal diameter of the proximal LAD and left circumflex (LCX) artery was measured before VSTENT˙ placement, immediately after implantation, and at 28 days post-implant. A bi-directional flow pattern is seen in the LAD immediately proximal to the conduit, with retrograde systolic flow from the LV to the LAD and large antegrade diastolic flow from the proximal LAD to the LV and distal LAD. Vessel wall morphometry indicated that the significant increase in luminal diameter was accompanied by a proportionate increase in wall thickness, suggesting that the remodeling response was accompanied by proliferation, which facilitated conservation of wall stress.

Conclusion: The initial results demonstrate significant coronary flow-mediated structural vessel remodeling in the LAD due to the presence of the LAD-to-LV conduit at 28 days post-implantation.

CV3.

Intrapericarial Paclitaxel Delivery Does Not Show Beneficial Effect on Neointimal Proliferation in the Porcine Coronary In-Stent Stenosis Model

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Background: Paclitaxel is a clinically-effective antiproliferative agent which has been shown to inhibit neointimal proliferation and promote positive remodeling after intrapericardial (IPC) space delivery in the porcine coronary balloon overstretch injury model. This study tested whether IPC delivery of paclitaxel would reduce coronary in-stent stenosis.

Methods: 20 mg (Treatment, n = 5) of paclitaxel bound to a carrier or 20 mg copolymer control carrier (Control, n = 4) was administered IPC after implantation of oversized stents in the porcine coronary arteries (stent:artery = 1.3 : 1). The animal was sacrificed at 28 days after repeat angiography and intravascular ultrasound (IVUS) were performed. Coronary arteries were perfusion fixed and processed using the plastic embedding method. Serial sections were evaluated by morphometric analysis.

Results: Arterial injury scores were no different between the two groups. Both IVUS and histologicial data revealed no inhibitory effect on neointimal growth 28 days post-injury.

Conclusion: Single-dose IPC delivery of paclitaxel does not reduce neointimal proliferation in this porcine coronary artery in-stent restenosis model.

CV4.

Ossabaw Miniature Swine as a Novel Model of the Metabolic Syndrome and Subsequent Cardiovascular Disease

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The metabolic syndrome is characterized by obesity, insulin resistance/hyperinsulinemia, glucose intolerance, dyslipidemia (hypertriglyceridemia and low HDL cholesterol), and hypertension. We present a novel porcine model to study the metabolic syndrome. Ossabaw and Yucatan miniature swine were fed a low fat diet, (8% kcal from fat, LF), or 2 versions of a high fat diet (46% kcal from fat, H46 or 75% kcal from fat, H75, both 2% cholesterol). Duration of the dietary treatment ranged from 20-40 weeks. Ossabaw fed either H46 or H75 had ~2-fold greater carcass adiposity vs. LF fed swine and Yucatan fed H46 ($p<0.05$). Intravenous glucose tolerance tests (IVGTT) showed higher peak blood glucose and insulin in Ossabaw vs. Yucatan (286 + 9 vs. 248 + 14 mg/dl, and 65 + 6 vs. 41 + 8 U/ml, respectively). Ossabaw on H75 had slower glucose clearance during IVGTT than Ossabaw on H46 or LF diets. Ossabaw fed H46 had a 115 % increase in serum triglycerides, whereas Yucatan fed H46 showed no increase compared to LF fed controls. Obese Ossabaw became hypertensive, whereas obese Yucatan did not. A stent was deployed in the circumflex coronary artery. After 3 weeks, mean neointimal hyperplasia in Yucatan fed LF was similar to Yucatan fed H46, whereas in Ossabaw fed H46 there was greater neointima vs. Ossabaw fed LF. CONCLUSIONS: Ossabaw miniature swine are a novel humanoid model of the metabolic syndrome uniquely suited for the study of coronary artery disease and interventional devices. Support: NIH RR13223, T32 HL07094, T32 AR48523, T32 RR07004, American Diabetes Association.

CV5.

Caspase-3 Levels are Elevated in Myocardium of Hypercholesterolemic Ossabaw Minipigs

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Ossabaw swine were isolated on a barrier island near Georgia ~500 years ago and developed a „thrifty‰ genotype to survive cycles of seasonal food shortage. We have previously shown that they will present with hypertension, dyslipidemia, impaired glucose tolerance and central (intra-abdominal obesity) when fed a hyperlipidemic diet. They therefore represent a novel humanoid model of the metabolic syndrome, which may further manifest into type 2 diabetes. We tested the hypothesis that Ossabaw minipigs fed a high fat/cholesterol (2%) diet would show evidence of lipotocity in the myocardium. Male Ossabaw minipigs were randomly assigned to 2 groups: control (C, n= 4) and hyperlipidemic (H, n=7). Control animals were fed normal minipig chow (8% kcal from fat) and H animals received minipig chow supplemented with extra fat (46% kcal from fat) and 2% cholesterol. Upon completion of the study (~44 weeks), cardiac tissue were prepared for both histochemistry and immunoblotting. Oil-Red-O staining resulted in a significantly higher accumulation of lipids in the myocardium of pigs in the H group ($P<0.05$). Western blots were performed for the pro-apoptotic protein Caspase-3. There was a significant increase in Caspase-3 protein expression in the myocardium harvested from the H group, ($P<0.05$). These data suggest that apoptotic events triggered by the accumulation of lipids in the myocardium might contribute to cardiac dysfunction resulting from insulin resistance.

CV6.

Anesthesia for Swine with Cardiovascular Compromise

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One of the most common uses of swine in biomedical research is the study of cardiovascular diseases. Disease conditions such as myocardial infarction and heart failure due to volume or pressure overload are produced by a variety of surgical procedures. Swine, especially domestic breeds, have a predisposition to the development of fatal cardiac arrhythmias. Management of resultant clinical signs associated with these disease conditions is essential for successful post-procedural maintenance of these models. In our laboratories, anesthesia and perioperative care protocols have been developed to prevent cardiac arrhythmias and death due to heart failure for these types of procedures. High dose opioid infusion with sufentanil increases myocardial oxygenation and is protective against arrhythmias and techniques have been developed to utilize this regimen for swine with heart failure. Ketamine/midazolam infusion techniques have proven to be protective against cardiac arrhythmias stimulated during intracardiac pacing and conduction system ablation procedures. Bretylium was utilized as a Class III antiarrhythmic agent for cardiac surgery, but it has been recently withdrawn from the market. New techniques involving amiodarone infusions have been developed to replace bretylium. Use of indomethacin and corticosteroids have proven effective in the prevention of postperfusion pulmonary hypertension following cardiopulmonary bypass. Consideration of the physiologic effects of analgesics and appropriate use of supportive drugs makes it possible to perform complex cardiac surgical procedures in swine.

CV7.

Comparing Two Modalities of Myocardial Gene Delivery: Percutaneous Retrograde Coronary Venous Delivery and Intramyocardial Injection

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ICVBM, Krannert Institute of Cardiology, Indiana University School of Medicine; Roudebush VA Medical Center, IN

Background: Myocardial gene therapy is emerging as a novel modality for treating heart disease. However, the optimal technique maximizing safety and efficacy for myocardial gene delivery is not well established. In this study we evaluated two delivery techniques in swine; percutaneous retrograde coronary venous delivery (RCVD) and direct intramyocardial injection (IM). Methods: RCVD was performed in the anterior interventricular vein (AIV) by an occlusion balloon catheter. A 10 ml of gWiz˙ plasmid encoding £]-gal (1mg/ml) was injected using either manual high-pressure (HP-RCVD, n = 5) or pressure wire guided low-pressure (LP-RCVD, n = 4). In the direct intramyocardial injection (IM) group (n = 4) £]-gal plasmid (5 mg/ml) was injected at 10 sites (200 µl/site) in the anterior wall of the left ventricle. Animals were euthanized after 5 days.

Results: The percentage of £]-gal expressing cells in the delivered region was higher in the HP-RCVD (0.26 ± 0.05%) than in the LP-RCVD (0.05 ± 0.03 %, p = 0.07) and IM groups (0.02 ± 0.01%, p = 0.01). Enzymatic activity in the myocardium of the high pressure RCVD group was also 7 and 17 fold higher than low pressure RCVD and DMI groups, respectively (P = 0.05 for both).

Conclusion: The results confirm the superiority of HP-RCVD in myocardial plasmid delivery compared to LP-RCVD and IM.

CV8.

Fate of Endothelial Progenitor Cells after Intramyocardial, Intracoronary, and Retrograde Coronary Venous Delivery; Implications for Current Clinical Trials

E. A.-S. Youssef, P. Zhang, P. Rogers, B. Johnstone, K. March and D. Hou.
Indiana University Medical School, Indianapolis, IN

Background: Several clinical studies are evaluating the therapeutic potential of progenitor and stem cells for treatment of injured hearts. Although a localized effect of the cells is desired for optimum safety and efficacy, the actual fate of delivered cells has not been thoroughly assessed. We evaluated the short-term fate of endothelial progenitor cells (EPC) after direct intramyocardial (IM), intracoronary (IC), and retrograde coronary venous delivery (RCVD) in an ischemic swine model.

Methods: 16 Yorkshire cross swine of mixed sex were randomly assigned to three groups. Myocardial ischemia (MI) was created in each animal by 45 min. balloon occlusion of the proximal LAD artery. Six days after ischemia, 10 million Indium-111 labeled human EPC were delivered via IC (n = 5), IM (n = 6), or RCVD (n=5) injections. The overall distribution of injected cells was assessed by whole body radioactive emission scans. For quantitative assessment, multiple organs were harvested and assayed using £^-emission counting, The MI area was determined by tetrazolium trichloride (TTC) staining of tissue sections.

Results: Whole body scans showed that the majority of EPC delivered by each modality are entrapped in the lungs. Within the myocardium, significantly more cells were retained following IM injection (27.5 ± 17%) compared to IC (5.1 ± 1%) (p = 0.02) and RCVD (6.5 ± 1%) (p = 0.02) infusion.

Conclusion: The clinical implications of these findings are potentially significant, as these proangiogenic cells may have undesirable effects in the lungs. Also, we found that IM injection is more efficient in EPC delivery than IC and RCVD techniques.

CV9.

Ossabaw Swine Having the Metabolic Syndrome Exhibit Greater Neointimal Hyperplasia After Coronary Stent Placement Than Lean Yucatan Swine

M. N. Zafar, S. Kaser, E. A. Mokelke, M. Alloosh, M. C. Dyson and M. Sturek

BACKGROUND: Most studies in porcine models show no greater restenosis after coronary stenting in hyperlipidemia vs. normolipidemia. HYPOTHESIS: Hyperlipidemic (H) Ossabaw swine will exhibit greater neointimal hyperplasia after coronary stent deployment than Yucatan H swine.

METHODS: Male swine were Yucatan on control diet (C, n=5) or 2% cholesterol diet (H, n=3) and Ossabaw C (n=6) and H (n=4). Coronary angiography and intravascular ultrasound (IVUS) were performed at 40 weeks. Multi-link bare metal stents were placed in with apposition verified by IVUS. IVUS was repeated three weeks later. Mean area of neointimal hyperplasia was calculated from the cross sectional area of internal elastic lamina minus lumen cross sectional area in the stented segment.

RESULTS: Both H groups were hypercholesterolemic, while only Ossabaw H had visceral adiposity, hyperinsulinemia, and glucose intolerance compared to Yucatan H. Mean neointimal hyperplasia in Yucatan C (1.63+0.59 mm2) was not different from Yuc H (2.26+0.50 mm2), whereas Ossabaw C (1.072+0.54 mm2) was less than H (2.64+ 0.72 mm2) ($p < 0.05$).

CONCLUSIONS: Hyperlipidemia resulted in greater neointimal hyperplasia in stented segments of Ossabaw swine, which may be a better large animal model of the metabolic syndrome for the study of coronary restenosis. Support: NIH RR13223, T32 HL07094, T32 AR48523, American Diabetes Association.

Cancer (CA)

CA1.

A Porcine Model for the Study of Tumorigenesis and Cancer

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Given differences in some aspects of the process of tumorigenesis between humans and mice, we sought to exploit the pig as an experimental and preclinical model for human cancer. Pigs offer the advantage of having a similar size, diet, metabolism, lifespan, and and anatomy to humans. To this end, we have shown that porcine cells can be genetically converted to a tumorigenic state, as assessed in immunocompromised mice, by expression of genes known to promote tumorigenic growth of human cells. Moreover, when such cells are returned to the host animal, tumors grew. However, this growth depended on suppression of the immune system, likely owing to the reliance on expressing human genes for this tumorigenic conversion. Future studies will determine whether porcine cells can be driven to malignant fates and the need for immunosuppression can be overcome in pigs through the use of porcine genes to drive tumorigenesis. Ultimately, if we are successful in creating a non-immunocompromised porcine tumorgenesis model, we will use the ability to clone pigs to create a tumor model system that employs genetically identical animals with genetically identical tumors for pharmaceutical screening of anti-cancer therapeutics.

CA2.

Use of the MeLiM Swine Model to Search for Novel Loci of Hereditary Cutaneous Melanoma

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[4]Faculty of Agriculture, Kagoshima University, Kagoshima 890-0065, Japan
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Human cutaneous melanoma has been one of the fastest-rising malignancies with a yearly increasing incidence over the past several decades, and accounts for about 10% of all melanoma cases. To search for new melanoma susceptibility loci, we started the genetic analyses of the MeLiM (melanoblastoma-bearing Libechov minipig) swine model, which may help to solve the difficulties encountered in human melanoma study.

We performed a genome-wide scan using 200 backcross animals after clinical and histological phenotyping, which were derived from affected MeLiM F1 pigs and healthy Duroc founders. Quantitative trait loci (QTLs) on Sus Scrofa chromosomes (SSC) 1, 7 and 10, were associated with melanoma development, as well as some regions on SSC 2, 6, 8 and 14 after linkage and association tests. Furthermore, after sequencing MC1R (melanocortin-1 receptor) coding region and then SSCP genotyping, black pigs with MC1R allele 2 were found to have predisposition to develop melanoma, after calibration of the coat color effect.

Massive radiation hybrid (RH) mapping comparatively localized orthologous genes on Homo Sapiens chromosome (HSA) 9p21, which was related with SSC1 chromosomal regions of some putative QTL(s). Our previous work and the characteristic composition pattern of SSC1 putative QTL peaks compared to HSA9p21 region suggested that novel putative gene(s) underlying melanoma susceptibility might be in these regions, which needs further investigation.

CA3.

National Cancer Institute - Mouse Models of Human Cancers Consortium

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Division of Cancer Biology, NCI

In 1999, the National Cancer Institute (NCI) confronted the need for improved model systems to inform basic, clinical, epidemiologic, and translational cancer research. The ability to manipulate the germline of mice and mouse genomics resources prompted the NCI to implement a collaborative cancer modeling project, the Mouse Models of Human Cancers Consortium (MMHCC). The initial program of 19 groups, with expertise in many aspects of human cancer research and mouse genetics, was recently enlarged to 25 to accommodate expanded goals that leverage advances in in vivo imaging and mathematical modeling, and in genomics of model organisms. The increase to 25 groups required the NCI to revisit how to manage the MMHCC to preserve the free exchange of ideas and spirit of cooperation that are great strengths of the program. The MMHCC members and the NCI convene numerous roundtables, hands-on workshops, and other open forums to promote mouse cancer science and its interface with other cancer models.

The 300-member MMHCC cooperates with the NCI Center for Bioinformatics to evolve a systems biology approach to human cancer research, providing the informatics platforms to blend descriptive information from model systems with comparable human disease data. The Center maintains Cancer Models and Cancer Images databases, to which any researcher may submit data, ensuring that these databases reflect the experience of all cancer researchers who explore how well model systems inform human cancer science. The eMICE website (http://emice.nci.nih.gov) provides access to the NCI's preclinical models programs, resources, databases, and the NCI Mouse Repository.

CA4.

Type I Collagen Synthesis is a Critical Parameter for Melanoma Progression

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The tumor microenvironment is a critical parameter of melanoma progression. Here we show that progressive stages of melanoma are each embedded within a unique stromal context: (i) superficial and micro-invasive melanoma that is confined to the papillary dermis displays de novo expression of type I collagen mRNA synthesis by fibroblasts juxtaposed to tumor cell nests and (ii) deeply invasive melanoma within the reticular dermis that does not evidence type I collagen expression and in which degradation of pre-existing collagen occurs. In addition, only melanoma cell lines that give rise to metastases in nude mice can inhibit type I collagen synthesis by dermal fibroblasts.

Type I collagen is the predominant extracellular matrix protein of skin and its remodeling is critical for angiogenesis. Furthermore, concurrent modulation of matrix protein synthesis and degradation by host and tumor cells enables tumor growth and invasion of most neoplasms.

In order to test if altered collagen turn-over affects melanoma progression, we have inhibited its synthesis using the pharmacological inhibitor Halofuginone during the development of melanomas in the Melanoblastoma-bearing Libechov Mini Pig melanoma model. Attenuated collagen synthesis attenuated angiogenesis, reduced tumor thickness and the occurrence of deeply invasive melanomas. These data indicate that changing the microenvironment of a developing melanoma modulates its progression.

CA5.

The Melanoblastoma-Bearing Libechov Minipigs: An Accurate Model to Improve the In Vivo Detection Of Melanoma and the Knowledge of Anti-Tumoural Self Defence

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Spontaneous animal tumours appear as suitable models to study human oncology and cancer therapy. Cutaneous melanoma is a highly aggressive form of skin cancer ; it is now the most rapidly increasing malignancy in the Caucasian population with a high mortality rate. The porcine skin presents morphological and functional characteristics similar to human skin in contrast to the rodent one. We have imported from Czech Republic the MeLiM model, a swine melanoma model, which was built from the Hormel strain as the Sinclair model. Clinically, pigmented lesions were flat, raised and polypoid. Swine cutaneous melanoma arise spontaneously in utero or shortly after birth and disseminate mostly in loco-regional lymph nodes and less frequently in visceral organs ; most of them spontaneously regress during the first year of life. Histologically, the lesions were distributed among intra-epidermal lesions until invasive Clark,s level V melanomas resembling those observed in humans; regressive melanomas are infiltrated precociously by highly pigmented histiocyte-like cells and later by lymphocytes and fibrosis. Depigmentation of hair, skin and iris followed the first signs of regression. We evaluated this swine model in in vivo imaging and showed that FDG PET is effective in the staging of cutaneous melanoma and the follow up of tumoural extension and regression in the MeLiM melanoma. The results obtained in this animal model show similar trends as the human cutaneous melanoma. Accordingly, this model may be useful to test new tracers specific for melanoma imaging and help to detect molecules expressed early during tumoural regression.

Clinical Models (CM)

CM1.

Down and Out with Porcine CFTR; towards a large animal model of Cystic Fibrosis

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Cystic fibrosis (CF) is the most common life-shortening disease in Caucasians, affecting between 1 in 2,000 and 1 in 4,500 individuals. The gene involved in CF, the transmembrane conductance regulator (CFTR), was identified in 1989. Although mouse models of CF have been generated which manifest some of the electrophysiologic characteristics of CF, their benign pulmonary phenotype renders these models somewhat irrelevant. We have undertaken the development of a porcine model of CF.

A two pronged approach has been adopted for ablation of porcine CFTR function; homologous recombination to introduce the ΔF508 mutation, and RNAi of porcine CFTR using the *Sleeping Beauty* (SB) Transposon system. Our cellular resources include traditional porcine fetal fibroblasts (PFF) and pig multipotent adult progenitor cells (pMAPC). pMAPCs may be ideal for the extended culture required for double selection for homologous recombination, as they can proliferate extensively in culture (>130 PDs) without obvious senescence or loss of differentiation potential.

We have used BAC recombineering to construct a series of replacement vectors harboring the ΔF508 mutation in combination with both positive and negative selection cassettes for homologous recombination. For RNAi, shRNA-expressing transposons have been developed and tested in a porcine cell line that expresses CFTR. CFTR mRNA knockdown (90-94%) was demonstrated by quantitative PCR, in agreement with an equivalent loss of function based on apical membrane Cl^- current. The status and charac-

terization of PFFs and pMAPCs transfected with constructs for both approaches will be presented, along with plans for developing a pig genetic model by nuclear transfer.

CM2.

Total Parenteral Nutrition Induces Sulfomucin Goblet Cell Expansion in the Small Intestine of a Neonatal Piglet Model

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Total parenteral nutrition is associated with complications of gastrointestinal (GI) development or function, though underlying mechanisms are poorly understood. We have observed in parenterally-fed piglets (TPN) elevated inflammatory indices in the small intestine (SI), including increased sulfomucin-positive goblet cells coinciding with local T-lymphocyte expansion and decreased enterocyte migration rates. Sulfomucins are resistant to microbial enzymatic-degradation, potentially augmenting GI resistance to bacterial translocation. Goblet cell (GC) differentiation toward a sulfomucin lineage may reflect direct effects of T-cell products or reflect proliferative dysfunction in intestinal crypts. To examine these postulates, ileal GC numbers and chemotype and T-lymphocyte (T-cell) numbers were compared in 100% enterally-fed (TEN), TPN- and PEN-fed (80% parenteral/ 20% enteral) 7-day-old piglets. Ileal histological sections were stained with alcian blue/PAS-hematoxylin and high iron diamine to detect sulfomucin goblet cells and with antibodies to porcine CD8 and CD4 to identify T-cells. All sections were normalized by area. Four-fold increases in villus sulfomucin GC numbers in TPN and PEN vs. TEN (P<0.02), and a 3-fold increase in crypt sulfomucin GC numbers in PEN vs. TEN (P<0.03) were observed. Villus $CD8^+$ and $CD4^+$ T-cell numbers increased in TPN vs. TEN (P<0.05), but were normalized in PEN vs. TPN (P<0.05). TPN also increased crypt $CD4^+$ T-cell numbers vs. TEN (P<0.05). Thus, enteral stimulation down-regulated TPN-induced T-cell inflammation in PEN without affecting GC numbers. Together these data indicate that TPN-associated GC responses may not be directly mediated by T-cells, supporting the alternative postulate that sulfomucin goblet cell expansion may reflect proliferative dysfunction in intestinal crypts

CM3.

The Genetics of Skin Wound Healing and Scarring˜A Porcine Model

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Swine have recently been introduced as models for skin wound healing research due to their anatomical similarities to humans. We have characterized the healing response of excisional wounds in juvenile female Yorkshire (Y) pigs, and have determined that healing closely resembles the normal process in humans. In contrast, identical wounds in female red Duroc (RD) pigs contracted significantly more, resulting in a 90% reduction in wound area. This hypercontraction was associated with marked hyperpigmentation at the wound margins, and was associated with an increased deposition of collagen, a disorganization of collagen fibrils and the formation of collagen nodules. Some features of the RD healing phenotype are similar to hypertrophic scar formation in humans.

Recent investigations have begun to elucidate the genetic transmissibility of the RD healing phenotype. Skin wounds were created on Y x RD F1 female animals (N=8), and while hyperpigmentation was not observed, the hypercontraction prevalent in the RD persisted in 100% of animals studied. Molecular analysis has indicated that some gene expression profiles of each breed are apparent in the F1 progeny. Subsequently, all F1 animals were bred to a single Yorkshire boar, producing a cohort of 20 female backcross animals. Healing in the backcross animals generally followed the Yorkshire phenotype with regard to wound contraction.

These studies have begun the process of elucidating the genetic basis for abnormal skin wound healing and scarring. Ongoing studies are using genomic and proteomic approaches to further elucidate candidate genes potentially involved in the RD healing phenotype.

CM4.

Chinese Experimental Pig Models for Human Disease and Xenotransplatation

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The Chinese Experimental Mini-Pigs (CEMPs) was originated from the Xiang pig of Chinese native pig located in the isolated mountain area of Guangxi and Guizhou provinces, and titled by the review panel from the Ministry of Agriculture as the first experimental mini-pig breed in China. There are 2 lines of CEMPs, one with white color and the other black- the original color, and 465 pigs (6 half-sib families) for Line I (black) and 95 pigs (2 half-sib families) for Line II (white). The inbreeding coefficient is around 0.71~0.73. It was 24.6±4.27kg at age of 6 months for the bodyweight, 90-115 days for the First mating age, 7.79±2.13 for the Litter size. 80 physiological and blood biochemical parameters were measured and more than 10 high-risk pathogen such as mouth, Pseualorabies, Salmonellosis, Swine Dysentery , Salmonellosis etc. were free in CEMPs. The CEMPs was used as the model for elucidating the gene pathway displayed by Clenbuterol, a kind of beta-adrenergic agonist, there were 8 qualitatively differential expressed proteins in silver stain gel, and 4 more protein were recovered, which were not identified through the MASCOT search. The Porcine endogenous Retrovirus (PERV) was the main obstacle for xenotransplantation from pig to human, we found 2 pigs without PERVs tested by PCR and Southern, and the proportion was about 1/67. The copy number of PERV in CEMPs is very low, just around 10-20. Pigs with both types of membrane protein were much more than that containing only one type of membrane protein, and large difference of PERV loading was found in Chinese native pig breeds, and showing rich genetic polymorphism in Chinese pigs. It is concluded that the CEMPs is effective and safe model for the medical researches, due to the characters of low copy number of PERVs, suitable bodyweight, sensitivity to the drugs and so on. Key words: CEMP/PERV/ Clenbuterol/Gene expression

CM5.

A Porcine Model for the Study of Atherosclerosis

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Atherosclerotic cardiovascular disease is the leading cause of death for both men and women among all racial and ethnic backgrounds in Western populations, accounting for nearly 1 million deaths in the United States annually. The development of atherosclerosis involves initial injury to the endothelial cell lining, followed by accumulation of macrophages, adherence of LDL, and accumulation of cholesterol. Apolipoprotein E plays a major role in the metabolisms of cholesterol and triglyceride by serving as a ligand for receptors that clear remnants of chylomicrons and VLDL cholesterol from plasma. The link between APOE, serum cholesterol levels, and the development of atherosclerosis has been well established in humans. Numerous animal models have been used to study the pathogenesis and potential treatment of the lesions of atherosclerosis. Mice are highly resistant to atherosclerosis and the vascular lesions differ in location and histology when compared to humans. Rats and dogs are not good models for atherosclerosis because they do not develop spontaneous lesions and require heavy modifications of diet to develop vascular lesions. Rabbits are highly responsive to cholesterol manipulation, but the lesions they develop are much more fatty and macrophage-rich than the human and their plasma cholesterol levels are extraordinarily high. Pigs, however, are very good models when fed high cholesterol diets because they reach plasma cholesterol levels and atherosclerotic lesions similar to humans. In addition, pig models of the disease initially revealed that monocyte infiltration was one of the primary cellular events in the atherogenic process. Therefore, we are investigating the potential of the pig as a model for atherosclerosis by utilizing sequence information to compare human APOE to pig APOE. We are comparing the coding region SNPs in exon 4 of human APOE to pig APOE, which account for the phenotypic differences. We are also comparing SNP frequencies of APOE in twelve animals from seven different pig breeds. Ultimately, we aim to develop a pig model of atherosclerosis that will be used for therapeutic intervention and prevention. We will accomplish this by making a point mutation in the pig at the location of the SNP that accounts for the high cholesterol phenotype in humans as well as create an apoE-knockout pig.

Genomics & Cloning (GC)

GC1.

Resources for Genomics and Functional Genomics Research in Pigs

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The dominance of the mouse as the experimental mammalian model of choice has been underpinned by the wealth of murine experimental reagents and data. Other non-primate species including pigs, however, may provide better physiological models of humans. The outputs from over a decade of genome research now facilitate the use of pigs where they offer advantages as the experimental model.

We have developed a range of resources to enable genomics and functional genomics research in pigs. These resources include normalised cDNA libraries representing transcripts from uterus, placenta, blastocyst stage embryos, ovary, brain, spleen, tonsil, thymus, lymph nodes, heart, kidney and macrophages. Over 50,000 expressed sequence tags (ESTs) have been generated from these libraries and submitted to the public databases (EMBL/Genbank/DDBJ). A genomic BAC (bacterial artificial chromosome) library providing about five-fold coverage of the pig genome has been developed. Clones from the BAC library have been incorporated into the clone-based physical map of the pig genome being developed by an international consortium including ourselves. The cDNA and BAC clones are available through the ARK-genomics centre based at Roslin (http://www.ark-genomics.org). Pig-specific microarrays fabricated from cDNA inserts or long oligonucleotides and the opportunity to conduct expression profiling experiments with the arrays are also available through ARK-genomics. We also developed database systems to manage and disseminate genomics data for pigs, e.g. the pig genome database, ARKdb-pig (http://www.thearkdb.org).

GC2.

Porcine Somatic-Cell Transgenesis In Vivo And in Vitro Using the Sleeping Beauty Transposon System

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Because the size of piglets approximates that of human neonates, they should provide excellent models for the development of gene therapy procedures. In addition, a potentially unlimited supply of pigs argues for the use of porcine tissue in therapeutic xenotransplantation. These diverse applications would benefit greatly from the development of technologies for efficient porcine somatic-cell transgenesis. We are using transposons and recombinases to engineer pig cells in vivo and in vitro, and will present current data regarding use of the Sleeping Beauty (SB) transposon system for improved efficiency and precision in transgene delivery and expression.

The effectiveness of in vivo delivery of transgenes to the liver (via the umbilical vein) and lungs (via tracheal nebulizer) of neonatal piglets using the SB transposon system are being investigated. Rates of transgene delivery and expression are being monitored using the Xenogen in vivo imaging system. The influence of functional SB transposase on transgenesis rates, short and long-term transgene gene expression, and the molecular architecture of transgenes are being analyzed and will be discussed.

We are also investigating the use of SB for transgenesis of isolated porcine islets to improve islet engraftment and tolerance. Transfection of islets with transposons encoding luciferase resulted in the uniform expression of 15,000 ˆ 30,000 molecules per cell. Transfected islets transplanted under the kidney

capsule rescued diabetic nude mice and maintained luciferase expression for at least 5 weeks. Transposons encoding immune regulatory molecules are being developed and will be tested for their ability to protect islets transplanted into immune-competent mice.

GC3.

Developing a Physical (BAC Contig) Map of the Pig Genome

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A physical cloned based map of the pig genome will provide a launch pad for finding genes of economic importance, sequencing of specific loci or the entire genome and the development of molecular tools for marker assisted selection. Large fragment genomic clones from a BAC library (pigE BAC and CHORI-242) have been characterised by HindIII DNA fingerprinting and BAC end sequencing.. Data analysis and contig assembly were effected using fpc. In initial analyses, fingerprints from almost 135,000 BAC clones were assembled at high stringency into 11,971 contigs. A total of 310,450 BAC end sequence (BES) reads have been obtained of which 263,883 passed the quality threshold. When 229,579 BES were subjected to WuBLASTN analyses against the human genome sequence 40,305 BES found matches with > 70% identity and scores > 700. The alignment of the BES reads with the human genome sequence has allowed the identification of BACs containing genes of interest. These data have been merged with those from other groups in Illinois and INRA to develop a comprehensive sequence ready map of the pig genome. (This project is funded by BBSRC, Defra, Roslin Institute and Sygen/PIC).

GC4.

The Sino-Danish Pig Genome Project and Genetically Manipulated Pig Models

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The first phase of the "Sino-Danish Pig Genome project" has been successfully completed. We now have some access to most pig genes and their common variants, which makes the pig all the more relevant for medical research. Thus, we have started to develop and analyse genetically manipulated pig models.

Pig fibroblasts are made transgenic by lipofection. Large numbers of in vitro-matured zona-free pig oocytes are bisected manually and halves containing no chromatin (cytoplasts) are selected. Transgenic fibroblasts are electrofused to pairs of cytoplasts and the resulting zygotes are activated and cultured in NCSU-23 medium.In pilot experiments with a CMV-promoter driven EGFP reporter gene, EGFP was expressed in all cells of blastocysts that developed from reconstructed embryos with an efficiency of at least 6%. Production of offspring from such blastocysts will soon be started.

Genome editing by INA-induced gene conversion is attempted in connection with the nuclear transfer procedure. The objective is to introduce dominant negative folding mutations in the keratin 14 gene leading to degenerative skin problems that can be studied with respect to the pathogenesis and prevention of tissue degeneration. Keratin 14 mutant gene constructs are also made for the production of transgenic pigs with the same objective. For analytical purposes, we are establishing post-hatching cultures of genetically manipulated embryos in agarose tunnels (and cell lineages from these). Oligonucleotide arrays for gene expression analyses are also produced.

GC5.

Pig Genome Resources at NCBI

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The National Center for Biotechnology Information (NCBI) integrates map and genome data from a variety of sources and makes it available to the scientific public as interactive web resources and as organized releases of bulk data for FTP. Genomic data is available from several resources including Entrez Gene, the reference sequence (RefSeq) collection, organism-specific pages such as Pig Genome Resources, and the Map Viewer. These resources are extensively cross-linked and facilitate navigation across a broad spectrum of biological information.

Entrez Gene is a gene-oriented database that includes protein-coding genes, genes encoding functional RNAs, pseudogenes, and QTLs. Gene currently includes more than 1500 protein-coding genes and over 780 QTLs for Sus scrofa. Gene provides accession numbers, map information, publications, and external links for each locus. It also supports GeneRIF (Gene References into Function), which allows users to contribute functional annotation in Gene.

The NCBI Map Viewer resource currently provides a display of the MARC genetic linkage map. It has the capability to include additional genetic maps as well as physical maps generated by research groups, cross-link the map data using common markers, and correlate maps to sequences and genes when assembled genome sequence, sequence-based primers, ESTs, cDNAs, or BAC end sequences are also available. Map Viewer includes similar data for many organisms, encouraging discovery by comparative genomic analysis.

More NCBI genome resources available for swine include clusters of related transcripts (UniGene), computed homologous clusters (HomoloGene), expression data (GEO), alignment analysis (BLAST, BLink), and publications. See NCBI Pig Genome Resources at http://www.ncbi.nlm.nih.gov/projecs/genome/guide/ pig/index.html.

GC6.

Construction of a High-Resolution Physically-Anchored Human-Pig Comparative WG-RH Map

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Comparative mapping information can be effectively utilized for the identification of candidate genes associated with genetically complex phenotypes. The successful use of such information relies upon the construction of a detailed genome map determining segments of conserved synteny and gene order, as well as evolutionary breakpoints. The recent availability of the complete human genome sequence and thousands of homologous porcine sequences provides a tremendous resource for the construction of such a map of the porcine genome. Using the INRA-Minnesota porcine Radiation Hybrid (IMpRH) panel, we have constructed a radiation hybrid map composed of nearly 2,274 markers, including 206 ESTs and 2,068 porcine BAC-end sequences. The average spacing between comparative anchor loci is 1.15 Mb based on human genome sequence. This radiation hybrid map has the highest resolution of any porcine genome map to date, and should greatly facilitate the positional cloning of porcine genes influencing complex traits. An increased understanding of the molecular basis of these traits should prove valuable for comparative biology and the development of porcine models.

GC7.

Sequenced Based Swine Model Building

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Comparative genome sequence information for humans and pigs is well established and provides the basis for comparative phenotypic maps between rodents, humans and pigs. In current models (*Drosophila*, yeast, *C. elegans*, and mice), functional genomics is supported by the ability to develop congenic inbred lines, clones, and knock-out or knock-in mutants. Significant progress has been achieved in the analysis of phenotypes using gene-driven mutagenesis approaches for the functional analysis of the mouse genome. We are using DNA-sequence information together with gene targeting technologies to resolve complex traits (diseases) in pigs. This approach is based on the hypothesis that recombineering and gene-trapping in porcine fetal fibroblasts coupled with animal cloning through nuclear transfer permits development of biomedical models and provide insights into gene function. These models can support the use of *comparative phenomics* between mouse, rat, dog, man, and the pig. We have developed a recombineering platform to introduce targeted changes in porcine fibroblast and other somatic cells. These studies were designed to: (1) demonstrate that larger, complex gene constructs could be developed and (2) that transient gene expression could be monitored following BAC transfection into somatic cells. We have been able to target deletions and insertions as well as introduce selection markers into specific genes. In addition, we have reduced the targeted BAC from 180 kb to 20 kb by gap repair rescue methods which increased transfection efficiency in the FF. Through gene-trapping of ES cells, a collection of random mutant lines can be developed as the BayGenomics project (NIH-sponsored) has done. They focus on a mouse ES gene-trap model for deciphering new gene function associated with cardiopulmonary development and related diseases (www.baygenomics.ucsf.edu). Thus, we are using gene-trapping technology in porcine fetal fibroblasts (FF) to develop cell lines for defining phenotypes. Both recombineering and gene-trapping approaches will provide community resources for addressing biomedical models of complex traits.

GC8.

Anchoring PERV Integration Loci on the Human Map

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Porcine Endogenous Retroviruses (PERVs) are considered as a potential risk in xenograft transplantation. PERV integration sites vary in number and chromosomal position according to breeds and animals. There are three PERV subtypes depending on their envelope sequence and referred to as PERV-A, -B or -C. We have already reported 26 distinct chromosomal loci for PERV-A or -B in the genome of a Large White pig using BAC clones and FISH mapping. PERVs were found on all chromosomes except chromosomes 6, 12, 15, 16 and 18. Our goal was to better characterize these loci and to anchor them to a reference map such as the human map. BAC end sequences were obtained for all PERV loci and provided PCR primers suitable for RH mapping (http://www.toulouse.inra.fr/lgc/pig/RH/IMpRH.htm). Thus we could refine the localization of 24 loci on the pig genome. Since PERV-containing BAC clones were derived from the INRA BAC library included in the Porcine Genome Physical Mapping Project (http://www.sanger.ac.uk/Projects/S_scrofa/), we searched for corresponding BAC contigs and identified contigs for 18 loci. Two redundant loci corresponding to distinct allelic forms of two other loci were eliminated. In order to find hit locations on the human sequence, we used PERV-containing BAC end sequences, PERV flanking sequences subcloned at 14 loci and information available through the Porcine Genome Physical Mapping Project. This last step allowed us to anchor a number of PERVs to the human build 35 and provided information on the genomic background of PERV integration sites.

GC9.

Pigs in Sequence Space: A 0.66X Coverage Sino-Danish Pig Genome Survey

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We have generated ~ 3.84 million shotgun sequences (0.66X coverage) of the pig genome. The non-repetitive fraction of the sequences was aligned to the UCSC human-mouse alignment and the resulting three-species alignments were annotated using the human genome annotation. Ultra-conserved elements and miRNAs were identified. The results show that for each of these types of orthologous data, pig is much closer to human than mouse is. Purifying selection has been more efficient in pig compared to human, but not as efficient as in mouse, and pig seems to have an isochore structure most similar to the structure in human. The addition of the pig to the set of species sequenced at low coverage adds to the understanding of selective pressures that have acted on the human genome by bisecting the evolutionary branch between human and mouse with the mouse branch being approximately 3 times as long as the human branch, and the joint alignment of the shot-gun sequences to the human-mouse alignment offers a rapid way for the investigator to define specific regions for analysis and resequencing.

GC10.

Lentiviral-Vectors Enable Efficient Transgene Delivery into the Pig Germline

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Transgenic livestock are an exciting adjunct to classical genetic approaches to understand and exploit biological variation. The technology overcomes the limitations of classical animal breeding regimes, where importation of genes by crossbreeding is limited to those traits already present within a given species. In addition it offers the potential to develop novel biotechnological applications, including models of human disease and drug development. New methods including the use of viral vectors to deliver transgenes have been developed. These vectors offer an advantage over more standard methodology in that they enable spectacularly efficient generation efficiencies. We have evaluated lentiviral-vectors as a tool to generate transgenic livestock and determined the efficiency of transgene delivery and expression in pigs; 25% of injected eggs resulted in a transgenic founder animal and 95% of the founder animals displayed green fluorescence. Germline transmission has been demonstrated and due to segregation of individual transgene integration loci, the final number of transgenic lines available is greater than that measured in founder animals. We propose that lentiviral transgene delivery may be a general tool for the efficient generation of transgenic mammals, including the production of disease models and enabling functional genomics in the target species.

GC11.

Current Issues in Using 70mer-oligo Chip: Oligo and Experimental Design, Image and Data Analysis

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Microarray-based analysis of gene expression provides the opportunity to simultaneously assess the expression of thousands of genes in porcine tissues. We will discuss technical issues encountered utilizing a >13,000 element 70-mer oligonucleotide microarray design by Qiagen to assess porcine gene expres-

sion. Issues to be discussed extend from oligonucleotide design to the statistical analysis of expression differences.

An attractive feature of oligonucleotide microarrays relates to the ease of probe design, not requiring full-length sequence information. However, retrospective analysis of a 70mer-oligonucleotide array designed against version 5 of the TIGR Porcine Gene Index revealed redundancy that could have been avoided *ab initio* by comparison to human and mouse genome sequences. In addition, subsequent accumulation of porcine transcript data has resulted in the coalescence of tentative consensus sequences. Current estimates indicate the oligo array to be >34% redundant. Although this redundancy caused less effective sampling of the porcine transcriptome, it has provided internal validation of data integrity. Future designs will target non-redundancy.

We will also discuss data capture, experimental design and statistical analysis for the identification of differentially expressed genes. We have implemented a loop design and have analyzed the expression data using pairwise (GeneSpring) and multifactorial (R/mannova) approaches. Because multifactorial analysis of a loop design effectively increases the number of replicates for a given condition, it delivered four times the number of significant results than a pairwise comparison with a $p < 0.05$. In addition, the multifactorial analysis permitted the revelation of factor-interactions not apparent by simple pairwise comparison.

GC12.

Technical Issues Encountered Using a Porcine 70mer-Oligonucleotide Microarray

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Microarray-based analysis of gene expression provides the opportunity to simultaneously assess the expression of thousands of genes in porcine tissues. We will discuss technical issues encountered utilizing a >13,000 element 70-mer oligonucleotide microarray design by Qiagen to assess porcine gene expression. Issues to be discussed extend from oligonucleotide design to the statistical analysis of expression differences.

An attractive feature of oligonucleotide microarrays relates to the ease of probe design, not requiring full-length sequence information. However, retrospective analysis of a 70mer-oligonucleotide array designed against version 5 of the TIGR Porcine Gene Index revealed redundancy that could have been avoided *ab initio* by comparison to human and mouse genome sequences. In addition, subsequent accumulation of porcine transcript data has resulted in the coalescence of tentative consensus sequences. Current estimates indicate the oligo array to be >34% redundant. Although this redundancy caused less effective sampling of the porcine transcriptome, it has provided internal validation of data integrity. Future designs will target non-redundancy.

We will also discuss data capture, experimental design and statistical analysis for the identification of differentially expressed genes. We have implemented a loop design and have analyzed the expression data using pairwise (GeneSpring) and multifactorial (R/mannova) approaches. Because multifactorial analysis of a loop design effectively increases the number of replicates for a given condition, it delivered four times the number of significant results than a pairwise comparison with a $p < 0.05$. In addition, the multifactorial analysis permitted the revelation of factor-interactions not apparent by simple pairwise comparison.

Immunology & Infectious Diseases (IID)

IID1.

Immunological Changes after FTY720 Treatment in Swine

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FTY720 (FTY) induces lymphopenia in humans and may be a breakthrough immunosuppressive agent for transplantation. It is unclear whether FTY induces lymphopenia in swine. This study assessed the immunological impact of FTY in a porcine model (outbred Yorkshire). Dose escalation impact on mixed lymphocyte reactions (MLR) and apoptosis (TUNEL) were performed. In addition, allogeneic response (MLR) was measured at day 0, 7, 14. Three groups (n=2) of pigs were sacrificed after oral FTY for 14 days at: 0, 0.01 (low), and 0.1mg/kg/day (high). Immunophenotyping was performed at intervals to monitor peripheral lymphocyte depletion. Secondary lymphoid tissue samples were stained hematoxylin and eosin. There was a dose escalation response in apoptosis induction (5ng/mL: 26±12%; 10ng/mL: 95±5%) which could explain the dose response reduction in the allogeneic response (5ng/mL: 33±25%; 10ng/mL: 88±5%). There was minimal allogeneic response in both treatment groups at day 7 and 14. Both doses led to peripheral B and T-lymphocyte depletion (Figure 1 and 2). Peyer's patches from treated animals demonstrated histological architectural changes especially lymphocytic hyperplasia. There were no appreciable changes observed in thymus, mesenteric lymph nodes and spleen. FTY leads to profound peripheral lymphocyte depletion with preliminary evidence of lymphocyte migration to Peyer's patches. Future studies will pursue use of FTY to develop calcineurin-free protocols in swine transplantation. Transplantation

IID2.

Fixation Of The Swine Major Histocompatibility Complex (SLA) By Rapid Assignment Of The SLA Class I And II Genotypes In Clawn Miniature Swine

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Inbred miniature swine with defined novel SLA haplotypes will be useful in allo- and xeno-transplantation studies, which can be carried out representing variable combinations of SLA haplotypes. Clawn miniature swine have been established from mating between the F1 pigs derived from Göttingen and Ohmini miniature swine, and derived from Landrace and Large Yorkshire pigs. Substantial breeding between two females and one male, and subsequent breeding within their progenies were carried out for several generations. As a result of this extensive raising among a few related individuals, only two haplotypes (c1 and c2) and one crossover haplotype (c3) have been assigned by nucleotide sequence determination of RT-PCR products of the three SLA classical class I genes and two SLA class II genes in Clawn miniature swine. To select SLA class I and II homozygotes in Clawn miniature swine individuals, we developed a rapid and simple SLA-class I and II-DNA typing method by a combination of polymerase chain reaction-sequence specific primer (PCR-SSP) and polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) techniques. This combination of PCR-SSP and PCR-RFLP methods facilitate the rapid identification of the three haplotypes and SLA class I and II homozygotes in individual Clawn miniature swine. Over 400 Clawn miniature swine per year are produced by selective breeding for fixation of the SLA genes in Japan Farm CLAWN Institute. Therefore, this miniature swine with known SLA haplotypes will be great benefit to allo- and xeno-transplantation experiments and subsequent analysis on immune response against conventional antigens or synthetic peptides.

IID3.

Porcine CD4+/CD3- Cells Secrete Interferon-Alpha, Have Plasmacytoid Morphology and Express SWC3, a Member of the Signal Regulatory Protein (SIRP) Family

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Interferon-alpha-producing plasmacytoid cells are a type of cells found in human blood with immediate dendritic cell precursor potential (pDC). These cells have the morphological appearance of B-lineage antibody-producing plasma cells, but lack most B-lineage markers and do not produce immunoglobulin. Upon exposure to either a virus or CpG-containing oligodeoxynucleotides (ODN), these cells are triggered to secrete copious amounts of IFN-alpha and differentiate into mature DCs. These plasmacytoid interferon (IFN)-alpha-producing cells have been termed pDC2, and represent the most potent IFN-alpha-secreting cells in the body. A potent natural IFN-alpha-producing cells (NIPC) present in the blood of pigs had been previously described. The porcine NIPC was characterized as a CD4+/CD3- cell that secreted IFN-alpha upon stimulation with the porcine coronavirus Transmissible Gastroenteritis virus (TGEV). Here we report that porcine CD4+/CD3-, freshly isolated from porcine blood are capable of secreting large amounts of IFN-alpha, have plasmacytoid morphology and acquire dendritic morphology upon stimulation with either TGEV. Type I IFN was also produced by these cells in response to stimulation with CpG-containing ODN, suggesting that they express on their surface TLR9. Moreover, the selected cells, which represent less than 1% of the total mononuclear cell population in the blood, express low levels of SWC3, a member of the signal regulatory protein (SIRP) family. Since they did not express detectable levels of CD11c, we concluded that the cell previously known as NIPC in the pig are functionally akin to human IFN-alpha/beta-producing plasmacytoid cells, also known as type 2 dendritic cell precursors (pDC2).

IID4.

Use of Experimental Porcine Models to Provide Biomarkers of Human Nutrient and Disease Interactions

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Species differences limit confident extrapolation of data on interactions between nutrition and immunology from experimental mouse models to humans, while porcine anatomy, physiology, and immunology are generally considered more similar to human. Three recent review articles list >100 major human-murine differences in immune system genotype, phenotype and/or function, and our own assessment reveals that the pig immune system is more similar to humans than mice for >80% of the variables compared and the mouse more similar to human than pigs in <10%. A pig experimental model for allergic airway inflammation, as an example, closely resembles several characteristics of human asthma including airway obstruction, eosinophilia and late-phase asthmatic reactions following antigen challenge. In addition, pigs provide good to superior models of human infectious diseases because of common cross species infections with many viruses, bacteria, and parasites. We have extensively profiled the immune response to the parasitic worms Ascaris suum and Trichuris suis, and the protozoan parasite Toxoplasma gondii by real time PCR arrays. Further, we have measured the influence of vitamin A and probiotic bacteria on the immune function in pigs, and developed an annotated bioinformantics database describing 2,400 genes related to nutrition and immunity to evaluate these responses. Our porcine gene database is unique because it links gene expression to gene function, identifies related gene pathways and interconnects with existing porcine gene databases. It contains information for >300 validated real time PCR assays of gene expression as well as information for protein reagents.

IID5.

Mucin 4: A Candidate Gene for Susceptibility Towards E. Coli F4ab/Ac Diarrhea in the Pig

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Enterotoxigenic Escherichia coli (ETEC) that express the F4ab or F4ac fimbriae (formerly known as K88ab/ac) are major causes of diarrhea and death in neonatal and young pigs. A locus controlling susceptibility towards ETEC F4ab/ac has previously been mapped to pig chromosome 13q41. A number of studies indicate that candidate genes for this trait could either be transferrin-like-genes or mucin-like sialoglycoproteins. Deductions based on linkage and comparative mapping data point to mucin 4 (MUC4) as a likely candidate. Incidentally, the gene lies in one of the candidate regions on Hsap3 (viz., Hsap3q29). We isolated pig BAC clones containing MUC4, and mapped the BACs by FISH to SSC13q41. FISH mapping on interphase nuclei positioned MUC4 between microsatellite markers Sw207 and S0283 while radiation hybrid mapping positioned it between SST and SWR2189. Initial screening for polymorphism in the gene revealed a SNP in intron 7 that shows complete co-segregation with ETEC F4ab/ac susceptibility in a Wild boar/Large White intercross. We report on the genomic characterization of the porcine mucin 4 gene spanning 25 exons and more than 31 kb. Comparison between the porcine mucin 4 gene and the human ortholog shows conservation between 58% and 89% on the nucleotide level of the different exons. Highly conserved non-protein coding sequences are also evident from the comparison. Alignment between porcine and murine MUC4 shows less conservation than the pig-human comparison. In addition to the genomic sequence of pig mucin 4 we also report on SNPs discovered in the porcine MUC4 sequence.

IID6.

Transfer o Cytokines from Sows to Newborn Piglets Via Colostrum and Milk

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The role of lactogenic cytokines in development of neonatal immune responses is undefined. Swine provide a unique model for such studies because the sow placenta is impermeable to macromolecules; consequently piglets may not acquire maternal cytokines transplacentally. We investigated the level of cytokines transferred in sow colostrum/milk to newborn piglets suckled for 14 days. The IFN-g (Th1), IL-10 (Th2), IL-4 (B cell suppressor in swine) and free form TGF-b1 (Th3) levels in piglets' serum and intestinal contents, and in sow serum and colostrum/milk were measured at 1-14 post-farrowing days (PFD). Hysterectomy-derived colostrum-deprived (HDCD) piglets were included to assess the presuckling cytokine levels (PFD0). No IL-4, IL-10, IFN-g or TGF-b1 were detected in HDCD piglets at PFD0 confirming absence of transplacental transfer. The cytokine levels in colostrum/milk (IL-4 and TGF-b1_IFN-g_IL-10) reflected the levels in piglets serum at PFD1-2. In piglet serum, IL-4, IL-10 and IFN-g decreased significantly from PFD2 to PFD9. In piglet intestinal contents, IL-4 and TGF-b1 levels were highest at PFD2 and decreased 2-3-fold by PFD7, although their levels remained high in sow milk, indicating intestinal degradation of these cytokines. The presence of high levels of regulatory cytokines TGF-b and IL-4 in colostrum/milk and in piglet serum might explain the maternal immunosuppression of neonatal T and B cell responses observed in both humans and pigs. To our knowledge this is the first report of the transfer of cytokines from colostrum/milk to offspring providing a basis for future studies of the influence of maternally-derived cytokines on development of the neonatal immune system.

IID7.

Molecular Analysis of Tachyzoite-Bradyzoite Stage Conversion in a Porcine in vitro Model for Toxoplasmosis

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The intracellular protozoan parasite *Toxoplasma gondii* interconverts between two major asexual forms produced during its life cycle: the rapidly dividing tachyzoite and the slowly dividing, encysted bradyzoite. Interconversion between the two stages is important in the establishment of a chronic infection and is responsible for disease reactivation in certain immunodeficiencies, particularly HIV/AIDS. Stage conversion between tachyzoites and bradyzoites of *Toxoplasma gondii* strain TS-4, was investigated *in vitro* in porcine kidney epithelial (PK13) host cells by shifting the culture incubation temperature from 370C to 430C over 72 hrs. Differentiation of tachyzoites to bradyzoites was assessed by immunofluorescence using a monoclonal antibody against P30 (a tachyzoite-specific surface antigen) and *Dolichos biflorus* agglutinin (a bradyzoite cyst wall-specific lectin). Stage conversion was also confirmed by transmission electron microscopy and RT-PCR analysis of stage specific genes. The tachyzoite-specific SAG1 gene was expressed by parasites maintained at 370C and 430C in all experimental time points, while the bradyzoite-specific SAG4 was only expressed at 430C at 6h, 24h, 48h and 72h. Stage-specific directionally cloned cDNA libraries, ave. insert size 2.4Kb; >99% recombinant were constructed from tachyzoite and bradyzoite derived mRNAs. To date, ~4000 expressed sequence tags (ESTs) have been generated from the respective cDNA libraries. As we continue to sequence, we expect this information to provide a better understanding of gene expression and the molecular basis of tachyzoite-bradyzoite interconversion. We also expect to be able to identify candidate genes and respective pathways as targets for the development of novel drugs against acute and chronic *Toxoplasmosis.*

IID8.

Full length sequence of the Swine Major Histocompatibility Complex (SLA):

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We completely sequenced the SLA complex and annotated 148 genes and pseudogenes. The SLA class I and class III regions encompasse 1.8 Mb on the short arm of chromosome 7 from the UBD gene to the NOTCH4 gene. Differences between the SLA and HLA arise only from the copy number of class I-related sequences and orthologous relationship between class I series. The centromere of chromosome 7 lies within a cluster of butyrophilin-like (BTNL) sequences with two BTNL loci on the class III side and three loci on the class II side, whereas in HLA a single BTNL locus exists adjacent to DRA. On the long arm of chromosome 7, the SLA class II region extends for 580 kb from DRA to RING1. Despite notable variation in the copy number of class II-related sequences, including the absence of DPA and DPB-like sequences, all genes have counterparts in HLA. Only one set of expressed genes are found for the SLA class II DR, DQ, DO, DM series. The end of the SLA extended class II region is yet to be fully sequenced. The physical map derived from BAC alignment indicates that 4 Mb separate the Tapasin locus from RING1 in pig whereas the two loci lie 78 kb apart in human. PCR-amplified cDNAs corresponding to all the genes from the chromosomal segment described above have been printed onto glass slides to study the transcription of MHC and non-MHC genes under different biological conditions.

IID9.

The Effects of Immune Challenge on Circulating Cytokines and Hypothalamic Gene Expression in Domestic White and Chinese Meishan Gilts

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Our laboratory is interested in identifying genes whose expression is altered in response to immune challenge, particularly those genes that may impact reproductive function. We have utilized a model whereby adult cycling gilts are challenged with an injection of Escherichia coli lipopolysaccharide (LPS). Animals injected with LPS show a characteristic increase in body temperature, sickness behavior and levels of circulating cortisol. These increase to peak values at approximately 3-4 hours post infection and then return to baseline by 8 hours. The LPS model therefore provides a well-defined window during which one can collect blood samples and tissues for gene expression studies. We injected 20 Chinese Meishan gilts and 20 Yorkshire/Crossbred gilts with LPS (5 ìg/kg BW) or saline. Blood samples were collected every hour until 4 hours after injection at which time the animals were euthanized and their hypothalami and uteri collected for RNA. Blood samples were assayed for cortisol, interleukin (IL)-1â and IL-6. Results showed that the Chinese Meishan gilts showed little increase in body temperature in response to LPS but had significantly higher cortisol levels than the white pigs. Both breeds showed similar increases in levels of interleukins.

A porcine cDNA microarray containing 5,097 genes was constructed using cDNA sequences from the porcine hypothalamus and uterus. Two cDNA libraries were constructed. Each library was tagged, normalized and 11,000 sequences underwent high-throughput sequencing. 5,097 genes were double-spotted onto glass slides and used for hybridization analysis. Microarray analysis identified 221 hypothalamic genes that showed altered expression in response to LPS.

IID10.

SLA Class I Typing of Several Breeds of Miniature Pigs

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Miniature pigs are a useful large animal model for transplantation, however most breeds have not been characterized for their swine leukocytes antigens (SLA).We have characterized 12 SLA class I haplotypes by cloning and sequencing cDNA from four breeds of miniature pigs (Yucatan, NIH, Sinclair and Hanford). We have also developed PCR-ssp primer sets to rapidly identify these haplotypes within these breeds.

Most SLA haplotypes have three classical class I genes designated SLA-1, SLA-2 and SLA-3. In three haplotypes a duplicated SLA-1 locus was found and in two haplotypes, either the SLA-1 or the SLA-3 locus was not detectable. Thus, SLA haplotypes can have between two and four classical class I genes. Full length cDNA clones are available for these alleles and the DNA sequences and a standardized SLA nomenclature are available at http://www.ebi.ac.uk/ipd/mhc/sla/index.html

We have developed a PCR based assay using site specific primers (PCR-ssp) and a positive control primer set derived from the alpha-actin gene to identify haplotypes within related kindreds of each of these breeds. We have standardized the primer design to work under the same set of PCR conditions, allowing additional primer sets to be added as needed to type any outbred pig.

DNA sequence based typing and PCR-ssp typing of SLA antigens is now possible in any kindred of pigs. This makes it feasible to design transplantation experiments with much more control over SLA matching. In addition, the availability of SLA class I clones and SLA typed pigs will make it possible to use new technologies such as MHC/peptide tetramers to study T-cell responses in pigs.

Nutrition (NU)

NU1.

Insulin-like Growth Factor-I and Piglet Intestinal Development in Biomedical Models and Transgenics

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The piglet is considered the best model in which to study the nutritional regulation of human infant intestinal development. Insulin-like growth factor-I (IGF-I) is a multifunctional growth factor that is present in the milk of all species. Our laboratory has employed three experimental piglet models to investigate the role of IGF-I in intestinal development. The first asked whether IGF-I would enhance intestinal development of neonates supported on total parenteral nutrition (TPN). Piglets on TPN were fed small volumes of formula +/- IGF-I for 7 d. The second investigated whether IGF-I added to formula would affect intestinal development of infants. Piglets were fed formula +/- IGF-I for 7-14 d. Lastly, whether transgenic over-expression of IGF-I in milk would impact intestinal development was studied over a 21-d lactation. A construct linking IGF-I to bovine ?-lactalbumin was used to create transgenic swine with mammary specific over-expression of IGF-I. In all studies, outcomes associated with enterocyte growth (villus height, protein, DNA) and differentiation (disaccharidase activity) were significantly upregulated by IGF-I in all piglet models. However, the magnitude of response to IGF-I was inversely proportional to the degree of intestinal compromise TPN > artificially reared > sow-reared, suggesting that IGF-I was most efficacious at modulating intestinal development and function in during a compromised condition (TPN). Taken together, these data demonstrate the enormous potential of the piglet as a biomedical model in which to explore the regulation of neonatal intestinal development (Supported by the NIH, USDA NRI and the Illinois Council for Food and Agricultural Research).

NU2.

Glucose Intolerance and Insulin Resistance in Ossabaw Compared to Yucatan Swine

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Ossabaw swine that were isolated on a coastal barrier island near Georgia ~500 years ago developed a thrifty genotype to survive cycles of seasonal food shortage. Previous investigators have noted obesity and decreased insulin sensitivity in Ossabaw swine, thus representing a novel model of the metabolic syndrome. We tested the hypothesis that Ossabaw swine fed a high fat diet would exhibit reduced glucose tolerance compared to similarly fed Yucatan swine. Male Yucatan and Ossabaw swine were assigned to 3 isocaloric diet groups: control (8% of kcal from fat) for 40 weeks or high fat/high cholesterol diets, H46 (46% kcal from fat and 2% cholesterol) for 40 weeks or H75 (75% kcal from fat and 2% cholesterol) for 20 weeks. Intravenous glucose tolerance tests (IVGTT) showed higher peak blood glucose and insulin in Ossabaw vs. Yucatan (285.9+ 8.71 vs. 247.9+ 13.8, $p<0.05$ and 64.6+ 6.42 vs. 40.8 + 8.09, $p<0.05$, respectively). Ossabaw on the H75 diet had a slower clearance of glucose during IVGTT than Ossabaw on H46 or control diets ($p<0.05$). The area under the blood glucose vs. time curve of Ossabaw on H75 diet was greater than that of Ossabaw on control diet ($p<0.05$). We conclude that Ossabaw manifest insulin resistance, and glucose intolerance compared to lean Yucatan swine and glucose intolerance is exacerbated in Ossabaw on a higher fat diet.

NU3.

Use of Computed Tomography to Evaluate Intra-Abdominal Fat Stores in a Swine Model of the Metabolic Syndrome

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Ossabaw swine were isolated on a barrier island near Georgia ~500 years ago and developed a thrifty genotype to survive cycles of seasonal food shortage. They represent a novel model of the metabolic syndrome, which is characterized by hypertension, dyslipidemia, impaired glucose tolerance and central (intra-abdominal obesity), often leading to cardiovascular disease. Computed Tomography (CT) is commonly used to asess abdominal fat content of human patients. We evaluated the use of abdominal CT scan to assess subcutaneous and intra-abdominal fat deposition in Ossabaw swine compared to traditional methods including back fat ultrasound and post mortem chemical composition analysis as the „gold standard‰. Female Ossabaw swine were fed either 500g of a chow with 7% kcal from fat (lean n=9) or ad libitum chow with 46% kcal from trans fat to induce obesity (high fat n=8). After 9 weeks animals were evaluated with ultrasound and CT, then euthanized for chemical analysis. CT analysis of animals on high fat diets showed significantly increased fat in subcutaneous and intra-abdominal (retroperitoneal and visceral) areas. CT data correlated significantly with back fat ultrasound (average back fat r=0.85) and chemical analysis (subcutaneous tissue r=0.85, total intra-abdominal fat r=0.94, retroperitoneal fat r=0.78, visceral fat r=0.92). Histologic analysis of coronary arteries for determination of neointima formation showed a higher intimal:media in the high fat group compared to the lean group ($P<0.05$). We conclude that abdominal CT is a valid and highly predictive measure of subcutaneous and intra-abdominal fat deposition in swine models of the metabolic syndrome. Support: NIH RR13223, HL62552, T32 RR07004, American Diabetes Association.

NU4.

Investigation of Human Obesity Candidate Genes in Porcine Fat Deposition QTL Regions

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Pigs have been intensively used as a biomedical research model for various human physiological conditions and pigs and humans share numerous physiological and phenotypic similarities for fat deposition and food intake. Therefore, identified chromosomal regions and genes that regulate lean growth and fat deposition in pigs might be applicable to the study of the genetic basis of human obesity and other related health problems. However, currently the gene map of pig chromosomes (SSC) is limited such that a pig QTL region cannot be clearly mapped onto the human gene map. We investigated obesity candidate genes in the pig growth and fat deposition QTL regions and phenotypic association of these candidate genes in several commercial pig populations. Comparative locations of these candidate genes localized in pig QTL regions can be used as anchor loci to clarify homologous human chromosomal locations and identify additional candidate genes. Phenotypic analyses of candidate gene polymorphisms also provide important insights into the role of biological candidate genes on obesity and other related metabolic disorders. Our study in the pig first identified that polar overdominance mode of inheritance is also associated with fat deposition and growth. These results demonstrate that pig is an excellent model organism to examine the genetic regulation of obesity. Development of the entire pig genome sequence will permit easy transfer of results to human studies.

NU5.

Early Weaning Increases the Small Intestinal Digestive Capacity for Sucrose in the Piglet.

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The small intestinal sucrase-isomaltase (SIM) is essential for the digestion of sucrose and maltose. Along with other brush border membrane enzymes, SIM has also been implicated in congenital and developmental disaccharidase deficiencies associated with malnourishment in children. Early weaning (EW) in piglets is characterized by low feed intake, villous atrophy and crypt hyperplasia. Weaning piglets serve as a relevant animal model to study the regulation of intestinal SIM expression. This study examined changes in the digestive capacity of intestinal SIM responses to EW stress. Eight Yorkshire piglets, with an average BW of 3 kg at the age of 10 d, were weaned from sows and fed a corn and soybean meal-based diet for 12 d in comparison with 8 suckling (SU) piglets. Kinetic experiments of SIM activity were conducted with six levels of D-sucrose (0-60 mM) at 37C and pH 6.0. Weaning increased ($P<0.05$) the SIM maximal specific activity (Vmax: EW, 40.11±0.20 vs. SU, 28.41±0.18, μmol/mg protein.min, n=48) by 41% and the SIM affinity (Km: EW, 18.74±0.44 vs. SU, 28.95±0.31, mM, n=48) by 35% primarily in the jejunum. Weaning also increased ($P<0.05$) 48% of the SIM digestive capacity (EW, 230.87±3.26 vs. SU, 155.97±2.87, mol/kg BW.d, n=16). Thus, early weaning increases the digestive capacity for sucrose by increasing both the SIM maximal specific activity and its affinity in the pig. Supported by OMAF.

NU6.

Mimicking Nutritional Modulation of Transient or Chronic Pancreatitis and Cholecystitis in Human Subjects Using a New Experimental Model of Pigs

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The objective of this study was to develop a reproducible, experimental pig model to mimic nutritional modulation of exocrine pancreatic dysfunction (transient or chronic pancreatitis)and biliary disorders (cholecystitis, cholecystectomy)in human subjects. For this purpose, in total 12 gilts were used with surgically modified pancreatic or biliary duct secretions to get transient or chronic symptoms of disfunction. These animals were fed fat-low or fat-rich diets to measure functional, histological and biochemical parameters. In conclusion, we found that these responses of pigs mimicked human pancreatitis (transient or obstructive). Also, cholecystitis and cholecystectomy in our new experimental model of pigs mimicked human's biliary dysfunction. Feeding fat-low versus fat-rich diets had a clear, modulatory impact on the response parameters at the pancreas or gall bladder dysfunction.

Physiology (PH)

PH1.

Induction of Overlapping Genes By Fasting and a Peroxisome Proliferator in Pigs: Evidence of Functional Peroxisome Proliferator Activated Receptor • In Non-Proliferating Species

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Peroxisome proliferator activated receptor • (PPAR•), a key regulator of fatty acid oxidation, is essential for adaptation to fasting in rats and mice. However, physiological functions of PPAR• in other species, including humans, are controversial. A group of PPAR• ligands called peroxisome proliferators (PPs) causes peroxisome proliferation and hepatocarcinogenesis only in rats and mice. To elucidate the role of PPAR• in non-proliferating species, we compared gene expressions in the pig liver from fasted and clofibric acid (a PP)-fed groups against a control diet-fed group. As in rats and mice, fasting induced genes involved with mitochondrial fatty acid oxidation and ketogenesis in pigs. Those genes were also induced by clofibric acid feeding, indicating that PPAR• mediates the induction. In contrast to rats and mice, little or no induction of genes for peroxisomal or microsomal fatty acid oxidation was observed in clofibric acid-fed pigs. Histology showed no significant hyperplasia or hepatomegaly in the clofibric acid-fed pigs, whereas it showed a reduction of glycogen by clofibric acid, an effect of PPs also observed in rats. The copy number of PPAR• mRNA was higher in pigs than in rats and mice, suggesting that peroxisomal proliferation and hyper-response of several genes to PPs seen only in rats and mice are unrelated to the abundance of PPAR•. In conclusion, PPAR• is likely to play a central role in adaptation to fasting in the pig liver as in rats and mice. This study reveals the pig as an appropriate model for understanding human lipid physiology and metabolism.

PH2.

Comparative Histological Analyses on Mammalian Brains from Different Species and Ages

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We performed a histological comparison of different mammalian brains such as human, swine and mouse. The materials included human brain (male) at 60 years old, swine (male), at 3 months old(3M) and at 10 years old(10Y), and mouse (male), at postnatal 21 days(P21) and at 8 weeks(8W). Paraffin-embedded sections were stained with hematoxylin-eosin, myelin and silver staining and immunostained for neurofilament, MAP2, several calcium-binding proteins, tyrosine hydroxylase and glutamate receptors to assess characteristic cytoarchitecture, development of axons, dendrites and synapses and neurotransmitter distribution. The cerebral cortices showed similar laminar structure in swine brain at 3M and mice brains at P21 and 8W, whereas clear lamination with more abundant neuropils was found in swine brain at 10Y. The human cerebral cortex showed much finer lamination with rich neuropils and synapses formation, especially in adult visual cortex. Olfactory lobe was well developed in swine brain but rudimentary in human. The basal ganglia and thalamus appeared similar in swine and mice. The motor neurons in brain stem showed more prominent in swine and mouse as compared to human. The cerebellar cortex showed similar structure in three species. The adult swine brain showed cerebral gyri with histological similarity to human cerebral cortex, which suggests that swine brain could be more useful than rodent brains for understanding integrated functions demonstrated in human brain.

PH3.

Effects of Semen Characteristics, Frozen-Thawed Sperm Viability and Serum FSH, LH, Estradiol-17 beta and Testosterone Concentrations Between Breeds and Among Seasons in Boars

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This study was carried out to investigate the effects of semen characteristics, frozen-thawed sperm viability and serum FSH, LH, estradiol-17 beta and testosterone concentrations between breeds and among seasons in boars. Yorkshire boars produced higher semen volume compared with Duroc boars among seasons. Semen volume on spring was higher compared with summer, autumn and winter between Duroc and Yorkshire boars. Sperm motility and normal acrosome of frozen-thawed sperm produced in spring were higher than those in summer, autumn and winter between Duroc and Yorkshire boars. Sperm motility of frozen-thawed sperm in Yorkshire boars was higher than that in Duroc boars among seasons. However, normal acrosome did not differ significantly between Duroc and Yorkshire boars. Serum FSH concentration in Yorkshire boars was lower than that in Duroc boars in all seasons. Serum LH and estradiol-17 beta concentrations did not differ significantly between Duroc and Yorkshire boars. Also, there were no significant differences in serum LH and estradiol-17 beta concentrations of Duroc and Yorkshire boars among seasons. Serum testosterone concentration in Yorkshire boars was higher than that in Duroc boars in all seasons. Also, Serum testosterone concentrations in Duroc and Yorkshire boars were higher in spring than in summer, autumn and winter. In conclusion, when serum FSH concentrations were lower and semen volumes were higher, and when serum testosterone concentrations were higher, sperm motility and normal acrosome of frozen-thawed sperm were higher.

PH4.

Transcriptional Profiling of In Vitro Versus In Vivo Produced Pig Embryos by Using a 15K Member Unigene Set Specific for Pig Reproductive Tissues and Embryos

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mRNA content was evaluated between in vitro and in vivo produced 4-cell and blastocyst stage embryos to determine key transcripts responsible for early embryonic development. Messenger RNA was purified from three pools of 34-100 embryos and oocytes and amplified using an aminoallyl system. Each pool was analyzed twice, resulting in three biological replicates and two technical replicates. Amplified cDNA was compared to cDNA from a reference sample. Labeled cDNA was hybridized to our pig reproductive tissue-specific 19,968 spot cDNA microarray. Microarrays were scanned using a Genepix scanner, and files were loaded into Genespring 6.2.1 for analysis. Lowess normalization was performed on all good spots. ANOVA ($p<0.05$) showed that there were 1,409 and 1,696 differentially detected cDNAs between the in vitro and in vivo produced embryos at the 4-cell and blastocyst stages, respectively. Real-time PCR analysis on four genes at the 4-cell stage (destrin, PAIP1, E4B, and NASP) showed an identical pattern of gene expression as found on the microarrays. Real-time PCR analysis on four of five genes at the blastocyst stage (HMG1, APT5a, snRNP, DNAj, and ALCAM) showed an identical pattern of gene expression as found on the microarrays (APT5a did not follow the same pattern). Thus only 1 of the 9 comparisons of the pattern of gene expression exhibited a deviation between the microarray and the real-time PCR. Numerous genes were identified that code for proteins that might be used to non-invasively determine embryo quality. These results illustrate the complex mechanisms involved in pig early embryonic development.

Speakers (S)

S1.

The Swine as a Model Organism for Human Cancer

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The transition from basic to clinical cancer research is hampered, to some extent, by the lack of model organisms similar to man. Specifically, animals that have a similar diet, metabolism, and anatomy would be of great valuable in assessing the effect of anti-cancer drugs on tumor development. One such animal is the pig, which being an omnivore, shares a similar metabolism with humans as well as having a comparable lifespan, body size, and anatomy. Indeed, the pig has long been used as a biomedical model for many human diseases, but has not been exploited for cancer research. We have recently found that many of the genetic events required to promote the tumorigenic growth of normal human cells in immunocompromised mice are likewise required for the same process in pigs. Moreover, such genetically modified porcine cells also formed tumors when returned back to the syngenic host pig. While the immune system of the host pigs needed to be suppressed, likely due to a reliance on using human genes to drive the porcine cells to a tumorigenic state, these experiments provide proof-of-principle that, as in mice, porcine cells could be manipulated in culture to yield cancer cell lines that could be returned to the host animal. With the advent of animal cloning, a single tumorigenic cell line generated from a cloned pig could even be returned to a host of cloned animals, greatly expanding the utility of this model. Along the same lines of reasoning, we are also inducing tumors in cloned pigs with chemical carcinogens to generate cancer cell lines that could also be injected into other cloned animals. This ability to grow established genetically defined or chemically-induced tumor cell lines in cloned pigs could provide a robust and malleable system to not only study human cancer, but also to serve as a preclinical model of this disease in an animal that is similar, in many respects, to humans.

S2.

Historical Perspective Biology of the Pig and Relevance to Medicine

L. Andersson
Department of Medical Biochemistry and Microbiology, Uppsala University and Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden

Pig domestication occurred about 9000 years ago. Genetic studies primarily using mitochondrial DNA have shown that pigs were domesticated both in Europe and Asia. Furthermore, it is clear that many modern breeds of domestic pigs are hybrids between European and Asian domestic pigs due to the introgression of Asian domestic pigs into European domestic pigs that occurred in the 18th and 19th century and possibly earlier than this. A consequence of this evolutionary history is that it is possible to find quite divergent haplotypes among domestic pigs.

The domestic pig is a relevant model for biomedical research because of the extensive genetic modifications that have been achieved by selective breeding the last 9,000 years. A number of phenotypic traits with relevance to human medicine have been altered during domestication and animal breeding. These include reproductive traits, immune response traits, host-pathogen interactions and all types of metabolic traits including appetite. In my talk I will exemplify the new knowledge that can be generated from biomedical research in the pig and how we should best utilize the emerging genome resources for the pig.

S3.

Bioinformatics for Phenotypic Models of Human Diseases

Janan T. Eppig
Jackson Laboratory, 600 Main Street, Bar Harbor, ME

Mammalian model organisms are critical experimental surrogates for studying human biology and disease. The mouse is an exceptional model in that all life stages can be readily accessed and there are myriad experimental tools available, including well-developed genetic maps, a wealth of inbred strains, a sequenced genome, and the technology to specifically alter its genome. The many new genetically engineered and mutagenized alleles being generated to create a "mutant for every gene" promise to further enhance our ability to study critical pathways and gene function.

The Mouse Genome Informatics Database (MGI, http://www.informatics.jax.org) supports biological knowledge building for the laboratory mouse by integrating and providing access to data ranging from sequence to phenotype. Core data include extensive gene characterization, gene function, mutant phenotype descriptions and associations with human diseases, DNA and protein sequences, gene expression data, strain and polymorphism data, genetic and comparative maps, and comparative gene data for mouse and other mammals.

Among the many challenges of developing and integrating phenotype and disease model data are the accelerating pace of data generation, the need to develop and use vocabulary standards, the implementation of human-friendly interfaces for querying and retrieval of information, and the support of computational analyses of comparative phenotypes, diseases, and other complex data. Key aspects of these challenges will be discussed, with examples of how successfully integrating these data can support hypothesis generation for models of human disease.

S4.

Genetically Modified Swine

E. J. Forsberg, Ph.D.
Infigen, Inc., 1825 Infinity Drive, DeForest, WI 53532

Until the advent of nuclear transfer technologies, mice were the only mammalian species that were easily amenable to gene manipulation using gene-targeting methods that are applied to mouse embryonic stem cells (ESC). The ability to add, delete and alter genes in mice has led to a wealth of information on the function of specific genes and the potential role of orthologous genes in human disease. However, there is increasing awareness that mouse models often do not replicate important features of human diseases and they often do not predict the success of proposed human therapies. Nuclear transfer technologies provide the opportunity to apply gene-targeting technologies initially developed for mouse ESC to cultured nuclear transfer donor cells. Thus, it is now possible to modify specific genes in any animal that can be cloned. Particularly attractive are animals such as swine whose anatomy, physiology and gene expression patterns more closely match those of humans. The use of genetically modified swine for the study and development of therapies for cardiovascular disease will be an important adjunct to the common use of non-genetically modified swine for the same purposes. Cardiovascular disease is the leading cause of morbidity and mortality in the US and coronary artery disease accounts for the majority of deaths. Most studies point to hypercholesterolemia as the principal cause of atherosclerosis. Advances in the understanding and treatment of atherosclerosis have relied, in part, on animal models, including rabbits, primates and swine that are genetically predisposed to develop the disease, swine that develop the disease when fed high-cholesterol diets and genetically modified mice. However, genetically modified mice that manifest hypercholesterolemia do not exhibit lesions typical of atherosclerosis in humans. In contrast, swine pedigrees that develop hypercholesterolemia as a result of naturally occurring genetic mutations that resemble those that occur in human hereditary hypercholesterolemia manifest arterial lesions similar to those seen in humans. Our goal is to introduce specific genetic alterations in miniature swine that reproduce the genetic and pathological characteristics of human atherosclerosis. These genetically modified miniature swine will meet a growing need of medical device and pharmaceutical researchers for uniform animal models of human pathologies that can help predict the outcome of human therapeutic interventions.

S5.

Minipig Models For Musculoskeletal Reconstruction Research

S. Hollister
University of Michigan

Musculoskeletal reconstruction and tissue engineering research requires large functional animal models to test procedures prior to clinical use. There are a range of requirements for these models including functional and anatomic similarity to humans as well as the availability of probes for molecular assays including PCR, immunohistochemistry and in situ hybridization. This talk will cover the use of minipigs as models for musculoskeletal reconstruction, with a primary focus on craniofacial, spinal, and bone-cartilage reconstruction. Examples of minipig models will be taken from the literature as well as the authors own research. Results from these studies demonstrate that minipigs are a viable model for musculoskeletal reconstruction research

S6.

The National Swine Resource and Research Center

L. K. Riley, J. K. Critser, R. Prather and T. Strauch
University of Missouri, Columbia, MO.

The National Swine Resource and Research Center (NSRRC) was funded by the National Institutes of Health (NIH) in September 2003. The goals of the NSRRC are: (1) to provide swine models to biomedical researchers, (2) to shift the burden for maintaining and distributing unique swine models from investigators to a National Resource Center, and (3) to perform research aimed at improving swine as animal models. Importantly, the NSRRC serves as repository and distribution center for swine models used in biomedical research. Functions of the NSRRC include importation, rederivation, health evaluation, genetic evaluation, production, cryopreservation, phenotyping, and distribution of swine models. In the 1st year of operation 10 swine models have been imported into the Center; most are in the process of being rederived to a pathogen-free status. Animals are currently being held in temporary facilities. The NSRRC will be permanently housed in a new facility being constructed with a separate NIH-funded construction award. The facility is scheduled to be complete in February of 2006 and will include state-of-the-art animal housing, surgery facilities, and laboratory space. In addition to serving as a repository and distribution center, the NSRRC creates genetically-modified swine models for NIH-funded investigators through a competitive application process. Research activities within the NSRRC include: (1) development of improved cryopreservation methods for swine gametes and embryos, (2) development of improved health and genetic monitoring approaches, and (3) development of improved efficiencies for creation of genetically-modified pigs. Additional information regarding the NSRRC is located on the web at http://www.nsrrrc.missouri.edu.

S7.

The Swine Genome Sequencing Project

J. Rogers[1] and members of the Swine Genome Sequencing Consortium
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A reference sequence for the swine genome will provide an invaluable resource for swine genetics and genomics, as well as for comparative studies with other mammals. A programme to obtain a reference sequence that will be publicly available is being developed by a consortium of laboratories from ten countries. The project is being developed in two phases. The first phase has focused on the production of a physical map of the swine genome in mapped, bacterial large insert clones. The map is based principally on a BAC library generated from a single female pig (CHORI-242), which has been end-sequenced and fingerprinted in Illinois and at the Sanger Institute, but also draws in additional end-sequences and fingerprints from three other libraries (CHORI-44, pigEBAC and INRA). The physical map is being assembled on the basis of overlapping fingerprints, using alignment of end sequences to the human genome sequence to help orient and order contigs and the radiation hybrid map of the swine genome generated at the University of Illinois, to position contigs on the swine genome. To date, the map contains around 1500 contigs and we hope to reduce this number to a few hundred, by summer 2005. Views of the BAC end sequences mapped to the human genome are displayed in Ensembl (http://www.ensembl.org) and pro-

vide a useful resource for researchers to identify clones containing genes of interest by homology with human genes.

The strategy that will be used to sequence the swine genome is a combined whole genome sequencing and mapped clone sequencing approach. The majority of the sequence data will be derived from the single animal used to generate the CHORI-242 BAC library. Data will be deposited in the GenBank and Ensembl trace archives as they are generated and sequence assemblies, display and automated annotation will be made available by the Sanger Institute and Ensembl.

S8.

The Value of Swine in Transplantation Research

D. H. Sachs, M.D.
Transplantation Biology Research Center, Massachusetts General Hospital and Harvard Medical School, Boston, MA

It would be difficult for any animal model to equal the mouse with respect to cost, ease of genetic manipulation or availability of information and reagents for detailed study of it genetics. Nevertheless, despite the enormous contributions which mouse models have made to the field of transplantation, there are certain disadvantages of mice that can only be overcome by use of large animals. These include 1) differences between mice and humans in the tissue expression of some molecules of importance to immune recognition; 2) size disparity as a surgical model; 3) comparability to humans in response to infections, immunosuppressive drugs and radiation; and 4) ease with which immunologic tolerance can be induced. For all of these reasons, we have chosen miniature swine as a large animal model for transplantation research. Among their advantages in this regard are 1) comparable size to humans; 2) ease of inbreeding, allowing establishment of MHC homozygous and recombinant homozygous herds, and the recent achievement of a line with >94% coefficient of inbreeding, leading to histocompatibility; and 3) a wealth of reagents for study of cell surface antigens that rivals that of murine and human models. These animals have proved extremely useful for studies of allogeneic transplantation and their suitability for genetic engineering make them very attractive as potential donors for clinical xenotransplantation. Current research in several of these areas will be reviewed.

S9.

Swine in Biomedical Research

K. Swanson[1], K. Kuzmuk[1], S. Meyers[1], J. Beever[1], S. Adams[2], C. Counter[2], R. Rogatcheva[1], E. Rochelle[1], M. Mazur[1], L. Rund[1] and L. Schook[1]
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Based on the pig's anatomical and physiological similarities to humans, swine models possess great utility in biomedical research. Because the pig and human genomes are structured with high similarity, the use of comparative mapping further solidifies its value as an animal model. Genomic and tissue microarrays are just beginning to be appreciated for their investigative power in animal models. As porcine-specific genomic information becomes increasingly available, characterization and adoption of pigs as human models in many disease categories will accelerate. There is still much to explore beyond the pig's already proven value in immunology, nutrition, cardiovascular disease, orthopedics, and dermatology/wound healing. Initiatives are warranted in new zoonotic diseases, biodefense, behavior/neurological imaging, and gnotobiology. The sequencing of the pig genome through an international consortium will provide sequence information that can be used to construct relevant biomedical models. By capturing sequence information and creating appropriate models to study pathogenesis, a new means of diagnosing and treating life style-related diseases using the pig as a model will become a reality. Furthermore, the increasing shortage of donor organs for transplantation has lead to a search for suitable xenograft organs. Pigs are now considered the primary candidate donor animal. It is generally accepted that natural antibodies and their complement play a major role in xenograft rejection. The ability to genetically modify and clone pigs provides a unique opportunity for understanding the mechanisms of graft rejection and to create clinical therapies. Thus, by effectively uniting enabling technologies and experimental de-

sign, the pig may be used to create ideal biomedical models for defining the disease process and intervention strategies.

S10.
Bob Young & Michael Young
SERI, 20 Staniford Street, Boston, MA

Our work involves the use of progenitor cells to repair the diseased retina. I will discuss our studies using embryonic brain and retinal progenitor cells from GFP+ pigs. These cells have been expanded and characterized in culture, and grafted into the matured, injured pig retina. The use of the pig as a model for human retinal disease has great potential for the development of therapeutic strategies to treat retinal degeneration in humans.

Transplantation (TX)

TX1.
Will Analysis of Gene Expression Profiles Elucidate Mechanisms of Delayed Xenograft Rejection?
A. Azimzadeh[1], H. Dawson[2], G. Wu[1], S. Pfeiffer[1], B. N. Nguyen[1], S. Kelishadi[1], C. Schröder[1], T. Zhang[1] and R. N. Pierson III[1]
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[2]USDA, Beltsville, MD, USA;

Background: Prior work has focused on antibodies against Galα1,3Gal and non-Gal epitopes as the main cause of delayed xenograft rejection (DXR).

Methods: The role of co-stimulatory pathways was evaluated in a pig-to-baboon cardiac xenotransplantation model, using pigs transgenic for human complement regulatory molecules to prevent hyperacute rejection.

Results: With conventional immunosuppression, induced antibodies against Galα1,3Gal and non-Gal epitopes were effectively blocked. Sparse T-cell, monocyte, and macrophage infiltrates increased in grafts as DXR progressed to graft failure. Expression of IFN-□ and costimulation molecules (CD80, CD86, ICOS) was also increased in grafts with DXR.

Conclusion: Histologic and molecular analysis showed that adaptive immunity, likely mediated by costimulation molecules, contributes to DXR when xenoantibody elaboration is inhibited. Screening for gene expression associated with activation of porcine endothelial or smooth muscle cells and macrophages (e.g., CAM-, TLR-, MMP- or other NFkB pathway-associated genes such as TNF) may reveal new therapeutic targets to control or prevent DXR.

TX2.

Galt K/O Pig Lungs are Protected from HAR by Human Blood

A. Azimzadeh[1], B.-N. Nguyen[1], C. Schröder[1], J. S. Allan[2], G. Wu[1], T. Zhang[1], H.-J. Shuurman[3], D. H. Sachs[2] and R. N. Pierson III[1]

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[2]TBRC and Massachusetts General Hospital, Boston, MA
[3]Immerge Biotherapeutics Inc, Cambridge, MA

Background: Pig organs might provide a solution to the organ shortage for transplantation. However, a rapid and violent reaction, called hyperacute rejection (HAR), leads to the destruction of a xenograft if it is not protected. We have shown that pig lungs are extremely sensitive to HAR, even with potent complement inhibition. Recently developed galactosyl transferase knock-out (GalT K/O) pigs offer an additional tool to prevent lung HAR (HALR). Here we evaluate the role of coagulation system activation in this context.

Results: Using a well established ex-vivo lung perfusion model, we demonstrate that GalT KO lungs exhibit significantly prolonged lung survival with preserved function, decreased complement activation, platelet activation and thrombin formation. However capillary congestion and thrombosis are prominent at demise.

Conclusions: Dysregulated coagulation plays a role in GalT K/O HALR, with evidence also implicating lung macrophages and other platelet receptors and leukocyte adhesion molecules.

TX3.

Transcriptional Profiling of Stress-Response in Cultured Porcine Islets.

M. Hårdstedt[a], C. M. T. Dvorak[b], H. Xie[c], M.Wang[c], A. C. Bauer[a], B. J. Hering[a], M. P. Murtaugh[b] and S. C. Fahrenkrug[c],

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Xenotransplantation with adult porcine islets could meet the increasing demand for cell-based diabetes therapy. A better understanding of the perturbations of the cell biology encountered during islet processing will aid the rational design of cytoprotective strategies aimed at improving transplant outcomes. Isolated adult pig islets were exposed *in vitro* to inflammatory cytokines (IL-1β: 2ng/ml; TNFα: 1,000 U/ml; and IFN-γ: 1,000 U/ml) and/or elevated glucose (5.5 vs 16.7 mM glucose). Islet gene expression profiles were assessed using a new porcine 70-mer oligonucleotide microarray and real-time PCR. Microarray data were analyzed by direct pairwise comparison between culture conditions and by loop design using GeneSpring and R/maanova, respectively. Our results revealed distinct gene expression patterns in response to exposure to cytokines and glucose for 48 h. Cytokine treatment resulted in increased expression of genes involved in stress, immune response (e.g. MHC-related genes), apoptosis, and cellular defense (e.g. MnSOD). Islets cultured under conditions of elevated glucose showed increased expression of genes involved in intracellular protein transport, glucose and lipid metabolism, and stress response. A decrease in intracellular ATP and insulin content was detected at 48 h in response to cytokines and at 96 h in response to high glucose. In conclusion, transcriptional profiling of the response of porcine islet beta cells to inflammatory and hyperglycemic conditions will help identify molecular targets that are likely to protect porcine islets during islet isolation and engraftment. MGI is supported by NIH grants HG00330, HG02273, HD33745, HL64541, CA89713.

TX4.

Use of Lentiviral Vectors for Combination of Transgenic Strategies to Facilitate Pig-to-Primate Xenotransplantation

R. Klose, A. Hofmann[#], E. Kemter, B. Keßler, H. Sebald[#], S. Ewerling, M. Weppert, T. Holy, A Pfeifer[#] and E. Wolf
Institute of Molecular Animal Breeding and Biotechnology, Gene Center, LMU
[#]Department of Pharmacy/Center for Drug Research, LMU

For realisation of successful clinical xenotransplantation it will be necessary to design multitransgenic pigs. However, costs for the production of transgenic livestock by pronuclear injection of DNA into zygotes are immense, due to the low transgenesis rates achieved.

We have adapted gene transfer by lentiviral vectors for the generation of transgenic pigs (Hofmann et al., EMBO Rep 4, 1054-1060, 2003). Expression of hDAF on porcine xenografts was shown before to be effective in controling hyperacute rejection (HAR) in xenotransplantation experiments. Using this highly efficient method we infected porcine fibroblasts with lentiviral vectors carrying human decay accelerating factor (hDAF) under the control of the phosphoglycerate kinase promoter and achieved expression of hDAF.

Recently, TNF alpha related apoptosis inducing ligand (TRAIL) was reported to inhibit the primarily T-cell mediated rejection of corneal allografts (Xie et al., Transplantation 76:1556-1559, 2003). As an approach to neutralize the attack of xenografted tissues by recipient immune cells, we generated transgenic pigs expressing human TRAIL under the control of the murine MHC class I promoter H-2Kb. Western blot analysis with an anti-human TRAIL specific antibody revealed different expression levels for TRAIL depending on the tissue and transgenic line examined. TRAIL-transgenic peripheral blood lymphocytes induced apoptosis in human Jurkat T cells showing biological activity of the transgene.
To combine strategies against HAR and human cellular immune responses to pig xenoantigens we will generate multitransgenic pigs by lentiviral transfer of hDAF into TRAIL-transgenic zygotes.

TX5.

Enhancement of Islet Yield by Inhibition of Apoptosis by Antioxidant, N-Acetyl-L-Cysteine

S. Ramachandran, N. Benshoff, Nicole Babisky, Martin Jendrisak and T. Mohanakumar
Washington University School of Medicine, Box 8109 CSRB 3328, 660 S Euclid Ave, St. Louis, MO 63110

Islet transplantation is considered a potential therapy for treatment of selected type I diabetic patients. Enzymatic digestion and mechanical disruption during the process of islet isolation has been hypothesized to induce oxidative stress and apoptosis in isolated islets, resulting in less than 30% of transplanted islets to be stably engrafted. Anti-oxidant therapy has been shown to inhibit apoptosis in cells. N-Acetyl-L-cysteine (NAC), a scavenger of free radicals in cells and is currently used in treatment of various diseases. In order to study the effect of NAC to inhibit oxidative stress induced apoptosis initiation during the isolation process by adding NAC to the enzyme preparation and collection media. Porcine pancreas was obtained from the slaughter house and transported cold on UW. Pancreas was divided into two halves and processed for islet isolation. One half was processed immediately and the other half was stored for an additional 6 hrs and then processed. For the second processing NAC (150mg/L) was added to the enzyme preparation as well as the collection media. Even with an increased cold storage of 6 hrs, addition of NAC during the isolation process inhibited apoptosis and yielded similar number of islets (224,000 I.E s) obtained from pancreas that was immediately processed (218,000). Moreover islets from both the preparations with and without NAC addition were able to restore normoglycemia in diabetic-SCID mouse with similar kinetics. This demonstrates that addition of NAC during the process of islet isolation inhibits apoptosis and increases the yield of isolated islets.

TX6.

Important Role of NKG2D in Activation of NK Cells and Costimulation of Activated T CELLS Exposed TP Porcine Aortic Endothelial Cells

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NK cells play an important role in xenograft rejection. NK cells express both activating and inhibiting receptors on cell surface. The aim of the study was to identify the activating receptor involved in activation of NK cells on exposure to porcine aortic endothelial cells and characterize the signaling cascade leading to cytotoxicity and cytokine secretion. Human NK cells were purified from PBMCs by Rossettesep NK cell enrichment kit. Expression profiles of activating receptors NKp46, NKp44, NKp30 and NKG2D were analyzed by RT-PCR, western blot and FACS. Signaling events subsequent to activation were analyzed by western blot. Purified human NK cells were able to significantly lyse (44.4%) PAEC compared to HAEC. Incubation of NK cells with PAEC resulted in increased expression of NKG2D, a receptor for stress induced ligands, both at mRNA and protein level. FACS analysis of activated NK cells showed 3-fold increase in number of NKG2D+ NK cells. Western blot analysis with anti-DAP10 and PI3-kinase antibodies showed increased recruitment of DAP10 adapter protein and activation of PI3 kinase in NK cells cultured with PAEC. Tretreatment with soluble MICA, a ligand for the NKG2D receptor resulted in significant inhibition of PAEC cytotoxicity. Ligation with anti-NKG2D antibody also showed significant increase in proliferation of T cells activated with PAEC. In conclusion, human NK cells recognize PAEC through NKG2D leading to recruitment of DAP10, activation of PI3 and MAP kinase pathway leading to cytotoxicity and cytokine secretion. NKG2D can also act as costiumlatory signal for activated T cells in xenotransplant setting.

TX7.

CD8+ CTLS Against Porcine Endogenous Retrovirus Peptides Contribute Towards Xenograft Recognition

S. Ramachandran, Y. Virkud, W. Chapman and T. Mohanakumar
Washington University School of Medicine, Box 8109 CSRB 3328, 660 S Euclid Ave, St. Louis, MO 63110

Xenotransplantation has potential to alleviate organ shortage. Porcine endogenous retroviruses (PERV) have been shown to infect human cells raising concerns regarding the safety of xenotransplantation. However, in patients exposed to porcine tissues no PERV infection was observed. This maybe due to natural anti-Gal∞Ñ1-3Gal antibodies or cellular immunity. This study was designed to determine the contribution of PERV-specific CTLs in rejection of transplanted xenograftsl. The envelope glycoprotein is essential for retroviral entry and cell tropism. Computer-assisted analysis identified nine PERV-envelope-derived 9-mer peptides with high affinity for HLA-A2 (Env-1-9). CTLs generated in vitro against peptide loaded T2 cells identified Env-5 peptide (303-311, KLFSLIQGA) and demonstrated cytotoxicity against PERV-infected human cells. Antibody blocking studies and cold target inhibtion assays further demonstrated that the T cell line generated was MHC-class I restricted and PERV-specific. In order to determine whether PERV-peptides are also processed and presented in natural porcine host, using tandem mass spectrometry, we identified peptide derived from retroviral transactivating regulatory (Tat) protein (AHQDPLPEQP) recognized by human CD8+ CTLs in context of SLA. These results indicate that PERV peptides are naturally processed and presented in the context of HLA and SLA, and human CTLs recognize this PERV-peptide-MHC complex. The SLA1z gene from PAEC was cloned into pcDNA 3.1 and used to transfect T2 cells. T2 cells expressing SLA1z were loaded with the peptides and used to generate human Cd8+ CTLs. Currently studies are in progress to passively transfer these PERV specific CTLs into SCID mouse transplanted with porcine islets to study their contribution in xenograft rejection.

TX8.

Co-Stimulation Blockade Targeting CD154 and CD28/B7 Modulates the Induced Antibody Response to a Pig-to-Baboon Cardiac Xenograft

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Background: Antibodies against Galα1,3Gal and non-Gal epitopes may contribute to the delayed xenograft rejection (DXR) of pig xenografts. Here we evaluate the role of the CD40/CD154 and/or B7/CD28 co-stimulatory pathways in the elaboration of the elicited antibody response to these antigens following pig-to-baboon cardiac xenotranplantation.

Results: The antibody response to Gal and non-Gal antigens was well-controlled in 10 animals treated with conventional immunosuppression, but with severe morbidity and mortality. CD154 blockade was associated with increased anti-□Gal Ab titers in 4 of 6 animals, and anti-non-Gal Ab titers in 3 of 6 animals. Anti-non-gal antibody response was absent in 2 animals treated with additional CTLA4-Fc, and without major toxicity.

Conclusion: CD154 inhibition incompletely prevents induction of Gal and non-Gal anti-pig antibodies, but additional CD28/B7 co-stimulation pathway inhibition is effective, and safer than conventional immunosuppression.