Ramil E Sapinoro

Education/Training

Post-Doctoral Research Fellow, University of Rochester, Rochester, NY, 2008-2010
Academic Concentration: Pulmonary Inflammation
Ph.D., University of Rochester, Rochester, NY, 2001-2007
Academic Concentrations: Microbiology and Immunology
Master of Science, University of Rochester, Rochester, NY, 2004
Academic Concentrations: Microbiology and Immunology
Bachelor of Science, Pacific University, Forest Grove, OR, 1997-2001
Academic Concentrations: Biology and Japanese

Professional Experience

Teaching and Management Experience:

Assistant Professor of Pharmaceutical Sciences, Present
St. John Fisher College, Wegmans School of Pharmacy, Rochester, NY
-Responsible for planning lecture lesson plans and execution of presentations, class activities, and designing and grading examinations for first- thru third-year students in the Biosystems and Systems Pharmacology course series.

Adjunct Professor, Spring 2008; Spring 2009; Spring 2010
St. John Fisher College, Wegmans School of Pharmacy, Rochester, NY
-Responsible for planning lecture lesson plans and execution of presentations, class activities, and designing and grading examinations for an introductory course in Microbiology and Immunology, with emphasis on microbial pathogenesis, the study of the molecular mechanisms by which microbes interact with their host to cause damage leading to disease, and host immune responses. Student audience: ~70 first-year pharmacy students.

Immunology Workshop Coordinator/Teaching Assistant, Fall 2009
University of Rochester, Dept. of Microbiology and Immunology, Rochester, NY
-Assisted in the development of "Peer-Group Recitations" for a graduate level Immunology course were problem-based learning and case-studies were applied

Scientist-Instructor, Fall 2006
Life Sciences Learning Center, Scientist-Instructor Program, Rochester, NY
-Responsible for teaching various hands-on laboratory exercises to middle and high school students. Development and presentation of an original laboratory case-study to area high school biology teachers entitled, "AIDS Vaccine Case Study."

Student Teacher, Spring 2005
Graduate Experience in Science Education, Rochester, NY
-Provided with the fundamental understanding of the theories, principles and concepts of effective science education, including learning styles, differentiated instruction, lesson-planning and course-planning skills, and instructional strategies for case-study teaching and problem-based learning

-Directed a DNA lesson activity to be implemented and executed at the Rochester Museum and Science Center's "DNA! Science Saturday"

Teaching Assistant, Spring 2003

Host Defense Medical Microbiology Laboratory, University of Rochester
-Managed a first-year medical student laboratory with a faculty instructor, and provided group and one-on-one peer tutoring for students

Graduate Student Mentor, 2003-2007

University of Rochester, Dept. of Microbiology and Immunology, Rochester, NY
-Supervised and managed undergraduate and graduate students in team-oriented or independent research projects

Research Experience:

Post-Doctoral Fellowship, 2008-2010

Department of Medicine, Pulmonary and Critical Care, University of Rochester
Fellowship Training Program in Environmental Toxicology, NIH/NIEHS
-Conducting research studying the molecular mechanisms of lung fibrosis/scarring and investigating novel therapeutics for lung diseases.
-Determine the efficacy of ω-3-PUFA metabolites in regulating the inflammatory milieu in the lung induced in a mouse model of cigarette smoke exposure.
-Mentored and directed an undergraduate Summer Scholar in the lab

Post-Doctoral Fellowship, 2007-2008

Department of Microbiology and Immunology, University of Rochester
Fellowship *Training Program in Viral Diseases, Vaccines, and Bio-Defense, NIH/NIAID*
-Conducted research using bacteriophage lambda vectors as a protein scaffold to display HIV-1 envelope proteins in a dense, repetitive array to increase envelope immunogenicity.
-Mentored and directed several undergraduate and graduate students in the lab

Ph.D. Student, 2001-2007

Department of Microbiology and Immunology, University of Rochester
-Conducted Ph.D. research thesis in the laboratory of Dr. Stephen Dewhurst. Research focused on strategies for HIV-1 vaccine development using antibody-mediated targeting of viral vectors to dendritic cells; dissecting the cellular effector pathways of FcγRs
-Performed three research rotations of 10 weeks each, from 7/01 to 4/02. Research topics include the production of "virus-like particles" for the use of gene delivery, phage display of peptides for the targeting of dendritic cells, and cloning of adenovirus containing cytokine genes for the study of lung fibrosis.
-Mentored and directed several undergraduate and graduate students in the lab

Undergraduate Researcher, 2000-2001

Department of Biology, Pacific University, Forest Grove, OR
-Performed summer research in 2000 on the effects of ethnobotanical plants on pathogenic microorganisms, under the supervision of Dr. Lisa Sardinia. Also conducted independent research in the Fall of 2000 and Spring of 2001 with Dr. Deke Gundersen, on environmental toxicology regarding endocrine disrupting chemicals in Columbia River White Sturgeon.

Awards and Honors

Pilot Collaborative Clinical and Translational Studies Grant
Clinical and Translational Science Institute, University of Rochester, Rochester, NY

Funding: $50,000; June 2010 – June 2011
Investigators: Patricia Sime, **Ramil Sapinoro**, Richard Phipps, Charles Serhan
"Anti-inflammatory and Pro-resolving Lipid Mediators: Their role in regulating lung inflammation"

American Thoracic Society Minority Trainee Travel Award 2010
ATS International Conference, New Orleans, LA, 2010

Post-Baccalaureate
Provost's Fellowship, University of Rochester, 2001-2007

Undergraduate
GPA = 3.7
Beta Beta Beta Biological Honor Society
Dean's List, Pacific University
National Residence Hall Honorary

Presentations/Speaking Engagements

Environmental Medicine Toxicology Retreat, Rochester, NY, 2010
"The Anti-inflammatory and Pro-resolving Lipid Mediator Resolvin D1 Regulates Lung Inflammation *In vitro* and *In vivo*"

American Thoracic Society International Conference, New Orleans, LA, 2010
"The Anti-inflammatory and Pro-resolving Lipid Mediator Resolvin D1 Regulates Lung Inflammation *In vitro* and *In vivo*"

Environmental Medicine Toxicology Retreat, Rochester, NY, 2009
"Anti-Inflammatory and Pro-resolving Lipid Mediators: A potential role in regulating lung inflammation"

National Association of Biology Teachers Annual Conference, Atlanta, GA, 2007
"AIDS Vaccine Case Study"

National Association of Biology Teachers Regional Workshop: Pox to Pandemics, Swiftwater, PA, Sanofi-Pasteur campus, 2007
"AIDS Vaccine Case Study"

American Society of Gene Therapy 8th Annual Meeting, St. Louis, MO, 2005
"Enhanced Transduction of Dendritic Cells by FcγRI-Targeted Adenovirus Vectors"

Graduate Student Seminar, University of Rochester, Rochester, NY, 2005
"FcγRI-Targeted Adenovirus Vectors: Implications or Vaccine Development"

Graduate Student Seminar, University of Rochester, Rochester, NY, 2004
"Development and Characterization of Dendritic Cell-Targeting Proteins: Implications for Viral Vector Delivery"

AIDS Vaccine, New York, NY, 2003
"Recombinant Murine Cytomegalovirus Vectors Abortively Infect Human Dendritic Cells, Leading to Expression of Encoded Antigens and Retention of DC Function"

AAAS Annual Meeting and Science Exposition, San Francisco, CA, 2001
"The Inhibition of Growth of Pathogenic Microorganisms by Ethnobotanical Plants from Costa Rica"

Murdock Charitable Trust Conference for Undergraduate Research, Tacoma, WA, 2000

"The Inhibition of Growth of Pathogenic Microorganisms by Ethnobotanical Plants from Costa Rica"

Publications

Sapinoro R, Levy E, Serhan CN, Phipps RP, Sime PJ. 2010. The Anti-inflammatory and Pro-resolving Lipid Mediator Resolvin D1 Regulates Cigarette Smoke-induced Lung Inflammation *In vitro* and *In vivo*. *(Manuscript in preparation)*

Hogan CM, **Sapinoro R**, Gurell M, Pollack SJ, Phipps RP, Sime PJ. 2010. Electrophilic PPARγ ligands attenuate IL-1β and silica induced lung inflammation in human lung fibroblasts. Am J Physiol Lung Cell Mol Physiol *(In press)*

Sapinoro R, Rodrigo S, Schlesinger J, Dewhurst S. 2007. Fc receptor mediated, antibody-dependent enhancement of bacteriophage lambda-mediated gene transfer in mammalian cells. Virology. 2008 Apr 10;373(2):274-86. Epub 2008 Jan 14.

Sapinoro R, Maguire C, Burgess A, Dewhurst S. 2007. Enhanced transduction of dendritic cells by FcγRI-targeted adenovirus. J Gene Med. 2007 Dec;9(12):1033-45.

Zanghi CN, **Sapinoro R**, Bradel-Tretheway B, Dewhurst S. 2007. A tractable method for simultaneous modifications to the head and tail of bacteriophage lambda and its application to enhancing phage-mediated gene delivery.
Nucleic Acids Res. 35(8):e59.

Maguire CA, **Sapinoro R**, Girgis N, Rodriguez-Colon SM, Ramirez SH, Williams J, Dewhurst S. 2006. Recombinant adenovirus type 5 vectors that target DC-SIGN, ChemR23 and alpha(v)beta3 integrin efficiently transduce human dendritic cells and enhance presentation of vectored antigens. Vaccine. Jan 30;24(5):671-82.

Fan S, Maguire CA, Ramirez SH, Bradel-Tretheway B, **Sapinoro R**, Sui Z, Chakraborty-Sett S, Dewhurst S. 2005. Valproic acid enhances gene expression from viral gene transfer vectors. J Virol Methods. 125(1): 23-33.

Wang X, Messerle M, **Sapinoro R**, Santos K, Hocknell PK, Jin X, Dewhurst S. 2003. Murine cytomegalovirus abortively infects human dendritic cells, leading to expression and presentation of virally vectored genes. J Virol. 77(13): 7182-92.

Graduate Research Summary

The central goal of the studies investigated in my thesis work was to explore Fc receptor targeting as an approach to enhancing virally-vectored gene transfer in mammalian cells. To approach this question, two sets of experiments were performed. First, studies were conducted to test the hypothesis that the efficiency of gene transfer to dendritic cells (DC) by recombinant adenoviral (Ad) vectors could be improved by targeting the vector to the high affinity Fc-gamma receptor I (FcγRI). The long-term goal of these experiments was to improve Ad-based vaccine delivery.

To 'retarget' Ad vectors to FcγRI, a targeting complex consisting of a trimeric adenovirus fiber-binding moiety fused to a single-chain antibody specific for FcγRI was generated. Transduction studies revealed that FcγRI-targeted Ad transduced primary monocyte-derived DC with greater efficiency than unmodified Ad vectors. In addition, FcγRI-targeted Ad elicited more efficient activation of antigen-specific autologous memory $CD8^+$ T-cells than unmodified Ad vectors. Thus, FcγRI-targeted Ad demonstrated enhanced antigen delivery to DC.

A second experimental model system was also used to study effects of Fc receptor targeting on virally-vectors gene transfer. In this case, a bacterial virus vector (recombinant bacteriophage λ) was decorated with intact antibody molecules, and the resulting immune complexes were then used to study the molecular mechanisms involved in FcγRI internalization and intracellular routing following receptor ligation. Bacteriophage λ immune complexes were found to transduce FcγRI expressing mammalian cells with approximately 20-50 fold greater efficiency than unmodified bacteriophage λ particles. The ability of other Fc receptor subtypes to support gene transfer by bacteriophage λ immune complexes was also assessed, and FcγRI was found to confer the greatest permissivity to phage-mediated gene expression. Pharmacologic studies revealed that actin microfilaments, which are important for clathrin-mediated endocytosis, are essential for efficient gene expression, following addition of bacteriophage λ immune complexes to FcγRI positive cells. In contrast, endosomotropic and microtubule-disrupting agents *increased* bacteriophage λ-mediated gene expression. Overall, these studies suggest that bacteriophage λ immune complexes bind to FcγRI and then enter mammalian cells through clathrin-coated pits; most of the incoming bacteriophage particles then appear to enter endosomes, with the majority of bacteriophage λ particles presumably becoming degraded in lysosomes.

Overall, these studies show that Fc receptor targeting can enhance gene transfer efficiency in a wide range of mammalian cell types, by both bacterial and eukaryotic virus vectors. This suggests that Fc receptor targeting may be a broadly useful approach to improve virally-vectored gene transfer to FcR-positive host cells such as dendritic cells.

Post-Doctoral Research Fellowship Summary

Anti-inflammatory and Pro-resolving Lipid Mediators:
A potential role in regulating lung inflammation

Prolonged lung inflammation due to injury from inhalation of toxicants such as cigarette smoke, oxidative stress, infection or immune dysfunction is central to the development of numerous pulmonary diseases, such as chronic obstructive pulmonary disease (COPD), tissue fibrosis and asthma. Following acute lung injury, lung fibroblasts produce pro-inflammatory mediators such as cyclooxygenase-2 (COX-2), prostaglandins (PGs) and cytokines, leading to the activation and recruitment of inflammatory cells into the lung, particularly macrophages and neutrophils. Unresolved inflammatory responses can lead to substantial tissue damage and loss of function. Resolution of inflammation was previously thought to be a passive process, but recent studies support a paradigm shift: resolution is an active process linking novel biochemical pathways involving omega-3 polyunsaturated fatty acids (ω-3-PUFAs) and the generation of chemical mediators that regulate resolution programs. Docosahexaenoic acid (DHA) is an essential ω-3-PUFA that generates potent endogenous lipid mediators that possess anti-inflammatory and pro-resolving properties. ω-3-PUFA-derived lipid mediators have been demonstrated to reduce inflammatory responses, both i*n vitro* and *in vivo*. We are interested in investigating the anti-inflammatory and pro-resolution effects of ω-3-PUFA metabolites in regulating the inflammatory milieu incited by pro-inflammatory mediators (IL-1β, cigarette smoke) in structural lung cells.

Using primary human lung fibroblasts as a model system, western blot analyses revealed that the expression of COX-2 is dramatically attenuated in lung fibroblasts treated with ω-3-PUFA-derived lipid mediators prior to IL-1β or cigarette smoke stimulus. In addition, levels of pro-inflammatory cytokines (IL-6, IL-8, MCP-1) and the prostaglandin PGE_2 were also reduced. In an *in vivo* model of cigarette smoke exposure, lungs of mice treated with ω-3-PUFA-dervied lipid mediators and exposed to inhaled cigarette smoke exhibit a significant decrease in neutrophil infiltrates in the bronchoalveolar lavage (BAL), as well as a significant reduction in the levels of neutrophil chemo-attractants measured in the BAL fluid. Experiments are underway to understand the underlying mechanism of action attributed to the anti-inflammatory and pro-resolving properties of these lipid mediators, including two pathways associated with oxidative stress-initiated inflammation: NFκB and the induction of heme-oxygenase-1.

Overall, these studies provide insight into the therapeutic potential of ω-3-PUFA-derived lipid mediators, and understanding the mechanism by which they counter-regulate inflammation may lead to the development of novel therapies used to treat chronic inflammatory lung diseases such as COPD.