

Julie Gosse, Ph.D., Research Abstract submitted in 2009

“Arsenic Perturbation of Allergy/Asthma Signal Transduction”

Millions of people worldwide are drinking water containing high levels of arsenic, including millions of United States citizens who obtain their water from unregulated, contaminated, private wells. Additionally, high levels of arsenic contaminate many foods as well as industrial and mining waste sites.

Recent epidemiological studies suggest that arsenic exposure elevates asthma incidence, but the underlying molecular and cellular mechanism(s) of action are unknown. The most common form of asthma is allergic asthma, and mast cells are major effector cells in allergy and asthma. Additionally, mast cells are important immune defense cells in the body, charged with fighting parasitic infections.

Arsenic is a known endocrine disruptor, and it was recently shown that endocrine disruptors can exacerbate mast cell degranulation (histamine and other-mediator release), thereby potentiating the allergic and asthmatic response. Also, arsenic can affect phosphorylation cascades and other components of signaling pathways similar to those comprising the pathway leading to mast cell degranulation.

If arsenic potentiates the allergic and asthmatic response in mast cells, this effect may be a mechanism by which arsenic elevates asthma incidence. If arsenic inhibits mast cell signaling processes, this effect may reflect a type of immune suppression caused by arsenic exposure. Our studies will determine whether arsenic affects the function of mast cells, using a variety of biochemical, molecular, and cellular techniques. Also, we will seek to determine the underlying molecular mechanisms of these effects.

Julie Gosse, Ph.D., Final Progress Report submitted to the PhRMA Foundation in February 2011

Brief Report of Activities:

Millions of people worldwide are exposed to arsenic (As). As exposure increases the risk of cardiovascular disease, various cancers, and other diseases. As is a potent endocrine disruptor, including of the estrogen receptor. It was recently shown that environmental estrogen-receptor disruptors can affect mast cell signaling. Mast cells are important players in parasite defense, asthma, allergy, and carcinogenesis. Antigen (Ag, allergen) crosslinking of IgE-bound receptors on mast cells leads to signaling which culminates in degranulation, the release of histamine and other mediators of allergy, asthma, and parasite defense. Because As is also an endocrine disruptor, we have tested whether As affects degranulation.

Using the rat basophilic leukemia (RBL) mast cell model, we have measured degranulation in a fluorescence assay which measures the release of one of these allergic mediators, β -hexosaminidase. As alone was found to have no effect on basal degranulation levels. However, **As strongly inhibits Ag-stimulated degranulation**. This inhibition is highly dependent on concentrations of both As and Ag and is not limited to single type of crosslinker. The concentrations of As effective at inhibiting degranulation are not cytotoxic, as measured by trypan blue exclusion, lactate dehydrogenase leakage, and colony-forming assays. This inhibition of mast cell degranulation by As may be a mechanism underlying the

traditional Chinese medicinal use of As to treat asthma. These data also indicate that As may inhibit humans' defenses against parasitic disease. **This work has been published.**

Because these data indicate that As is a strong inhibitor of mast cell degranulation, the underlying molecular mechanism may reveal a useful drug target for the treatment of asthma or allergy. Thus, we are investigating this mechanism. We have submitted a grant to the National Institutes of Health proposing to investigate this molecular mechanism, and we plan to submit a manuscript on these data.

Additionally, during the two years of this grant, we have tested the effects of other endocrine disruptors on mast cell degranulation. Interestingly, we have found that the industrial chemical 4-tert-octylphenol (at 10-20 uM, non-cytotoxic doses) significantly *stimulates* mast cell degranulation. Also, we have found that the potential endocrine disruptor triclosan strongly *inhibits* mast cell degranulation.

Triclosan (TCS) is a broad-spectrum antimicrobial that is added to hundreds of products, such as toys, cookware, and most commonly in hand soaps. TCS is an antimicrobial agent that acts through inhibition of bacterial fatty acid synthesis. In addition, an anti-inflammatory role for TCS has been detected in human skin, including for dermatitis elicited after intradermal injection of histamine (Kjcerheim et al., J Clin Peridon 1994). Symptoms of atopic dermatitis can be alleviated with TCS (Breneman et al., Cutis 2000). We have further investigated the anti-inflammatory potential and mechanism of TCS using the endpoint of mast cell degranulation. Following exposure to TCS we found a strong dose-dependent reduction in degranulation, stimulated either by DNP-BSA antigen or by anti-IgE antibodies. Importantly, these same concentrations were found to be non-cytotoxic to RBLs in lactate dehydrogenase, trypan blue exclusion, and clonogenic cytotoxicity assays. Confocal images of suppressed membrane ruffling support these data. We are currently investigating the molecular mechanism by which **triclosan inhibits mast cell degranulation**. Interestingly, TCS strongly inhibits calcium ionophore-mediated degranulation; these data strongly suggest that **TCS's target is late in the signal transduction cascade**. Our finding that TCS can inhibit mast cell degranulation may explain the clinical data mentioned above and suggests that TCS may be an effective treatment for allergic disease such as eczema.

Papers, Manuscripts in press, Abstracts, Presentations, Invited Seminars:

Gosse Manuscripts Published or in Progress 2009 and 2010:

1. "Inorganic Arsenite Inhibits IgE Receptor-Mediated Degranulation of Mast Cells," Lee M. Hutchinson, Bennett M. Trinh, Rachel K. Palmer, Christopher A. Preziosi, Jonathan H. Pelletier, Hannah M. Nelson, **Julie A. Gosse***, *Journal of Applied Toxicology*, 2010, doi: 10.1002/jat.1585, *in press* (accepted 7-27-10; published online) *corresponding author
2. "Activation of Hedgehog signaling by the environmental toxicant arsenic may contribute to the etiology of arsenic-induced tumors," Dennis Liang Fei, Hua Li, Courtney D. Kozul, Kendall E. Black, Samer Singh, **Julie A. Gosse**, James DiRenzo, Kathleen A. Martin, Baolin Wang, Joshua W. Hamilton, Margaret R. Karagas, and David J. Robbins, *Cancer Research*, first published on February 23, 2010 as doi:10.1158/0008-5472.CAN-09-2898.
3. "Chronic exposure to arsenic in the drinking water alters the expression of immune response genes in mouse lung," C.D. Kozul, T.H. Hampton, J.C. Davey, **J.A. Gosse**, A.P. Nomikos, P.L. Eisenhauer, D.J. Weiss, J.E. Thorpe, M.A. Ihnat, J.W. Hamilton, *Environmental Health Perspectives*, vol.117, 2009, pp.1108-1115.

Gosse Abstracts, Presentations, Invited Seminars 2009 and 2010:

1. "Antibacterial Agent Triclosan Inhibits IgE Receptor-Mediated Mast Cell Degranulation," Rachel Palmer, Benjamin Burpee, Lee Hutchinson, Zsolt Kormendy, Emily Tupper, Jonathan

- Pelletier, Ethan Malay, and **Julie A. Gosse**, *The Toxicologist* (supplement to *Toxicological Sciences*), March 2011, *in press* (Poster at the Society of Toxicology meeting in Washington, DC, March 2011)
2. "Arsenite, Monomethylarsenite and Dimethylarsenite: Chemical Properties and Interaction with the DNA-binding Domain of Steroid Receptors." Anne M. Spuches, **Julie A. Gosse**, Colette F. Quinn, Jack E. Bodwell, Dean E. Wilcox. Poster presented (by Dean Wilcox) at the Superfund Research Program Annual Meeting 2010 in Portland, Oregon; Nov. 10-12, 2010.
 3. "Diametric allergic responses following short-term exposure to environmental phenols: OP enhances while triclosan inhibits mast cell degranulation," Rachel Palmer, Benjamin Burpee, Jonathan Pelletier, Lee Hutchinson, Zsolt Kormendy, Emily Tupper, and **Julie Gosse**; poster presented by graduate student Rachel Palmer at the annual meeting of the Graduate School of Biomedical Sciences, University of Maine, 9-10-10
 4. "Investigating endocrine disruption through allergic signaling perturbation: short-term exposure to the xenoestrogen 4-tert-octylphenol enhances mast cell degranulation." Rachel Palmer, Jonathan Pelletier, **Julie Gosse**. Poster presented at 37th Maine Biological and Medical Sciences Symposium at the Mount Desert Island Biological Laboratory in Salisbury Cove, ME; April 23, 2010.
 5. "Inorganic arsenite inhibits IgE receptor-mediated degranulation of mast cells," L. Hutchinson, B. Trinh, R. Palmer, J. Lyons, C. Preziosi, J. Pelletier, H. Nelson, **J. Gosse**. Abstract presented as an invited talk at the 37th Maine Biological and Medical Sciences Symposium at the Mount Desert Island Biological Laboratory in Salisbury Cove, ME; April 23, 2010.
 6. "Arsenic perturbation of allergy/asthma signal transduction in mast cells," Lee M. Hutchinson, Benett Trinh, Hannah Nelson, Jonathan Pelletier, Elizabeth Brochu, Christopher Preziosi, **Julie Gosse**; poster presented by graduate student Lee Hutchinson at the Graduate Expo, University of Maine, 4-15-09
 7. "Effects of chronic low dose arsenic exposure on hormone-regulated global gene expression in normal human lung fibroblasts," **J.A. Gosse**, E.R. Macari, C.D. Kozul, T.H. Hampton, M. Wungjiranirun, J.E. Bodwell, J.W. Hamilton, *The Toxicologist*, March 2009, Vol.108, No.1, p.367. (Poster at the Society of Toxicology meeting)
 8. "Arsenic perturbation of allergy/asthma signal transduction in mast cells," L.M. Hutchinson, B.M. Trinh, **J.A. Gosse**, *The Toxicologist*, March 2009, vol.108, No.1, p.412. (Platform talk at the Society of Toxicology meeting)

An update on Dr. Gosse and her research

A complete listing of Dr. Gosse's publications is available at her UMaine website:

<http://umaine.edu/biomed/faculty/julie-gosse/> . A pubmed search for her papers, including links to the articles (including links to freely-available articles) is found at <http://www.ncbi.nlm.nih.gov/pubmed/?term=gosse+ja> .

Her research is currently funded by a National Institutes of Health R15 AREA grant entitled "Mechanism of Triclosan Disruption of Mast Cell Function." Her team is collaborating with Dr. Samuel Hess (also at UMaine), who invented the super-resolution microscopy technique "FPALM," or Fluorescence Polarization Activation Localization Microscopy, to probe questions about the antimicrobial triclosan's molecular effects in mast cells.

The project also very recently discovered that the antimicrobial agent triclosan is a proton ionophore uncoupler of mitochondria in living rat and human mast cells and in primary human keratinocytes, and

this work is in press at the *Journal of Applied Toxicology*. This finding holds implications for the safety of triclosan in consumer products (known mitochondrial uncouplers have been pulled from the market in past cases) and also for potential medical uses of triclosan (mitochondrial uncouplers recently have been studied for their controlled usage in the treatment of various diseases such as cancers).