Communicating Research to the General Public

At the March 5, 2010 UW-Madison Chemistry Department Colloquium, Prof. Bassam Z. Shakhashiri, the director of the Wisconsin Initiative for Science Literacy (WISL), encouraged all UW-Madison chemistry Ph.D. candidates to include a chapter in their Ph.D. thesis communicating their research to non-specialists. The goal is to explain the candidate's scholarly research and its significance to a wider audience that includes family members, friends, civic groups, newspaper reporters, program officers at appropriate funding agencies, state legislators, and members of the U.S. Congress.

Over 50 Ph.D. degree recipients have successfully completed their theses and included such a chapter.

WISL encourages the inclusion of such chapters in all Ph.D. theses everywhere through the cooperation of Ph.D. candidates and their mentors. WISL is now offering additional awards of $250 for UW-Madison chemistry Ph.D. candidates.

Wisconsin Initiative for Science Literacy

The dual mission of the Wisconsin Initiative for Science Literacy is to promote literacy in science, mathematics and technology among the general public and to attract future generations to careers in research, teaching and public service.

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The Role of Oxylipins at the Intersection of Rhinovirus Infection and Asthma

By

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Chapter 5: Communicating to non-scientists

Author: Nicole M Lane Starr

I would like to begin this chapter by thanking the Wisconsin Initiative for Science Literacy (WISL), without whom this chapter would not have happened. I have always felt strongly that scientists must be able to communicate their research to the broader public, not just to other scientists in their field. As scientists, we are all working to better understand the world and to make it a better place for each other, in a myriad of different ways. However, we tend to spend so much time in the minutia of our research, talking to other people in the same niche topic, that we forget to reach out to a broader audience. We know why our research is going to save the world, but if we can't translate these lofty ideas into something understandable and meaningful to those outside our field, why should they care? And why should they, as taxpayers, keep funding our research? I am reminded of a story my father liked to share. One day when he was in graduate school for his PhD, his mother called him up, complaining that she had seen on the news some lab was researching why crickets are able to jump so far! Why should we care about how crickets jump as far as they do? What does it matter? So my dad, being a diligent grad student, did some research. And he found out that this lab was trying to understand how crickets were able to use their muscles so efficiently (allowing them to jump great distances without massive muscles), in an attempt to help people who had lost muscle mass regain mobility. Now doesn't that seem like something worth pursuing? My grandma thought so, after hearing more. But what about all the grandmothers, fathers, friends, neighbors who don't have a PhD student in their lives to tell them why they should care how far a cricket can jump? That is why it is so important for us as scientists to learn how to tell the public about our science. If we don't, it either won't get out there, or the media will run with it, and that rarely ends well either. So no, I haven't cured cancer, or even the common cold, but I hope that this chapter will help everyone

understand a bit more about what I've spent the last several years of my life doing, and how it might be built on to one day help the world.

Asthma, Rhinovirus, and Oxylipins—Oh my!

I want to start with some background to make sure we are all on the same page. I'm sure you've all heard of asthma; you likely know someone who has it. You're also no doubt familiar with Rhinovirus, even if you don't know it by name—it causes the common cold. You might not have heard of lipids, but you likely have heard of cholesterol, a lipid important in forming cell membranes. The next three subsections will talk in more detail about each of these.

Asthma, more than just shortness of breath

Asthma affects almost 8% of the US population, so chances are you or someone you know has it. It's slightly more prevalent in children than adults and in females than males. In those with asthma, almost half report having an attack (also called an exacerbation), and 10% die from asthma-related causes. That amounts to the deaths of over 3000 people in the US from asthma each year. Asthma is characterized by a narrowing and thickening of the airways, muscle contraction around the airways, chronic inflammation in the lungs, and an increase in mucus production, leading to the hallmark symptoms of difficulty breathing, shortness of breath, wheezing, coughing, and chest tightness. A cartoon depicting the differences in healthy and asthmatic airways can be seen in Figure 1, clearly illustrating how much smaller the airways are in asthmatic patients.

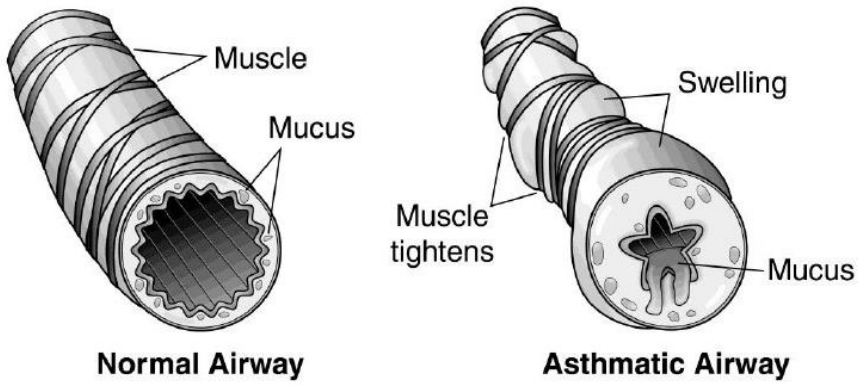

Figure 1: Normal vs Asthmatic Airways

A normal, healthy airway is depicted on the left, while an asthmatic airway is depicted on the right. The hallmarks of an asthmatic airway are highlighted, including mucus overproduction, thickening of the airway, and muscle contraction. All these result in a much narrower passage for airflow, leading to breathing difficulties. Figure taken from UW Health "Health Facts for You".

Asthma was first characterized in ancient times—with both Egyptian and Greek authors describing the hallmark symptoms. Since then, various scholars have put forth a number of suggestions on the causes and treatments of asthma. Today, it is increasingly understood as a broad spectrum of disorders. Both genetic and environmental influences play a role in the development of asthma. Low air quality is also associated with asthma development and severity, likely contributing to the increased asthma rates seen in low-income communities. Other factors like allergens, exercise, and aspirin can also trigger asthma. It wasn't until the start of the 1900s that modern treatment methods were employed. Apart from minimizing exposure to triggers, current treatment options are inhaled corticosteroids and long-acting beta agonists. Short-acting beta-2 agonists, anticholinergics and oral corticosteroids are used to treat rapidly-worsening symptoms. Recently, biologics, drugs derived from organic sources that target specific molecules within the body to mitigate inflammation associated with asthma, have been used more often.

Rhinovirus, aka the common cold

Rhinovirus is the most common cause of the common cold. Although symptoms are not typically severe, they can linger for 3 weeks. The average adult gets a Rhinovirus infection 2-3 times every year, while kids experience even more. This is estimated to have an annual economic impact of billions of dollars due to lost work/school and health care costs! Like many respiratory viruses, Rhinovirus is spread through aerosolized droplets from coughing and sneezing and through touching contaminated surfaces and then your face. Following infection, symptoms usually peak within 2-3 days. Currently, there is a lack of treatment options once the infection is underway. The CDC's recommendations are rest, water, and over-the-counter medications to manage symptoms.

There are three species of Rhinovirus—A, B, and C. A and C are associated with more severe infections, in adults and children, respectively. The three species also vary in the cell surface receptors they use to enter cells in the lung. Rhinoviruses typically target ciliated epithelial cells in the upper airways. Epithelial cells are critical in maintaining the barrier function of the lung, with the ciliated cells working to clear debris (including viruses) from the lung. Once inside cells, the virus replicates, bursts the cell, and spreads to the neighboring cells. This causes areas of disruption to the epithelial barrier that protects the lung, as depicted in Figure 2. However, the infection is patchy. It is unclear why some cells become infected and others do not.

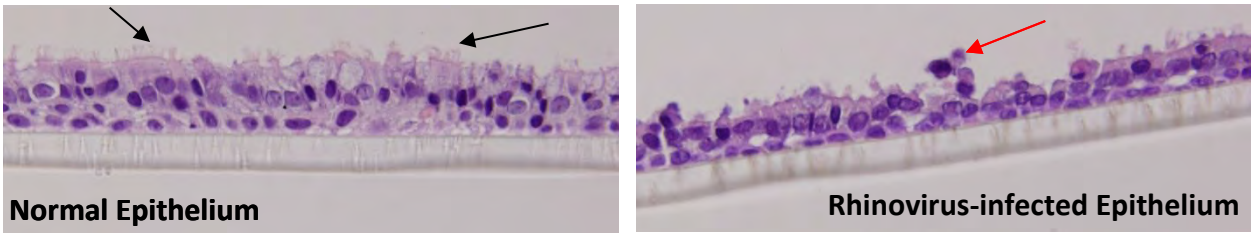

Figure 2: Uninfected vs Rhinovirus Infected Epithelium

I grew epithelial cells on a membrane and then infected some with Rhinovirus. On the left is an example of uninfected epithelium, showing the cilia (black arrows) and normal epithelial morphology. On the right is Rhinovirus infected epithelium. There is a notable lack of ciliated cells, the epithelial layer is thinner, cells are being shed (indicating they are dying; red arrow), and the morphology is altered. This disruption begins within hours of the infection and persists for days.

Lipids, more than just cholesterol

Many people are familiar with their cholesterol levels—it is regularly measured at doctor visits. Cholesterol is one example of a lipid. Lipids are a very large category of molecules which serve an equally broad range of functions. Lipids, like cholesterol, are important in forming the membranes that surround cells. They keep cell membranes flexible and help control the passage of molecules through the membrane. Membrane lipids can also be broken down into oxylipins, which are a type of lipid that function as locally-acting signaling molecules. These oxylipins help maintain homeostasis (the normal equilibrium within the body to keep everything functioning optimally) through complex signaling networks. The various oxylipins produced by these networks can have opposing effects. For instance, some work to increase inflammation, while others work to decrease inflammation. This balance is controlled by the number of each type that are produced.

So, how do these three things connect?

The link between asthma and Rhinovirus is well established. Childhood Rhinovirus infections are associated with asthma development. Additionally, Rhinovirus infection in people with weakened immune systems (such as asthma) causes more severe respiratory infections, often involving the lower airways. More worryingly, Rhinovirus infections can lead to asthma exacerbations. In fact, some 80% of attacks in kids, and 60% in adults, are linked to Rhinovirus infections (1).

In addition to maintaining homeostasis, oxylipins are important in fighting infections. They are one of the first signals of an infection and trigger immune cells to move into the site of infection and release pro-inflammatory factors. Viruses disrupt the levels of various oxylipins to impair this inflammatory response. Rhinovirus in particular alters levels of some of the proteins involved in the production of oxylipins (2).

Baseline oxylipin levels are different in people with asthma than in people who are healthy and vary between people with mild and severe asthma. In asthmatics, oxylipins associated with bronchoconstriction are elevated, while oxylipins involved in airway repair and injury recovery are diminished (3, 4).

What have I spent the past several years doing?

My professor's lab studies asthma. I joined the lab with an interest in viral infections and how patient factors play a role in the severity of infections. Thus, the reason for the more severe response seen in asthmatics to Rhinovirus infection was a compelling project for me to investigate. Existing studies have found few differences in asthmatic and healthy patients in the

cell counts and inflammatory markers following Rhinovirus infection. But no one had looked at lipids in this setting. Thus was born my hypothesis that oxylipins are involved in the differential response to Rhinovirus infection observed in healthy and asthmatic patients. The pipe dream is that we will be able to identify oxylipins that are either too high or too low in asthmatic patients which can be targeted to prevent their more severe symptoms and exacerbations following Rhinovirus infections. We might also be able to mitigate symptoms in healthy patients and speed recovery if a good oxylipin candidate is identified. We were lucky enough to already have access to a number of samples from both healthy and asthmatic patients, with and without virus infections (and information on whether or not the virus was Rhinovirus) to do this research.

I analyzed some snot

Yup, you read that right. Those samples I just mentioned, they were snot samples. Science is super glamorous. I started by analyzing samples (doesn't that sound more science-y than snot?) from healthy humans who were experimentally infected with Rhinovirus A. Yes, people actually volunteer to get a cold. Yes, they do get paid. No, I don't know how much. This kind of study is great because we know exactly when each person was infected and how much virus they received (one of 3 viral doses or a mock dose). Everyone enrolled in the study kept a twice-daily record of their cold symptoms, starting about a week before the infection and going out at least 2 weeks or until their symptoms cleared, whichever was later. During this period, they also gave samples (aka blew snot into a tube) just before the infection, and 2, 3, 4, 7, and 21 days after the infection. These samples were analyzed to determine viral load and oxylipin levels. Viral load was calculated using polymerase chain reaction, which measures the amount of specific genomic signatures (in this case, for Rhinovirus). Oxylipin levels were measured both

through assays that detect a specific oxylipin and through mass spectrometry, which is able to determine the relative levels of a bunch of molecules at the same time using their masses and electrical charges.

With the mass spectrometry data, we identified 71 oxylipins across 4 time points. Subjects recorded 17 separate symptom scores twice a day for at least 3 weeks. We had information on Rhinovirus levels for 4 different inoculation groups across 6 time points. This amounts to a lot of data with some pretty tricky analysis. In fact, I had to develop a new analysis method! I calculated an upper airway recovery score (since Rhinovirus infection in healthy people is confined primarily to the upper airways) based on the subject recorded scores. I then used a combination of machine learning techniques (designed to minimize the rates of false positives) to identify oxylipins involved in recovery. Using this method, I was able to show that a specific oxylipin, 15-keto-PGE_2, was associated with recovery. 15-keto-PGE_2 is a metabolite of an oxylipin that is involved in switching from pro-inflammation to pro-resolution signals. Figure 3 depicts the results of the analysis.

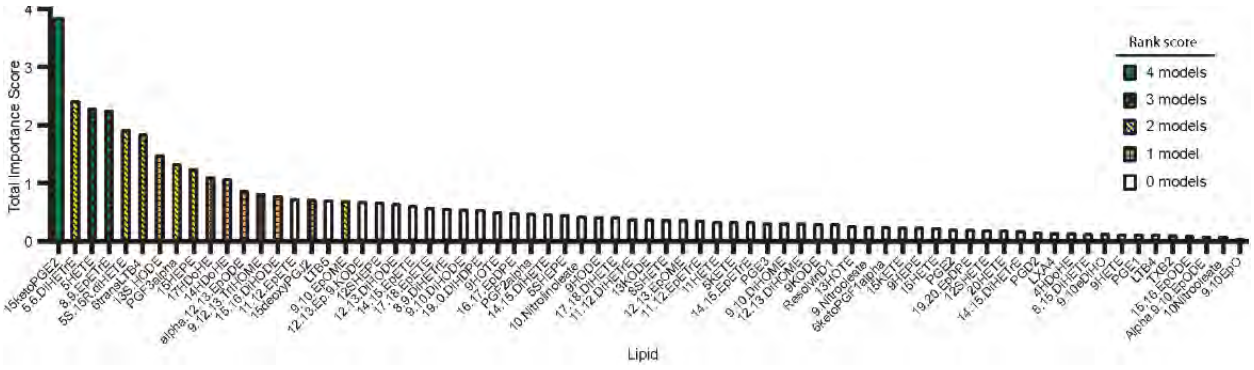

Figure 3: Statistical analysis of oxylipins involved in recovery

This depicts the oxylipins that were identified in the mass spectrometry analysis, ordered from most to least involved in recovery. Note that 15-keto-PGE_2 is notably more important than the next highest oxylipin. Colors designate the number of models in which that particular oxylipin was identified by. 15-keto-PGE_2 was the only one identified in all 4 statistical models used.

I also showed that 15-keto-PGE_2 was elevated following Rhinovirus infection in an inoculation-dependent manner, suggesting that this oxylipin is responding to the levels of the virus. I also modeled recovery scores using 15-keto-PGE_2 levels, with subject demographics taken into account. This confirmed that the negative relationship between 15-keto-PGE_2 levels and recovery was not confounded by differences in patient characteristics. Finally, I mapped relationships between all of the oxylipins and all of the symptom scores. This showed that 15-keto-PGE_2 was negatively associated with headache and nasal discharge, specifically. The negative relationship suggests that when 15-keto-PGE_2 levels are high, these symptoms are low, and vice versa. This may mean that 15-keto-PGE_2 helps with improved recovery following Rhinovirus infection.

I analyzed some more snot

Yes, lucky me, we had more snot to analyze! I say this only somewhat sarcastically. Access to human samples, especially ones with so much relevant patient data and over multiple timepoints, is really exciting and not something that many researchers are able to use. I just focused on that and not what the samples were… This time, I used samples (you know what that means) from asthmatic patients who had community acquired infections. This means that I did not have data on the exact date of infection. Instead, I had one sample each from before infection, at some unspecified point during infection, and after recovery from infection. I also had information on whether or not each patient was using medication to control their asthma, their lung function and cell counts, measurements of how severe their asthma and cold symptoms were, and whether the virus was detected at each of the time points.

With these samples, we focused on specific oxylipins—3 pro-inflammatory and 3 anti-inflammatory, rather than the broader mass spectrometry approach I had used previously. This was in the interest of time (the mass spectrometry results took 9 months to get back from the lab we sent samples to!), expense (like I said, mass spectrometry is expensive), and to have a more focused experiment since we already had mass spectrometry data from non-asthmatic patients during a Rhinovirus infection. Thus, I used the assays designed to detect specific oxylipins (the same type I mentioned using with the healthy samples) on these samples.

Once I had results from these assays, I again compared oxylipin levels with patient-factors to determine if any oxylipins were associated with changes in patients. Severe asthmatics had lower levels of oxylipins across the board than those with less severe asthma. Oxylipin levels also decreased slightly during viral infection. When results were grouped by both severity and viral presence, the differences became even more pronounced, with viral-infected severe asthmatics having the lowest lipid levels. This effect, which can be seen in Figure 4, was particularly notable in PGE_2, which is the precursor of 15-keto- PGE_2 and is believed to have anti-inflammatory effects.

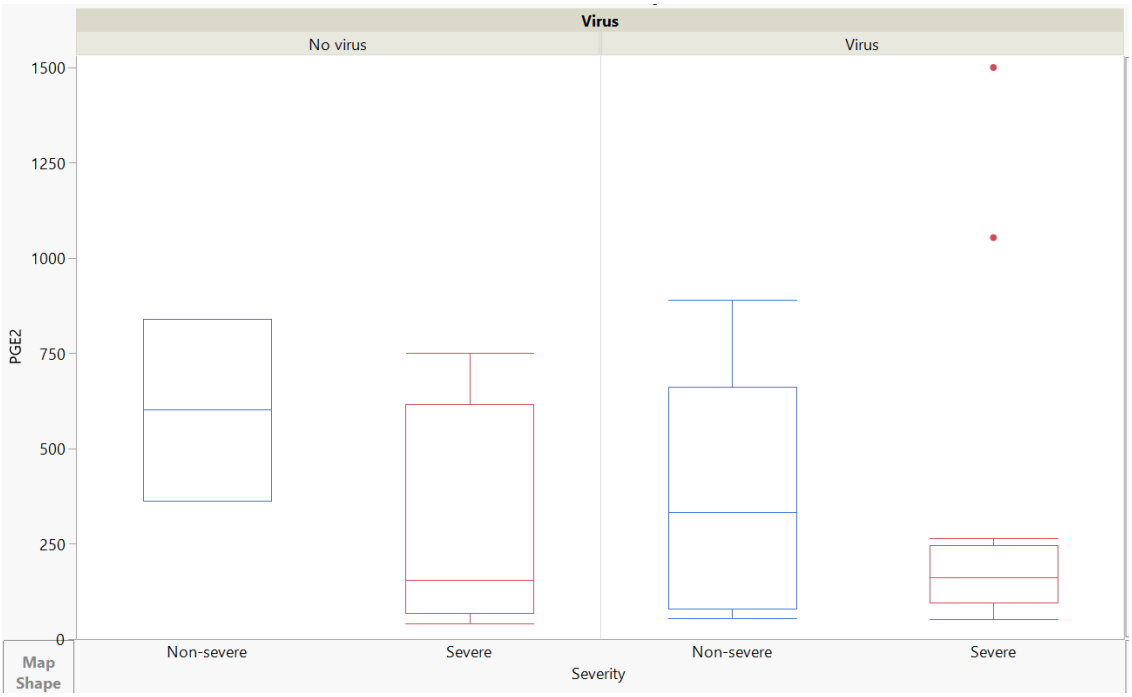

Figure 4: PGE_2 levels by asthma severity and presence of virus

This graph depicts the levels of PGE_2 in patients with either severe or non-severe asthma, based on whether or not they had virus detected. Regardless of virus presence, severe asthmatics had lower levels of PGE_2. A similar trend was seen in other oxylipins we investigated.

I grew a bunch of cells

To explore how Rhinovirus-infected epithelial cells are responding to these oxylipins, I grew human bronchial epithelial cells from both healthy and asthmatic subjects in a culture method called air-liquid interface. This requires the cells to grow on a special plate for a month, allowing them to mature to better emulate what exists inside airways, complete with ciliated cells, mucus-producing cells and basal cells. This is important when studying oxylipins since different mature cell types produce different oxylipins from each other and from immature cell types. Furthermore, Rhinovirus can have difficulty infecting immature cells, so using the air-liquid interface culture allows for a more accurate Rhinovirus infection as well.

Once the cells were mature, I treated some with one of the oxylipins I was interested in, while others were left untreated. The oxylipins I treated with included 15-keto-PGE_2 (from the research in healthy patients) and its precursor PGE_2 (from the research in asthmatic patients). Once the cells had had time to respond to the oxylipin, I infected some of the cells with Rhinovirus and again left others untreated. Figure 2 shows the results of this infection on my epithelial cells. This results in wholly untreated controls, cells that received just the oxylipin, cells that received just the virus, and cells that received both. In culture, the infection peaks quicker than it does in a human, usually around 24 hours. Thus, I focused on this time point, although I did collect samples from other time points as well.

These experiments showed that infection with Rhinovirus resulted in increases in the PGE_2 pathway. In this pathway, the enzymes which produce PGE_2 were elevated in infection and PGE_2 itself was elevated. PGE_2 and its metabolites play a role in producing mucus, so I also analyzed mucus levels in these cultures (bet you thought I was done with snot, didn't you?). Mucin was elevated in cells that were treated with PGE_2. Mucus is an important component of the lung's ability to clear viruses that are inhaled by trapping the virus before they can reach and infect cells and has also been shown to be increased by 15-keto-PGE_2 (5). Cells from asthmatic donors had only slightly lower levels of induction of this pathway than cells from healthy donors. If, as my results suggest, PGE_2 is important in recovery from infections, this difference could help explain why asthmatics experience more severe colds than non-asthmatics.

What should you take away from this?

Oxylipins are important! More specifically, my research supports that oxylipins, and specifically those in the PGE_2 pathway (including the relatively unstudied 15-keto-PGE_2) (simplified pathway summarized in Figure 5), are involved in recovery from the common cold (Rhinovirus infection). These oxylipins seem to work by aiding in the production of mucus, which helps protect the lung epithelium from infection. It is possible this same pathway is involved in other viral infections, but that will have to be confirmed. If this pathway can be targeted with a drug, this could be a new way to treat the common cold that might actually improve recovery, rather than just treating symptoms.

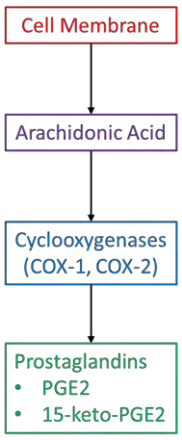

Figure 5: Simplified oxylipin pathway

Simplified pathway showing the production of Prostaglandins (green), including PGE_2 and 15-keto- PGE_2, from arachidonic acid (purple) derived from the cell membrane (red) via the cyclooxygenase pathway (blue).

Furthermore, if the differences in asthmatic and healthy patients are confirmed, this could serve as a target for improving the response to Rhinovirus infection in asthmatics. Here comes the big "BUT…". Confirming all of these and showing that such a treatment is effective and safe in humans will take a lot of work, and A LOT of money. This is why so many of the "cures" heralded by the media never amount to an actual drug. Before companies invest so many resources in developing a new drug and going through the very long and expensive FDA approval process, they want to be reasonably confident it will work—that the science supporting

it is there. And that's where my research comes in. My results show that, in humans, oxylipins in the PGE_2 pathway are likely involved in recovery from Rhinovirus infection, adding support to the growing body of research showing that oxylipins are important in infection. And companies are starting to notice. There are already some drugs targeting enzymes in this pathway. Research like mine shows that these drugs may be useful in treating a broad range of diseases and warrant further exploration.

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