[redacted]

Personal Statement, October 2016

Because of my R00 grant, my appointment at MSU has been 80% dedicated to research so the majority of my growth and activity has been in this area. However, I have also expanded with respect to teaching and service, which will be discussed toward the end of this statement.

The research program. My research focuses on how the cells of the immune system normally function and how these processes become dysregulated during the course of disease, such as autoimmune disease and allergy. I am also very interested in how toxicants and drugs modulate immune cell function. One of my long-time research interests has been in how certain proteins that become active in response to cell stress, such as Nrf2, NFκB and HIF1α, modulate immune cell function with the majority of my research focused on Nrf2. Nrf2 (nuclear erythroid 2 related factor 2) is a transcription factor that is activated by various types of cellular stress, including oxidative stress. My postdoctoral advisor, [redacted], was interested in the protective role of Nrf2 in the metabolism and detoxication of various toxicants. My interest in Nrf2 was sparked by the development of autoimmune disease resembling systemic lupus erythematosus (SLE, also called lupus) in Nrf2-null mice, which suggested a possible role for Nrf2 in regulating the immune system. As a result, the burning question for me in my postdoctoral research was to determine why Nrf2-null mice develop autoimmunity. My first step toward pursuing this mystery was to define the role of Nrf2 in regulating T cell function. My rationale for investigating T cells specifically was derived from substantial evidence implicating T cells in numerous types of autoimmune disease, including Type-I diabetes, multiple sclerosis, ulcerative colitis, etc. In addition, numerous T cell subtypes have specifically been implicated in SLE (lupus).

My postdoctoral research revealed an important role for Nrf2 in modulating the function of CD4 T cells. CD4 T cells, when activated (by pathogens, for example) produce a number of different proteins, called cytokines, which function to signal other immune cells and trigger an immune response. With respect to this, my studies showed that Nrf2 inhibits early production of certain cytokines (such as IFNγ) that are known to be critical to providing protection against various pathogenic bacteria and viruses. Interestingly, I also found that a number of different environmental contaminants, such as cadmium and arsenic, and food preservatives, such as tBHQ and BHA, markedly increase Nrf2 activity in T cells, which ultimately results in suppression of early cytokine production by T cells. These findings were the basis of several manuscripts and an NIH K99/R00 grant that was initially awarded in 2010 (I applied for and was awarded the second R00 phase of this grant in 2012).

In implementing the studies in this grant, the data suggested that the role of Nrf2 was likely more sophisticated than I initially realized. Specifically, I discovered that although Nrf2 inhibits early cytokine production by T cells, it has differential effects on cytokine production at later time points. I observed that Nrf2 inhibited certain cytokines, such as IFNγ, which are associated with Th1 cells, a specific subtype of CD4 T cell. Conversely, I found that Nrf2 increases production of other cytokines, such as IL-4, IL-5 and IL-13, which are associated with Th2 cells, a different CD4 T cell subset. These observations were important for a number of reasons. 1) They suggested that Nrf2 inhibits Th1 cell activity, which is known

to be important for host defense against a number of different pathogens. 2) They also suggested that Nrf2 promotes the activity of Th2 cells, which are strongly associated with the development of allergy and asthma. 3) I showed that Nrf2 is activated in immune cells by tBHQ, a widely-used food preservative, as well as by certain environmental contaminants, including cadmium and arsenic. These studies generated a lot of interest when presented at national meetings and were published in *The Journal of Immunology* in 2012. In addition, these studies also provided important preliminary data for an R01 grant that I submitted and was awarded in 2016. The next step in this project is to determine the downstream consequences of increased Th2 differentiation by Nrf2 on the development of allergy, since Th2 cells are strongly associated with allergy and asthma. This has been a major goal for me as an independent investigator.

Coming to MSU. I started my position as an assistant professor in the Department of Pharmacology & Toxicology at MSU in 2011. I was excited to join MSU because it is a research-intensive university with an outstanding reputation in the area of pharmacology and toxicology. In addition to that, I felt certain I could accomplish a number of my research goals at MSU. These goals included the development of an animal allergy model to complement my findings in isolated immune cells, incorporation of primary human immune cells into my studies, and expansion into novel, powerful methodologies, such as genome editing (which was SA3 of my K99/R00 proposal). Thus, my transition to an independent investigator has allowed me to build on and to greatly expand my postdoctoral research. In addition to my growth as a researcher, I have been provided with great opportunities to develop as a teacher and mentor for medical, veterinary, graduate and undergraduate students, which is a very fulfilling part of my professional development. Each of these areas of growth is discussed in further detail below.

Development of an in vivo allergy model. Arguably, the most important and most exciting research accomplishment for me has been the development of an animal allergy model and the use of that model to test my hypothesis. Because activation of Nrf2 promotes Th2 differentiation (which is linked to allergy), I needed to develop a model of allergy to determine whether Nrf2 activation promotes allergy. I was delighted to find (with the help of one of my mentors, [redacted] an investigator in the Dept of Food Science and Nutrition who had developed a unique model of food allergy, [redacted]. [redacted] model was unique in that it did not require adjuvants (chemicals that stimulate immune response, but are often toxic in and of themselves) and also did not require injections. [redacted] model is different in that he exposes the animals to antigen transdermally during the sensitization phase (analogous to using a patch to deliver a drug). He then administers the allergen orally, shortly after which the animals go into anaphylactic shock. [redacted] and I determined that this model would be useful for determining the role of Nrf2 in food allergy, so we decided to adapt it for my studies. Because [redacted] was using fairly expensive antigens (hazelnut and sesame), I adapted the model to ovalbumin, a food antigen which is cheaper, but highly relevant (as one of the proteins that causes egg allergy). It took about a year to establish the model to ovalbumin.

Our initial pilot studies using this novel model of food allergy show that mice exposed to the food preservative, tBHQ, through diet are more sensitive to ovalbumin (egg allergen) than mice on a diet that lacks tBHQ. Mice on the tBHQ diet demonstrated a greater change in body temperature (which occurs during anaphylaxis), higher serum levels of IgE and IgG1 (both associated with allergic response) and

exacerbated clinical symptoms in response to ovalbumin, including changes in respiration and activity. This finding was consistent with our hypothesis that activation of Nrf2 (by tBHQ) promotes Th2 differentiation and thus exacerbates allergic response. These findings are also consistent with our previously published data in isolated T cells.

The development of this model provided key data that supported SA3 of the R01 grant application (ONES award) awarded in 2016. The ONES (Outstanding New Environmental Scientist) award is an R01 grant that is offered by the National Institute of Environmental Health Sciences (NIEHS) to promote cutting-edge research by new investigators.

Complementary studies in primary human cells. Another major goal for me as an independent researcher was to perform studies in primary human immune cells to complement our studies in mouse immune cells. This represents a major advantage of conducting research in immune cells: the availability of human blood for immune cell isolation. Because I had no experience in working with primary human cells prior to arriving at MSU, it took a period of trial and error to determine the best source for human blood; ultimately, the source that can deliver the blood most quickly is optimal (within 24 h is ideal). Fortunately, another faculty member in our department, [redacted], was already working in primary human immune cells and was able to advise me in this regard.

Our studies in primary human CD4 T cells have largely been conducted by a graduate student in my laboratory, [redacted] observed that human immune cells seem to be more sensitive to the food preservative, tBHQ, than mouse immune cells. tBHQ suppresses many of the events following activation of human T cells, including IL-2 and IFNγ production and upregulation of CD25 and CD69. Overall, these studies suggest that the effects of tBHQ and the role of Nrf2 in immune cells are potentially relevant to humans and warrant further investigation. These studies are the focus of research manuscript that was published by the journal, *Cytokine*.

Genome editing. One of the specific aims of the K99/R00 proposal entailed mutating the Nrf2 gene in a human T cell line by genome editing. In the R00 grant, I proposed to use the recently-developed technology of zinc finger nucleases to accomplish this. Shortly after starting at MSU, however, the zinc finger nucleases were rapidly being replaced by "TALEN" technology (which stands for Transcription Activator-Like Effector Nuclease), which emerged as a practical (and less expensive) way to accomplish the same thing. As we prepared to initiate the TALEN studies, the CRISPR/CAS9 technology emerged as an even more efficient way to edit the genome. We shifted tactics again and are now using CRISPR/CAS9 in our laboratory to mutate Nrf2. Using molecular approaches these days is not for the faint of heart—the technology changes rapidly and is continually improving. I am grateful to have colleagues inside and outside of MSU who are happy to share their knowledge and experience with various methodologies; these conversations greatly improve my research.

Using CRISPR/Cas9, we successfully produced multiple mutant clones of Jurkat cells in which the Nrf2 gene is knocked out. These studies were conducted by another outstanding graduate student in my lab, [redacted]. Our data show that a number of actions of "known" Nrf2 modulators are mediated by

Nrf2 but others are not. These studies are the focus of a manuscript nearing completion. We are very excited by our ability to generate these clones and plan to generate more in the future.

Broadened technical capabilities. In addition to the methodologies and models listed above, my laboratory has broadened its technical capabilities in many other ways as well. One of the graduate students in my lab, [redacted], has successfully transfected primary human CD4 T cells (meaning she has successfully inserted foreign DNA into these cells using electrical energy). We considered this a major success because this cell type is notoriously difficult to transfect and thus most people prefer to use viruses to insert DNA into these cells (which has its own drawbacks). Her ability to transfect primary CD4 T cells, opens up many possibilities for manipulating gene expression in human lymphocytes. We have also initiated studies in primary B cells, which is the basis of a poster presentation that an undergraduate student (now recently hired as a research technician) in my laboratory, [redacted] presented at the national SOT meeting the last 2 years. We have also started to explore mucosal immunology of the gastrointestinal tract. An undergraduate student in my lab, [redacted], has become proficient at isolating lymphocytes from the gastrointestinal tract and has begun characterizing these populations in the Nrf2-null mice. Lastly, we have recently developed an infectious model of influenza in my lab through a collaboration with [redacted] in the Dept. of Food Science and Nutrition at MSU. Another graduate student in my lab, [redacted], is using this model to determine the effect of Nrf2 activators in host defense against influenza.

Training and advising students. A vital part of my job is to train and advise graduate and undergraduate students; this is complementary to the research goals of the laboratory. Thus, the two missions of teaching and research are difficult to separate. Like many other investigators, I find that training and advising students is the most fulfilling part of my work. For first year graduate students, I tend to design fairly structured research projects. I communicate regularly with the first year students and am heavily involved in experimental design (particularly in the beginning). As the student gains more technical expertise and a greater understanding of our research, he/she will start designing his/her own studies. By the time the student is in the second year, he/she will be designing most, if not all, of their own studies. At this point, I meet regularly with the student to discuss data (and if need be, the design of the study) as well as next steps. With every year of experience, a student should be gaining a proportional amount of independence in my view. My goal is to graduate poised, accomplished and overall, independent students. What I have told my students is that when they start their postdoctoral fellowship, their advisor should be able to give them a project and expect that they (as a new postdoc) will be able to do all the necessary literature searches and come up with a plan to implement that project (not to say that the postdoctoral advisor won't have input or won't modify the plan, however).

I emphasize to the students that they should be looking at their years in graduate school as an integral part of their career, rather than as simply "a training experience". I urge them to publish early and as often as possible and I try to enable this. This means looking at one's data for "publishability" at an early stage and designing studies with the intent to publish. I try to keep unpublishable pilot studies to a minimum (although they are of course sometimes necessary). I strongly advocate teamwork among the lab members. I think this facilitates productivity and problem-solving. I also think this is a vital attribute to working in research—both in industry and in academia.

In my opinion, the students in my laboratory are currently on the right trajectory for success. They will have strong credentials by the time they graduate and will be poised for outstanding postdoctoral positions.

Teaching. I have already written elsewhere in this application about my teaching philosophy and thus will not repeat that here. In my opinion, one of the strengths of our department is that the faculty are truly committed to the education of our graduate, medical and other professional students. The department provides outstanding teaching toward that end. I feel a strong sense of responsibility to continue that tradition. In the last couple of years I have broadened my teaching responsibilities. In addition to teaching CHM PBL (problem-based learning) classes, I have also lectured in PHM 552 (Veterinary Pharmacology) for the last 2 years. I also lecture in the Pharmacology classes for the graduate students, PHM 801, PHM 802 and PHM 816. Like the other faculty in our department, I get a great deal of satisfaction from my teaching activities.

Service. In addition to teaching and research, I have also broadened my service responsibilities. Most of the service I have done outside the university is related to my research and teaching in some way. Because my laboratory presents research at the national meeting of the Society of Toxicology every year, I support this society in particular with service. Specifically, I have served as a councilor in the Immunotoxicology specialty section for the past 2 years and also serve on several committees for this specialty section. I was honored to receive the Outstanding Young Immunotoxicologst award from this specialty section in 2014, which was for research, teaching and service in the area of Immunotoxicology. In addition, I have also served as a councilor for the Michigan chapter of SOT. I have also expanded my service to the university as described previously in this application.

Conclusion. From my own perspective, my growth as a researcher and as a teacher has increased exponentially in the five years I have been at MSU. This is not to say that every experiment is a success, but I typically learn quickly from my missteps, as do the members of my lab and we use the information from such studies to revise and optimize experimental design and/or methodology. In general, our overall momentum is always going forward as evidenced by steady publications, presentations at national meetings and a number of awards received by myself and the members of my laboratory.