Randall G. Worth, PhD

Research Interests

One important biologic question is the role of macrophages and neutrophils in mediating the immune system's paradoxical roles. First, is the positive role that these cells play in inflammation during clearance of pathogenic organisms to clear the body of infection. Secondly, I am interested in the mechanisms by which specialized pathogens are able to survive and prosper within macrophages. One important family of receptors on macrophages and neutrophils are the Fcγ receptors which recognize and bind the Fc portion of IgG. IgG is responsible for opsonizing pathogens and is also one causative agent in inflammatory responses. Signaling pathways responsible for killing IgG coated pathogens and those responsible for inflammation have been described to be quite similar. Understanding how these cells function and the signaling networks involved in these processes will help us model the signaling dynamics and enhance responses toward pathogens.

I foresee my research program progressing in two major directions: 1) understanding signaling networks involved in calcium initiation, propagation and routing and 2) delineating the role of calcium in membrane fusion. Specifically, I will investigate the role of calcium in phagosome-lysosome fusion leading to degradation of pathogens and the ability of certain pathogens to circumvent this process by inhibiting membrane fusion. Comparison of the signaling networks involved in these different responses will help to define the molecules involved and shed light into the players required for membrane fusion.

To accomplish the cell biology of signaling pathways I am maintaining my collaboration with Dr. Howard Petty. I have had the opportunity to help develop and use state of the art microscopy systems in Dr. Petty's laboratory (Figure 1a,b) whereby we obtain spectral data (e.g. resonance energy transfer) and acquire images using a high-speed image capture system to observe signaling and metabolic events in real time with

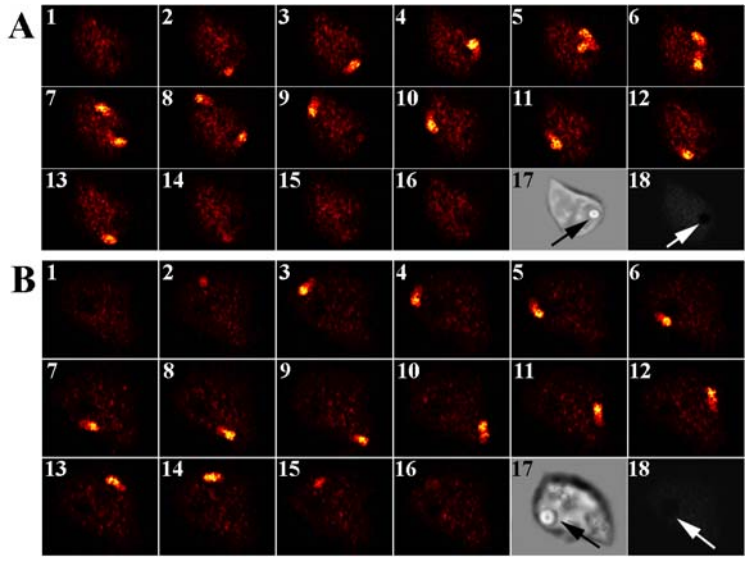

Figure 1. Traveling calcium waves encircle phagosomes from transfectants expressing wild-type FcγRIIA but not FcγRIIA (AAA). CHO cells were loaded with Indo-1(AM). Cells were examined with high-speed microscopy using an exposure time of 100 nsec and a duty cycle of 30 msec. Experiments were carried out using wild-type FcγRIIA (panel A) and FcγRIIA (AAA) (panel B). For transfectants expressing the wild-type FcγRIIA, the calcium wave was found to split near the phagosome with one wave traveling intracellular while the original wave propagated about the periphery of the cell. However, FcγRIIA (AAA)-expressing cells did not display the second calcium wave propagating in the phagosome's vicinity. (x800)

spatiotemporal dissection in the range of 50 nanosecond exposure times with a 20 millisecond duty cycle. These techniques allow the resolution of second messenger signaling parameters such as the observation of calcium traveling within a cell during

events such as phagolysosome fusion. Recently, we have utilized traditional molecular biology techniques to introduce mutations into receptors in model systems so that signaling events affected by these mutations can be studied. Using the system described above we have observed signaling lesions found when the LTL motif of FcγRIIA is mutated to AAA (Figure 1a vs.b).

The calcium lesion triggered by the mutant receptors suggests that FcγRIIA is able to form a supramolecular complex most likely with calcium indicators (calmodulin, calnexin, etc) or a calcium channel (SERCA, ryanodine, etc) to mediate propagation of this signal. I will investigate the molecules involved in directing the calcium wave to the phagosome and specifically how this can be a model for other calcium signaling networks. Additionally, the role of endoplasmic reticulum as a calcium source is of particular interest. We hypothesize that the ER composes the phagosome and maintains a conduit between the phagosome and plasma membrane. This would in effect act as an "extension cord" for calcium to travel the 1-2μm from plasma membrane to phagosome. I will utilize advanced imaging techniques as described above, traditional molecular biology to engineer signaling molecules, and yeast two-hybrid screening to determine the molecules capable of interacting with FcγRIIA. These three components will allow me to describe the molecules, define their interaction and characterize the effect on calcium signaling during membrane fusion. I will then model this process for translation into other systems which has the potential of shedding light on infectious diseases, autoimmune diseases and general cell biology in terms of signal networks and disease pathogenesis.

There are many unanswered questions regarding how unopsonized pathogens can be recognized, internalized and degraded or in some circumstances how pathogens evade degradation machinery. Receptors responsible for recognizing different pathological organisms and apoptotic cells have been identified. My experience with IgG-mediated phagocytosis and phagolysosome fusion will mold nicely into research involving many other receptors such as Toll-like receptors and scavenger receptors to determine the role(s) played by each in integrating a response. I will begin these studies *in vitro* using cells that I have engineered to express various receptors and *ex vivo* examining the role of neutrophils/macrophages in these processes.

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Teaching Philosophy

A quote posted on my desk states, “Knowing and not doing is the same as not knowing at all”. Much is yet to be learned about the science of teaching. As noted in a news feature last year in *Nature* (425:234, 2003), novel methods of teaching are developing rapidly. In order to be the best teacher possible one must envelop three important traits. First, a good teacher must be current on new advancements in teaching styles and methodologies. Secondly, a great teacher must be imaginative so as to envision new ways to connect to students on various levels at the same time. Lastly, an outstanding teacher must not only think of new teaching styles but must implement them and be interested in the reaction of students.

My desire to teach stems from two experiences. The first is a teacher I encountered in high-school in English Literature and Creative Writing. Mr. Bond had an ability to make a subject like English Literature the most enjoyable class I had that year. His excitement about the class and the development of students made him the most influential teacher I have had. I want to have the same affect on my students. To this day I am still in touch with Mr. Bond. I look forward to speaking/writing with him purely for the enthusiasm he has about life and the achievements of his students. The second experience that turned me on to teaching was my undergraduate Microbiology and Immunology professor. He was so casual about teaching but was able to make his point and raise interest in the subject just by speaking. He knew the subject well enough to show up for class with a piece of chalk and a cup of coffee. His lectures were the most organized and flowing lectures I have experienced to this day. He could stop mid-thought to start a discussion and get back to the same point later without missing a beat. The students in our class are to this day amazed by his abilities. He is the major reason I decided to study immunity due to his encouragement and understanding. I want to make impressions on peoples lives similar to these experiences.

I believe in an interactive teaching environment where students are involved in problem solving and the professor acts as more of a guide than a traditional lecturer. I am evaluating new ways of teaching Molecular Biology & Genetics at Penn whereby my class of ~55 students is broken into small groups on the first day of class. After every lecture, the groups are posed with different problems which they solve and present to the class as a group. Results of this technique are forthcoming but the students seem to enjoy the challenge and the nontraditional role that I play in the classroom as a consultant. Additionally, I have enjoyed the increased student understanding and awareness that comes from this experience.

I truly enjoy teaching Advanced Cell Biology. My class of 15 students is ideal to hold a discussion on various topics in cell biology. The students prepare a 45 minute presentation on a topic followed by a discussion and critique of the science, the hypothesis and the ways they proved the hypothesis, and most importantly designing new experiments to prove the hypothesis better. I have

had fantastic feedback from students in this course and I look forward to each January when I can be part of another group of young budding scientists.

I enjoy motivating students and discussing exciting advances in science to fresh minds in hope that they will continue developing into scientists. I have had extensive experience teaching students at various levels. I have taken advantage of many technological benefits available to teachers including the Blackboard program at Penn. Lectures, supplemental information such as URL's and chat rooms are posted on the Blackboard site for students to download or view on line. Blackboard also makes communication with students easy regarding projects or questions about material. I can post answers so the entire class can have access.

My commitment to novel teaching techniques also carries over to my participation in the Harvey Project (www.harveyproject.org). Though a Physiology based project, there are immune and cell based components being added and I look forward to contributing more topics and information. In addition to Advanced Cell Biology and Molecular Biology and Genetics, I have taught traditional undergraduates for 3 years in laboratory sections of Anatomy and Physiology.

Being part of a developing curriculum would be challenging and satisfying. I would enjoy taking part in Biology classes and designing bridge courses that could be taken by senior level students wanting to get into Biocomplexity. The field of Biocomplexity is an excellent mixture of physics, biology, chemistry, mathematics, and computer science that will allow students to integrate their knowledge in terms of biochemistry, biophysics and physical chemistry with a mathematical application. The knowledge generated in this program would define the future of science and stimulate numerous students of various disciplines to collaborate as your faculty does. In a more biologic basis, I feel complementary courses could be added to your curriculum. A class on biologic imaging/analysis would be an excellent starting point to combine science from different disciplines into a single course. Types of microscopy imaging such as electron, brightfield, fluorescence and confocal would allow scientists from all disciplines to showcase their work while tying them all together. A second addition may be a course on membrane biology that would cover aspects of membrane organization, structure, signaling as well as other aspects of membrane research. This particular discipline has become a major area of interest as proteomes and interactomes have come to the forefront of biology.

I strive to see my students succeed in their future endeavors. Science is often viewed by students as an overwhelming, eccentric, and often boring subject. I strive to make science as fun to students as it is to me. I love to see students take an interest in science. If I communicate information in an effective manner they will be inspired, energized and motivated to do their best wherever their career paths take them (even if it turns out to be something other that science). That is the true measure of a good teacher.