Jonathan Kagan: A cell biologist's view of immunity

Marie Anne O'Donnell

Kagan studies how organelles control innate immunity.

Wrestling was Jonathan Kagan's greatest passion while growing up in Farmingville, Long Island, but his interest in biology got the upper hand during his graduate studies with Craig Roy at State University of New York Stony Brook. Kagan ultimately found grappling with the cell biology of when and where pathogens are tackled by the immune system to be an equally engaging fight.

Location has influenced the direction of Kagan's research at each stage of his career. As no virologists were willing to let Kagan rotate in their laboratory, he switched his interest to bacteria, joining Roy's laboratory to study how *Legionella* customizes phagosomes into specialized organelles for microbial replication. As Kagan explains, this turned out to be "the best decision of my professional career, as I consider Craig to be the best experimentalist I have ever met. He provided me with a perspective on how to approach a problem that transcends any specific scientific topic, which is a skill that cannot be overvalued." After Kagan's first year of graduate school, Roy's laboratory moved to Yale's new Department of Microbial Pathogenesis. This move placed Kagan adjacent to the Cell Biology and Immunobiology Departments, which helped shape his thinking of host–microbe interactions from a cell biological perspective. During his graduate studies, Kagan discovered that *Legionella* phagosomes intercept vesicular traffic from endoplasmic reticulum exit sites and identified the GTPase ARF1 as the first host factor required for *Legionella* to generate a replicative organelle.

Toward the end of his graduate studies, Kagan met Ruslan Medzhitov, who along with Charles Janeway was pioneering research into how the Toll-like receptors (TLR) control innate and adaptive immunity by recognizing conserved microbial products. Kagan joined Medzhitov's laboratory for his postdoctoral studies to investigate how signaling by the TLRs was organized in time and space within individual cells. Kagan identified key cell biological processes that control TLR signaling, in particular how specialized sorting adaptors determine the subcellular location of TLRs and the signals they evoke after endocytosis (1, 2). "Ruslan's lab was an amazing place to work, and I learned so much from him during my postdoc. Most importantly, I learned how to consider my research in the context of the biggest picture possible, and to think deeply about how my studies may explain other aspects of biology and how to communicate these ideas to a broad audience. These skills are essential for long term success." With these skills, Kagan moved to Boston Children's Hospital and Harvard Medical School, where his independent research program has continued adding groundbreaking detail to the cell biological map of innate immune signal transduction by TLRs and the related RIG-I–like receptors (3). We contacted him to learn more.

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What first drew you to study TLR signaling?

When I finished my studies in Craig Roy's laboratory, I was becoming more interested in the host immune response to infection, and Yale was the place to be. Medzhitov's research group was systematically analyzing the ability of TLRs to promote inflammation and adaptive immunity in mice. However, we knew almost nothing about where TLRs and their associated signaling proteins were localized and my initial goal was to create a map of the intracellular sites of TLR signal transduction. In my own lab, we have continued these efforts and we have recently expanded our work to include *Drosophila melanogaster*. In this system we provided the first in vivo evidence for the importance of our cell biological map of signaling for host defense. Perhaps most importantly, we identified the first example of a broadly used cellular response to bacterial lipopolysaccharide (LPS) that proceeds independently of TLR4. TLR4 was thought to be the sole receptor for LPS capable of controlling downstream cellular responses to this cell wall component. Other LPS receptors were presumed to function merely as chaperones delivering LPS to TLR4. However, we found that TLR4 does not promote its own endocytosis but requires signaling triggered by a distinct LPS receptor called CD14 and TLR4 is merely cargo for this pathway (4). Once TLR4 enters endosomes, it induces the expression of hundreds of immunomodulatory genes. The importance of CD14-inducible endocytosis for TLR4 signaling highlights the amazing connection between trafficking and signaling pathways. Our findings were quite timely for the field, as subsequent studies from other laboratories identified additional TLR4-independent cellular responses to LPS. We now face the challenge of understanding how several LPS receptors operate to control distinct and overlapping cellular responses that are collectively important for host defense.

Jon Kagan.

What is your laboratory actively working on?

How the immune response operates in complex environments is a question that excites us. We recently identified how the innate immune system uses coincident detection of microbial products and tissue damage to "hyperactivate" immune cells. We are

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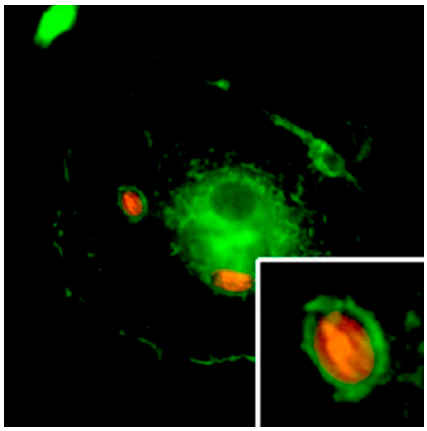

Confocal micrograph of a macrophage after it has phagocytosed a fluorescently labeled yeast particle (red). The macrophage has been stained to identify the TLR adaptor protein TIRAP (green), which is present at the plasma membrane and on the phagosomal membrane. Combining this type of cell biological approach with genetics, the Kagan laboratory has helped elucidate the importance of the subcellular positioning of TIRAP and other innate immune regulatory proteins in inflammation and host defense.

interested in understanding how these cells promote host defense (5). We are also studying the biochemical basis of innate immune signal transduction at distinct intracellular sites. Many proteins operate at these locations but we are just beginning to understand how they are organized. For example, we have identified the endogenous myddosome in macrophages, a receptor proximal protein complex that is the principal site of TLR signaling (6). The myddosome is just one example of several large oligomeric protein complexes that control inflammation, which we have dubbed supramolecular organizing centers (SMOCs). Similar SMOCs control inflammasome and RIG-I–like receptor signaling events, and we are interested in defining the mechanisms and consequences of SMOC activation as these organizing centers are the decision-making organelles of the immune system.

What kind of approach do you bring to your work?

My approach to our projects is very much concept driven. We often discuss questions in the field that may be overlooked based on current dogma or experimental methodologies. In other words, we try not to initiate projects that are obvious, as these projects are usually highly competitive. Rather, I prefer to work on questions that others may not (at the time) find noteworthy. The hope is that whatever we learn may shift the perspective of our colleagues and spur them to follow up our observations.

What did you learn during your PhD and postdoc that helped prepare you for being a group leader?

I learned how to be a scientist, in every sense of the word, during my graduate and postdoctoral studies. I believe the hardest part of being a group leader is maintaining high standards of work performed by several trainees. By working with such excellent mentors and laboratory-mates, I learned the best way to design experiments, to truly test a hypothesis, and to communicate these findings to a broad audience. I try very hard to pass these lessons on to my trainees. I was unprepared for how hard it is to judge talent when considering postdoctoral applicants. There is no perfect way to determine who is going to be a star, and it is a constant struggle for me to make such decisions when hiring new people. After all, you meet an interviewee for a single day, and decide whether you want a years-long relationship with him/her. It is almost like speed-dating . . . no, speed-marriage! For this reason, I hire very few postdocs, and the ones I do hire are selected very carefully.

"Stay excited about your work, and never, ever, get complacent. There is always a new challenge ahead."

What has been the biggest challenge in your career so far?

The biggest challenge of my career has been to stay true to my interests in basic science. These days, there is a tremendous push to work on translational aspects of biology, with the goal of identifying clinical significance. The incentive to shift our research focus into one that is less basic and more translational is huge, as it is easier to obtain grants, publish papers, and establish oneself in the community by following this path. However, I did not get into science to cure a disease. I got into science to understand life. I look at the human body as a clock, and I just want to take it apart and see how it ticks. I have tried very hard to stay true to my interests in basic science, and this approach allows me to truly love all the projects in my laboratory.

What is the best advice you have been given?

The best advice I ever received was from my parents. I was encouraged to focus on my passions and to work hard. My parents led by example and their day-to-day choices made it clear that they valued hard work and consistency. There was rarely a discussion of finances as a critical determinant of career path, but there was often a discussion of doing what you liked and hoping for the best. Students often feel that they need a path to success that is more tangible than "be happy, work hard, and hope for the best." But I believe that my mentors, and most successful people I know, lived by this mantra in their formative years. Of course, it is good to plan for the future, but never let that plan come at the cost of doing what you love. Because without that passion for your work (or your life), you will be unhappy.

What hobbies do you have?

These days, my hobbies are to play with my three wonderful kids (ages 7, 4, and 2) and spend time with my beautiful wife, who is also a scientist. We are a very active family, with much laughter and craziness. Most of my free time is spent embracing the joys that come with early parenthood. Prior to having children, my hobby was poker. I have many fond memories of driving to the local casino near Yale on a weeknight, talking about experiments and card games, and then doing the same many hours later on the way home.

Any tips for a successful research career?

Follow your heart, meet lots of people, stay excited about your work, and never, ever, get complacent. There is always a new challenge ahead, and the fight is what keeps life interesting.

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A Kagan family outing in Boston. Depicted are Jon Kagan, Lorri Marek-Kagan, and their children—Max, Amelia, and Madeline.