Recognition for an Innate Explorer

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On June 5, 2013, Ruslan Medzhitov received the Else Kröner-Fresenius Immunology Award. This recognition goes to an individual who has had such an influence on basic and medical immunology that it is almost difficult to recall a time before his discoveries were made. But in reality, that time was not long ago. To mark this celebratory event, I highlight the conceptual revolution spurred by his work, which continues to inspire excitement today.

Janeway's Legacy

Most experts agree that the modern fields of immunology began with the awarding of the 1908 Nobel Prize in Medicine to Ilya (Elie) Mechnikov for his discovery of cell-based immunity (i.e., phagocytes and phagocytosis) and to Paul Ehrlich for his work on soluble mediators of immunity (now known as antibodies). These two discoveries founded distinct research areas, with some scientists favoring Mechnikov's ideas on phagocytosis as the principle mediator of immunity but many more considering Ehrlich's ideas on antigen-antibody interactions most important.

For the next 80 years, Ehrlich's ideas developed into what would now be considered the field of adaptive immunity, which focuses on understanding the biology underlying the function of B and T lymphocytes that express antigen-specific receptors. Mechnikov's ideas, in contrast, developed into what would be considered the field of innate immunity, which seeks to understand the function of phagocytes in capturing and killing microbes. It was generally believed that, during infections, innate and adaptive immune responses acted independently, with innate phagocytes functioning to merely keep an infection under control until the more sophisticated (but slower acting) adaptive immune response could be unleashed. Because the antigen specificity of an immune reaction was determined by T and B cells, adaptive immunity became the "interesting" arm of the immune system to study. In contrast, much less attention was paid to the earliest stages of an immune response, wherein innate phagocytes were thought to act nonspecifically to capture and kill microbes that they encountered.

Studies in immunology in the early and mid-20th century focused heavily on understanding how the exquisite antigen specificity of immune responses was achieved. These studies revealed that the choice of which T or B cells to activate was determined by their unique receptors and that each of the ~2 trillion lymphocytes in the human body could (in theory) detect a distinct antigenic peptide. Although the remarkable repertoire of antigen-specific lymphocytes would allow the immune system to detect any peptide sequence, there was no intrinsic means by which a T cell could determine whether its receptor was specific for self, nonself, or microbial nonself molecules. Making this distinction was critical for human health, as the only adaptive immune responses that should be generated are against microbial antigens. How did the immune system only permit activation of microbe-specific lymphocytes?

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What was largely ignored by the community at this time was the fact that, in order for protein antigens to induce robust T and B cell responses, these antigens needed to be administered as a mixture with bacterial products (known as adjuvants). Why this mixture was needed for immune responses to occur was not considered in any biological context until 1989, when the late Charles Janeway Jr. proposed an unconventional idea. In a landmark essay published by Cold Spring Harbor Laboratory Press, Janeway predicted that the innate immune system (namely phagocytes) does not operate independently of the adaptive immune system. Rather, the innate immune system functions to instruct the adaptive responses of T and B cells—effectively determining which antigens are of microbial origin. In Janeway's model, the function of phagocytes was not simply to kill microbes and present their antigens to T cells via major histocompatibility complex (MHC) proteins. Rather, their function would be to deliver signals that ensure that only microbe-specific (not self-reactive) T cells ever become active. Moreover, Janeway proposed that these innate immune cells could distinguish between broad classes of microbes like bacteria and viruses, such that the ensuing immune response would be best suited to fight that particular type of pathogen.

After posing such a provocative idea, the real question became how to prove it. The fundamental prediction of Janeway's model was that phagocytes had the capacity to identify microbes specifically and distinguish them from self-antigens. If correct, then phagocytes should express receptors that evolved to detect microbes, and these receptors should be able to induce signals that promote activation of adaptive immunity. Janeway recognized that the task of detecting microbes is not unique to humans but is a fundamental challenge faced by all multicellular organisms. As such, he speculated that all multicellular organisms would encode receptors that detected common features that define microbes uniquely. These microbial signatures were called pathogen-associated molecular patterns (PAMPs), and their proposed receptors were termed pattern recognition receptors (PRRs). Proposing what the PAMPs were was easy. There was already ample evidence that microbial products such as bacterial lipopolysaccharides (LPS) and viral infections induced responses in phagocytes that result in the secretion of immunostimulatory factors such as interleukin 1 (IL-1), the first cytokine that was discovered in the early 1980s by Charles Dinarello. What was entirely unknown was the identity of the PRRs. How many were there? Do they even exist at all? Are they really conserved through evolution, as Janeway proposed?

To many students in the field today, the idea that the innate immune system controls adaptive immunity does not sound unconventional at all. In fact, this idea is oft-considered obvious and expected. Of course, most good ideas are considered obvious in hindsight. It is a huge testament to Janeway's legacy that his ideas have become so ingrained in our understanding of immunity that it is hard to even conceive of a time when these ideas were not commonly discussed. As will be described below, there are few in the field who can take more credit than Ruslan Medzhitov for turning Janeway's ideas into reality.

Toll-like Receptors in Pattern Recognition

Ruslan Medzhitov joined this story in 1993, when as a graduate student at Moscow State University, he read Janeway's theory on innate immunity and began an international correspondence that led to a conceptual revolution. Medzhitov contacted Janeway, and the two began a discussion that culminated in the offer of a postdoctoral appointment in Janeway's lab at Yale University. Once at Yale, Medzhitov embarked on a mission to identify Janeway's proposed PRRs. Many possible approaches could have been taken to identify these receptors, but what bore fruit was an approach that would typify Medzhitov's scientific inquiries over the next 15 years. That approach built on his remarkable ability to place several seemingly unrelated observations into a context that generates a novel hypothesis. These "unrelated" observations included the following. (1) As shown by Michael Levine and colleagues, the fruit fly *Drosophila melanogaster* utilizes the transcription factor NF-κB to control immune responses to microbial infections. Because NF-κB was already known as an immune transcription factor in mammals, these data supported Janeway's contention that an evolutionarily ancient signal transduction pathway would be activated by infections in diverse organisms. (2) The *Drosophila* Toll receptor, a developmental regulator cloned by Kathryn Anderson and colleagues, was found to contain a cytoplasmic signaling domain similar to the mammalian IL-1 receptor. This domain, now known as a Toll/IL-1 receptor/resistance protein (TIR) domain, was notable because it suggested that the Toll and IL-1 receptors were related evolutionarily. The IL-1 receptor was identified by scientists at Immunex Corporation and became interesting because it was found to activate NF-κB-mediated gene expression upon binding to its ligand IL-1. Thus, the immune pathways in flies and the IL-1 receptor pathways in mammals both activated NF-κB. Based on these data, Medzhitov hypothesized that the mammalian PRR would be a transmembrane protein that contained a cytosolic TIR domain (like the IL-1 receptor). Toll was not considered a candidate PRR, however, because it was thought to only function as a regulator of development, not immunity. Using the conserved TIR domain to screen EST databases and splenic cDNA libraries, Medzhitov identified a human protein that did indeed contain a transmembrane domain and a cytosolic TIR. To his surprise, this protein turned out to be a human homolog of the *Drosophila* Toll protein, which today we refer to as TLR4. Medzhitov went on to demonstrate that the function of human TLR4 was to induce the expression of cytokines and costimulatory molecules that were known to regulate T cell differentiation and activation, thus providing the first evidence for a protein that fulfilled the criteria of a PRR. While Medzhitov's studies were still underway, work published by Bruno Lemaitre and Jules Hoffmann demonstrated that, in addition to its developmental functions, the *Drosophila* Toll protein functions to detect microbial infections and induce NF-κB-dependent protective responses. Thus, although it remained unclear what the mammalian Toll receptor detected, it was hypothesized by Medzhitov and Janeway in a Review published in *Cell* in 1997 that it functioned downstream of PAMPs. This prediction was then proven in rapid fashion over the next several years, most prolifically by the lab of Shizuo Akira. Today we know that Medzhitov's human Toll protein is one of many mammalian TLRs that function to detect the presence of bacterial, viral, or fungal PAMPs.

Unleashing a New Age of Immunological Research

In 1999, Medzhitov obtained a faculty position in the Immunobiology Department at Yale University School of Medicine. Over the next several years, he undertook a diverse series of research endeavors to comprehensively test the predictions of Janeway's Pattern Recognition Hypothesis. Perhaps the most important question to address was whether TLR signaling was truly involved in activation of adaptive immune responses in vivo. This question was addressed in a landmark study in which Medzhitov and colleagues used mice genetically deficient in the protein MyD88. MyD88 is a TIR-domain-containing adaptor protein that controls signal transduction pathways activated by the IL-1 receptor and TLR families. Medzhitov's lab showed that TLR signaling is essential for certain adaptive

immune responses to immunization. These experiments provided the first genetic evidence that PRRs are critical for activation of adaptive immunity in living animals, thus proving the most critical point of Janeway's theory—that the innate immune system controls the adaptive immune system. Also in this study, Medzhitov's group demonstrated that TLR signaling is a potent activator of dendritic cells, the primary antigen-presenting cells of mammalian immune systems. TLR-induced signals activated dendritic cells to express cytokines and costimulatory molecules that promote T cell activation and differentiation. It therefore became commonly accepted that TLRs promote adaptive immune responses by providing the signals needed to activate antigen-specific T cells.

While the work described above was of fundamental importance in establishing the link between TLRs and adaptive immunity, subsequent work from Medzhitov's lab highlighted the remarkably diverse means by which TLRs accomplish this task. For example, over the past decade, we have learned of the importance of regulatory T cells (Tregs) in preventing activation of autoreactive T cells. The question of how Tregs permitted microbe-specific T cell activation while preventing self-reactive T cell responses was very much unclear. This problem was solved, at least conceptually, when Medzhitov and colleagues found that TLR-induced cytokines produced by dendritic cells render microbe-specific T cells refractory to suppression by Tregs. This study therefore established that TLR-induced cytokines not only promote T cell activation directly, but also make them refractory to the suppression by Tregs.

It had therefore become clear that TLRs activate cellular responses in dendritic cells (and their highly related macrophage cousins) in order to influence multiple aspects of T cell differentiation. But it was not clear what the role of TLRs was in the function of other types of cells. From 2003 to 2005, seminal studies were published by Medzhitov's group, demonstrating the importance of TLR signaling in B cells for the production of T-cell-dependent antibodies, as well as the importance of TLR signaling in intestinal epithelia for the maintenance of intestinal homeostasis. This latter point was most intriguing because it highlighted the important role that TLRs play in the interactions between our immune system and the trillions of commensal bacteria present in the intestine. These discoveries helped to establish the idea that the mammalian immune system uses TLRs to both fight infections and maintain a healthy mucosal environment through interactions with the intestinal microbiota.

The studies described above highlighted the importance of understanding the signal transduction pathways that are activated by TLRs, and Medzhitov's group played a critical role in establishing several principles that drive the field today. For example, his group co-discovered the first cytosolic signaling protein (TIRAP, also known as Mal) that distinguishes the TLR pathways from the IL-1 receptor pathways. Subsequent to this work, several other related proteins were identified that now define the molecular basis for the specific pathways activated by TLRs. Medzhitov's group also provided some of the first insights into the cell biological aspects of TLR function, and he identified the means by which TLRs control the antimicrobial and antigen-presenting activities of dendritic cells. These early studies have expanded dramatically, and today entire subfields of immunology are devoted to defining the signaling pathways that TLRs activate to control cytokine expression, antigen presentation, and host-microbe interactions. All of these subfields operate under the conceptual framework put forth by Janeway, which were proven and expanded on by Medzhitov and the community at large.

A Vision into the Future

In recent years, as the fields of pattern recognition matured, Medzhitov diversified his interests by asking deep unanswered questions associated with immunology and inflammatory disorders. For example, he has recognized the important fact that, although TLRs control innate and adaptive responses to microbes, they do not contribute to similar responses that target multicellular parasites or allergens. These so-called type 2 immune activators trigger robust T- and B-cell responses, but the molecular basis for their detection is not explained by any of the known PRR families. Medzhitov and colleagues have pursued the idea that parasites may be recognized indirectly, for example, by the innate sensing of enzymatic activities of proteins they secrete. It is interesting to consider this work in the context of Medzhitov's early work on TLRs. In some sense, by focusing on understanding type 2 immune responses, he has reset the immunological clock back to the early 1990s, when virtually nothing was known about innate triggers of adaptive immunity to microbes. If past performance is any predictor of future behavior, we can expect new ideas to emerge from Medzhitov's lab on this front as well.

Most recently, Medzhitov has taken perhaps his broadest approach yet to understanding immunology in the context of human health. Reaching back to his ability to take (seemingly) unrelated observations and synthesize a unifying theme, he has promoted the idea that there is an inflammatory component to virtually all human ailments, even those not typically associated with infection. He has highlighted the fact that, in many instances, the inflammatory responses within a given tissue are more damaging than the initial insult, even if this insult is microbial replication. With this principle in mind, it is possible to consider treatments to diverse human ailments that increase tolerance to inflammation-induced tissue damage, rather than treatments that target the initial insult (e.g., antibiotics to treat infection). Though the hypotheses outlined in this section have yet to be proven at the molecular level in terms of receptor/pathway identification, it is clear that Ruslan Medzhitov has a vision of immunology that extends far beyond the details of TLR signaling.

Final Thoughts

The Else Kröner-Fresenius Award sought to identify a scientist whose achievements have had both an intellectual and practical impact on human society. With this in mind, it is worth noting that examination of any immunology textbook today highlights the critical role of TLRs in controlling multiple aspects of innate and adaptive immunity. These principles were in no small part established by the discoveries made by

Medzhitov and his lab. Although it has only been about 15 years since TLRs exploded onto the immunological scene, numerous drug companies now operate within the conceptual framework of pattern recognition, and a synthetic TLR ligand (monophosphoryl lipid A) has been approved by the United States government for widespread use in vaccinations. Few scientists have had a greater influence in shaping immunological thinking over the last several years than Ruslan Medzhitov. He has given us a wealth of ideas, some yet to be tested. He has seeded the careers of a remarkable number of independent investigators and has inspired a new generation of scientists hoping to follow in his very large footsteps. He is a professional is every sense of the word and is well respected by his colleagues. He is a world-class mentor, a world-class orator, and a good friend. Congratulations Ruslan. We could not be happier for you and cannot wait to learn what you will teach us next.

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