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Innovating immunology: an interview with Ruslan Medzhitov

Ruslan Medzhitov was inspired to become a researcher in immunology on reading a 1989 paper written by Charles Janeway that outlined a new theory for immune system activation. Just a few years later, having achieved a postdoc position in Janeway's lab, he carried out the experiments that confirmed the theory, re-igniting interest in the field of innate immunity and launching his own career. Here, he discusses this early discovery and explains what he considers the three most important questions facing immunologists today.

Two decades ago, the mechanisms that led to the activation of the adaptive immune system were largely unknown. Adaptive immune cells – such as T and B cells – and the cytokines that they produce had been characterised in some detail, yet how an invading pathogen could induce their actions was a mystery. In 1989, Charles Janeway proposed in a landmark paper (Janeway, 1989) how this might take place: invariant molecular structures expressed by invading pathogens would activate pattern recognition receptors on innate immune cells, which in turn would trigger the expression of co-stimulatory molecules and important signalling pathways to instruct the activation of adaptive immune cells. However, the experimental evidence to prove this theory was lacking until Ruslan Medzhitov joined Janeway's lab as a postdoc a few years later, and they published a seminal paper describing the capacity of a human Toll-like receptor (TLR) to activate both innate and adaptive immune responses (Medzhitov et al., 1997). This discovery, together with others in the then-quiet corner of the field, led to an explosive interest in innate immune signalling. Since then, investigators including Ruslan Medzhitov have mapped out a myriad of different receptors and pathways that are involved in the mechanisms of pattern recognition.

Ruslan Medzhitov grew up in the former Soviet Union in a large city called Tashkent, where he enrolled in college at the age of 18. Owing to unusual demographics at that time, the government extended its compulsory army service to college students for a short period, meaning that he had to spend two years serving in the Russian army before returning to complete his college education.

How did your time in the army influence your academic training?

Two years in the Russian army can be very damaging – they were probably the most miserable two years of my life. The Russian army is very different than in other countries: it's extremely anti-intellectual, and it's just horrible. On coming back to college, students were essentially completely unable to continue their studies, or found it extremely difficult. In fact, by the time I returned to college, university professors had started protesting the fact that students were being recruited, because returning students were all failing in their studies. They protested that recruiting students was a huge mistake, and that continuing the practice would mean that the country would become depleted of scientists, which would have implications for national defence and so on. (This was still at the time of the Cold War.) They eventually convinced the government to stop the practice.

Anyway, when I got back from serving in the army, I could not simply continue in my second year of college – it was like everything had been erased. I had to go back to 7th grade chemistry and physics textbooks to start remembering things. Overall, it was very unfortunate – the time when you're supposed to be most productive in terms of learning, and it was completely wasted for me.

What led you to pursue a research career in the United States?

After I graduated from college I went to Moscow University to do my PhD in 1990. That was unfortunately when the Soviet Union broke up into different countries, and a time of economic crisis. It was not a good time to do experimental science as there was no funding. At that time I was interested in various theoretical conceptual questions, mostly related to the evolution of molecular recognition. But I couldn't do experiments,

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because there was no funding, so I would just go and sit in the library and think. Towards the end of my PhD, I became interested in immunology because it has a lot to do with molecular recognition. I'd never taken a formal immunology course, and much of the literature I was reading was very confusing or outdated – it was prior to molecular biology and molecular definition of various cytokines and so on. But, by chance, I read Charlie Janeway's famous article where he proposed the pattern recognition theory of innate immunity in the proceedings of *Cold Spring Harbor Symposia* (Janeway, 1989). Reading that paper changed the course of my career: it was at that point I decided that I would like to study that particular area, because I believed that understanding the concept that Charlie had proposed would sort a lot of things out. It was one of those transformative moments. Email had just become available, and I initially contacted Charlie that way, and we exchanged a few emails.

During the last year of my PhD I went to UCSD [University of California, San Diego] on a fellowship to study bioinformatics (which at that time was called protein evolution) in Russell Doolittle's lab. He connected me with local immunologists, including Dick Dutton, who at the time was the President of the American Association of Immunologists. Dick Dutton helped to convince Charlie to take me on as a postdoc, despite the fact that I had no experience in immunology, or even in experimental biology.

While working in Charlie's lab, you generated evidence to confirm his pattern recognition hypothesis. That must have felt like a wonderful achievement.

I think I was just in the right place at the right time – anyone else in the same position would probably have done exactly the same thing. I was just interested in that particular question: identifying the hypothetical receptors that might detect microbial products and then activate antigen-presenting cells and induce immunity and inflammation. And Charlie's lab was exactly the right environment for that project: it was not driven by quick publications, and we were not restricted by resources, for the most part. The fact that we had certain expectations on theoretical grounds about what these kinds of receptors might do – that they would activate antigen-presenting cells and induce the expression of co-stimulatory molecules and inflammatory cytokines – that helped us to look for the right type of receptors. Of course, there are always prior discoveries that guide you in your work, and what helped us tremendously was the fact that there was already some information available about inflammatory signalling: the signalling pathways of NFκB [nuclear factor-κB] and the IL1R [interleukin-1 receptor] were known, and the TNF [tumor necrosis factor] pathway was being characterised at the time. The similarity between IL1R and *Drosophila* Toll had also already been recognised, as had the fact that both of those receptors could activate NFκB. From there, at least in retrospect, it was natural to think that the receptors that we were looking for would be something that would be similar to IL1R and Toll and would activate NFκB. So, that all happened during the period when I was a postdoc, and it was very exciting.

At that time, you set out to address a key question about the mechanisms linking innate and adaptive immunity. What do you consider to be three key questions in immunology today?

We now know a lot about pattern recognition: in addition to TLRs, there are several other families of receptors – both transmembrane and intracellular receptors – that function by recognising conserved microbial structures. But there is a growing suspicion that there are additional types of innate immune sensing mechanisms that are not based on pattern recognition but rather on other principles. It's likely that the kind of principles involved are similar to the 'guard theory' described in the plant immunity field by Dangl and Jones (Dangl and Jones, 2001; Van der Biezen and Jones, 1998), whereby the immune system senses the consequences of some stereotypic function of a pathogen or virulence factor. That is, instead of directly sensing the microbial structure, these sensors detect unusual suspicious activities associated with the microbial virulence apparatus, such as disruption of the cytoskeleton or of endocytic trafficking. This kind of sensing would complement pattern recognition mechanisms.

The second area where I think there are a lot of important unanswered questions relates to how protective immune responses are induced. We now know a lot about immunogenicity: you need ligands for innate receptors, you need antigen and you need the proper context to induce an immune response. However, what we *don't* know is how to induce a protective immune response – one that will provide protection from a given pathogen. And, because we don't know that, the majority of vaccine candidates don't work. In cases where vaccines do work, I think it's just a lucky coincidence, because in reality we don't know how to design them. The exception to that is protective immunity mediated by neutralising antibodies, which work well for flu vaccines and some other antiviral vaccines. But when it comes to protective immunity mediated by other arms of the adaptive immune system, we don't know the rules. Protective immunity may involve its own set of signals, cytokines, processes and mechanisms, or there might be something different about how the system is engaged initially. It may have a set of characteristics that currently we simply just do not understand. A protective immune response is induced during most natural infections, which means that the mechanisms controlling protective immunity can be naturally engaged. But, when we immunise, we probably only mimic immunogenicity and not those protective mechanisms, which is why we often don't induce protective immunity with a vaccine.

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Third, questions related to allergy are on the list – in fact, probably even before the other two questions, because we don't even understand first principles when it comes to allergy. When I said before that we understand immunogenicity, I should qualify that: we actually understand it in the case when pattern recognition receptors are involved in triggering the immune response. When it comes to allergens and parasitic worms [helminths – the types of parasites that induce type 2 immunity, which contributes to allergic responses], we don't really understand immunogenicity. This issue is complicated by the fact that there is more than one distinct pathway that can activate type 2 immunity. In my opinion, there are at

least four distinct pathways, and these correspond to different purposes of type 2 immunity. And, accordingly, there are at least four different classes of allergens that activate these four different pathways. A conceptual problem in understanding allergens is that they belong to all sorts of biochemical classes: some are enzymes, such as proteases, some are lipid-binding proteins, some are synthetic molecules, some are xenobiotic, and some don't seem to fit into any of these categories. I think these classes correspond to the different pathways that I'm referring to. But there has been very little progress recently in understanding the mechanisms of how allergens work. For every example of an allergen, you can find examples of at least three classes of allergens that do something different. I don't think there will be one discovery that explains allergy: there would need to be characterisation of the many different pathways that lead to allergy. And at this time we do not even understand one of them.

One pathway that we are close to understanding the rationale of is that involving allergens that mimic the activity of helminth-derived products, particularly helminth-derived proteases. The idea is that the host senses helminths not by pattern recognition but through a mechanism that is described by guard theory, which I mentioned earlier, whereby the immune system senses abnormal activities associated with worm infection. One of these is secretion of highly active cysteine proteases that all worms secrete as part of their infectious cycle. It appears that the host has protease sensors that detect this protease activity by virtue of being cleaved, and that cleavage then activates these sensors. The identity of these sensors is not known; this is something we've been working on for several years now. So, this hypothetical sensor that the host uses to sense parasite infection might be triggered inadvertently by protease allergens and perhaps environmental allergens, such as pollen. This idea is probably correct because there is a lot of experimental evidence supporting it, but it certainly doesn't explain all allergies. There are other types of allergens that don't fit that model.

Your lab has many interests. How do you choose what to focus on?

It's true that I'm interested in many different questions. My lab generally works as one person per project, so we cannot be doing the same things as a lab that specialises only on one area. For example, we work on flu, but we're not a flu lab; we work on transcription, but we're not a transcription lab. Projects in the lab are determined by the type of question one can ask. We don't ask the same questions as labs that are experts on flu or transcription: rather, we ask questions that are as simple as possible, and that are different from the questions that are being actively pursued in the field. The idea is to find a question that is not obvious in the field and that addresses a conceptually novel question – and ideally something that is simple. The answer may not be simple, of course, but ideally the project begins with a simple question. These are my favourite types of questions. There are actually many simple questions that are unaddressed. Obvious questions are being addressed to saturation. Obvious questions are fashionable, and those fields tend to be very crowded. Simple questions are not always obvious (although some are) – and those are the ones we like. We can afford to have one student or one postdoc working on that question.

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One consequence of this approach, and one of my goals, is that we actively avoid competition. I like almost everything about science, but one thing I don't like is competition. It's counterproductive. Competition can be healthy and can provide a self-correcting mechanism. If you're the only one working on something, you'll never be sure if you're right or wrong. People are only sure about something when multiple labs reproduce it. But what I mean is that I don't like to be involved in competition in addressing an obvious question. I try to avoid competition for two main reasons: first, because if you're involved in competition in addressing questions that ten other labs are working on, what you're doing is redundant. And, second, I hate the fact that the people that do the work can end up being scooped, which can be devastating if that project involved years of hard work and dedication. In the end if they get scooped, that can have a very negative effect on everyone. I've been scooped myself a couple of times, and very quickly I developed the attitude of not wanting to be involved in anything competitive. So, I just try to ask questions that are not obvious.

If you could switch gears and go back to the bench and start a completely different project, what would you do?

The question that I am very interested in is innate stereotypic behaviours. I'm referring to behaviours that are genome-encoded, that are hard-wired. There are many types of behaviour like this: aggression, mating behaviour, territorial behaviour and so on. You can study this in flies and *C. elegans*, but some of this work can also be done in mammals.

When it comes to model organisms, what do you think is one that is understudied, and that could teach us a lot about biology?

The one that I would definitely study is amoeba – a single-celled soil amoeba like *Amoeba Proteus*, which is an incredibly beautiful organism. I've tried to study it – I'm still trying to convince someone in the lab to work on it! I could stare at it through the microscope for hours, it's so beautiful. These single-celled organisms eat bacteria, and they're a little bit like macrophages, which is my favourite cell type. I think there are a lot of interesting questions about cell biology, signalling, gene expression, behaviour and so on that could be studied in those animals. And I'm interested in *Amoeba Proteus* because this is a truly beautiful animal – it's just spectacular!

Excerpts from this interview can be heard in the podcast associated with DMM Vol. 4, Issue 4 at http://www.biologists.com/DMM/podcasts/index.html. DMM greatly appreciates Ruslan Medzhitov's willingness to share his unique thoughts and experiences. He was interviewed by Sarah Allan, Scientific Editor for DMM. This piece has been edited and condensed with approval from the interviewee.

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