Kenneth A. Jacobson, Ph.D. and Donald Cook, Ph.D.

Behind the Mask

October 24, 2022

Barr: Good afternoon. Today is October 31, 2022. My name is Gabrielle Barr, and I'm the archivist at the Office of NIH History and Stetten Museum. Today I have the pleasure of speaking with Dr. Kenneth Jacobson and Dr. Donald Cook. Dr. Jacobson is a senior investigator in the Molecular Recognition Section in the Laboratory of Bioorganic Chemistry at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). Dr. Cook is a senior investigator in the Immunity, Inflammation, and Disease Laboratory, is the head of the Immunogenetics Group within the Laboratory of Respiratory Biology, and is an adjunct Assistant Professor in the Department of Immunology at Duke University. Dr. Cook is part of the National Institute of Environmental Health Sciences (NIEHS). Thank you both for being with me today. To a lay audience, can you please define what the purinergic hearing system is?

Jacobson: Yes, the purinergic system is a collection of receptors and enzymes that process or modulate signals related to extracellular purines—those being adenosine and various nucleotides. These compounds are important molecules found in all living cells. They're actually building blocks used by the cell to chemically form RNA inside the cell, and they have other biological roles inside the cell. But what we're interested in is what they do outside the cell as signals, like neurotransmitters. This is a ubiquitous and evolutionarily ancient signaling system, as these purinergic receptors are found in some combination in every cell in the body. When these compounds exit the cell, they can send a signal back to the same or neighboring cells to correct an imbalance. These biochemical signals are part of the body's response to stress, whether it be from infection, lack of oxygen, or other cause, such as low levels of inflammation that underlie many chronic disease conditions. Thus, the purinergic signaling system is one way the body restores health to tissues and organs that are pathologically challenged in organisms having immune systems that may be out of balance, either excessively activated or attenuated.

Barr: You said there was a very ancient system found in almost all cells? Is it both in eukaryotic and prokaryotic?

Jacobson: There are examples in prokaryotic cells, but it's mostly been studied in eukaryotic cells, and it's throughout the animal kingdom. That's why I say it's evolutionarily ancient because it goes back to the beginnings of the animal kingdom.

Barr: Is it in plants, too, or mostly animals?

Jacobson: I'd call that controversial.

Barr: Why is that controversial?

Jacobson: Well, the receptors would have to be very different in plants, and they haven't been identified like they have been in mammals. In mammals, we're talking about 19 different receptors. This is a huge collection of various receptors that are either G [guanine nucleotide-binding] protein coupled or ion channels. It's as vast as acetylcholine as a neurotransmitter, and it wasn't recognized until recent years as being an important signaling system.

Barr: That's really interesting. Will you elaborate as to why purinergic signaling is important to understand in regard to COVID-19? I have read that P2X7 has possibly contributed to the cytokine storm some COVID patients have experienced, inflammation from NLRP3 activation, and increased levels of P2Y1 that activate the blood coagulation pathway. Are there other potentially harmful effects?

Jacobson: The purinergic receptors and their enzymes play an important role in the immune system, both the innate and the adaptive immune systems. We surmised that these receptors could be utilized to tone down the excessive immune response leading to the cytokine storm and acute lung injury accompanying severe COVID-19 diseases. We included P2X7 receptors in our study for the reason you mentioned, and also P2Y14 receptors that respond to certain pro-inflammatory nucleosides and nucleotides, and A3 adenosine receptors that respond to adenosine. The adenosine receptors are known to be anti-inflammatory. It's like a balance—the nucleotides induce inflammation when it's needed. We don't want it to get out of hand—we only want it to go so far, and that's when the anti-inflammatory adenosine receptors kick in to tone down the inflammation. We want to modulate this balance in COVID models.

Cook: With regard to your question about whether there are potentially harmful effects, that's always the case whenever you perturb a biological pathway, and especially immune pathways, which is one reason why it's very important to do the experiments. Dr. Jacobson can confirm if he likes, but as far as I know, none of the drugs that we've used so far has caused any overt harm to the animal, so that's a promising sign.

Jacobson: That's correct. We have examined in some toxicity testing to determine whether our drugs are safe. At this point of preliminary testing, they appear to be very safe.

Barr: That’s good. How and when did you all team up to work on the purinergic intervention for COVID-19 using small molecule drugs?

Jacobson: Extending the known beneficial effects of purinergic modulators of the immune response to COVID-19 seemed to us like an obvious step in light of our established collaboration, funded by a joint project between our two institutes, NIDDK and NIEHS. We began collaborating on the P2Y14 receptor seven years ago on a project directed toward using synthetic antagonists—that means blockers—as potential therapeutics for asthma. When COVID started around mid-2020, we submitted a project proposal for funding of COVID related research, initially from the NIH Director’s Fund. Unfortunately, our proposal was declined, but nevertheless, we thought it was important enough to do the work. We forged ahead on our existing lab resources. We were also impressed with the urgency to identify effective treatments for COVID. Note that these are not antiviral drugs. They're not related to vaccines directly. These are just treatments for people who might have serious COVID.

Cook: I would just clarify that it was actually Dr. Jacobson's idea to pursue this line of research, not mine. He and I had worked together already on a different, very fruitful project. It made sense, but it was actually his idea. Also, it's important to know that even though there's been great work done in the area of vaccines, they can lose their efficacy over time. Also, some people elect not to receive the vaccine. When those people get sick, the vaccine is not helpful for them, and we need to have therapeutic drugs. The more therapeutic drugs we have in our arsenal, the better it's going to be because some might work better on certain types of patients than others do.

Barr: Can you speak a little bit about the underlying methodology and principles behind your research, such as which receptors you hope to target with these small molecule drugs and why?

Jacobson: I can start by addressing the chemical aspects. We are medicinal chemists, so we design new molecules that have interesting biological activities. We have a long history of discovering small molecule drugs that interact with the purinergic system. In fact, four molecules that we've invented or characterized in our lab are currently in clinical trials for various diseases. One of these drugs that's already reached phase three clinical trials for psoriasis is called IB MECA. It's a selective A3 agonist. The company that's working on that has also initiated a clinical trial on COVID-19 patients. Other people are thinking along the same lines as we are. Also, there's a trial of inhaled adenosine, which seems to show some benefit in humans that have COVID-19. Many other labs are interested in using purinergic signaling, either through the adenosine receptor agonists or P2 receptor antagonists, as a potential medical intervention to reduce the hyper-inflammatory state of COVID-19.

Cook: As Dr. Jacobson has outlined, his lab has done a lot of excellent work in terms of generating and characterizing antagonists and agonists of various purinergic receptors, whereas our lab specializes more in in vivo biology—in particular, immunity. The model that we came up with was based in part on our knowledge that vertebrates have developed mechanisms to sense and respond to viruses. One of the replicative stages that the virus goes through is to generate double-stranded RNA [ribonucleic acid]. It's a single-stranded RNA virus, but it replicates by generating double-stranded RNA. This is quite unusual in the host and so they've adapted immune systems to seize upon this and use this double-stranded RNA to generate inflammatory responses to clear the virus. Of course, inflammatory responses can help clear pathogens, but they can also cause tissue damage. One of the things we'd like to do is fine tune the inflammatory response so that it's able to clear the virus, but not so strong that it compromises lung function. We used a mimetic or analog of this viral RNA called poly IC, which mimics the effect of this double-stranded RNA. We instilled that into the airways of mice, which then generated immune responses. But we also wanted to have a foreign protein that would mimic the effects of accumulating viral protein in the cell. For that, we used ovalbumin, which on its own is non-immunogenic, but when combined with the poly IC, it generated immune responses. We found that with these daily installations of poly IC plus ovalbumin, the mice develop pulmonary inflation, which increased over the course of the one-week long experiment. The primary cell type we found that came into the lungs were neutrophils, which is very similar to what's seen in COVID-19 disease. Then we use some of the agonist and antagonists of these various purinergic receptors—that Dr. Jacobson had provided us—to test their ability to dampen the inflammation.

Barr: Can you talk a bit more about how you would go about selecting which compounds you would test and how many?

Jacobson: Well, actually, the compounds we used, with one exception, have been tested in other disease models, and they're efficacious. We know they're selective for their target receptor, so they should be good pharmacological probes, both in vitro and in vivo. It was natural to try these particular compounds.

Cook: We test the ability of the compounds to limit the inflammation. There are various models out there for COVID and each have their own advantages and disadvantages. One of the major advantages of this model is that there's no live virus—there's no virus of any sort. All of the components are very safe. It can really be done in any laboratory.

Barr: There are obviously some disadvantages to that not being a live virus, right?

Cook: Oh, definitely. One of the things that the virus does is adhere to and penetrate epithelial cells that line the lung, and so that generates an inflammatory response on its own, which is probably different in some ways than just instilling the analog of double-stranded RNA. There are definitely some disadvantages to this model—as there are to most models. Pulling the knowledge from our experiments, with the knowledge gained in other approaches, is really what makes science go forward.

Barr: Definitely. Can you talk a little bit about what some of your findings have been to date from this study?

Cook: The compounds were injected intraperitoneally, so there would be a systemic spread throughout the body. We found that antagonists of the purinergic receptor P2Y14R, which we'd actually used before in a different model, worked to suppress inflammation in this model of COVID-19. These antagonists included a parent drug and another drug that had to be modified enzymatically in the animal. In addition, we found that agonists of the adenosine receptor A3AR also reduced inflammation. As Dr. Jacobson was saying before, some agonists and some antagonists can, acting through different receptors, suppress inflammation—so we could confirm that. We also found that mice lacking the P2X receptor also had reduced inflammation in our model. We found targeting multiple purinergic receptors diminished the inflammation that we saw in the animals.

Barr: What are some of the next steps with this work?

Cook: One of the things that the Jacobson lab wants to do—and he can speak more on this than I can—is to try and modify the compounds to possibly increase their bioavailability. He also wants to test some different receptors, including different types of adenosine receptors, such as the ATA subtype. Then ultimately, we'd like to see if some of these drugs can have an additive or even synergistic effect when delivered to animals or, ultimately, people. Ken, is that accurate and do you have anything else to add?

Jacobson: That's accurate. I'd just like to add that in general, the public should understand that the process of making a drug takes many years and is very expensive. It's certainly beyond the means that we have in the NIH Intramural Program. What we have to do is show that our molecules are safe enough and promising enough in animals that perhaps some drug company could come to us and ask to license the technology. Then they would

carry it forward beyond what we've done. We can't really develop drugs here. We just do the discovery part. We let the professional drug developers in pharma license it officially from NIH, so that it can actually get into patients eventually—but that takes many years. It's a high-risk endeavor because there are so many reasons why liability can arise when examining any particular drug molecule.

Cook: Some of these drugs that their lab is developing we are now testing in a model that we're calling a model of COVID-19; it's really a model of pulmonary inflammation. There are other viruses that could be completely unrelated to coronaviruses that cause similar types of inflammation in the lung. Of course, the vaccines that we have now are useless against these completely different viruses. But some of the compounds that the Jacobson lab are developing could potentially be useful in a number of different types of viral infections.

Barr: That's a good point. That's promising.

Jacobson: And I should add that Dr. Cook is really an expert because he worked in the pharmaceutical industry before he came to NIH.

Barr: That's a good partner for you, then.

Jacobson: Absolutely.

Barr: What were there any challenges or roadblocks that you all have faced or experienced with this study, and were there any surprises or things that you found very interesting?

Cook: One of the things that didn't work out quite as well as we'd hoped is that we haven't yet seen any synergistic interaction with the different drugs. There's a number of potential explanations for that, but one of the things that we'd like to get to going forward is that if we add and we target more than one of these receptors, will it have an additive or synergistic effect? That'll be a challenge.

Jacobson: Another challenge that is more in the research domain is that you can show that a drug works in an animal model, and then it may not work in higher species like dogs or primates because there are biological differences in the roles of the receptor. There are also differences in the way the drugs interact with different species of receptors. There are so many barriers that would have to be overcome to make this a practical treatment. But that's our long-term goal.

Barr: Have you all been involved in any other COVID related research, or do you plan to be involved in other COVID-19 research?

Cook: Not me. I don't have any other projects that are directly aimed at mimicking COVID-19.

Jacobson: I don't either.

Barr: What was it like for you both to continue to run your labs during the pandemic?

Cook: Speaking from the animal side of things, it's been trying because the animal facility was pretty much running at capacity. When the pandemic came, the people that were working in the animal facility had to really try isolate themselves from others, and that dramatically reduced their ability to keep the colony going. Since my lab's research is almost entirely based on work with animals, it really took a hit. In addition, even in the lab people were not able to work closely together, so we had to stagger work shifts. That definitely slowed us down.

Jacobson: Yeah, I can echo that, too. My style is to be very interactive with my staff, and I want them to be interactive with each other. That was extremely difficult during the pandemic. We could only have a limited number of people at any given time. We also depend on other labs in the institute for exchange of materials and information. This was all very difficult. We're happy that we finally published the work from our project, which was initiated under very negative conditions.

Barr: Yeah, definitely. In addition to being scientists, you're also people who've been living through the pandemic. What have been some personal challenges and opportunities COVID-19 has presented for you?

Cook: We all know people that have gotten COVID, and some of them got sicker than others, including friends and family. It's always tough to see people get sick, but that's true for everybody.

Jacobson: It was difficult for me to see so much misinformation spreading that doesn't come from validated medical authorities. Everybody became an armchair expert on COVID and its treatment. A lot of that information was just somebody Googling that thought they had the solution. Or they thought something was wrong with the solutions offered by medical science. I hope that as time goes on, we can more adequately inform the public on what our tremendous efforts are doing for public health.

Cook: Up until now, people went to the doctor and got information from the doctor, believed what the doctor said, and acted accordingly. But the percentage of people that do that now is declining and continues to decline with the explosion of information and misinformation on the internet—to the point where some people don't believe doctors. They would rather believe someone else, and it's unfortunately cost a lot of lives and hardship. We're all aware of that.

Barr: That's definitely true. Is there anything else that either of you would like to share about your research or your experiences during the pandemic?

Cook: There's nothing else that's unique from my perspective. I appreciate your interest in our work, though.

Barr: Yeah, it's very interesting—so many different angles of people looking at therapeutics at NIH.

Jacobson: Don and I have worked together beautifully on multiple projects. I'm grateful.

Cook: As am I.

Barr: Wonderful! Thank you both. I wish you continued health and success. It will be interesting to see where this goes.

Cook: Thank you. Good luck to you in your endeavors as well.

Barr: Thank you very much.