PEDIAPOD SEPTEMBER 2021 TRANSCRIPT

Geoff Marsh
Hello and welcome to Pediapod for September 2021. This month the role of inhibitory receptors in the pathogenesis of oligoarticular juvenile idiopathic arthritis.
Juvenile idiopathic arthritis is one of the most common inflammatory joint diseases in children. Previous studies have shown that in the oligoarticular subtype of this disease, T cells play a central role in pathogenesis. T cell inhibitory receptors, also known as immune checkpoints, seem to play an important role in the development of tolerance and recognition of self and non-self antigens. Ligands binding to these checkpoint proteins inhibit T cell function and modulate the course of the immune response. When there's continued antigen exposure this gradual loss of T cell effector function has led to these cells being referred to as 'exhausted'. In children with inflammatory disease such as this subtype of juvenile idiopathic arthritis, less is known of their immunological response to the use of inhibitory receptors.
In this episode, we meet Erdal Sag, a paediatric rheumatologist at Ankara Training and Research Hospital in Turkey. He and his team designed an *ex vivo* disease model to examine the effects of different co-inhibitory receptors on the pathogenesis of oligoarticular juvenile idiopathic arthritis. Erdal started off by telling me about his career in paediatric rheumatology and how it started in Turkey.

Erdal Sag
Hi. I am Erdal Sag and I am from Turkey. I have my medical education at Hacettepe University which is in Ankara, the capital city of Turkey. When I was doing my paediatric residency, I started to study pediatric rheumatology with the guidance of Professor Seza Ozen. I visited University College to Lucy Wedderburn's lab during my paediatric residency, and I visited the Deleuran's lab in Denmark Aarhus, where I did all my research for the current paper.

Geoff Marsh
Do you have much personal experience in Turkey with juvenile idiopathic arthritis? Do you see this a lot?

Erdal Sag
Juvenile idiopathic arthritis is the most common inflammatory arthritis in childhood. It accounts for nearly half of our daily clinic in Turkey. It is not such a severe disease but if you did not give treatment it may cause the child to become more restricted or to have chronic pain. So it's our primary goal to decrease the burden of the disease, of course, and improve the child's health. Juvenile idiopathic arthritis, especially the oligoarticular subtype, is an autoimmune disease. When a naive T cell encounters an antigen or enter an inflammatory environment, they turn into functional primary memory cells and their capacity for cytokine production is very high in that state. However, if the antigen presentation or the inflammation becomes chronic, these cells begin to run out and the checkpoint proteins work here. They make the T cells exhausted. In 2018, two scientists won the Nobel Prize.

They blocked this exhaustion step and made these T cells become non-exhausted and make them again, proliferative so they start to fight with the cancer cells again. But with these treatments, they've seen that there are a lot of autoimmune side effects. That's the point we started thinking about the pathogenetic mechanisms of these proteins in the autoinflammatory or autoimmune diseases because there should be a link between cancer and autoimmune diseases.

Geoff Marsh
So tell me about the current study. How did this come about?

Erdal Sag
When I started my rheumatology fellowship, I had seen a lot of juvenile idiopathic arthritis patients. In the oligoarticular subtype we usually drain the inflamed synovial fluid from the joints and we used to throw that away. But I decided to compare the synovial fluid with the peripheral blood of the same child to decide what happens in this microenvironment. So, I started to collect synovial fluid and at some point I got in touch with Professor Bent Deleuran. He is working on the synovial fluid in adult patients. So we discussed it with him and we were trying to find a new pathway, a new molecule. We performed a new *ex vivo* arthritis model because the current models in the rheumatology field usually mimics the adult rheumatoid arthritis but that is pretty much different from the childhood disease. The first thing we have done is to show that our model is working and thankfully it worked.

Geoff Marsh
So you looked at the plasma and the synovial fluids from your patients. Where did you see most activity of the checkpoint analogues, the inhibitory receptors?

Erdal Sag
The inflamed joint is the major site of inflammation. We do not see that much inflammation in the peripheral blood. But when we compare the cells and the cytokines in the synovial fluid, we see a great increase. So we thought that these cells, they go to the exhaustion phase because there's chronic inflammation in there. In this synovial microenvironment, the T cells were not totally exhausted. We can still push them to become more pro-inflammatory. If they were not exhausted we tried to give them checkpoint protein analogues.

Geoff Marsh
Essentially, you were trying to study the effects of different inhibitory receptors?

Erdal Sag
The idea is that if you give the locus of checkpoint proteins the T cells start to aggravate the inflammation, because you rescue them from apoptosis. But if you give the analogue of these checkpoint proteins you tell them to stop working and you make them exhausted so they stop working and they block the inflammation. The story was very similar with the CTLA4 analogue, Abatacept, so we decided to move on with LAG3 hopefully for a new therapeutic agent. We used the synovial mononuclear cells. We directly put them into the flasks and grew some fibroblasts. The fibroblasts are

really important cells in pathogenesis of rheumatoid arthritis actually, but they were not studied well in juvenile idiopathic arthritis. Then we add the same patients PBMCs or synovial mononuclear cells to the fibroblasts, and we co-cultured them. In the end, we found that if you co-cultured fibroblasts with the PBMCs or SFMCs, they start to aggravate the inflammation. If you grow fibroblasts and PBMCs in a separate wells you get some inflammation. But if you grow them in the same flask you get really high inflammation. So the main idea is to block this inflammation at the checkpoint protein leven. Then the idea comes up to block their activity with the checkpoint analogues. When we block checkpoint proteins in our co-cultures, the inflammation goes very high. So we decided that these cells are not totally exhausted,and we can still make them much more active. But when we add the analogue of LAG3, we block the inflammation and make the secretion of several cytokines decreased.

Geoff Marsh
So LAG3 was the key checkpoint analogue

Erdal Sag
According to our study, yes, LAG3 is the key checkpoint analogue in oligoarticular juvenile idiopathic arthritis.

Geoff Marsh
What does that mean for the potential for using LAGG3 as a therapeutic target?

Erdal Sag
If you give LAG3 analogue to fibroblasts plus T cells, we've seen that we are blocking the inflammation, because we hypothesise that we make the T cells exhausted. We make them go into the apoptosis or the exhausted phase of the T cells so in the end the inflammation goes away. That's our hypothesis but I mean we have to prove it in animal models first. And then in patients of course.

Geoff Marsh 08:37
Are there worries that there would be side effects of exhausting T cells?

Erdal Sag
Sure but we are still taking CTLA4 analogue into account and there is evidence that that is working quite safely. That has been proved with randomised control trials in juvenile idiopathic arthritis patients and there are no serious side effects. I don't know how, I don't know why. But I think at some point, there is another control mechanism that blocks them from going to cancer I think. But again, it's a pilot study so we have to move on with the animal models first.

Geoff Marsh
Okay, so is that your next step, to move this from *ex vivo* cultures to some sort of animal model?

Erdal Sag

The first thing we are planning to do is to prove it in a larger cohort with different subtypes of juvenile idiopathic arthritis. But the next step will be the animal models.