

**SPEAKER 1:** So the next big question is, how does a single therapy ECP have its bidirectionality, its symmetry, turning on an immune reaction when needed against a cancer, and off an immune reaction which is actually causing its own disease. So let's take a look at perhaps a clinical circumstance that we all have experience with. I for example during my internship became a positive PPD reactor, since I was exposed to a patient who had tuberculosis.

But I don't have tuberculosis. I'm simply immune to it. So what we now know, and we use this in my own laboratory when we need the cells, is if you give me an intermediate strength PPD skin test, my entire arm will swell up within 48 hours to a tremendous reaction, suddenly loaded with and triggered by PPD sensitive T-cells, all originally triggered by an antigen presenting cell set that processed the PPD and told them to go have a reaction.

But it turned out, of course, it's a false alarm. If it weren't a false alarm, and my immune system didn't know it was a false alarm because the antigen is no longer there, I would be overrun within a week, perhaps, by that tremendously overwhelming reaction. So what's happening physiologically in ECP, we learned by having to do something rather difficult. We needed to make an ECP miniaturized, actual apparatus for mice.

And I won't go into that in detail. But the question that I'm answering here about how ECP has its bidirectionality comes back to the [INAUDIBLE] oxsoralen being activated by the light, to again piggyback or jump into what is a normal physiologic mechanism of how to shut off a reaction. Here's what we now know in a nutshell, and what is really probably happening inside my own PPD reaction when it knows to get turned on and it knows to get turned off.

What's happening in my reaction, presumably, is this. Antigen presenting cells specifically dendritic cells, when they process the antigen, engage the specific T-cells for which they are able to recognize and respond to that antigen, do it by direct contact with the T-cell receptor and costimulatory molecules on the responding T-cell. And they are capable, these dendritic cells that are antigen loaded, in my case with PPD, but it could be any antigen, to turn on lots of T-cells quickly, all antigen responsive in a very selective way.

But then what happens is the T-cell, through a tumor necrosis factor like system, signals back to the dendritic cell, you are going to undergo slow programmed cell death, or apoptosis. We're going to get rid of you. You are like a bee that can sting, but then will die. And so what happens is a very dynamic relationship between the antigen presenting cell, which stimulates T-cells, and T-cells, which stimulate the end of the life of that dendritic cell.

So if the antigen, this is the key, if the antigen runs out, in my case with the PPD, there's no more tuberculin. The dendritic cells all die, and the immunologic stimulation stops, but something else also happens. When that dendritic cell dies, and it dies after having already contained and processed the PPD, we use that just as a metaphor for all antigens, it is now recognized as a dying dendritic cell, and it is taken up by naive, healthy dendritic cells, and in ways that are not yet entirely clear, now sends a signal to the new dendritic cell, you are a suppressive dendritic cell, and you are suppressive to that particular antigen.

You are a tolerizing dendritic cell, again in an antigen specific way. So what's happening, and a lot more work needs to be done on this, but we can show functionally that this is what's going on, is that if we put the relevant antigen, and it can be a tumor antigen against melanoma, for example, as we reported recently in cancer research, if we put that into an ECP induced healthy dendritic cell, that healthy dendritic cell will stimulate specific T-cell responses to all the antigens that that animal or human's T-cells can recognize.

However, if we expose that same dendritic cell to lethal quantities of photoactivated 8-MOP, that photoactivated 8-MOP slowly kills in a very controlled way, mimicking the reaction that normal T cells give through tumor necrosis factor. In those dendritic cells with that antigen in it, become tolerizing. So the key here, after all of these years, is this.

ECP makes in the current apparatus that is so widely used, it does two things broadly at once to dendritic cells. Some of the dendritic cells get exposed in that plate to very little light, and they remain healthy, and they turn on immune reactions against the antigens that they process. Other dendritic cells, the ones that creep along the surface of the plate, get exposed to large amounts of photoactivated 8-MOP, and they become apoptotic or dying dendritic cells with the same antigen inside them, and they become tolerizing dendritic cells.

So essentially, what's coming out of the plate in ECP are two broad sets of dendritic cells. All may have the same antigen inside them, or set of antigens. On the one hand, the healthy, only slightly affected by light activated drug dendritic cells are immunogenic. The others are tolerogenic, so it's essentially a seesaw, just like the normal immune system is a balance of on switches and off switches.

And what we see when we see a clinical response in cutaneous T-cell lymphoma is a net effect. The heavier side of the seesaw is the immunization side. When we see a tolerizing specific effect in graft versus host disease, or an organ transplant rejection, it's the other side, it's a tolerizing side, that is dominant.

But what we need to know is that in the current ECP apparatus, both things are happening at once. And they serve as breaks for the alternative one, and it now a rheostat that we can easily, easily affect towards the poll that we would like to. All we need to do now, and we showed this in our experimental melanoma, colon cancer and ovarian cancer systems in mice, all we need to do to maximize the on switch is to turn the light off, make only immunogenic dendritic cells.

And all we need to do to make only antigen specific tolerogenic dendritic cells is to expose all of the passage dendritic cells to enough light activated drug to make them all tolerogenic. So we really have a therapy with a controllable rheostat, and all we need to do right now is reduce this to practice and test these concepts in humans.