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Episode name: Apoptosis-mediated ADAM10 activation removes a mucin barrier promoting T cell efferocytosis

People: Quentin Sattentau, Paul Klenerman

Transcript

Paul: Well, today we're talking to Quentin Sattentau and, Quentin, welcome to the podcast which is called To Immunity and Beyond.

Quentin: Thank you, Paul. Thanks very much. Nice to be here.

Paul: So, Quentin, so we're going to talk about your beautiful paper, but the first question that we're asking everybody is just to give us a bit of background about you. So maybe you could just start and explain how you kind of, your long and illustrious career, how it's gone and the twists and turns it's taken.

Quentin: Yeah. So I did my PhD in London at the Middlesex Medical School and then got a job, fortunately, with Robin Weiss and Peter Beverley working on HIV, and this was in 1985, so it was just after they'd worked out that it was caused by a virus, that AIDS was caused by a virus. And I worked on HIV for almost 40 years since then, and my lab has moved from initially London, then I went to Marseilles, from Marseilles, New York, and then from New York back to London and then London to Oxford, and I've been at the Dunn School of Pathology for 20 years.

Paul: Fantastic. Thanks very much. So obviously the thread through that was HIV and T-cells, or T-cells and myeloid cells, I guess. So was that the basis sort of that led you out to this paper, or how did you end up studying this particular aspect of T-cell biology?

Quentin: Yeah, it's exactly HIV that led us to this paper. What we discovered was that HIV infection of T-cells causes them to die. Actually we didn't discover that, that's been known for some time, but we were looking at how those HIV-infected T-cells were cleared by macrophages, and what we noted was that HIV-infected T-cells were incredibly tasty to macrophages. They would scurry around and eat them preferentially compared to other cells in the culture, and we couldn't figure out what was going on, and so this started a project trying to work out what the signals were that led HIV-infected cells to be preferentially eaten by macrophages, and that led to this paper, which is all about cell death.

Paul: Okay, great. So the paper's based around kind of eat me and don't eat me signals, so I'm sort of vaguely familiar with that, and there was another one which was sort of, I think you gave it another name which was don’t come close to me, so perhaps can you just give us a bit of context on those? I think that's important for understanding the paper.

Quentin: Yeah, absolutely. So all cells die, except active cancer cells, which are immortalised, and they need to be cleared when they die, and so there need to be signals that are exposed on the surface of dying cells that can be recognised by phagocytes, so that those phagocytes, mostly macrophages, can eliminate them. If those dying cells are not cleared quickly, then they start to disintegrate and release all kinds of inflammatory contents from the cell, and you get an inflammatory problem, and lack of clearance of dying cells leads to multiple pathologies, including cardiovascular disease, neurodegenerative disorders, and other inflammation-related problems.

So the clearance system of dying cells has to be efficient, and T-cells in particular die a lot. They become activated by antigen exposure, they do their job, and they proliferate enormously, and then they have to die so that they can be cleared, otherwise you'd fill up with T-cells. So there's a lot of T-cell death going on, there's particularly a lot of T-cell death going on in HIV-infected individuals, because the virus induces cell death in T-cells, and so understanding the mechanisms by which macrophages recognise dying T-cells and eliminate them is quite important.

Paul: Okay, that's brilliant. So we've got an idea for kind of the biological context, but then why don't you just explain a little bit about the biochemistry and the mechanisms that you uncovered, and why you went down that path. I mean, it's not obvious to me why you took that path, but maybe it was obvious at the time.

Quentin: Yeah, it was one of those lucky observations, really. One of my past PhD students called Niloofar was looking at dying T-cells inside macrophages, and she stained those T-cells with a number of antibodies against different surface markers just to see what they looked like. And she noticed that one molecule called CD43, which is a long molecule covered in sugar, it's called a mucin-like molecule, and it's part of the glycocalyx, disappeared when she looked at the T-cells inside the macrophage. And the HIV-infected dying T-cells, she then noted, basically had no CD43. And that's a striking observation, because CD43 is extremely highly expressed on normal healthy T-cells. There's somewhere between 10,000 and 100,000 molecules per cell, and they make up what's called the glycocalyx, part of the glycocalyx.

So the glycocalyx is a sugar coat around the cell, which stops nonspecific interactions. And CD43 is a major component of that. So we then looked a bit more carefully at the different molecules that might also disappear in that family. We looked at other mucins, and we found that three highly expressed mucins disappear when T-cells die by apoptosis, in this case. And so we went on to try and find what the mechanism was for that disappearance. And we discovered that it's triggered by an enzyme called ADAM10, which is a bit like a Pac-Man on the surface of T-cells. And when it's activated, it zooms around the membrane, chewing off all of these molecules and spitting them out in a soluble form. And it seems to have a real taste, ADAM10, for mucin-like molecules when the T-cell starts to die. And what we worked out was that the T-cell was losing about 50% of its glycocalyx over a period of a few hours due to ADAM10 chewing off these mucins.

Paul: Okay, so what's the first thing that happens then? So the cell is starting to die, and then you've got all these things that happen that make it tasty for phagocytes. So just rehearse the order of events, and then what is the phagocyte actually recognising or does it just stop recognising a don't-eat-me signal?

Quentin: No, that's a great question. So the glycocalyx, if you like, is a sort of non-specific biophysical barrier. It stops cells sticking to each other and interacting in ways that are unhelpful and non-specific. And it's about 50 nanometres tall on average, depending on the cell type. And it hides lots of molecules that are very close to the cell membrane because other cells can't get close enough to

that cell membrane to recognise them with their receptors. The molecule that is most known as an eat-me signal for macrophages is called phosphatidylserine, and that's a phospholipid. Phosphatidylserine on a healthy cell is almost entirely on the inner leaflet of the plasma membrane, so it can't be seen from the outside. When cells undergo apoptosis, apoptotic cell death, or even other forms of cell death as it turns out, phosphatidylserine is flipped to the outer surface of the membrane. And it turns out that that flipping of phosphatidylserine is a signal to switch on ADAM10.

It induces a conformational change in ADAM10 which brings the active site of the enzyme right down close to the plasma membrane where it can engage with its substrate, these mucins, and start chewing them off. So the sequence of events is the induction of apoptosis by activation of an enzyme, intracellular enzyme called Caspase-3. Caspase-3 activates a scramblase, which is an enzyme that flips phosphatidylserine from the inside to the outside of the membrane. That in turn activates ADAM10. ADAM10 goes about its business, releases all these mucins, and suddenly your T cell is almost bald and macrophages can then really easily identify and engage phosphatidylserine on the surface and start to gobble them up.

Paul: Thanks. So that's very clear. So is it a macrophage specific interaction or are there other cells that are gobbling up certainly in vivo, gobbling up the HIV infected or otherwise apoptotic T cells? Presumably it's not quite so specific.

Quentin: Yeah, I mean there are other phagocytes. There are dendritic cells. There are probably not neutrophils because they're a little bit small. I think they'd probably choke to death on a T cell unless it's already fragmented. And that's a great question because it brings us back to the beginning. Does this have any relevance for HIV? And it turns out that when an HIV infected T cell is engulfed by a macrophage via this mechanism we think, this shedding of mucins and this recognition of phosphatidylserine, the macrophage doesn't do the job that it's designed to do which is to destroy the virus that's inside the dying T cell. Actually the virus jumps from the dying T cell and infects the macrophage. So it turns out that it's a very efficient mechanism for HIV to infect macrophages and set up a sort of long-lived reservoir in the myeloid compartment.

Paul: That's really fascinating. So how much of the macrophage infection in HIV is occurring through this sort of secondary engulfing and how much do you think is direct infection of the macrophages?

Quentin: Yeah, that's a tough one to answer. You can do experiments in vitro, which is what we've done, and if you just put what we call cell-free virus, so virions, onto macrophages, they're mostly pretty resistant to infection. You need quite a lot of virus particles to infect a macrophage and the kinetics of infection are very slow. But if you dump some HIV infected T cells on the macrophages, the kinetics are about ten times faster and you get a lot more virus into the macrophage and you get many more macrophages infected. So we think probably in vivo this is a way to infect macrophages quite effectively.

Paul: So then, the way the HIV field has moved from what I see is from a study of active infection really to a study of what's happening under drug suppression and what is the latent pool and how do we best achieve cure through getting rid of the latent pool. So do you think this process is contributing to the ongoing latent pool and do you think that potentially the myeloid reservoir for the virus is more important than perhaps we'd consider?

Quentin: Yeah, that's a very debated question and we still don't have an answer to it. We don't really have very good models to address that. My reading of the literature over the last few decades is that T cells are definitely the primary target cell for HIV. HIV replicates to higher levels, infects T cells more readily and kills them. But nevertheless, there is evidence that macrophages can be

infected and some of the studies done in macaques and in humanized mouse models show quite clearly that macrophages are infected in vivo.

Is there HIV latency in macrophages? Nobody has shown that. There may be or there may not be. Of course, if you can infect tissue resident macrophages, the thinking is that they are self-renewing and can live for a long time, potentially years. Interestingly, unlike T cells, which are killed by HIV infection quite rapidly, macrophages are not. Certainly, in tissue culture, if you infect a macrophage, it can live for months with a virus in it, no problem. Sometimes they fuse to form multinucleic macrophages, but they live for a long time. My feeling is macrophages are very likely to play a role in maintaining low levels of virus even under antiretroviral therapy suppression. The most worrying tissue, I think, is the brain because there is evidence that microglia can be infected and that's problematic for getting drugs across the blood-brain barrier and it's problematic for inflammatory consequences and it's problematic as a reservoir altogether.

Paul: Great. That was super clear. Thanks so much. The other questions I sort of had were outside HIV because, I mean, this is a general process. Obviously, HIV is driving it like crazy, but did you sort of look at other areas where this was likely to be important biologically?

Quentin: Yeah. There's not much on the role of the glycocalyx in immune cell function. People have thought of it over the last 50 years as a sort of nonspecific barrier that doesn't really have any specific function. I don't think that people have shown that inside-out signalling can change the glycocalyx in immune cells. We were excited by this because what we showed was that a signal which would sell death could actually strip the glycocalyx off the cell. We've gone on to show that not only apoptosis does this, but so does pyroptosis, which is an inflammatory form of cell death. That also flips phosphatidylserine via a different standard and you end up, again, shedding the mucins off the surface of the cell.

In terms of cell death, it's pretty clear that this is a fairly general phenomenon, but there's more to it than that. It turns out that when T cells become activated, they transiently flip PS to the outer leaflet and then put it back inside again, presumably before they get eaten by macrophages. We have shown, this is unpublished, we've shown very recently that during that period of PS exposure, the T cells lose a very specific set of mucins on the surface. Our hypothesis is that this allows the T cell, early on in activation, to interact more closely with an antigen-presenting cell because otherwise the mucins would be in the way. It makes the T cell activation process more effective. We haven't directly shown that final aspect, but that's our working hypothesis.

Paul: Thanks. That makes a lot of sense. Very interesting. One of my questions reading it was that it seemed ... I'd sort of imagined it was dangerous for an antigen-presenting cell to bump into an activated T cell because they'll present, but they may also be killed. Your paper's shown more the opposite. It's bad for the T cell to bump into the antigen-presenting cell because it gets eaten. Are you thinking then that during the process of T cell engagement for activation and the burst of response to an antigen or memory formation, that these modulations in the glycocalyx could sort of tune that function then?

Quentin: Yeah. The loss of mucins on activated T cells is not as dramatic as upon cell death. I think there's a threshold at which the T cell becomes a meal rather than becoming a sort of partner in activation with an antigen-presenting cell. That's very fine-tuned. The other thing we've noticed is that there's one particular, very specific mucin-like molecule called PSGL1, which is one of these checkpoint molecules for T cell activation. That is very specifically and highly cleaved off. It's as if this

is a mechanism to allow the T cell to be fully activated by cleaving off very specific molecules in the glycocalyx during activation that we believe are activated by phosphatidylserine.

Paul: Okay. There were a couple of other questions. One, I was trying to tease other people a little bit because you don't get this when you read the papers. What the kind of issues were in getting it published and what pushback you got and what extra bits and pieces you needed to solve how the process went?

Quentin: Yeah. Well, it was, of course, frustrating. We sent it to... Can I mention journal names?

Paul: Yes, of course.

Quentin: We sent it to Science Immunology and they said...

Paul: Yes. Other journals are available.

Quentin: Right. Right. Science Immunology and they said it's too cell-biological. And so we sent it to, I think, Nature Cell Biology and they said it's too immunological. And so we ended up sending it to Nature Communications and they actually... It walked in. It was one of the easiest rides that I've had. The four or five reviewers were all strangely positive. And so in this particular instance, we did a couple of minor experiments that they asked for and it was a very easy ride. I think the only substantial criticism we had was that one of the reviewers said, you go on a bit about how this might have therapeutic implications, but do you actually believe that? If not, take it out of the discussion. So we took it out of the discussion.

Paul: I think the discussion was quite short.

Quentin: Yeah. It was very short. Yeah. Yeah. We turned it.

Paul: But do you think there are some... I mean, it's hard to speculate in a paper in a really solid way. But since you're on a podcast, perhaps you could speculate about that.

Quentin: Yeah. ADAM10, which is the enzyme at the heart of this process, has a number of well-defined polymorphisms in humans and they are associated with interesting diseases, atherosclerosis, neurodegeneration. Some loss-of-function mutations are associated with improvements in some neurodegenerative disorders and some gain-of-function the other way around. So it's very complicated and it's very hard to work out what's going on.

But you can modulate ADAM10 function. There's a couple of very specific inhibitors that have been in man, not too toxic. So one could imagine messing around with ADAM10 to fine-tune certain elements of this. Ideally we would have liked to have had an agonist so that we could induce ADAM10 activation and speed up the phagocytosis of dying cells, for example, because if people have a problem with that, that might be a way to solve it. But there are no good agonists, so we sort of stop there. The final thing I would mention is that the glycocalyx, these mutants in the glycocalyx are highly upregulated in many leukaemias. The literature strongly suggests that this is an immune evasion mechanism. The bigger the sugar coating in the glycocalyx, the harder it is, for example, for a killer cell to latch on and kill that tumour cell, or for a phagocyte to recognize the receptor that it needs to recognize and eat it. So the idea of somehow targeting ADAM10 on tumour cells so that you can strip off these mucins and make it a target, a better target, is quite enticing. We just don't know how to do it.

Paul: Great. I guess your next step is, or what did you have, where is it going to, there's so many open questions here, what are you going to do next with it?

Quentin: Yeah, yeah. So at the moment we're focusing on showing that pyroptosis also induces this phenomenon and that when a pyroptotic cell is taken up by a macrophage, we're just confirming that that switches the macrophage into a highly pro-inflammatory type of cell. And so it has implications for inflammatory outcomes. But we're also very interested in this T-cell activation component, and we're collaborating with a few people, Omer Dushek, for example, to look at that. And that may go quite a long way. The cancer element is really interesting, but I don't know if we have the tools yet to be able to really probe that properly. But it's kind of on the cards. We're thinking about it.

Paul: Great. Thank you very much. That was absolutely wonderful and very, very clear. And yeah, you're in the first group of three people on the podcast, and we're going to release them all simultaneously so people can enjoy them all together. And we're very grateful for you to come along today. Thank you very much.

Quentin: Great fun. Thanks Paul.