Transcript

Paul

Hello, my name's Paul Klenerman. I'm a professor at Oxford and I'm the host of this podcast on immunology called To Immunity and Beyond. So this is just to say that what we're putting forward with the podcast is a scientific discussion and it's really just for information and it isn't in any way medical advice. So if it's medical advice you're after, please go and talk to your doctor or some other medical professional. Meanwhile, enjoy the podcast. Great. Well, it's a pleasure to introduce another episode of To Immunity and Beyond. And I've got a couple of authors with me. I'll get them to introduce themselves. And we're going to have a chat about Ebola this week. So over to you, Miles.

Miles

So my name is Miles Carroll and I run a lab at the Centre for Human Genetics, also in the Pandemic Sciences Institute. And we work on many viruses, including Ebola virus and especially Ebola virus persistence. So let's introduce my co-author.

Oliver

Hello, thanks for having us. My name is Oliver and I'm a six year medical student. and I got involved with Miles' project on this Ebola virus persistence in my third year. So it's been going on for a few years now and I'm excited to talk about it.

Paul

Great. Well, it's a lovely review. But first of all, perhaps Miles, you could explain that this obviously didn't come out of the blue. You've worked in Ebola fairly heroically for quite a long time. So perhaps you can explain all the background to that and how people actually do this sort of research, just to give it some context.

Miles

So my first interest in Ebola was when I joined was Public Health England back in 2008 and then consistently then, especially after the 2013-2016 Ebola epidemic in West Africa. And so Public Health England and myself were involved in both responses in Guinea and in Sierra Leone. But we carried on a project there and we introduced something called real-time molecular epidemiology by introducing the minION sequencing system. And that led us to discover these very strange transmission cycles that we later worked out were due to sexual transmission. So the virus was in the semen. But then there's many numerous studies, including some that we were involved with, that were looking at how long the virus

could last in semen. And then this is also extremely important for the public health response for WHO and their vaccination strategies around male survivors to stop this reinitiation of infection.

Paul

Great. So perhaps before we get into the persistence bit, give us a context of the actual scale of that epidemic and so how many people were infected and what was the mortality. And what was actually given as an intervention at that time, just to kind of contextualise it a little bit.

Miles

So the outbreak, we believe, by sort of retrospective epidemiology, happened in December 2013 in a small village in the forested region of Guinea called Meliandou. It spread rapidly from the forested region in Guinea over the border into Liberia and Sierra Leone and estimated about 24/25,000 cases. with about 11/12,000 deaths, so mortality rate of around 40/50%.

Paul

And at that, there was a rapid development of vaccines at that point. So what point did those come in, the monoclonals and so forth?

Miles

So the VSV vaccine, which was initially developed by the Canadian government and then later taken on by Merck, that was trialled in a ring vaccination smallpox type approach in Guinea. That was in 2015 and that rapidly got emergency use licensure and played a small role in the final stopping of the epidemic. Though local public health precautions also massively reduce it by basically isolation. But there was a drug that was trialled that we were involved in that with MSF up in Gueckedou called Favipiravir that actually showed some really exciting antiviral promise as well. And the monoclonals, they came later, but it was the Favipiravir that showed some really interesting promise.

Paul

Good. And just last one on the sort of background to this. So there have been subsequent outbreaks, different strains, but in Uganda, I think, and possibly elsewhere, perhaps just update us on what's happened in that area.

Miles

Yeah, so Ebola virus is in the same family as Marburg virus, and it's in the same genus as Sudan Ebola virus. This is Zaire Ebola virus that we're talking about. And there's been numerous outbreaks in different sizes, especially in Democratic Republic of Congo, as you say, Uganda and Tanzania and the recent Marburg outbreak in Rwanda, which is quite sizable.

Paul

Okay, so it's a live issue, but what you picked on in this nice review was one specific element. And I'll pass over to Oliver now. What were you trying to address with the review?

Oliver

So we looked at the persistence of the virus. So persistence isn't a new phenomenon related to Ebola, but it's seen in viruses such as measles that can cause encephalitis years down the line. And that's highly fatal. And Ebola virus itself, we know that persistence in this virus has been happening since 1967 with its relative Marburg virus, where there was sexual transmission of the virus. But with the Zaire Ebola virus, we only realise this more in the recent Western Africa outbreak in 2013, because there was a huge amount of cases of the virus that had never been seen before. So you had an unprecedented amount of survivors. And so there's a huge population of people that, where the virus persists in these people that managed to survive the initial infection. So these people, they clear the initial infection from the bloodstream, but the virus can remain dormant, hidden in certain sites in the body. So these include the brain, the eyes and the testes. And the person may be asymptomatic for months to years until the virus becomes reactivated or maybe is shed into certain bodily fluids. And in terms of Ebola virus, these are immune privileged tissues where the virus persists. And it means that the immune system has a harder time getting rid of the virus from these certain sites. And this is particularly worrying for the testes in particular because the Ebola virus is shed into the semen. And so you now think of Ebola virus as almost a sexually transmitted infection that can be transmitted for months to years down the line that can lead to infection of a naive female that hasn't seen the virus before. And so through this, you can start to get new outbreaks of the virus years after the original outbreak has been declared over.

Paul

Thanks. So that's, I mean, there were clearly these very well documented events. And in your paper, which people can read, you put in a nice graph showing the approximate rates of carriage. So the, there's, you know, with the scale, it's not just a one-off event. This is clearly part of the features of the disease. But what I'm interested in then, perhaps, for the immunology audience is you talk about immune privilege sites. So what's actually going

on? So I think the implication from the review is that the virus is at a very low level and not replicating much. Is that really the case? Or what's the evidence one way or another that it's sort of essentially latent versus persistently replicating versus some other state?

Miles

Yeah, we must be really careful in the wording because obviously latency, we think of herpes viruses. So we don't think Ebola virus is latent. It doesn't have the machinery or any of the genetic signatures to do that, nor does nor for integration for HIV. Think more that the virus is replicating slowly, as you said, and the evidence would be that the mutation rate in these immune privileged sites, like the testes, is one-tenth the mutation rate that we see in blood. So suggesting that there's a relationship between mutations and replication, which suggests that the virus replicates at one-tenth the level it does in the blood. But there's still many unknowns about it. How is it able to persist in the testes without complete testicular destruction, for example, over that five year period, which is the suggestion of the 2021 sexual transmission flare up in Guinea. So we think that up to five years, that virus was persisting. And then for a number of reasons, a new naive partner, as Oliver said, that we saw the actual transmission flare up. And that's something that we're doing in the lab and going forward, a postdoc with Ola Diebold and a collaborator, Phalguni Rath, we're trying to create a teste in a test tube to look at that.

Paul

Yeah, that was going to be one of my questions. So if you can make an organoid, can you, from a testis? Is that the case?

Miles

Well, Phalguni can, because he's a bit of a genius, and he has made a preliminary one already. And with the work with Ebola, we're trying to use a combination of a genetic helper to allow us to use Ebola at CL2, so we can study more easily this testicular organoid.

Paul

Cool. So in the absence of that yet, there are some data from animal models that you mentioned in the review. And perhaps you could give us a bit of a sense of which animal models have been looked at and what they've told us.

Oliver

Yeah, of course. So originally all the data came from these clinical studies of the survivors. But as the outbreak is now over in this time where there's no outbreaks, animal models have been looked at. So particularly looking at rhesus macaques, that's where these

animal challenge studies were carried out. And when the main studies came from this retrospective looking at tissues, so they weren't originally looking at persistence, but they had archived these tissues and then they decided to sequence them to see where the Ebola virus persisted. So in these monkeys, there were high levels of the virus found in the eye, the brain and the testes. So this is where most of the animal models have been. And so I think the macaque has been quite a gold standard for Ebola virus persistence research. And there's other animal models out there, but some are harder to replicate, in terms of being equivalent to a human. So they've looked at mice models, but the problem with those is mice are resistant to Ebola naturally. So they've developed mice adapted Ebola virus. So it's a bit different to the virus that infects humans, but that means it's susceptible to infect mice, which in terms of an ethical and financial perspective, those studies are a bit easier. Although mice aren't as obviously related to humans, so you have to be careful of looking at the data. A more recent model has been with ferrets, because these are almost a middle ground between the resistance of mice to Ebola virus, but the fatality of monkeys is really high. And the ferrets sit in the middle. So these are susceptible to the virus, but don't die almost straight away. So I think these have been used not as much, but in more recent years, they've become a bit more popular. So it'd be interesting to see if they get used more in the future. But the main problem with the macaque model is that it's almost universally fatal to the animal when given Ebola virus. And so they don't survive for very long. So when you look at persistence models, you may be thinking of days as opposed to the time frame of months to years that are in humans. So it's hard to see if it's fully representative of that persistence.

Miles

So just adding to that, as Oliver said, the fatality is there for both actually the ferret and the NHP model. And then that retrospective study Oliver mentioned, those animals lived because they were given a therapeutic antibody. So you could use a combination of a therapeutic to stop the animal dying, but the incidence of persistence is relatively low. So it would be really impractical to set up an NHP model because you'd have to use so many of them for long-term persistence. So short-term persistence might be doable.

Paul

Great. So all of this is on a background of essentially what seems to be quite robust immunity in the rest of the body. It's just that they're these hidden areas where you can't really eliminate the last vestiges, which is not unique to Ebola, but is very striking. But perhaps you could, there's a bit in the review where you talk about essentially the maintenance of immunity post-infection. Perhaps you could just expand on that because that seemed quite interesting.

Miles

Yeah, so we've been studying Ebola disease survivors for 10 years now, looking at their immune responses. And we find, we looked at this concept that the response is really strong for a long time. And is that due to virus leaching out of these immune privileged sites and re-stimulating the B-cell and T-cell response? But our data suggests that the virus does not re-stimulate the antibody response because that is a continuous linear decay and not with these sort of peaks and troughs that you'd expect from re-stimulation. And some work that Tom Tipton did in my lab for a Lancet ID paper back about four years ago showed that the T-cell phenotype, memory phenotype, was that of distant memory, stem cell-like memory rather than affecting memory. So again, suggesting there isn't recent re-stimulation of the T-cell response either.

Paul

And if you, in these cases where it's truly, you definitely absolutely demonstrated persistence in the semen, do their T-cell responses look anything different to people where it's apparently lost?

Miles

So in our long-term studies, we didn't also marry up the semen analysis because we didn't have the resources to do that at CL4. But other papers have been published where they have looked at the, there was a relationship with maybe a longer and higher antibody response in those that were semen positive. But that might just be because people that are semen positive for a longer period had a more intense infection and the virus had more chance to reside and get to all the immune privilege sites and establish itself. And rather those more transient infections would lead to a lower and less robust immune response.

Paul

Great. So we talked a bit about the immunology. So there's clearly a lot to learn and perhaps some implications we can come back to for other viruses. But there's also a nice bit in the paper where you discuss what the therapeutic implications, what would you do about it? So Oliver, over to you.

Oliver

So I think in the ideal world, you would develop a drug that can clear these sites of persistence. And the problem is we don't know the mechanism of the persistence. We know that it's replicating at a very low rate. We don't know the mechanism behind that. In the paper, we speculated a couple ideas. But so if you did know the mechanism, you could

tailor a new drug that targets that mechanism to clear these sites of virus dormancy. But there's been trials, phase two trials, of remdesivir and favipiravir. And they've looked fairly promising at clearing these sites of persistence. In particular, remdesivir's phase two trial looks quite good at clearing this. And I think if there was a future Ebola virus outbreak, then a phase three trial of that remdesivir could be quite useful. The main problem is that it's quite hard to penetrate into these sites. So if you're thinking about the pharmacokinetics and dynamics of the body, because it's such a small amount of virus that's there and it's got such a low replication rate, you might need quite a high dose of drug for a longer time period compared to say something like COVID, which you've treated. So I think in short, using these small molecule antivirals to clear these sites of persistence is probably the way forwards. Maybe using these ones that can be repurposed for Ebola as opposed to developing new ones, which would require more understanding of the underlying mechanisms of the persistence.

Miles

Just to add, I think the way that we could look at the potential of remdesivir or favi for these longer-term persisters is if we had that human organoid model, we could then treat with drug and look after and then see how many days, weeks, whatever it takes to finally stop the virus. Because yeah, as it's replicating 1/10th the rate, so we think, you really have to change the protocol. So normally treating acute Ebola from treating a persistent Ebola infection.

Paul

So your organoids could also give you some indication of what state the virus is in, which cellular compartment it's in and essentially that. So that would be super helpful. Do you think there's any immunological tools we could use to kind of promote this apart from vaccinating the potential recipients of this? Or is it sort of going to be just a virological problem?

Miles

I mean, I don't know enough about the immunology of the testes, but that would be something we should be looking at. Is there a drug that could also stimulate that local immunity? That could be looked at as well.

Paul

And you mentioned that your top pick for a cell would be a Sertoli cell, which has got some stem cell kind of flavour to it. And there's something about the duration of the persistence that actually would support that idea.

Oliver

Yes, so you've got the Sertoli cells which form almost the tubules and the testes. And then you also have just behind them the spermatogonia, which are the regenerating stem cell population that form the spermatids and then you get your sperm. So it's this spermatogonia that's been found in other infections that the persistence kind of is in a similar time frame to the amount of time it takes for the spermatogonia to renew. So that could be a potential site of persistence. But I think the Sertoli cell itself is quite well placed because the spermatids migrate through there. And if the Sertoli cell is the reservoir, then they could infect the spermatid as it passes through into the tubule.

Paul

Great. So just one last question then. I think this is probably one for Miles because you've got other things to do probably, Oliver. But Miles, what are you going to do next with Ebola? What's the, what are the next frontiers? Just thinking more broadly. I mean, this is like a really focused effort, but there's plenty of other things that we need to fix.

Miles

Well, to tell the truth, this is my most interesting project for me to look at, to understand that persistent mechanism and then how it relates to other viruses. We talked before in our preamble prep about hantavirus that can persist for at least six years in a male survivor who caught it in the Andes on holiday in Chile and come back to Switzerland and has been monitored year on year. So I think it's looking at broader, it's bigger than virus persistence, it's bigger than just Ebola. Another area we're really interested in is actually spillover and mechanisms spillover of emerging viruses in general, but especially filoviruses in a cohort of wildlife hunters in West Africa, which we're doing with a former PhD student, a Guinean national, Joseph Akoi Bore. And to follow that up with Grace Hood, a current PhD student, who is now looking at the gap between the 2017 wildlife hunter, blood sampling, where Joe found immune profiles of Ebola outside those villages that were ever reported for Ebola. So almost definitely the virus was spilling over before the 2013-2016 epidemic. And also looking at immune signatures from other filoviruses like Thai forest or Sudan or even Marburg signatures, which are definitely in Guinea because there was a fatal case in 2021, 2022. And then looking at the sero-epi of Marburg virus, we've seen real strong clustering of positive responses in wildlife hunters. So understanding that spillover event and can we develop early warning systems, which we're doing with Kim Fornace, who did some of the meta-analysis on the virus and semen for this study as well.

Paul

Brilliant. So this, I think it's a fascinating read and I think it's also really clever to be able to link this at a very dramatic, acute, horrible event with the sort of much later sequelae, which has still got lots of potential and clearly lots of scientific interest. So hopefully people will enjoy reading that. So thanks very much for coming on today. I think we'll draw it close. Thank you.

Miles

Thank you.

Oliver

Thanks for having us.

Paul

You're welcome and hopefully anybody who also wants to promote their research, they're very welcome to come on and at any time and we'd be happy to have a podcast with you.