Presenter: Professor James Whisstock, Monash University - *2015*

Title: How can carnivorous mushrooms and the "Angels Glow" help us understand immune killing? (17:53)

Time	*Dialogue*
00:07 00:41	Good evening, So I'm going to talk to you about killing and immune killing and try to persuade you that a little bit of killing is good for you but too much is a bad thing and how we are using structural biology to try and understand how the immune system kills. So this is actually a 100 year old mystery and it started I guess in 1898 with one of the fathers of biology, Jules Bordet. What he found was that if he took blood plasma and added it to foreign cells or certain types of bacteria something really remarkable happened. On the right if you watch the video you can see I hope these little blobs disappearing and that is cells bursting in response to a lytic component present in blood plasma. He won the Nobel prize in 1921 for this work but the actual mechanism of how these immune killing machines work has remained really mysterious up until very recently.
01:22 02:03	So what do I mean by lysis? The big black blob on the screen is a cell and the stuff spraying out of it is essentially a cell being lysed. It's being burst and the contents being emptying. If you can imagine a cell like a balloon full of water and putting a hole into it and the contents spill out. The way the immune system does this is by punching lots of holes in cells. So you can see on this slide here I hope all these round donut shaped things are actually holes in the cell surface. Now the immune system is a pretty interesting machine and it is not quite just as simple as making a hole in the cell … because what the immune system does is not only does it make a hole it actually inserts something through that hole, a toxin which actually even if the cell can repair itself, which it often does, the toxin is already inside the cell and it is all too late and the cell dies.
02:26 02:52	And that is essentially a wonderful way of basically of making sure that when your immune system attacks something it really does end up dying as intended and these class of proteins that I am talking about today are called perforin type proteins, and they are called perforin type proteins because they perforate things … like cells. So you might say why do I care about perforin like proteins? Well one reason is if you don't have them you can get very very sick. So certain people who lack aspects of the immune system such as the compliment system are far more susceptible to getting very very serious bacterial infections, like for example bacterial meningitis or septicaemia and if you ever wake up with a rash like this on the screen you should go to hospital very very fast.
03:17 03:46	In addition people who lack perforin itself which is produced by immune cells to kill virally infected and cancerous cells, individuals who don't have perforin develop a very serious immunoproliferative disease usually in the first 6 months of life so these are babies and they develop something called HLH (*Hemophagocytic lymphohistiocytosis*) or FHL (*Familial hemophagocytic lymphohistiocytosis*) and basically what happens in this disease is the immune system can't kill properly so the immune system encounters a virally infected cell and it tries to kill it and it can't kill it so what it does is it sends out signals for more immune cells to come along and try to kill the cell and they can't kill it and they send out more and more and more signals and we end up actually with something called 'cytokine storm' which eventually there is so much immune activity that the immune system turns on the individual and if untreated they die.
04:18	So the only treatment for HLH is a bone marrow transplant which is eventually giving someone a new immune system that does contain perforin. Just as an aside it may interest you to know that Ebola for example doesn't kill you necessarily because of the Ebola virus but it is actually the cytokine storm of your own immune system trying desperately trying to wipe out the Ebola virus that actually ends up kills you.

Time	Dialogue
04:46 05:06	OK, so killing is good and killing is also bad, and in this particular slide here we can see and individual injecting him or herself with insulin because they are a type 1 diabetic and the tissue damage of the cells that basically produce insulin in autoimmune diabetes is perforin mediated, and so basically in this instance it is an unwanted activity of perforin. Similarly too much compliment, Jules Bordet's discovery in blood plasma. If you don't control compliment properly you can end up again with serious diseases and in this case this is a disease where the compliment system, the perforin like protein in the complement system is attacking red blood cells and as a consequence the cells are bursting, releasing haemoglobin, and that ends up being secreted in the urine and so you get blood stained urine and eventually kidney failure and death.
05:47 06:16	And so we are very interested in developing inhibitors of these proteins because we think they are going to be of use in improving the success of both solid organ and bone marrow transplantation and also in controlling the immune system in certain disease states. So how do we go about doing this? Well I moved to Australia in 1997 and I met my partner, now my wife in 1998, Michelle Dunstone. She is a scientist as well and we decided that we wanted to work on something together and we decided that we were going to work on trying to solve the atomic structure of a perforin like protein, the despite the fact that this family of proteins had been known for over 100 years we had no idea how they work.
06:38 07:09	I'm a structural biologist so what I do is determine the actual atomic structure of a protein and the reason that is useful and interesting is because that proteins are like tiny machines and if you can see where all the atoms are then you can start to work out how they work and really from a structural biology perspective it's like turning on a light in a dark room with a machine in the middle of it and suddenly - Oh I can see how that functions. So we started basically and for ten years or around ten years we got absolutely nowhere. We tried hundreds of different things and this is a common tale that many scientists will tell you. It takes a long time to actually make a break through and so we had pretty much almost given up when a very talented Post-Doc in my lab at the time, Carlos Rosaldo, found this perforin like protein in an organism called Photorhabdus luminescence.
07:36 08:08	And photorhabdus luminescence is a rather interesting bug. It's a bacteria and it infects insects and as its name suggests it's luminescent. So you can see on the screen here insect lava infected with photorhabdus and it is glowing in the dark. Now this is a special bacteria because it produces a lot of other toxins, one of which is a perforin like protein but it produces a lot of other, if you like antibiotics. We think probably to prevent other bacteria from coming along and feeding on its dinner. Once it gets into its' insect it wants this all to its self. It doesn't want to give it to anything else. So it's' a bacteria that produces a lot of antibiotics and it's a very famous bacteria because in the American Civil War, which was a horrific war, people rolling around in mud with very, very serious wounds and those wounds sometimes got infected with photorhabdus.
8:34 09:06	And when they got infected the wounds glowed which you imagine was a pretty unpleasant experience for the person infected with it but … the bacteria in the wound is producing natural antibiotics which prevent worse, more dangerous bacteria from get in and colonising the wound and then killing the individual. And so it turns out that the doctors and nurses at the time of the civil war noticed that the people whose wounds glowed survived longer or survived where as those who didn't were more likely to die and so they nick named it the "Angels Glow". But anyway this protein has a perforin like protein and (we were very) Carlos was able to grow crystals of this protein and we used an instrument a little like the Australian Synchrotron but we had to go overseas for this particular experiment because we didn't have a Synchrotron back in 2006 as it was still being built.

Time	*Dialogue*
09:33 10:02	And using the Synchrotron we were able to collect the diffraction data needed to determine its structure. So all of the spots on the left hand side of the screen that you see is like our raw data and we can use that information to back calculate the position of all of the atoms in the protein that is present in the crystal. Now I first saw this data actually in the Monash Medical Centre maternity hospital because it so happened that we solved this structure at the same time as our first child was born – Charlotte. This was not a problem because new babies are generally pretty sort of sleepy things but the midwives were reasonably appalled to see myself and Michelle with computers on our lap and sort of like this with our child on one hand, typing away and looking at the crystal structure but this is what we saw.
10:30 10:57	And so on the left hand side of the screen is our structure of a perforin like protein from this photorhabdus bacteria and when we saw it, it was I think the most exciting moment of my life because we could immediately see this wasn't a new type of pore forming machinery at all. This was actually a very very ancient fold that had been well studied in another system, but it is so distantly related that no one had picked it up before. So on the right is a bacterial cytolysin which is a class of pore forming proteins, and I hope if you look carefully at these two images you can see that they are sort of the same shape. They have that sort of bent (betas) that bent structure in the middle.
11:15 11:45	They are quite square shaped and to cut a long story short these two proteins are related. They separate but are like cousins separated by around 2 billion years. Now the great thing about bacteria cytolysin is a lots known about them and remember we really had no idea how a perforin like protein formed a pore in a cell. But a group of scientists and in particular Helen Sable (*Helen J.K. Sable*) and Rod Tweten (*RK Tweten)* in the US … and Helen Sable in London had done an enormous amount of work on how the cytolysins' work and they (cytolysins) like our immune system cause tissue damage, so when you get a sore throat, if you've got a strep throat for example, one of the reasons that you are in such pain is because there is a pore forming toxin being secreted by that bacteria causing damage to your tissue.
12:08 12:36	So how do bacterial cytolysins work? They are like giant hole punching machines. What happens is at first they bind onto the surface of the cell and then they start to coalesce and bind to one another to form a giant ring shape as you can see in the video here. And once they form this ring something remarkable is going to happen. The whole assembly is going to collapse towards the membrane surface and it is going to punch a great big hole about 100 angstroms wide (~10 nanometres) which in biological terms is pretty big. Now everyone always asks what happens to the bit in the middle? We don't know. We assume it just gets spat out into the extra cellular environment. And here's this conformational change happening now. A nice big cookie cutter cutting a hole in the cell.
13:03 13:36	And so this is how cytolysins work and we hypothesise that the perforin like proteins because they have the same sort of machinery, likely work in the same way, but we are scientists so we need to test they hypotheses and we need to demonstrate that hey work in the same way because don't forget these two groups of protein are separated by a very long time indeed. So how do we do that? Well at this point Michelle had a stroke of genius and she worked out that the oyster mushroom had a perforin like protein that we felt would be useful for studying the structure of the actual pore form itself. Now why does an oyster mushroom have a perforin like protein? We don't really know the answer to that but one really interesting thing about oyster mushrooms is that they are nice to eat. They are great in risotto but the mushroom actually isn't a vegetarian.

Time	*Dialogue*
14:04 14:42	The mushroom likes to eat nematodes. So for all vegetarians out there who like eating mushrooms what you are eating is actually something that likes eating worms … which is cool. It is how it gets its nitrogen. And here we've got fungal hyphae, if you like a filament basically wrapping around the nematode trying to trap it so it can basically use the resources within that little worm to grow and survive. So we think one reason the fungi might have perforin like protein is to help it in killing its prey. So this time again we used a combination of the Australian Synchrotron and we also in collaboration with Helen Sable in London; one of the great things about being a scientist is you get to travel a lot and work with a lot of different and great people and Helen's group in London used electron microscopy a little like the instrument shown here on the left here to basically solve the pore form we solved the crystal structures and we put everything together.
15:14 15:48	And what did we see? We could see actually at the highest resolution to date the structure of a perforin like pore in a membrane. And you can see it's this giant barrel shaped molecule with a big hole in the middle about 80 angstroms (~8 nanometres) in diameter which is well big enough to take a nice toxic cargo into the cytoplasm, into the cell it is trying to basically kill. Michelle went one step further though and she also made a lot of if you like intermediate structure on route which we also solved the structure of, so we could actually build up a molecular movie of how this protein changes in shape as it assembles on the membrane and enters the cell. So taken together this actually gives us a picture of how perforin like proteins function when an immune cell encounters a virally infected cell they first have to make sure that it is a genuinely foreign infected cell rather than a harmless cell.
16:24 17:02 17:32	It releases cytokines to bring in new immune cells into the action and then it releases perforin and toxin granzymes into the synapse. The perforin assembles into a pore and then through the pore the toxic granzyme molecules entre the target cell. And even if the cell manages to repair itself it's too late because the toxin is inside and the cell dies and the immune cell goes off to find another target and to kill that. So I guess over the last 10 years we have gone from knowing really very very little about how this family of pore forming proteins work to building up a bigger picture of how these molecules bind to and assemble on the surface of cells, undergo conformational change, or changes in shape, allow other toxic granzymes and other toxins into the cell and thereby causing death and this is now reforming our drug discovery program. Thank-you very much.