COVID Information Commons (CIC) Research Lightning Talk

Transcript of a Presentation by Gregory Bix (Tulane University) October 10, 2023

Title: SARS-CoV-2 mediated neuroinflammation and the impact of COVID-19 in neurological disorders

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Transcript

Slide 1
Thank you Lauren, I really appreciate that.

Slide 2
All right, well, today I'm going to talk to you about some work that I've been doing over the last couple of years since the pandemic started. I was looking at novel interactions that the virus has with receptors that we've identified in the body and then how we can therapeutically target that and the impact that that has on the brain.

Slide 3
Our work is really focused on the spike protein. I'm not sure if you can see my pointer as I move it? Okay, excellent. So our work is really focused on the spike protein which the virus uses to bind to cell surface receptors and ultimately invades cells.

Slide 4
We identified pretty early on in the pandemic - we and others - that the amino acid sequence of the spike protein contained a particular three amino acid structure called RGD. This is a particular binding motif in protein structures that recognizes a particular class of cell surface receptors called integrins. What was particularly interesting about this is twofold: one, that SARS-CoV-2 was relatively unique amongst other coronaviruses that you see listed on the screen

in that it had this binding motif and others did not. It was speculated pretty early on in the pandemic, in early 2020, that the presence of this integrin binding domain on coronavirus spike proteins might convey some of the virulence that the virus has unleashed upon all of us.

Slide 5
With that being said, we and others looked at what was happening to this particular spike protein as the various variants emerged over the last couple of years. Sure enough, with each mutation in the spike protein and mutations this integrin binding motif, this RGD, which was already fairly prominently expressed at the tip of the spear, so to speak, was even more prominent with various mutations. Here you're looking at the UK variant, but subsequent variants have have borne this out. So there is a specific receptor binding domain on the tip of the spike protein of coronavirus that conveyed a unique ability to bind to this class of receptors known as integrin.

Slide 6
All right, so integrins, very basically, they are composed of two subunits: an alpha and a beta. They bind to something called extracellular matrix. Extracellular matrix is essentially the glue that helps hold cells together. It forms base membranes. Every cell in the body expresses integrins of different complements. These subunits are referred to as alpha and beta, depending upon which alpha bonds to which beta or associates with which beta you get receptors that have different affinities for different components of the extracellular matrix.

Slide 7
We were particularly interested in some that are expressed in the brain. This schematic essentially shows integrin subunits that help form what's called the blood-brain barrier. I'll be getting back to this in a little bit, but essentially there's a barrier that isolates blood and vascular compartments from the brain and the brain brain parenchyma. This is tightly regulated and viruses and other infectious agents can impact the blood-brain barrier. As a result there can be infection in the brain or swelling, inflammation that sort of thing. These integrins that I was referring to help regulate the tightness of this blood-brain barrier, which will become very important in a minute.

Slide 8
Then, we've spent a lot of time in my lab looking at specific modulators or inhibitors of integrin and one that I'm going to talk about a fair bit. This is something called ATN-161. This is a peptide that can bind to integrins such as alpha 5 beta 1, which we have since identified as a COVID receptor and we've done a lot of work to look at whether or not this inhibitor peptide might actually have therapeutic efficacy against coronavirus.

Slide 9
This is a summary of a couple of years of work, but suffice it to say, here you're looking at the spike protein of the virus. It interacts with known receptors. ACE-2 is one that you've probably heard and associated integrins. This interaction facilitates the the virus getting taken up into a cell and then all the bad things that happen as a result of that post-cell Invasion.

Slide 10
We've done a lot of work to demonstrate, essentially, that if one takes just the spike protein and puts it on cells and culture - in this case, these are brain endothelial cells, we looked at a number of different indicators of blood-brain barrier integrity. We see that the spike protein by itself can actually change the expression patterns of these proteins associated with the blood brain barrier. It reduces them, which is a clue (and since validated by others) that the virus can actually impact vascular barriers.

Slide 11
We did a lot of work early on in the pandemic to essentially to show that these spike proteins can bind to integrins and that we could block this interaction by co-incubating with this peptide I referred to before - ATN-161. This is a study that was done pretty early on in COVID-19.

Slide 12
We showed, I think equally as importantly, that if we added cells - the virus, rather, live virus - to cells, we could block a lot of the viral replication by adding the this inhibitor to alpha 5 beta 1 Integrins. That's shown by the graph here.

Slide 13
And here, you're just looking at the cells that were co-incubated with the virus. All these little round balls or plaques are cells that are impacted by the virus. When we treat it with our ATN peptide, we can largely suppress these round cell formations, which is synonymous with viral replication.

Slide 14
In animal models - there are number of different animal models for looking at COVID-19 - my lab's taken advantage of a number of different animal models as they become available. In one particular model, you can make a normal lab mouse express the human form of ACE-2, which is a known receptor for the virus. At the time, this was the only way to make a standard lab mouse susceptible to infection. If we inoculated these mice with coronavirus and then treated them with ATN-161, we could significantly suppress viral load in the animals.

Slide 15
In a different model where instead of making the mice express human ACE-2 with the virus, we could actually use a transgenic mouse, something called K-18, that expressed human ACE-2 everywhere - similarly, if we infected those mice, we could actually block the infection by add - by treating with ATN-161.

Slide 16
But a better tool that emerged in the last year or so is a version of coronavirus that was most adaptive. This was a SARS-CoV-2 that was passaged in standard laboratory mice, put into a mouse. Allowed to infect, taken out of the mouse, passaged into a different animal, etc., etc. After ten passages, the virus could actually infect wild type mice. This really facilitates coronavirus research in labs because now one can work with with standard mice that most labs have access to.

Slide 17
So we took advantage of that and we did a number of studies and when we infected mice, we just waited two or three days and started looking at markers in the brain. We noted that those same type junction proteins - these are proteins that stabilize, facilitate the tightness of the blood-brain barrier. Here, you're looking at claudin-5 is one - we see a significant reduction in the levels of this protein. Again, that's important to blood-brain barrier stability. We also see a significant increase in inflammatory cells and astrocytes in the brain with coronavirus infection. Now, I should point out - the virus is put into the lungs, so we're looking in the brain three days later. This is in the absence of virus actually getting into the brain. We're seeing changes in the brain that indicate blood-brain barrier disruption and neuroinflammation three days after infection.

Slide 18
This is just another example - we're looking at a stain from microglial cells, which are the brains resident immune cells. There's a significant increase in immuno-reactivity of microglia three days after coronavirus infection in standard mice.

Slide 19
This is just another schematic again where we infected mice, standard lab mice, with MA10 and then we also treated with that ATN-161.

Slide 20
Very nicely, we saw effects on various markers of inflammation. This is interleukin-6, proinflammatory cytokines in the brain. We saw that ATN-161 could significantly reduce the proinflamatory cytokine in the brain. We saw similar effects on blocking the reduction in claudin-5, which you get as a marker of blood brain-barrier integrity.

Slide 21
Very briefly, I think everyone on this call is familiar with long COVID, so I won't belabor this slide.

Slide 22
But suffice it to say, one of the changes that have been noted in clinical long COVID is a reduction in blood flow to the brain.

Slide 23
There's a number of different reasons why this may occur, but we decided to model this in those standard laboratory mice by putting coils on the carotid arteries - these are major blood vessels that feed blood to the brain - and if you restrict, but don't eliminate the blood flow, typically what happens in a standard mouse is after a couple of weeks there's a white matter injury in the brain. After about a month, these animals begin to develop cognitive impairment. So it's a chronic hypoprofusion of blood to the brain resulting in a cognitive decline.

Slide 24
This is a so-called vascular dementia. We did a number of studies and essentially showed that if you do this, wait about a week after you put these coils on and then infect the mice, their brains are much worse than if you just infected them alone or if you just put the coils on by themselves. This is an alpha 5 integrin which is a receptor we've identified for coronavirus - levels in the brain go through the roof.

Slide 25
We saw changes in microglia. We saw pretty significant changes in GFAP, the astrocyte marker again in the brain.

Slide 26 - 27
I'm going to skip ahead. Essentially, if one looks months later after an infection in laboratory mice, we see clear indications of neuroinflammation that are chronic. So this is 30 days post-infection and we see changes around blood vessels in the brain that persist. This is an experimental long COVID.

Slide 28
We see a significant drop out of neurons in parts of the brain where you don't want neurons to go away. In this case, the hippocampus which is important for the consolidation of new memory into long-term memory. This is 60 days after infection and in a number of the mice we saw a significant neuronal cell dropout.

Slide 29
This is just showing signs of inflammation, again, that were chronic two months after infection.

Slide 30
I want to leave you with this idea that we're now at the stage where we can nicely model coronavirus infection in standard laboratory mice. Unfortunately, in a number of these animals, days to weeks to months after infection, we see changes in the brain which are made worse when we superimpose, reduce blood flow, when we model cognitive decline. However, we think there are particular receptors that make coronavirus particularly virulent that we could also modulate, in this case, with ATN-161 which we are also studying as a potential dementia therapy. I think because this receptor may play a role in both dementia and vascular integrity in coronavirus infection, it may serve a dual purpose in both treating the virus as well as the vascular [inaudible] associated with it.

Slides 31-32.
Hopefully, I haven't gone too far over. I'll just acknowledge a team that helped do the research.

Slides 33-34
And my collaborators. I guess we'll wait for the very end for your questions. Thank you very much for your attention.