COVID Information Commons (CIC) Research Lightning Talk

Transcript of a Presentation by Kristen Funk (University of North Carolina, Charlotte)
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Title: Neurotropic Viral Infection in CNS Aging and Alzheimer's Disease COVID-19 Supplement

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YouTube Recording with Slides

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Transcript

Slide 1
All right, thank you everybody for being here. I'm excited to tell you about the work that I've been doing in my Lab, investigating CD8+ T cell immunity in the aged brain in response to a respiratory coronavirus infection, and particularly the context of cognitive impairment.

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We've known for a long time that advanced age impacts the severity of viral infections. Viral infections occur in all age groups, but the most severe outcomes disproportionally affect those who are over the age of 60. We've seen this previously. My work has been on the West Nile virus neuroinvasive disease, and then, of course, more recently with the coronavirus outbreak. We want to understand how age impacts the antiviral immune response in the central nervous system. Previously, we looked at West Nile virus and that was published a couple of years ago. More recently, what I'm going to talk you about today, was using a mouse coronavirus, MHV, which has been posted to BioRxiv and is currently in revision for publication.

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We know that aging broadly affects the immune system. "Inflammaging" is this term that we use to denote this highly inflammatory environment. In particular, we see increased CD8+ T cells but we see a reduction in the naive t- cell pool as well as a reduction in t- cell receptor diversity, which we think impacts the ability to respond to new challenges. My Lab is interested in

understanding how this affects the antiviral immune response in the brain and how it might affect post-infectious cognitive recovery.

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In order to study this, my lab has established this model - this mouse model - using MHV-A59. We inoculate intra-nasally 8 week or 18 month old C57 black six male mice with 10^3 plaque forming units (10 to the third infectious units) or with HBSS. We watch these mice over a period of 30 days. This model is really established by Dr. Katie Regan in my Lab who's a post-doc who left recently. Using this model, we showed that aged mice are more susceptible to lethal viral infection, which was not surprising. They also might experience a more severe disease course, so greater weight loss as well as higher clinical scores that we can measure.

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We wanted to understand how this correlated particularly with the cellular immune response in the brain. We did this by spectrometry, looking at the CD4 and the CD8+ T cells in the brain. Notably, under normal conditions, we have very few to know T- cells in the brain. During infection, we see this recruitment of these T- cells to the brain. Here, we're looking at 12 days post infection and at 30 days post infection. We see that aged mice have higher levels of these CD8+ T cells in the brain both at 12 and 30 days post infection. We also investigated in the lungs, the kind of primary source of infection, as well as cervical lymph nodes, mediastinal lymph nodes, and spleen. You see these higher levels of T- cells across the board in these aged mice.

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However, when we look at the viral specificity of these T- cells ,we saw that a decreased percentage of these T- cells in aged mice are specific to this MHV virus that we're infecting them with, both at 12 and 30 days post infection. This is suggesting to us that we have this influx of T-cells to the brain, but a lower percentage of them in aged mice are specific to the virus and contributing to clearing the virus.

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We wanted to understand how that might play out in in a spatial learning model. We have previously seen in our West Nile model that post infectious mice have these cognitive deficits. We tested that using the Barnes Maze that we're depicting here - it's basically a circular table that you put a mouse in the middle of. You test them twice each day for five consecutive days. This target hole is put in the same location every day and they eventually learn where this target hole is. This is basically just a "hidey box" that the mouse can go in to escape anxiety causing task that we have them on. And we're doing this at day 25 to day 29 post infection - that's about two weeks after the virus has cleared from the brain. During this test - we test them twice each day for five consecutive days - that's what we're seeing here. The black dotted line is our mock

infected adult mice and the black solid line is our our MHV-infected adult mice. You can see that both groups of mice improve over that 5 day time course. We really don't see a significant difference between those mock versus infected groups. When we look at our mock infected aged mice, which is this red dotted line, they do improve over that 5 day period, but there's a little bit of a bump here on that day 2. However, when we look at this infected age group - the line is this red solid line - the line is really totally shifted upward, suggesting a significant spatial learning deficit in these mice. We can collapse this down basically to one datapoint using this latency, or this area under the curve that we've normalized. The mock it's basically taking this bump that we see in aged mice, normalizing that effect. Again, each of these dots here represents a single mouse that we've tested on this paradigm. We see a significant increase in latency in these adult mice / aged mice post-infection. It's particularly on days 2, 3, and 4 that we see this this cognitive decline.

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We wanted to understand the cellular mechanism leading to this effect. In our West Nile model, we've seen that this is due to microglial-mediated synapse elimination, basically impairing the communication between nerves. Demyelination is also known to occur in these MHV models. However, we saw no evidence of either of those things happening in our system. Rather, we saw evidence of neuronal death, particularly in the hippocampus, which we know is important for spatial learning. Here, I'm showing you these are 8 week old mice and then 18 month old mice. Particularly in the CA3 region of the hippocampus, we've seen in blue is DAPI and green is NeuN, which is our neuronal nuclei, and then in red is our tunel staining, which is indicative of apoptosis. We've quantified them each individually. Here's our NeuN staining and our tunel staining and then our co-localization using Pearson correlation coefficient and Manders coefficient. What we see is that particularly in our Mock infected animals we see a little bit of elevated tunnel staining. But at 12 days, we see it elevated in both our 18 month and our 8 week old animals and we can see that co-localization is being highlighted by these arrows in each of these groups. We see that elevated at this acute time frame for both ages, and then it seems to recover in both age groups, although it remains a little bit elevated in the aged group. This suggesting that we're having this this cognitive decline which is likely mediated by this neuronal death within the tri-synaptic circuit that we know is important for spatial learning.

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We wanted to understand the cellular mechanism causing this neuronal apoptosis so we established a cell culture system in which we took primary neurons from mice and we left them either uninfected or we infected them with NHBA59. We did that on it's own. Again we're staying with NeuN in green and tunel is in red and then co-localization is highlighted in this yellow by these arrows. The the virus on its own didn't really seem to kill the neuron, however when we co-cultured them with CD8+ T cells that had been isolated from MHV infected mice at 7 days post-infection, we saw a much stronger co-localization in which we're seeing neuronal

death. This suggests that the virus on its own didn't kill our neurons, but the T cells from these infected mice did.

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So we wanted to know whether this was an antigen specific response or antigen independent. We again took our co-culture system of uninfected neurons or infected neurons and cultured them either alone or co-cultured them with naive CD8+ T. These are from an uninfected mouse or T cells that had been bulk simulated with PMA and Ionomycin. Again, we're staining with NeuN and tunel. I think we can appreciate that the PNA/Ionomycin bulk stimulated T- cells cause reduced neuronal nuclei staining and increased tunel staining co-localization. This is suggesting to us that it's not necessarily an antigen specific response, it's more just these activated T- cells are likely causing this neuronal death phenotype. We think that's important in that aged group in particular, which had that infiltration of those T- cells that were not necessarily antigen specific or not specific to our virus.

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With that, my conclusions are that we think that viral infections are causing death to these neurons via this CD8+ T cell response. We're trying to understand more about this mechanism mediating this response.

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We're ultimately interested in the progression of Effector T cells and Memory T cells and what factors may be influencing that during aging. We're also interested in how viral infections or T cells may promote AD-related pathology, including genotoxic stress.

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With that, I'd like to thank the people who did this work, particularly Katie Reagan. If you're interested, here's that link to that BioRxiv submission. Thank you!