elucidate the cellular and molecular mechanisms underlying DN T cell responses and its potential therapeutic role in lung IRI during transplantation.

Webcast

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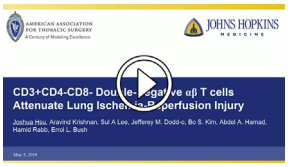

Conflict of Interest Statement

Authors have nothing to disclose with regard to commercial support.

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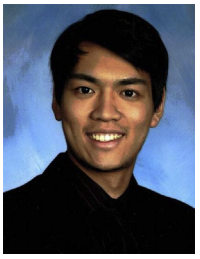
Key Words: lung, ischemia-reperfusion injury, T cell, double-negative T cell, IL-10, adoptive transfer

Discussion

A. Sasha Krupnick *(Charlottesville, Va)*. This is a very nice presentation. Obviously as discussed at this conference and lung session, it's a very big problem; ischemia-reperfusion injury (IRI) injury occurs in about one-third of patients, leads to significant morbidity and mortality. Thus, your efforts to understand the basic mechanism and improve it are critical. I have 2 questions based on your data, and thanks for providing the manuscript.

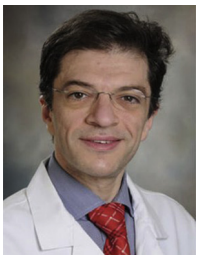
The first question regarding the origin of these double-negative (DN) T cells. Because double-negative T cells are one of the earliest stages of T cell development in the thymus, do you think these are early thymic T cells that haven't matured, that whatever the signals that IRI causes that home to the lung and do the thing that they do? In other words, is this a thymic origin early cell that's kind of brought to the lung early based on some signals?

And the second question I have regarding interleukin (IL) 10, that's critical data, especially given the fact that the Toronto group has shown in human trials that overexpression of IL-10 can ameliorate IRI in the lung graft. So do you think IL-10 acts directly in these cells to cause them to do whatever it is that they do to decrease injury or do you think IL-10 affects the whole lung milieu, which then brings in fewer DN T cells. Thus is it a cause-and-effect type of relationship?



Dr Joshua Hsu *(Baltimore, Md)*. Thank you for these excellent questions. In regard to the origin of DN T cells, we haven't shown this directly, but the influenza A study showed that athymic mice showed similar levels of DN T cells in the lungs when compared with mice with a thymus. This might suggest that DN T cells arise from an extrathymic origin. However, and also preliminary data from our studies, we have also looked at specifically the alpha/beta DN T cells in the thymus of humans and mice, and they exhibit little to no levels of DN T cells. However, after lung IRI, this rapid expansion of DN T cells, those occur in the lung, which may suggest that it is trafficking from other sites. So further experimentation will be conducted with mice without thymus or kidneys or with the spleen to see where they are trafficking from.

In regard to the second question, the exact mechanism of IL-10 in our study still remains unclear. From this presentation, DN T cells secrete robust levels of IL-10 and inhibition of IL-10 seems to attenuate this expansion and attenuates the apoptosis signaling pathway. We think that the IL-10 secreted by DN T cells could contribute to the induction of other anti-inflammatory cells, such as regulatory beta or T cells. We have shown that other cytokines, such as IL-2 and interferon gamma, do act on DN T cells to proliferate and somehow function in this anti-inflammatory state. So further in vitro experiments will be conducted to dissect this out, the mechanism, and the role they play in lung IRI.



Daniel Kreisel *(St Louis, Mo)*. I have 2 questions for you. You show that the adoptive transfer of DN T cells attenuate this lung IRI. If I read your abstract correctly, you isolated those cells from *gld* Fas ligand-deficient mice, which makes sense because you have an abundance of them, but there are articles in the literature that show that DN T cells can mediate proinflammatory effects through Fas ligand.

Can you comment on that?

Dr Hsu. In the literature and also as shown by others, there are proinflammatory as well as anti-inflammatory effects of double-negative T cells. With our intracellular staining we have also seen DN T cells secrete proinflammatory cytokines, and the exact function of this is still very unclear. However, in our own hands the adoptive transfer of these specific alpha/beta DN T cells from *gld* mice attenuate lung as well as renal injury.

Dr Kreisel. And the second question I have, you show reductions in cleaved caspase-3 as a marker of apoptosis. Recently an article showed that treatment with necrostatin, which inhibits necroptosis, which is a different pathway of regulated cell death which is inflammatory, plays a major role in lung IRI in rats, as I recall. Can you comment on that? Do you think the attenuation of apoptosis is how these cells mediate their beneficial effect?

Dr Hsu. Well, certainly, I can't comment much about that other study. However, cleaved caspase-3 is a measure of readout in terms of lung injury and cellular death in our study, and we correlate that with the kinetics of DN T cell expansion and adoptive transfer and so forth. But additional studies do need to be conducted to really dissect the mechanism, because there are multiple pathways that can lead to cellular death, and if it's cytokine mediated, if it's oxidative damage, we are not sure of how this is working.