Recipient Classical Monocytes Promote Retention of Donor Nonclassical Monocytes That Mediate Multiorgan Dysfunction After Lung Transplant

Stephen Chiu, MD; Emilia Leucona, PhD; Mahzad Akbarpour, PhD; Ramiro Fernandez, MD; Qiang Wu, PhD; Zhikun Zheng, MD; Alexander Misharin, MD, PhD; G.R. Scott Budinger, MD; Ankit Bharat, MD

Background
Multiorgan dysfunction syndrome (MODS), involving lung and kidneys, is a life-threatening complication after solid-organ transplantation. Lack of mechanistic insights into the pathogenesis of MODS has prevented effective therapies. Using the murine single lung transplant model, we identified a previously undiscovered role for donor nonclassical monocytes (NCM) in mediating MODS.

Methods
For in vivo experiments:
1. Allogeneic murine single lung transplant
2. CX3Cl1-/- donor lungs for identification of donor-NCM
3. Two-photon microscopy for identification of GFP-expressing NCM
4. Multi-channel flow cytometry for cell sorting
5. RNA sequencing of donor NCM to determine transcriptional profiling
6. Intravenous adoptive transfer of freshly sorted NCM from lung and spleen into syngeneic N4at1-/- mice
7. Pharmacologic depletion NCM in donor lungs using I.V. clo-lip
8. Genetic depletion of NCM using N4at1-/- donor mice
9. CX3Cl1+/+ recipients to measure expression of CX3cl1 in endothelial cells
10. CD31 magnetic bead sorting of endothelial cells isolated from lungs

For in vitro experiments:
1. Lung endothelial cells were co-cultured classical monocytes (CM) and NCM
2. R848 was used as a TLR7 agonist
3. qPCR was used to measure mRNA expression
4. Multiplex ELISA was used to measure levels of cytokine production

Conclusions
CM produce TNFa in a TLR7-dependent manner, which is necessary for post-transplant increase in endothelial CX3CL1 and retention of donor NCM in remote organs. Donor NCM drive neutrophil influx, mediating MODS. Neutralization of TNFa may be a clinically feasible treatment to prevent MODS.