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"Uncovering Mechanisms of Dysfunction in Plasmacytoid Dendritic Cells and T cells: Part 1: Transcriptional Regulation of Plasmacytoid Dendritic Cells Part 2: Immunosenescent CD8+ T cells in Healthy Aging and COVID-19"

by
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Wednesday, July 28th, 2021
11:00 A.M.
Webex link: https://rutgers.webex.com/rutgers/j.php?MTID=m7e1121af150b93190b11113415b131b5

Meeting number (access code): 120 014 4711
Meeting password: a57TkE4XMdt

ABSTRACT

Part 1: Plasmacytoid dendritic cells (pDCs) are the main producers of interferon-alpha (IFNα). pDCs are transcriptionally controlled by transcription factor E2-2, which governs pDC development and function. Stimulation of pDCs decreases E2-2 expression, contributing to a change in function and allowing pDCs to present antigen. We investigated E2-2 regulation by stimulating peripheral blood mononuclear cells (PBMCs) or enriched pDCs with TLR agonists for up to 24 hours. In PBMCs, R848 and viral stimulation significantly decreased E2-2 expression after maximal IFNα production; LPS induced the greatest decrease in E2-2 expression. Enriched pDCs did not downregulate E2-2 in response to any stimuli. Since pDC function can be modulated by cytokines and TNFα in particular is known to diminish pDC IFNα production, we treated pDCs with TNFα and monitored E2-2 expression. We found significant E2-2 reduction in response to TNFα . By removing monocytes from PBMCs, we revealed that monocytes were the main producers of TNFα and that without monocytes, E2-2 was not downregulated. We have established the kinetics of E2-2 downregulation during *in vitro* stimulation of human pDC with TLR7, 9, and 4 ligands and determined IFNα is not responsible for E2-2 downregulation. Elucidating regulation of E2-2 expression furthers our understanding of the role E2-2 plays in transitioning from interferon producing cell to antigen presenting cell and how this is impacted during chronic viral infection and pDC dysfunction.

Part 2: Aging brings a decline in the immune system, termed “immunosenescence.” Cellular senescence is defined by persistent cellular growth arrest and overall loss of function. If immune cells undergo cellular senescence, and if it has a role in immunosenescence, remains controversial due to the lack of specific markers to identify senescent immune cells. Human peripheral blood mononuclear cells from cord blood, younger and older donors were labeled for cell-surface markers and with a fluorescent senescence-associated β-galactosidase (SA-βGal) substrate. We identified CD4+ and CD8+ T cells, B cells, natural killer cells, pDCs, and monocytes within PBMCs and found increasing SA-βGal activity with age in both CD4+ and CD8+ T cells. Cord blood had the lowest percentage of CD8+ T cells with high SA-βGal activity (fSA-βGal high) while older donors had up to 88.2% fSA-βGal high CD8+ T cells. Furthermore, SA-βGal activity increased as CD8+ T cells differentiated from naïve to memory cells. Since the most drastic age-associated change in SA-βGal activity was in the CD8+ T cells, we sorted CD8+ T cells based on fSA-βGal signal and analyzed by qRT-PCR, RNA sequencing, and microscopy. We found an increased percentage of CD8+ T cells with high SA-βGal activity (fSA-βGal high) in the older cohort. fSA-βGal high CD8+ T cells displayed p21, p16INK4a, and inflammatory cytokine transcripts. They also had greater DNA damage response foci and $p16^{INK4a}$ protein as measured by immunofluorescence imaging and flow cytometry and had deficient proliferation in response to anti-CD3 and CD28 stimulation. RNA sequencing revealed that fSA-βGal low, medium, and high cells had distinct profiles. In addition, fSA-βGal high CD8+ T cells had larger mitochondrial mass than fSA-βGal low cells as measured by MitoID staining. Extracellular flux analysis validated that senescent CD8+ T cells had decreased mitochondrial fitness despite retaining the ability to efficiently switch between oxidative phosphorylation and glycolysis. There was also an increase in the proton leak into the mitochondria, indicative of the presence of reactive oxygen species that can cause DNA damage. Together, these data demonstrate that SA-βGal activity is an indicator of cellular senescence in CD8+ T cells as well as other PBMC populations. Investigating the correlation between age and COVID-19, we found that COVID-19 patients had a higher proportion of senescent CD8+ T cells. This may contribute to older individuals’ increased susceptibly to severe SARS-CoV-2 infections. Overall, we found that measurement of SA-βGal can be leveraged to elucidate the role of cellular senescence in immunosenescence.