Akt1 deficiency in neonatal macrophages reinforces their bactericidal activity against Streptococcus by modulating autophagy and oxidative stress

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ABSTRACT

Streptococcus Group Beta (GBS) is a commensal of the human microflora in healthy adult individuals. In infants, however, GBS is the leading cause of serious health complications, such as sepsis and meningitis, thus making it one of the most life-threatening pathogens. Lacking developed adapitive immune system, newborns are highly dependent on their innate immune system and the activity of professional phagocytic cells, macrophages and neutrophils to successfully fight bacterial infections.

Macrophages tightly regulate the process of inflammation and are classified as classically activated (M1) and alternatively activated (M2). According to previous studies from our group, ablation of Akt1 kinase is known to polarize macrophages towards M1 phenotype, resulting in increased oxidative burst, pro-inflammatory cytokines and enhanced bactericidal capacity.

Based on the aforementioned evidence, we speculate that Akt1 silencing in immature neonatal macrophages will reinforce the generation of robust oxidative burst and will activate their autophagic machinery to promote killing of GBS.

To delineate the mechanism controling GBS infection in adults and neonates and the role of Akt1 in it, we infected adult Wild-Type (WT) and neonatal macrophages of WT and $Akt1^{loxP/loxP}$,$LysM^{Cre}$ origin, with a hyper-virulent strain of GBS (serotype III). Our data demonstrated that adult cells had limited bacterial load compared to neonatal counterparts. Using both Transmittion Electron and confocal microscopy we showed that neonatal macrophages had more proliferating GBS bacteria in their cytoplasm compared to adult cells, in which GBS were primarily localized inside single membrane vacuoles. By knocking down the autophagic components, Rubicon and Atg5, intracellular GBS load was only increased in adult and $Akt1^{-/-}$ cells, indicating a deficiency in neonatal autophagy pathway. We showed that WT neonatal cells have decreased levels of Atg5 and miR-155, a microRNA contributing to TLR signalling, and upregulated production of the anti-inflammatory cytokine IL-10, known to limit immune responses in neonates. Finally, we showed that $Akt1^{-/-}$ cells expressed increased levels of Nox2, known to facilitate ROS generation within GBS-containing phagosomes, accompanied with elevated Reactive Oxygen Species (ROS).

Overall, our findings suggest that Akt1 deficiency in neonatal macrophages results in increased bactericidal capacity against GBS compared to WT cells, a process controlled by autophagy and

ROS production. Our work sheds light on the molecular machanism of GBS-host interaction and proposes a potential therapeutic role of Akt1 inhibition against neonatal GBS-borne diseases.