The role of neutrophils in a neonatal *in vivo* model of Group B *Streptococcus*-induced haematogenous meningitis.

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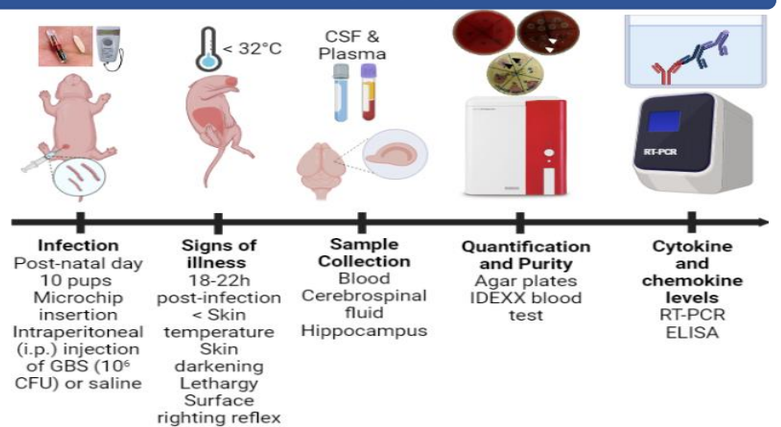
Introduction

Meningitis associated with neonatal invasive Group B *Streptococcus* (iGBS) disease, arises from haematoproliferative bacterial invasion into the central nervous system. Neutrophils are the first line of defence against infection forming neutrophil extracellular traps (NETs), which are extracellular DNA fibers that can bind and accumulate cytokines or infiltrating pathogens. Neurotoxic properties have been assigned to proteases and decondensed DNA released from neutrophil granulocytes after brain endothelial transmigration. Thus, the inflammatory response of neutrophils to GBS can lead to neuronal damage evidenced by neurodevelopmental impairments noted in survivors of iGBS disease.

Aims

To establish a neonatal rat model of GBS-induced sepsis and meningitis and to investigate the underlying role of neutrophils and NETs using serotype III which is the predominant serotype reported in South Africa

Methods



Results

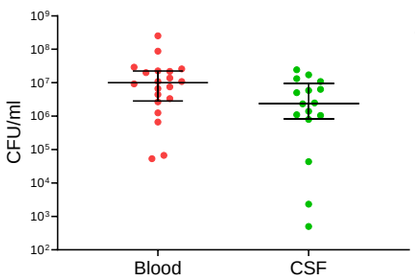


Figure 1: Distribution of GBS in the blood and CSF of postnatal day 10 rat pups injected with GBS or saline. Saline pups showed no sign of illness and growth of GBS. The bacterial load within the blood was higher than the CSF. The bacterial load within brain was similar to the blood (x10^7 CFU/g).

Table 1: Cytokine and chemokine response in the hippocampi of postnatal day 10 rat pups injected with GBS or saline. The hippocampus is a brain region associated with learning and memory. – no change. ↑ significantly different to saline $P < 0.01$.

Markers	Plasma protein levels	Hippocampus mRNA levels	Hippocampus protein levels
Pro-inflammatory cytokines			
IL1-β	↑	↑	↑
TNF-α	↑	↑	-
INF-γ	↑	-	↑
IL-6	↑	↑	-
Anti-inflammatory cytokines			
IL-10	↑	↑	-
Chemokines			
Cytokine-induced neutrophil chemoattractant (CINC) 1	↑	↑	↑
CINC 2	↑	-	↑
CINC 3	↑	↑	↑

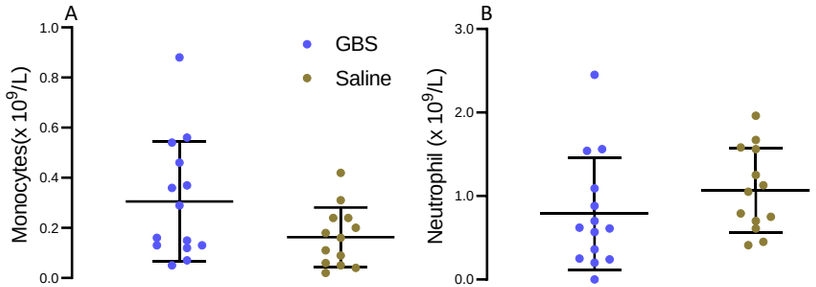
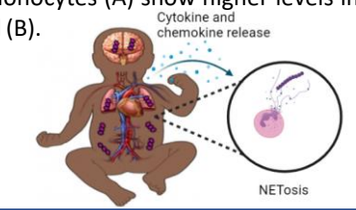


Figure 3: Immune cell response in the plasma of postnatal day 10 rat pups injected with GBS or saline. Monocytes (A) show higher levels in GBS groups when compared to neutrophil (B).



Discussion and conclusion

Our results showing the presence of GBS in the blood, CSF and brain confirm we have developed a neonatal rat model of iGBS disease. Our results show signs of sepsis indicated by the high bacterial loads and the elevated levels of cytokines and chemokine. For the immune response we expected a high level of plasma neutrophils, due to high level of CINCs. In line with literature, our data shows neonates immature innate immunity through neutrophilia which may lend to the progression of GBS. Taken all together, we hope to use this model in order to tease out the underlying mechanisms leading to neuronal injury.

References

Dangor et al, 2015. *PloS one* 10(4): e0123014.
Carey et al, 2014. *The Journal of Immunology* 192(4):1718-31.

Acknowledgements

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