Title: Inflammatory CD16+ CD163+ Monocytes localize to sites of inflammation in Necrotizing Enterocolitis

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Background: Necrotizing enterocolitis (NEC) is a devastating complication of prematurity. Advances in diagnosis and treatment have been limited and current therapy is non-specific. We hypothesized that in-depth single cell analysis of small intestine can identify differentiating phenotypes of NEC and specific therapeutic targets.

Methods: Small intestine (SI) from initial surgery for NEC (sNEC, n=12, gestational age (GA) 23-39 weeks, wks) were compared to neonates with non-immune congenital anomalies (Neonatal n=4, GA 31-33 wks) and discarded fetal intestinal tissue (n=3, GA 16-20 wks). Single cell RNA sequencing (scRNAseq) coupled with suspension (CyTOF) and imaging (IMC) mass cytometry was performed on SI. CyTOF analysis was performed on immune cells isolated from peripheral blood obtained from infants with medical NEC (mNEC, n= 15, GA 24-41 wks), and surgical (sNEC, n=3, GA 28-39 wks) and healthy age-matched neonates (n= 24-41 wks).

Results: We identified a population of NEC enriched monocytes/Mf co-expressing CD16+ CD163+ markers. These monocytes/Mf were abundant in the NEC tissue, found adjacent to blood vessels in the intestinal mucosa, and present in the peripheral blood of infants with sNEC, suggesting that they likely translocate from the periphery. scRNA-seq analysis established CD16+CD163+ monocytes/Mf to be highly inflammatory, transcribing genes including TREM1,
IL1a and IL1b, IL8 and calprotectin. Gene set enrichment analysis identified pathways involved in chemotaxis, migration, phagocytosis, toll-like receptor activation, reactive oxygen species and cytokine signaling to be upregulated in these monocytes.

**Conclusions**: We have identified a novel subtype of inflammatory monocyte/Mf present in blood and mucosa of patients with NEC that has pathogenic potential and can serve as a putative biomarker and therapeutic target in NEC.

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