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All on “CHIP”: using microfluidics to study neutrophil ontogeny

AMIL ALLEN and HITESH DESHMUKH

Division of Neonatology and Pulmonary Biology, Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio
Perinatal Institute, Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio

Bacterial sepsis is a significant cause of morbidity and mortality in neonates.¹ The incidence of severe neonatal sepsis in the United States exceeds 10,000 cases/year. The total hospital cost for treating sepsis in neonates exceed \$1.7 billion a year,² representing a major public health problem in the United States.³

The innate immune system is the primary defense against bacterial infection.⁴ As a major component of innate immunity, neutrophils are responsible for engulfing and destroying pathogenic bacteria during infection. As early as the 1900s, it was known that opsonization and phagocytosis were limited in neonatal granulocytes.⁵ Matoth in 1952 demonstrated that neonatal granulocytes were deficient in their ability to degrade phagocytosed material.⁶ In the 1980s, Anderson and Rider correlated the insufficient generation of hydroxyl radicals by neonatal neutrophils.⁷ Technical and conceptual barriers limited further advances in the understanding of neutrophil development in the newborn. The advent of newer technologies, like flow cytometry– based functional assays in the early 2000s led to studies by Nupponen et al, demonstrating decreased production and release of antimicrobial proteins and peptides by neonatal neutrophils.⁸ More recently, Yost et al. reported that neonatal neutrophils have reduced the ability to generate DNA-based neutrophil extracellular traps (NETs).⁹ The above quantitative and qualitative deficiencies in neonatal neutrophils contribute to increased morbidity due to bacterial infections in the neonates.

Despite technological advances, investigating the molecular basis of neutrophil deficiencies in the newborn remains challenging (Table I). Due to their short gestation period and extensive catalog of immunological reagents,¹⁰ neonatal mice have been used to model neutrophil ontogeny. However, extensive species-specific differences in immune cell development between human and murine newborns¹¹ limit the translational relevance of murine studies. Studies using nonhuman primates may overcome some of these limitations by more closely modeling human immune responses,¹² but such studies remain expensive and limited by the availability of immunological reagents. *In vitro* study of human neonatal immune responses can overcome limitations inherent in animal studies. Cord blood assays have been used to study the ontogeny of neutrophils in the human neonates.¹³ Such studies using flow cytometry and multiplex cytokine assay has expanded our understanding of

Reprint requests: Hitesh Deshmukh, Cincinnati Children’s Hospital Medical Center, 3333 Burnet Avenue, MLC7009, Cincinnati, Ohio 45219; Hitesh.deshmukh@cchmc.org.

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neutrophil development in the neonates.¹⁴ Nevertheless, access to peripheral blood is limited in the neonatal period. It is also clear that neutrophils circulating in the peripheral blood may have distinct responses to those in bone marrow and target tissues,¹⁵ for example, the lungs.¹⁶ The aforementioned limitations have prevented a comprehensive understanding of neutrophil responses in the neonates.

In this issue of Translational research, Raymond et al.¹⁷ describe a microfluidic-based approach¹⁸ to circumvent aforementioned limitations. Their novel assay captures an enriched neutrophil fraction from less than 400 microliters of whole blood. This obviates the need for ex vivo enrichment of neutrophils, a procedure known to activate the granulocytes.¹⁹ The captured neutrophils were used for chemotaxis assay or whole genome transcriptomic analysis. Neonatal neutrophils had diminished the ability to migrate in response to microbial ligands. Limited blood volume prevented additional assessments of neutrophil function, such as phagocytosis, oxidative burst, and bacterial killing. The whole genome transcriptomics revealed repression of genes involved in apoptosis, pathogen recognition, cytokine production, and antimicrobial activity in neonatal neutrophils as compared adult neutrophils, recapitulating the observational studies comparing neonatal and adult neutrophils.²⁰ These¹⁷ and previous observations by the same group²¹ suggest that defects in neutrophil migration may be just one component of a more generalized program of transcriptional repression involving genes directing inflammation and innate immunity.

Recent reports implicate neutrophil heterogeneity in differential response to bacterial pathogens in children,^{22,23} raising the possibility that neonates may harbor a distinct neutrophil subpopulation as compared to children.²⁴ Methodological constraints prevented the authors from evaluating heterogeneity in neutrophil populations in neonates. Understanding neutrophil heterogeneity may be instrumental to develop novel therapies for neonates without compromising immunity.²⁵

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Table I

Approaches to study neutrophil ontogeny

Approach	Limitations of approach
Newborn mice	Limited samples mass/volume. Species-specific development of the immune system.
Bone marrow–derived neutrophils	Species variation in the development of the immune system, Addition of recombinant cytokines and growth factor. Highly labor intensive. Specialized housing requirements and training
Human neonatal/infant whole blood assay	Access to neonatal/infant peripheral blood limited. Peripheral leukocytes may have distinct responses to those in tissues