ORIGINAL ARTICLES

Neutrophil chemotaxis and transcriptomics in term and preterm neonates

STEVEN L. RAYMOND, BRITTANY J. MATHIAS, TYLER J. MURPHY, JAIMAR C. RINCON, MARÍA CECILIA LÓPEZ, RICARDO UNGARO, FELIX ELLETT, JULIANNE JORGENSEN, JAMES L. WYNN, HENRY V. BAKER, LYLE L. MOLDAWER, DANIEL IRIMIA, and SHAWN D. LARSON

GAINESVILLE, FLA, AND BOSTON, MASS

Neutrophils play a crucial role in combating life-threatening bacterial infections in neonates. Previous studies investigating neonatal cell function have been limited because of restricted volume sampling. Here, using novel microfluidic approaches, we provide the first description of neutrophil chemotaxis and transcriptomics from whole blood of human term and preterm neonates, as well as young adults. Ex vivo percent cell migration, neutrophil velocity, and directionality to N-formylmethionyl-leucyl-phenylalanine were measured from whole blood using time-lapse imaging of microfluidic chemotaxis. Genome-wide expression was also evaluated in $CD66b^+$ cells using microfluidic capture devices. Neutrophils from preterm neonates migrated in fewer numbers compared to term neonates (preterm 12.3%, term 30.5%, $P = 0.008$) and at a reduced velocity compared to young adults (preterm 10.1 μm/min, adult 12.7 μm/min, $P = 0.003$). Despite fewer neutrophils migrating at slower velocities, neutrophil directionality from preterm neonates was comparable to adults and term neonates. 3607 genes were differentially expressed among the 3 groups ($P < 0.001$). Differences in gene expression between neutrophils from preterm and term neonates were consistent with reduced pathogen recognition and antimicrobial activity but not neutrophil migration, by preterm neonates. In summary, preterm neonates have significant disturbances in neutrophil chemotaxis compared to term neonates and adults, and these differences in phenotype appear at the transcriptional level to target inflammatory pathways in general, rather than in neutrophil migration and chemotaxis. (Translational Research 2017;190:4–15)

Abbreviations: CLEC2B = C-type lectin domain family 2, member B; dChip = DNA-Chip analyzer; FCC = focal chemoattractant chamber; fMLP = N-formylmethionyl-leucyl-phenylalanine; GBS = Group B streptococcal; HBSS = Hank's Balanced Salt Solution; IL = interleukin; iNOS = inducible nitric oxide synthase; IPA = Ingenuity Pathway Analysis; NF-kB = nuclear factor-kB; PI3 = peptidase inhibitor 3; PPBP = pro-platelet basic protein; PROM = premature rupture of membranes; RNASE6 = Ribonuclease RNase A family, k6; SAM = significance analysis of microarrays; TCR = T-cell receptor; TLR2 = Toll-like receptor 2; WBLC = Whole-blood loading chamber

From the Department of Surgery, University of Florida College of Medicine, Gainesville, Fla; Department of Molecular Genetics and Microbiology, University of Florida College of Medicine, Gainesville, Fla; Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Shriners Burns Hospital, Boston, Mass; Department of Pediatrics, University of Florida College of Medicine, Gainesville, Fla.

Submitted for publication May 22, 2017; revision submitted July 8, 2017; accepted for publication August 11, 2017.

Reprint requests: Shawn D. Larson, Division of Pediatric Surgery, Department of Surgery, University of Florida College of Medicine, MSB N6-10, PO Box 100119, Gainesville, FL, 32610-0119; e-mail: shawn.larson@surgery.ufl.edu.

1931-5244/$ - see front matter

http://dx.doi.org/10.1016/j.trsl.2017.08.003

AT A GLANCE COMMENTARY

Raymond SL, et al.

Background

Neutrophils play a crucial role in combating life-threatening bacterial infections in neonates. Despite their importance, our understanding of neonatal neutrophil function is limited. Recent advances in microfluidics have provided a unique opportunity to study this vulnerable population using small volume whole-blood samples.

Translational Significance

This study provides the first description of neutrophil chemotaxis and transcriptomics in both preterm and term neonates. These findings will aid in the development of reliable tests using microquantities of blood to diagnose defects in immune function, to evaluate potential therapies for infectious complications, and to establish criteria for which babies would benefit from these treatments.

INTRODUCTION

Despite advances in neonatal critical care medicine, mortality remains significant among neonates. The daily mortality rate during the neonatal period is almost 30-fold higher compared to the postneonatal period with the absolute highest risk of mortality occurring among preterm neonates (gestational age less than 37 weeks).[1] Infections including neonatal sepsis account for the greatest percentage of neonatal mortality worldwide, which exceeds 1 million neonatal deaths each year.[1] Bacteria are the predominant pathogens associated with neonatal sepsis with gram-negative bacteria accounting for 38 percent of neonatal septic shock cases and 63 percent of neonatal sepsis mortality.[2] To recognize and combat these life-threatening bacterial infections, neonates rely predominantly on their innate immune system.[3] As such, neutrophils play a crucial role in neonatal survival. Neutrophils act as early responders to pathogens and are the most predominant immune cells in human blood.[4] Despite their central role in the recognition and early response to infections, our understanding of human neonatal neutrophil function is limited. Recent advances have provided a unique opportunity to study low birth weight preterm population using whole-blood assays in the first few days of life. In the past, such studies would have been impossible to perform because of the required large blood volumes, which have now been overcome with the use of novel microfluidic approaches.[5] These approaches circumvent the need for neutrophil purification, thus allowing the investigation of neonatal neutrophils from a single 400 μL unprocessed whole-blood sample. In addition, functional assays that utilize whole blood better reflect the intravascular milieu and thus should more closely approximate neutrophil capabilities in vivo. In this study, neutrophil chemotaxis and transcriptomics of both term and preterm neonates were characterized using whole-blood samples obtained in the first few days of life, as well as whole-blood samples from healthy young adults.

METHODS

Study design. This prospective observational study was conducted between February and September 2016 at UF Health Shands Children's Hospital, a 202-bed tertiary medical center. The study was approved by the Institutional Review Board before initiation and carried out according to The Code of Ethics of the World Medical Association (Declaration of Helsinki). All neonates admitted to the Neonatal Nursery and Neonatal Intensive Care Unit were screened for inclusion. Neonates born greater than 24 weeks gestational age were eligible for participation. Neonates were excluded from the study if the sample collection did not coincide temporally with a required clinical blood draw. Healthy adults aged 21–45 years were also recruited for participation. Adult subjects were excluded from enrollment if any of the following was reported: severe pre-existing organ dysfunction, positive for human immunodeficiency virus, recent use of oncolytics, current use of steroids, or history of autoimmune disease. Written informed consent was obtained from all adult subjects and from the parents of neonatal subjects before sample collection.

Sample collection. Whole-blood samples were collected from adults, term neonates, and preterm neonates. In adult subjects, a 4-mL blood sample was collected. In neonatal subjects, a single 700-μL blood sample was collected once at the time of a clinically required blood draw. Blood was collected for term neonates in the Neonatal Nursery before discharge between 24 and 36 hours of life. Blood was collected for preterm neonates in the Neonatal Intensive Care Unit on day 4 of life as not to interfere with the intense clinical management in the first 48 hours and to allow the family an appropriate time to absorb the trauma of a premature birth before discussing study enrollment.

Patient data collection. Patient data including maternal age, Group B streptococcal (GBS) colonization, prolonged premature rupture of membranes (PROM), clinical chorioamnionitis, antepartum