seek help for symptoms (presentation delay) and health care providers to be unaware of the presenting symptoms and not order confirmatory laboratory tests (diagnostic delay).

REVIEWER COMMENTS. When young children present with ataxia, an easy and inexpensive test to obtain is α-fetoprotein (AFP), which is rarely normal in A-T; this may improve earlier diagnosis of A-T.

URL: www.pediatrics.org/cgi/doi/10.1542/peds.2017–2475NNNN

Mary V. Lasley, MD
Seattle, WA

Risk Factors and Clinical Significance of Lymphopenia in Survivors of the Fontan Procedure for Single-Ventricle Congenital Cardiac Disease

Morsheimer MM, Rychik J, Forbes L, et al. *J Allergy Clin Immunol Pract*. 2016;4(3):491–496

PURPOSE OF THE STUDY. To determine the clinical significance of immunologic laboratory anomalies (lymphopenia and hypogammaglobulinemia) commonly seen in survivors of the Fontan procedure for single-ventricle congenital heart disease.

STUDY POPULATON. The study included 178 patients ages 3 to 26 years with congenital single-ventricle cardiac anomaly (status: postcompletion of surgical repair) with Fontan who had established outpatient care in the Single Ventricle Survivorship Program (SVSP) at the Children's Hospital of Philadelphia.

METHODS. This was a retrospective chart review of the immunologic parameters of patients enrolled in the SVSP. Data on demographics, diagnoses, surgical interventions, immunologic laboratory data, and medications were gleaned from the electronic medical records. SVSP and immunology consult notes were reviewed for infectious history, absolute lymphocyte count (ALC), and IgG levels. A P value of $<.05$ was considered statistically significant.

RESULTS. Most SVSP patients had some degree of lymphopenia. Those with protein-losing enteropathy (PLE) had lower median ALC and IgG levels (672 cells/μL and 200 mg/dL, respectively) than those without (1610 cells/μL and 868 mg/dL, respectively). Approximately 12% of those in the non-PLE group had significant asymptomatic lymphopenia (ALC $<$1000 cells/μL). In a logistic regression analysis, PLE and increased number of years after Fontan were found to be the only significant risk factors for lymphopenia. Despite lymphopenia in the majority, few participants were significantly clinically affected; 24% had a delayed clearance of cutaneous viral infections, 63% had atopy, and 1 died of EBV-associated Hodgkin lymphoma. Severe opportunistic infections typical of cellular immune defects were not observed, even among those with significant lymphopenia.

CONCLUSIONS. Patients with repaired single-ventricle physiology often demonstrate T-cell lymphopenia and hypogammaglobulinemia. The most common clinical manifestation was a delayed clearance of cutaneous viral infections. Significant systemic opportunistic infections were not seen despite laboratory abnormalities and a lack of antimicrobial prophylaxis or immunoglobulin replacement.

REVIEWER COMMENTS. Patients with repaired single-ventricle physiology often demonstrate T-cell lymphopenia and hypogammaglobulinemia. The exact mechanism by which this occurs is unclear, but it is seen in patients both with and without PLE. A proposed mechanism that is unique to this population is chronic venous hypertension leading to chronic low-level lymph loss in the gut with resultant lymphopenia. Further studies will be needed to better understand the impact of congenital heart disease on lymphocyte development, function, recirculation, and long-term survival. This study provides reassurance that morbidity associated with these immunologic changes is limited.

URL: www.pediatrics.org/cgi/doi/10.1542/peds.2017–2475OOOO

Edith Schussler, MD
Scott H. Sicherer, MD
New York, NY

CRISPR/Cas9 β-globin Gene Targeting in Human Haematopoietic Stem Cells

Dever DP, Bak RO, Reinisch A, et al. *Nature*. 2016;539(7629):384–389

PURPOSE OF THE STUDY. Sickle cell disease (SCD) is caused by a single nucleotide mutation (A to T) in the β-globin *(HBB)* gene leading to a Glu6Val amino acid change. The purpose of this study is to show that this gene can be effectively edited in human hematopoietic stem and progenitor cells (HSPCs) using the CRISPR/Cas9 system and to develop a method to enable efficient engraftment of edited human cells in mice.

STUDY POPULATION. HSPCs from mobilized peripheral blood of healthy donors and SCD patients were used for gene editing ex vivo. Immune-deficient NSG mice were used as recipients of edited HSPCs.

METHODS. Cas9 protein can generate double-strand breaks in DNA that can then be repaired by nonhomologous end joining or homologous recombination (HR) if a donor strand is provided. HSPCs were electroporated with Cas9 protein combined with sgRNA (Cas9 RNP) targeting the *HBB* gene. Cells were then infected with recombinant adeno-associated viral vectors of serotype 6 (rAAV6) carrying a donor sequence with a single nucleotide mutation (A to T) in the *HBB* gene and a green fluorescent protein (GFP) sequence or truncated nerve growth factor receptor (tNGFR) expression cassette. tNGFR is expressed at the surface of transfected cells and allows for enrichment of transduced cells by utilizing magnetic bead–based

Downloaded from www.aappublications.org/news by guest on October 23, 2019

separation technology. Gene-edited HSPCs enriched for high expression levels of GFP or tNGFR were transplanted into NSG mice and reconstitution of human hematopoietic cells was evaluated. Glu6Val mutation was corrected in SCD patients' HSPCs by using this same method.

RESULTS. On average, 29% of HSPCs that were electroporated with Cas9 RNP and provided with a GFP-tagged donor strand were positive for GFP expression. Mice transplanted with GFP^{high} cells had a median of 90% GFP^+ human cells at week 16 after transplant, with a proper distribution within the myeloid and lymphoid compartments. On-target integration was confirmed by sequencing GFP^+ cells. Mice transplanted with bulk $tNGFR^+$ cells showed 7.5% of edited cells 16 weeks after transplant, while mice transplanted with enriched $tNGFR^+$ cells showed 10% to 75% of edited cells. *HBB*-targeted sickle cell patients' HSPCs reverted an average of 50% of disease-causing variant alleles to wild type. When using an antisickling *HBB* cDNA-EF1α-tNGFR donor, 11% of tNGFR-positive cells were achieved. These cells were able to differentiate into erythroid cells in vitro. Expression of corrected β-globin was assessed by RT-qPCR. Erythrocytes differentiated from bulk $tNGFR^+$ expressed 20% of corrected (HbAS3) out of total β-globin mRNA (HbAS3), and those differentiated from $tNGFR^{high}$ expressed 70% HbAS3 mRNA.

CONCLUSIONS. The *HBB* gene can be targeted by using CRISPR/Cas9 system to correct SCD-causing mutations in HPSCs. Introducing a tNGFR cassette allowed the enrichment of corrected cells. The methodology used for enrichment allowed a fivefold increase in the engraftment of gene-edited cells. SCD patients' HSPCs can be corrected using this strategy, and edited cells are able to differentiate into erythrocytes that express adult β-globin.

REVIEWER COMMENTS. This is a preclinical study in which the authors show effective gene editing of the *HBB* gene in HSPCs and efficient engraftment of these cells in a mouse model. CRISPR/Cas9 gene editing of HSPCs enables the replacement of a disease-causing mutation with a wild-type sequence integrated in the genome under the physiologic promoter, therefore avoiding complications of other methods for gene therapy. The strategy presented for enrichment and expansion of edited HSPCs is particularly exciting, as these strategies may be required to optimize gene editing as a therapeutic option. This study not only sets the ground for gene therapy for SCD but also for other congenital hematologic and immune diseases.

URL: www.pediatrics.org/cgi/doi/10.1542/peds.2017–2475PPPP

M. Cecilia Poli, MD
Jordan Orange, MD, PhD
Houston, TX

SECONDARY IMMUNODEFICIENCY (HIV)

Maternal HIV Infection Influences the Microbiome of HIV-Uninfected Infants

Bender JM, Li F, Martelly S, et al. *Sci Transl Med*. 2016;8(349):349ra100

PURPOSE OF THE STUDY. Multiple studies have demonstrated that perinatally HIV-exposed but HIV-uninfected (HEU) infants experience greater morbidity and mortality than HIV-unexposed, uninfected infants (HUU). Newborn acquisition of the maternal microbiome provides metabolic and immunologic health benefits. The purpose of this study was to examine the effects of HIV infection on the maternal microbiome and breast milk oligosaccharides and the subsequent impact these may have on the microbiomes of their HEU infants.

STUDY POPULATION. A total of 50 Haitian mother-infant pairs were studied. All infants were breastfed, and no infants were on antibiotics at the time of the study. A total of 25 HIV-positive mothers and their infants were studied and compared with 25 HIV-uninfected mothers and their infants. Notably, all HIV-positive mothers were on combination antiretroviral therapy, and most had low HIV viral loads and normal CD4+ T-cell counts.

METHODS. Demographic and clinical data were obtained from patient records. Selected body sites were sampled for bacterial microbiological analysis from each subject. 16S ribosomal DNA was analyzed by using a previously described method. Breast milk oligosaccharides were characterized with high-pressure liquid chromatography.

RESULTS. Surprisingly, the microbiomes of the 2 groups of mothers did not differ appreciably. However, the microbiomes of the HEU infants had significant differences compared with the microbiomes from the HUU infants. Uninfected infants born to HIV-infected mothers showed lower microbial diversity and a different taxonomic composition. For example, HEU infants had an increased proportion of Pseudomonadaceae and less mature bacterial communities. HUU infants had an increase in Prevotellaceae and a greater maturity of their bacterial communities. This study examined 1 potential cause for this difference in the infants' microbiomes. Infants cannot digest human milk oligosaccharides, which may act as prebiotics to influence the infants' microbiomes. The data presented showed that normal breast milk oligosaccharide composition is altered by maternal HIV infection, and this was in turn associated with changes in the infants' gut microbiomes.

CONCLUSIONS. The gut microbiome in HIV-exposed, uninfected infants differs from that of HIV-unexposed and uninfected infants, and human milk oligosaccharides were associated with specific bacterial species. It was speculated that this dysbiosis may contribute to the increased risk of illness in HEU infants.

REVIEWER COMMENTS. It is not unexpected that the presence of a chronic infection would alter individual microbiomes.

Downloaded from www.aappublications.org/news by guest on October 23, 2019

CRISPR/Cas9 β-globin Gene Targeting in Human Haematopoietic Stem Cells

M. Cecilia Poli and Jordan Orange

Pediatrics 2017;140;S226
DOI: 10.1542/peds.2017-2475PPPP

Updated Information & Services	including high resolution figures, can be found at: http://pediatrics.aappublications.org/content/140/Supplement_3/S226.2
Permissions & Licensing	Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: http://www.aappublications.org/site/misc/Permissions.xhtml
Reprints	Information about ordering reprints can be found online: http://www.aappublications.org/site/misc/reprints.xhtml

American Academy of Pediatrics
DEDICATED TO THE HEALTH OF ALL CHILDREN™

Downloaded from www.aappublications.org/news by guest on October 23, 2019

PEDIATRICS®
OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

CRISPR/Cas9 β-globin Gene Targeting in Human Haematopoietic Stem Cells

M. Cecilia Poli and Jordan Orange

Pediatrics 2017;140;S226
DOI: 10.1542/peds.2017-2475PPPP

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://pediatrics.aappublications.org/content/140/Supplement_3/S226.2

Pediatrics is the official journal of the American Academy of Pediatrics. A monthly publication, it has been published continuously since 1948. Pediatrics is owned, published, and trademarked by the American Academy of Pediatrics, 141 Northwest Point Boulevard, Elk Grove Village, Illinois, 60007. Copyright © 2017 by the American Academy of Pediatrics. All rights reserved. Print ISSN: 1073-0397.

American Academy of Pediatrics
DEDICATED TO THE HEALTH OF ALL CHILDREN™

Downloaded from www.aappublications.org/news by guest on October 23, 2019