

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-EF1 α -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.

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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.

However, in this study, the changes in the microbiomes of HIV-infected versus HIV-uninfected mothers are relatively subtle. It is possible that the effective treatment of the HIV-infected mothers allowed relative normalization of their microbiota. Despite this, there were still variations in the infant microbiota depending on their exposure (or not) to HIV. A majority of the studies that have reported increased morbidity and mortality in HEU infants come from sub-Saharan Africa. Additionally, study subjects from France who demonstrated this increased risk were largely composed of mothers also from sub-Saharan Africa. In a study from Denmark, there was no significant difference in the hospitalization rate among HIV-exposed compared with HIV-unexposed children when admission for infectious diseases was analyzed. Finally, in the French cohort, the increased risk for serious bacterial infections in HIV-exposed, uninfected infants was related primarily to maternal immunosuppression. If the limited differences in the microbiomes between HIV-infected mothers and HIV-uninfected mothers in the current study is related to effective maternal antiretroviral therapy, the end result (regardless of the mechanism) is most favorable to HIV-exposed infants.

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Nonprogressing HIV-Infected Children Share Fundamental Immunological Features of Nonpathogenic SIV Infection

Muenchhoff M, Adland E, Karimanzira O, et al. *Sci Transl Med*. 2016;8(358):358ra125

PURPOSE OF THE STUDY. HIV-infected, nonprogressing adults appear to control viremia with effective adaptive immune responses. In contrast, the natural primate hosts of simian immunodeficiency viruses (SIVs), such as sooty mangabeys, remain disease-free despite ongoing viral replication. The purpose of this study was to examine the immunologic features and the degree of immune activation in nonprogressing, HIV-infected children (pediatric nonprogressors [PNPs]).

STUDY POPULATION. 170 HIV-infected children >5 years of age who met the following criteria were studied: antiretroviral (ART) naïve, maintaining normal-for-age CD4+ T-cell counts, and clinically nonprogressing. At the time of enrollment, ART was limited to patients whose CD4+ T-cells declined to a specified threshold that the subjects in this study had not reached (CD4+ T-cells <500). These nonprogressing children were compared with children with HIV disease who were clinically and immunologically progressing.

METHODS. Levels of plasma HIV RNA were measured longitudinally, and flow cytometric measurements of

multiple lymphocyte subsets were made. Also addressed were circulating levels of iFABP (intestinal fatty-acid binding protein) and soluble CD14 (sCD14) as measures of intestinal damage and microbial translocation, HIV neutralizing antibody levels, and anti-HIV-specific CD8+ cytotoxic T-cell functions.

RESULTS. PNPs maintained normal CD4+ T-cell counts despite a plasma viral load set point of 20 000 to 30 000 viral nucleic acid copies per mL of plasma. PNP CD4+ T-cells expressed very low levels of activation markers (CD38 and DR). Levels of iFABP and sCD14 were lower in PNPs than in progressors, indicating less intestinal damage and reduced microbial translocation into the systemic circulation. Relatively high numbers of naïve T-cells, lower effector memory T-cells, and reduced T-cell PD1 expression were noted as previously reported. Increased T-cell differentiation, exhaustion, and dysfunction in T-cells were apparent in progressors. PNPs generated broadly neutralizing antibodies and HIV-specific CD8+ cytotoxic T-cell responses, but these were not associated specifically with nonprogression. Finally, long-lived T-cells in PNPs expressed reduced CCR5 levels, indicating a reduction in the susceptibility to infection.

CONCLUSION. Once again, children are not down-sized adults. The mechanisms of maintaining nonprogression in PNPs share common immunologic features with nonpathogenic SIV infection in natural primate hosts rather than with nonprogressing human adults.

REVIEWER COMMENTS. Great strides have been made in the treatment of HIV disease. However, the ability to induce clinical nonprogression of the disease remains elusive, and this primarily rests on the inability to eliminate the viral reservoir present in the secondary lymphoid tissues of infected individuals. The first of two strategies that have been proposed to address the latent viral reservoir has been called “kick and kill.” This approach holds that by activating latently infected cells, the resulting viremia will then be susceptible to an antiviral drug attack. A second method of addressing the latent virus includes “soothe and snooze,” which aims to inhibit the pathways that lead to reactivation of latent virus.

Chronic immunologic activation drives HIV pathobiology. As demonstrated in the current study, the ability of a select group of HIV-infected children to remain disease free rests in their ability to ignore the presence of the virus, control immune activation, and maintain normal immune functions. Perhaps a third approach to inducing long-term nonprogression would be the maintenance of “blissful ignorance.”

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Maternal HIV Infection Influences the Microbiome of HIV-Uninfected Infants

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