Comment on Liem et al, page 723, and Bello et al, page 799

Negative studies shape the state of sickle trait

Sophie Lanzkron and Rakhi P. Naik

In this issue of Blood, 2 important studies by Liem et al and Bello et al take advantage of robust phenotypic and genotypic data from well-established cohorts to demonstrate that sickle cell trait (SCT) is not associated with an increased risk of cardiovascular disorders.\(^1,2\)

Currently in the United States, people with SCT may be identified through mandated state newborn screening programs or athletic association screening.\(^3\) However, despite widespread screening recommendations, clear guidelines regarding follow-up, counseling, and management of individuals with SCT do not exist secondary to a paucity of data from well-designed studies. This research void in SCT is particularly evident in cardiovascular and exercise-related outcomes, where SCT has been the subject of rampant speculation. In extreme conditions such as military training and high-intensity sports, SCT has been linked to an increased relative, but very small, absolute risk of sudden death.\(^4,5\) This has led to the hypothesis that SCT may be associated with impaired exercise tolerance and adverse cardiac events in the general population. Using measures of cardiac fitness and heart failure (HF) from large population-based cohorts, the 2 studies in this issue add important and rigorous data to this understudied area.

To explore whether racial disparities in cardiopulmonary fitness are associated with SCT, Liem and colleagues examined changes in fitness in 1995 African Americans (136 with SCT) followed over 25 years in the Coronary Artery Risk Development in Young Adults cohort. They found that baseline fitness and changes in fitness over time were no different for individuals with SCT compared with those without the trait. They also found no association of SCT with hypertension, diabetes, and metabolic syndrome. Bello and colleagues performed a meta-analysis of 4 different population-based cohorts and similarly found no association between SCT and the risk of incident HF in 15,364 African Americans (1,211 with SCT).\(^2\) Additionally, they found no difference in left ventricular size, wall thickness, or systolic function between those with SCT and those without.

SCT has long been considered a clinically benign recessive trait with the exception of the rare reported complications of extreme exertion-related sudden death and renal medullary carcinoma. However, recent studies, using similarly large cohorts as in the Liem and Bello studies, have shown an increased risk of chronic kidney disease\(^6\) and venous thromboembolism\(^7\) among African Americans with SCT. In addition, a recent study found an association of SCT with exertional rhabdomyolysis, but no demonstrated increased risk of sudden death in a contemporary military cohort followed after the institution of universal precautions.\(^8\)

Unlike in sickle cell disease (SCD), erythrocyte sickling and hemolysis do not normally occur in individuals with SCT.\(^9\) However, red cell changes have been documented in SCT in the context of severe dehydration, low oxygen tension, or exercise that mimic those observed in SCD. Increased whole-blood viscosity,\(^10\) erythrocyte stiffness,\(^11\) and overt sickling\(^12\) have all been demonstrated to occur in individuals with SCT in the setting of stress. Although these alterations in erythrocyte rheology and activity offer a biologically plausible mechanism for the development of end-organ damage, it is important to note that, in stark contrast to SCD, clinically relevant red cell changes in SCT likely only occur in local areas with extreme conditions, such as in the hypoxic environment of the renal medulla or within anaerobic muscle tissue during strenuous exercise. For this reason, chronic arterial complications would not be expected in individuals with SCT. The absence of an association between SCT and cardiovascular fitness or HF in the investigations by Liem et al and Bello et al, therefore, not surprising but is nonetheless exceedingly valuable.

That these studies are negative only increases their importance for the medical community and the 300 million people worldwide with SCT. We can now offer some reassurance that carrying SCT does not increase the risk of HF and the development of factors known to increase the risk of cardiovascular disease.

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REFERENCES

Hematopoiesis is regulated in part by extrinsic regulators, such as growth factors and cytokines, and in part by intrinsic epigenetic and transcriptional regulators that, in concert, orchestrate differentiation of stem cells via a series of progenitor cells into all types of fully mature blood cells.

In their study, Josefsdottir et al demonstrate that broad-spectrum antibiotic treatment of mice for >2 weeks depletes the intestinal microbial flora, which ultimately leads to a decrease in numbers of stem and progenitor cells in the bone marrow and concomitant anemia, leukopenia, and marked panlymphopenia. The authors provide substantial experimental evidence that these changes are not a toxic effect of antibiotics on hematopoietic cells, but rather are related to depletion of gut microbiota by antibiotic treatment.

Consistently, the effects of antibiotic treatment were phenocopied in germ-free mice and reversed by fecal microbiota transplantation.1

The molecular mechanisms by which commensal gut microbiota control proper immune function and hematopoiesis were recently shown to partially rely on microbial compounds such as lipopolysaccharides, which sustain steady-state production of neutrophils and their constitutive priming against bacterial infections through Toll-like receptor/MyD88-mediated signaling (see figure).2-4

Importantly, Josefsdottir et al were able to demonstrate that the effects of broad-spectrum antibiotic treatment on hematopoiesis were phenocopied in Stat1 knockout mice, suggesting that microbiota sustain steady-state hematopoiesis through activation of Stat1 signaling. However, further investigations are needed to unravel the type of cells experiencing direct or indirect activation of Stat1 signaling mediated by the commensal gut microbiota.

The novel findings by Josefsdottir et al extend the series of recent studies on host-microbe symbiosis, demonstrating that the gut microbiota is a critical extrinsic regulator of innate and adaptive immunity as well as hematopoiesis, which ultimately maintains the vigilance of the immune system against bacterial and viral infections (see figure).3,5,6 Consistently, perturbation of the balance and diversity in the composition of gut microbiota, referred to as dysbiosis, is associated with higher susceptibility to infections.5,6 Importantly, dysbiosis was also demonstrated to impair clinical

**Gut microbiota sustains hematopoiesis**

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In this issue of *Blood*, Josefsdottir et al provide substantial evidence that commensal gut microbes regulate and sustain normal steady-state hematopoiesis.1

**Comment on Josefsdottir et al, page 729**

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