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Short communication

# *Saccharomyces cerevisiae*-derived virus-like particle parvovirus B19 vaccine elicits binding and neutralizing antibodies in a mouse model for sickle cell disease

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ABSTRACT

Parvovirus B19 infections are typically mild in healthy individuals, but can be life threatening in individuals with sickle cell disease (SCD). A *Saccharomyces cerevisiae*-derived B19 VLP vaccine, now in preclinical development, is immunogenic in wild type mice when administered with the adjuvant MF59. Because SCD alters the immune response, we evaluated the efficacy of this vaccine in a mouse model for SCD. Vaccinated mice with SCD demonstrated similar binding and neutralizing antibody responses to those of heterozygous littermate controls following a prime-boost-boost regimen. Due to the lack of a mouse parvovirus B19 challenge model, we employed a natural mouse pathogen, Sendai virus, to evaluate SCD respiratory tract responses to infection. Normal mucosal and systemic antibody responses were observed in these mice. Results demonstrate that mice with SCD can respond to a VLP vaccine and to a respiratory virus challenge, encouraging rapid development of the B19 vaccine for patients with SCD.


## 1. Introduction

In parvovirus B19 infection, erythroid progenitor cells of the bone marrow are targeted, leading to their destruction. Brief interruption of red blood cell production does not cause significant anemia in normal persons due to the long survival of erythrocytes in circulation. However, in patients with sickle cell disease (SCD), parvovirus B19 infection causes a precipitous drop in hemoglobin concentration due to the short supply and abbreviated longevity of red blood cells (15–20 days versus the normal 3–4 months). The consequent disease outcome, transient aplastic crisis, can be life-threatening and frequently requires hospitalization and blood transfusions. Potential sequelae include stroke with permanent neurologic deficits, glomerulonephritis, and cardiac dysfunction. It is noteworthy that individuals with SCD do not usually suffer hospitalizations upon repeat exposures to parvovirus B19, demonstrating the protective efficacy of the immune response [1–3].

Decades of research have been expended in the development of a vaccine candidate to protect children with SCD from parvovirus B19-induced transient aplastic crisis. Virus-like particle (VLP) vaccine candidates have been produced in baculovirus-infected insect cells, but significant local adverse reactions observed in clinical trials prevented product advancement [4,5]. More recently, a yeast-based VLP was shown to be immunogenic in wild type mice when co-administered with the adjuvant MF59 [6]. This vaccine was produced by expression of viral proteins (VP) 1 and 2 from a single construct. The dual expression strategy improved control of the

*Abbreviations:* i.n., intranasal; i.m., intramuscular; VLP, virus-like particle; SeV, Sendai virus; NW, nasal wash; BAL, bronchoalveolar lavage; SCD, sickle cell disease; HET, control, heterozygous mice; DPBS, Dulbecco's phosphate-buffered saline.

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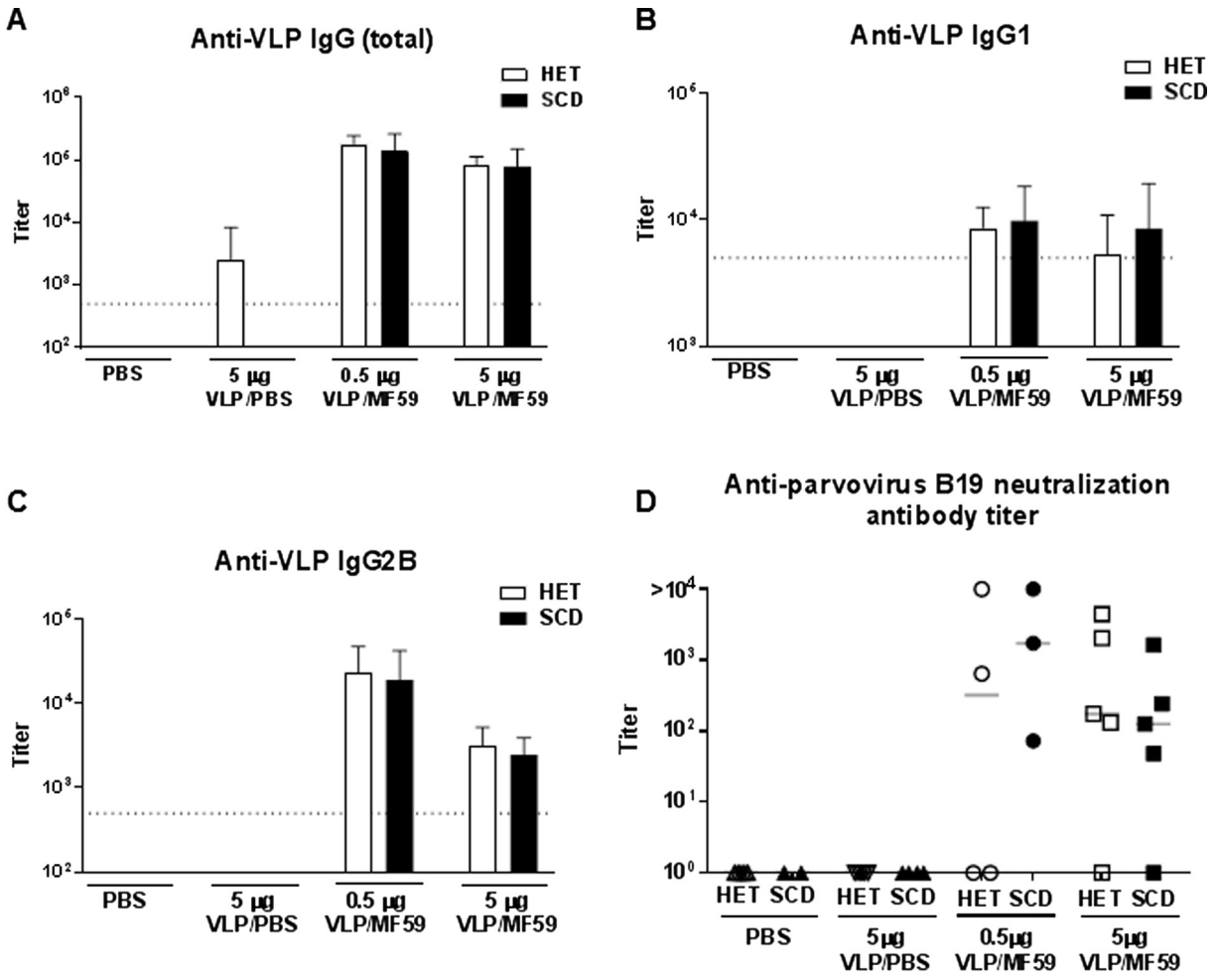


**Fig. 1.** Robust anti-parvovirus B19 antibody responses follow VLP with MF59 immunization of mice with SCD. Mice were vaccinated 3 times at 1 month intervals with either PBS (negative control), 5 µg VLP in PBS, 0.5 µg VLP in MF59, or 5 µg VLP in MF59 by the i.m. route. Three months after the first immunization, animals were sacrificed, and sera were tested for parvovirus B19 VLP-specific (A) IgG (all subtypes), (B) IgG1, and (C) IgG2B (N = 4–5 animals per group). Clear and black bars represent heterozygous littermate controls (HET) and animals with SCD, respectively. Titers shown represent the dilution of sera corresponding to an OD 405 reading of 0.1 within each assay. (D) Residual sera, when available, were also tested for neutralizing antibodies. Individual heterozygous and SCD mouse sample results are shown as open and solid symbols, respectively. The numbers of mice tested for neutralization in each group were as follows: 5 HETs and 2 SCD for PBS only; 5 HETs and 4 SCD for VLP in PBS; 4 HETs and 3 SCD for 0.5 µg VLP in MF59; 5 HETs and 5 SCD for 5 µg VLP in MF59. Samples failing to neutralize virus growth by 75% at the lowest dilution evaluated were assigned a titer of $10^0$. Dotted lines indicate the lowest dilution of sera evaluated.

VP1/VP2 ratio in the VLP. The vaccine also included a point mutation in VP1 that eliminated its phospholipase A2 activity, a potential cause of the adverse reactions observed in earlier clinical trials.

In the study described here, rather than testing wild type mice, we employed a transgenic mouse model of SCD (Berkeley; BERK) to test the usefulness of the yeast-derived VLP vaccine candidate in a clinically relevant model. These animals express human α, $\beta^S$, and γ globins, and have homozygous deletions of murine α and β globin genes [7,8]. Control littermates express the same human genes, but retain a mouse β globin gene that rescues the wild type phenotype. Mice with SCD exhibit many of the major genetic, hematologic and histopathologic features of humans with SCD in that: (1) red blood cells are irreversibly sickled; (2) mice suffer from anemia and multi-organ pathology; (3) mice suffer from exaggerated inflammatory responses; and (4) mice are at a risk of invasive disease due to encapsulated bacterial infections [7–10].

Here we show that mice with SCD respond well to vaccination when parvovirus B19 VLPs are co-administered with MF59, and respond well to a viral challenge by the respiratory route, the typical point of entry for B19.

## 2. Methods

### 2.1. Mice

All animal experiments were performed in accordance with the Institutional Animal Care and Use Committee protocols at St. Jude. Heterozygous BERK mice were obtained from a colony maintained at St. Jude and bred to produce homozygous mice with SCD. At 3 weeks of age, mice were bled, and SCD status was determined by both hemoglobin gel electrophoresis and complete blood count analysis. Heterozygous littermates were used as controls. Generally, 4–5 mice were allocated per test group. Due to the frailty of BERK mice, some animals did not survive until study completion. Both female and male mice were used in the study.

### 2.2. Vaccination

Parvovirus B19 VLPs were generated from a yeast cell line that expresses VP1 and VP2 in a fixed ratio [6]. At 8–12 weeks of age, mice were vaccinated intramuscularly (i.m.) with phosphate-

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