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Genomic Analyses of Pneumococci from Children with Sickle Cell Disease Expose Host-Specific Bacterial Adaptations and Deficits in Current Interventions

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SUMMARY

Sickle cell disease (SCD) patients are at high risk of contracting pneumococcal infection. To address this risk, they receive pneumococcal vaccines, and antibiotic prophylaxis and treatment. To assess the impact of SCD and these interventions on pneumococcal genetic architecture, we examined the genomes of more than 300 pneumococcal isolates from SCD patients over 20 years. Modern SCD strains retained invasive capacity but shifted away from the serotypes used in vaccines. These strains had specific genetic changes related to antibiotic resistance, capsule biosynthesis, metabolism, and metal transport. A murine SCD model coupled with Tn-seq mutagenesis identified 60 noncapsular pneumococcal genes under differential selective pressure in SCD, which correlated with aspects of SCD pathophysiology. Further, virulence determinants in the SCD context were distinct from the general population, and protective capacity of potential antigens was lost over time in SCD. This highlights the importance of understanding bacterial pathogenesis in the context of high-risk individuals.

INTRODUCTION

Streptococcus pneumoniae (pneumococcus) is a major cause of childhood illness worldwide, causing approximately 14 million episodes of invasive disease and 1 million deaths per year. The first step in invasive pneumococcal disease (IPD) is nasopharyngeal (NP) colonization, but asymptomatic carriage is common, especially in early childhood (~30%–50%) (Daw et al., 1997). Colonization is usually established by a single pneumococcal strain and persists for 1–2 months before clearance (Ghaffar et al., 1999). Pneumococci lack clustered regularly interspaced short palindromic repeats (CRISPR) systems to protect genomic content and are naturally highly transformable, permitting rapid genetic response to evolutionary pressures (Bikard et al., 2012; Croucher et al., 2011). For example, introduction of the 7-valent pneumococcal conjugate vaccine (Prevnar; PCV7) in the USA in 2000 resulted in near-complete elimination of vaccine serotypes and emergence of nonvaccine serotypes (NVT) in colonization and IPD in the general population (Croucher et al., 2013a; Halasa et al., 2013).

Sickle cell disease (SCD), a hemoglobinopathy characterized by chronic hemolysis and sickled red blood cells, is the most common genetic disorder worldwide, with 300,000 affected infants born each year. Children with SCD have a 600-fold increased risk of potentially fatal IPD compared with the general population, despite similar colonization rates (Overturf, 1999). The increased risk is related to hyposplenism, complement deficiency, and chronic vascular inflammation promoting upregulation of the ligand for pneumococcal invasion (Miller et al., 2007; Rosch et al., 2010). To address this risk, children with SCD receive long-term penicillin prophylaxis, frequent empiric antibiotic treatment, and pneumococcal vaccines. Pneumococci colonizing children with SCD have been previously shown to develop antibiotic resistance in response to the selective antibiotic pressure and demonstrate capsular serotype switching in response to vaccines (Adamkiewicz et al., 2008; Steele et al.,

Table 1. Demographic and NP Colonization Data

Patient Information	2004–2011	1994–1995 (Daw et al., 1997)
Evaluable patients	195	312
Median months on study (range)	48 (1–59)	NA
Total number of nasopharyngeal swabs	1,372	312
Pneumococcal colonization, n (%)	98 (7.1%)[a]	42 (13.5%)
Penicillin resistance[b]		
High level: nonmeningitis (PRP)	1 (1%)	3 (5.4%)[c]
Low level: meningitis (PNSP)	53 (54.1%)	29 (51.8%)[c]
Median age at enrollment, years (range)	5.0 (0.1–17.7)	4.3 (0.3–5.4)
Median age at time of swab, years	6.9	4.3
Female gender	98 (50.3%)	141 (45.2%)
Black race	193 (99.0%)	312 (100%)
Sickle cell disease type		
HbS/β-thalassemia	17 (8.7%)	24 (7.7%)
HbSS disease	178 (91.3%)	195 (62.5%)
Hydroxyurea use (ever during study)	106 (54.4%)	NA
Penicillin prophylaxis (ever during study)	134 (68.7%)	208 (67%)
Empiric antibiotic (ever during study)	195 (100%)	NA
Vaccine coverage (ever during study)		
Pneumovax: PPV23 (≥1 dose)	186 (95.4%)	NA
Prevnar 7: PCV7 (≥1 dose)	193 (99.0%)	0 (0.0%)
Infection episodes; n (events/1,000 patient years)		
Invasive pneumococcal disease	2 (2.8)[d]	NA
Acute chest syndrome	95 (133)	NA
Hospital admissions for infection	200 (280)	NA

NA, not available.
[a]Pneumococcal colonization rate in 2004–2011 adjusted to median age of 4.3 years = 13.4%.
[b]Includes data for an additional 12 isolates from the same cohort.
[c]Clinical and Laboratory Standards Institute susceptibility breakpoints.
[d]Post-PCV7 rate in healthy children <5 years old = 0.26/1,000 patient years; pre-PCV7 rate in SCD <5 years old = 24/1,000 patient years(Adamkiewicz et al., 2003; Hicks et al., 2007).

1996). The SCD host and the pneumococcus represent a unique paradigm for understanding how pathogens adapt to both clinical interventions, including vaccination and antibiotic pressure, as well as unique aspects of host pathophysiology underlying heightened infection risk. Due to the combination of clinical and host factors, we hypothesized that pneumococci found in the SCD population would develop unique genomic adaptations from the selective pressures imparted not only by clinical interventions but also the SCD host environment itself.

To characterize the pneumococcus emerging in the pediatric SCD population between 2004 and 2011 and re-evaluate current disease risk for these vulnerable children, we undertook the largest longitudinal study of pneumococcal colonization in children with SCD. We compared the results with a cohort from 1994–1995 to ascertain the impact of deployment of the conjugate PCV7 vaccine in 2000. We also acquired a broad range of IPD isolates from healthy and SCD children from across the United States and obtained sequence data for contemporary NP isolates from the general population (GP) (Croucher et al., 2013a). Whole-genome sequencing of 322 isolates, one of the largest data sets assembled thus far, defined overall gene content and identified genes with differential abundance between isolates from SCD and the GP as well as between historical and contemporary eras. A murine model of SCD coupled to Tn-seq whole-genome mutagenesis and vaccination experiments were used to identify and evaluate pneumococcal gene networks under selective pressure in this host. These data provide a comprehensive analysis of the influence of both clinical interventions and the SCD host environment on the pneumococcus, resulting in unique genetic selection and specialized contribution of genes to virulence in these high-risk patients.

RESULTS

Modern Colonizing SCD Strains Retain Invasive Characteristics and Evade Interventions

Pneumococcal strains were obtained from three sources: (1) 63 NP SCD isolates from 1994–1995 (Daw et al., 1997); (2) 186 IPD SCD isolates from the CDC ABC bacterial surveillance core, SJCRH patients, and published collections (McCavit et al., 2011); and (3) 98 NP SCD isolates from a longitudinal study spanning 2004–2011 in 195 SCD children followed for up to 4 years with serial NP swabs (Table 1). Children with SCD received PPV23 vaccine after 2 years of age, penicillin prophylaxis from birth to at least 5 years of age (Gaston et al., 1986), and frequent empiric antibiotic therapy. PCV7 vaccine was given only in the contemporary group. Multivariate analysis of the 2004–2011 NP data showed that younger age was significantly associated with increased risk of colonization (OR = 0.783 per year of age increase, $p < 0.0001$) and that, despite interventions, patients had a colonization rate of 7.1%, a value unchanged from 1994–1995 after correction for age (the adjusted colonization rate estimate for median age of 4.3 years was 13.4% in 2004–2011 and 13.5% in 1994–1995). The rates of acute chest syndrome and pneumonia remained high despite prophylactic interventions (133 episodes/1,000 patient years).

Penicillin resistance in contemporary SCD NP isolates was unchanged compared with the historical era for both low-level (MIC > 0.06 μg/ml; 54% versus 51%; $p = 0.87$) and high-level resistance (MIC > 2 μg/ml; 1% versus 5.4%; $p = 0.14$). Antibiotic prophylaxis appears to have maintained pressure on pneumococci in the SCD population, leading to a sustained high rate of penicillin resistance.

Capsular serogroup was compared between strains from pre (n = 112) and post (n = 225) PCV7 vaccine (Figure 1). After introduction of PCV7, there was the expected marked decrease in prevalence of PCV7 serotypes for both SCD NP and IPD isolates, similar to previous studies in both SCD and normal hosts (Adamkiewicz et al., 2008; Dagan and Klugman, 2008; Halasa et al., 2013) and reflecting the national trend following PCV7 introduction of serotype (Croucher et al., 2013a; Wroe et al., 2012). Unexpectedly, there was also a marked shift away from the additional serotypes in the not-yet-deployed PCV13 (Figure 1C; $p < 0.0001$). This indicates that the majority of serotypes found in