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Pneumococcus Adapts to the Sickle Cell Host

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Children with sickle cell disease (SCD) have significantly increased risk of invasive pneumococcal disease. In this issue of *Cell Host & Microbe*, Carter et al. (2014) report that pneumococcal strains from SCD children have genetic mutations associated with the unique SCD environment, which need to be considered in developing new vaccines.

Streptococcus pneumoniae (the pneumococcus) colonizes the nasopharynx of humans and in some circumstances can cause invasive diseases such as pneumonia, meningitis, and bacteraemia. This organism is genetically variable and can exchange DNA by the process of genetic recombination with the occurrence of more than 90 different serotypes, reflecting the ability of the organism to exchange capsular gene loci. Selective pressures on the pneumococcal population, such as use of antibiotics and vaccines, drive the emergence of genetic variants with altered capsule type or antibiotic targets. The pneumococcus is therefore highly adaptable to its environment and can change rapidly in response to interventions. The use of clinical interventions is known to have a marked effect on pneumococcal population structure (Croucher et al., 2011). It is also recognized that even single-nucleotide polymorphisms may have an effect on the ability of the pneumococcus to cause disease. A single nucleotide difference in the genomes of pneumococci taken from the blood and cerebrospinal fluid (CSF) of a patient with meningitis altered the expression of an ABC transporter. This mutation changed the virulence of the organism in mouse models of disease and also its ability to grow in different environments in vivo (Croucher et al., 2013). Genetic variation of bacterial pathogens is therefore an important facet of the dynamic host pathogen interaction and of great consequence for the rationale of design of new vaccines and treatments for infectious diseases. The host environment, and the interventions used to prevent or treat disease, drives genetic variation of the pneumococcus. The host environment can be very different in some populations, and susceptibility to pneumococcal infection is known to vary in these populations.

Children with sickle cell disease (SCD) are at extremely high risk of fatal pneumococcal infection, having a 600-fold increased risk of fatal invasive pneumococcal disease (IPD) (Overturf, 1999). The increased infection risk has been related to functional asplenia and complement deficiency, but SCD patients also have altered plasma levels of zinc, iron, purines, amino acids, and carbohydrates (Darghouth et al., 2011; Prasad et al., 1976). Given the high susceptibility to pneumococcal infection, children with SCD are routinely immunized with pneumococcal vaccines and administered prophylactic antibiotics alongside. The pneumococcus therefore encounters a very different environment in the sickle cell patient to that found in the general population (GP) in terms of host metabolism and exposure to interventions.

In this issue of *Cell Host & Microbe*, Carter et al. (2014) analyze and compare the genomic sequences of more than 320 pneumococcal strains isolated from carriage and disease in the GP and in children with SCD. This analysis reveals that the different host environments encountered in SCD lead to specific pneumococcal adaptations associated with colonization or disease in the SCD host, as depicted in Figure 1. The vaccines used to prevent pneumococcal disease protect against a limited number of serotypes chosen based on their likelihood to cause disease in humans. It is well known that use of these vaccines drives a shift away from these types and an increase in prevalence of capsule types not included in the vaccine (so-called non-vaccine types, or NVTs). NVTs are considered to be less able to cause disease in the GP. A shift toward NVTs was observed when vaccines were used in both SCD and GP. The invasive potential of NVT isolates was examined in a mouse

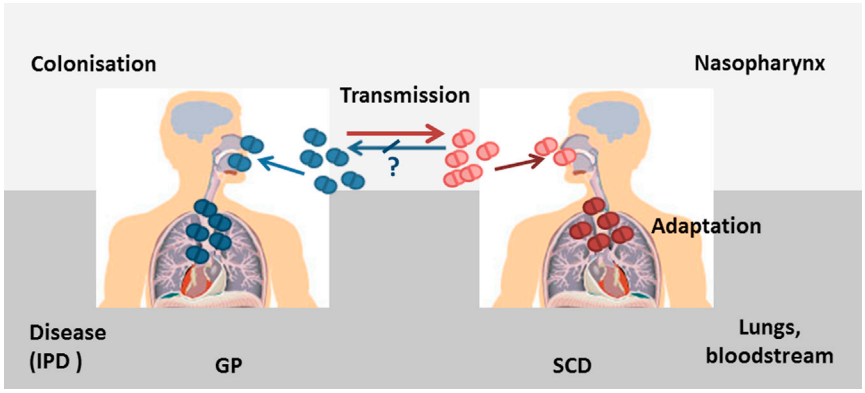

Figure 1. Diagram Illustrating How Microevolution of the Pneumococcal Genome Affects the Epidemiology of Carriage and Disease Isolates between Presumed Healthy Individuals of the General Population and Sickle Cell Disease Patients

S. pneumoniae of established genotypes colonize, and cause invasive disease, in the normal host, as depicted left. In colonizing the sickle cell disease (SCD) host, various adaptive mutations have been described by Carter et al. (2014). Comparative genome analysis indicates accumulation of more mutational changes in strains isolated from pneumococcal disease in SCD patients. Some of these mutations are detected in the same genes observed in genomes from SCD carriage such as PiaA, as shown in Figure 5 of Carter et al. (2014), in this issue of *Cell Host & Microbe*.

model of SCD. NVT strains showed variable virulence in normal mice but were all highly virulent in SCD mice, confirming that NVT strains selected by the vaccine are more virulent in the SCD than WT background.

Comparison of genome sequences and gene content of historical and contemporary pneumococcal strains showed that about half of the strains had undergone some gene loss in SCD and others had undergone intragenic recombination to produce mosaic genes. These genetic events were preferentially observed in the SCD population, suggesting that they may confer some advantage in SCD but are outcompeted in the GP. The differences were observed in four key groups of genes responsible for penicillin resistance, capsule biosynthesis, metabolic pathways, and metal ion uptake. Mutation in genes for capsule biosynthesis and antibiotic resistance are expected due to the selective pressure of antibiotic therapy and vaccination in SCD patients as noted above.

The mutations in genes involved in metal ion uptake suggest these mutations are not only beneficial in the SCD host environment but can only be tolerated in this host environment, but not in the normal host. In order to test this hypothesis, a panel of mutant strains was tested in a mouse model of SCD using Tn-seq, which is a technique that allows thousands of tagged, loss-of-function mutants to be generated and then screened in wild-type and SCD mice. If a particular loss-of-function mutant is less able to survive in the mouse, it will be represented in lower abundance when the strains are recovered and quantified by DNA sequencing of the identifying tag. Sixty genes were shown to confer differential fitness between the two hosts, reflecting the different environments in WT and SCD mice. The functions of genes under selective pressure in SCD mice correlate strongly with aspects of SCD pathophysiology in humans, including abnormal iron homeostasis, purine metabolism, and complement function. Importantly, six of the genes identified to be associated with SCD in the mouse model were either altered or absent in clinical isolates from human SCD patients, showing the relevance of the mouse study to the clinical situation.

One of these six genes identified by Tn-seq analysis in mice is involved in iron transport into the pneumococcus. The iron transport complex in the pneumococcus is highly immunogenic, and loss of this protein may be advantageous in avoiding the immune response. It seems that the iron transport process is not required or is even detrimental to the bacterium in the iron-rich SCD host. This raises the general question about protective immunity in the SCD host. The ability of the bacterium to thrive despite the loss of antigenic proteins could compromise protective immunity in specific host environments. Loss of immunogenic proteins is only possible if the function of the protein is no longer required due to the specific environment.

The principle that vaccine efficacy may be compromised by the host environment is demonstrated experimentally in this study. The iron transporter protein (PiaA) and the complement-degrading enzyme (CppA) are potential targets for inclusion in new protein-based vaccines against pneumococcal diseases. Vaccination of wild-type and SCD mice with these proteins stimulated antibody responses. Subsequent challenge of the animals with *Streptococcus pneumoniae* showed that WT animals were protected from challenge by the vaccination but SCD mice were not. This is presumably due to lack of expression of PiaA in the high iron environment and a lack of requirement for antibody-mediated neutralization of CppA in the low-complement environment of SCD. These are very important observations in terms of vaccine design and highlight the need to consider the environment within the population targeted for vaccination. This also raises another issue for consideration when refining the selection of vaccine targets, that of gene expression. The prediction arising from the work of Carter et al. (2014) is that the different environment within the SCD patient will have a marked effect on bacterial gene expression during infection, that is likely to be different to that in the GP. Exposure to high levels of iron in SCD patients, for example, would be expected to alter the pneumococcal transcriptome during IPD in SCD patients. Some of the genomic changes observed in strains from SCD may also result in subsequent alteration of gene expression.

The use of well-characterized strain collections and high-throughput sequencing is a powerful approach to understanding the interaction of pathogen and host. The importance of the microenvironment within the host on pathogen variation in patients with SCD is clearly demonstrated in this study. Bacteria are adaptable and are able to live in a whole range of environments. These include those encountered by pathogens during infection. The study by Carter et al. (2014) highlights how different environments in various patient cohorts can affect the bacterial population

and how important it is to take this into account when planning vaccine or intervention strategies. Several intriguing questions remain regarding pneumococcal carriage, transmission, and evolutionary adaptation. Are carriage strains of *S. pneumoniae* in SCD patients continuously reseeded from strains carried by the GP, or are these transmitted from SCD patient to patient? If the latter is true, this would mean that the pneumococcus is undergoing parallel but separate linear evolutionary pathways in these two populations. The use of "designer" vaccines to target these populations should be considered.

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Endosomes as Platforms for NOD-like Receptor Signaling

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The NOD-like receptors (NLRs) were among the first innate immune receptors discovered, yet a clear understanding of the basic principles underlying their mechanisms of action is lacking. Two recent studies provide important cell biological insights into the subcellular sites of NOD1- and NOD2-dependent signaling.

The modern field of innate immunity is centered on the study of several families of pattern recognition receptors (PRRs). These receptor families include the Toll-like receptors (TLRs), NOD-like receptors (NLRs), C-type lectin receptors (CLRs), RIG-I-like receptors (RLRs), and AIM2-like receptors (ALRs). Each of these receptor families functions to detect microbial products in a multicellular host and induces one or more antimicrobial responses that prevent infection. As such, much work in this area has focused on understanding how PRRs detect microbial products and the mechanisms of subsequent signal transduction. Despite being one of the first PRR families discovered (Philpott et al., 2014), insight into the means by which NLRs detect their microbial products lags far behind the other families. Two recent studies have addressed these deficiencies in our knowledge (Irving et al., 2014; Nakamura et al., 2014), providing us with a much clearer understanding of the earliest events associated with the activation of NLR-dependent innate immune responses.

NLRs are a structurally related family of proteins, with individual family members serving distinct biological functions (Philpott et al., 2014). For example, some NLRs act as transcription factors in the nucleus to promote the expression of major histocompatibility complex (MHC) genes, and others act as central regulators of inflammasome activation (Davis et al., 2011). The assignment of NLRs as PRRs came from studies of the NOD1 and NOD2 proteins, which were found to activate NF-κB-dependent cytokine expression during infections with bacteria that enter the cytosol. Reductionist studies subsequently revealed that NOD1 and NOD2 can be activated by specific components of the bacterial cell wall, such as d-glutamyl-meso-diaminopimelic acid (iE-DAP) in the case of NOD1 and muramyl dipeptide (MDP) in the case of NOD2 (Philpott et al., 2014). Despite this knowledge, several confusing aspects of NOD1 and NOD2 biology remained. For example, it has been difficult to detect direct interactions between these receptors and their proposed ligands, whereas similar inquiries into receptor-ligand interactions with other PRR families have been successful. This issue has been further complicated by the recent discovery that some NLR family members do not bind to microbial products directly, but rather interact with upstream proteins of the NAIP family that bind to specific microbial ligands (Kofoed and Vance, 2011). These findings raised the possibility that at least some NLR family members are not actually PRRs, but are adaptor proteins that facilitate the signaling functions of upstream PRRs. A second confusing aspect of NLR biology has emerged from studies demonstrating that natural NOD ligands can trigger NF-κB activation when added to the extracellular media of cultured cells (Kaparakis et al., 2010). This finding was surprising, since the NODs are cytosolic proteins. Thus, it has long been suspected