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Determinants of Invasiveness Beneath the Capsule of the Pneumococcus

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Interventions provide unique opportunities to learn about the natural history of infectious diseases, and one such intervention is the global rollout of pneumococcal conjugate vaccine. This is a vast experiment in natural selection, during which specific capsular types contained in 10–13-valent vaccines are increasingly being prevented from circulating in the human population. These types were chosen on the basis of their prevalence among invasive pneumococcal disease isolates, so by definition they represent a selection of the dominant invasive pneumococcal serotypes. The remaining ≥80 serotypes are then increasingly free to compete in an environment without their most invasive competitors. We can measure the invasive potential of these “replacement” strains most simply by comparing their prevalence in collections of carriage strains versus invasive strains. This allows the development of an invasiveness index [1–6], which has until now been based entirely on capsular types.

Thus, serotypes 1, 5, and 7F, which are rarely detected among carriage isolates and almost exclusively found in collections of invasive disease isolates, have the highest invasive potential, which is simply a ratio of their occurrence in collections of invasive versus carriage strains. A caveat of this approach is that these studies on carriage have used culture only, so as molecular or immunologic methods are developed to identify low density carriage, these invasive indices may change.

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There are some further caveats to these comparisons, such as taking the invasive strains and carriage strains from the same population in time and place, and these fundamentals are met in the article by Browall et al [7], from the Karolinska Institute in Sweden. They examined a collection of 550 carriage strains and 165 invasive strains, all recovered from children <18 years of age in the Stockholm area between 1997 and 2004, presumably before the introduction conjugate vaccine in much of that population. The mean age of 2.5 years among subjects with invasive pneumococci and 3.3 years among those carrying pneumococci means that only a fraction of the subjects in each group were <1 year old, the age when most mortality due to pneumococcal infection occurs in children in developing countries. Nonetheless, these data offer a useful comparison between invasive and carriage strains in the same community in a developed country.

The most important new contribution of the study is that invasiveness was not only an attribute of serotype, although Browall et al detected typically high odds ratios (ORs) of the invasive potential of serotypes 1, 7F, 14, 18C, and 19A. Intriguingly, among 107 isolates, all of sero-type 6B and clonal complex 138, the authors found 3 different pulsed-field gel electrophoresis (PFGE) types with differing levels of invasiveness. One PFGE sub-type, comprising 7 strains, was significantly associated with invasiveness (OR, 20.6; 95% confidence interval [CI], 2.5–949.7), whereas no such association existed for the other two. Similar observations were made for a specific PFGE type within clonal complex 113, a complex predominantly of serotype 18C, which conferred an invasive potential OR of 17.1 (95% CI, 1.9– 809.2), and a single invasive PFGE type within clonal complex 124, which was predominantly serotype 14 (OR, 5.7; 95% CI, 1.1–37).

To characterize further the properties of the invasive lineages within complex 138, they did whole-genome sequencing on 4 strains, including all three PFGE subtypes. This provided clues to suggest that the invasiveness may be due to the presence of specific proteins, but despite gene-specific polymerase chain reaction analyses, enzyme-linked immunosorbent assays for protein expression, and even virulence assays in mice, in no instance was there a single protein or variant allele that specifically differentiated invasive strains from carriage strains. It is likely, therefore, that the observed phenotypic differences are due to a combination of factors—(1) the presence of multiple genes and/or their genetic variants and (2) their levels of expression—that could not be fully discerned by the current analysis.

What these data confirm is that within a highly recombinatorial species such as the pneumococcus, our current definitions based on capsular type, multilocus sequence type, or PFGE type are insuffi-cient to explain the invasiveness of individual strains and that more general application of whole-genome sequencing will be essential if we are to identify genes-or, perhaps more importantly, combinations of genes, the expression of which confers invasiveness. We may well find that there are multiple routes and multiple solutions to an invasive genotype. Why does it matter? We need to know much more in general about pneumococcal invasiveness, but at least 2 further steps will help us understand the risk of the emergence of replacement invasive strains in a world in which pneumococcal conjugate vaccine is widely used. First, using whole-genome sequencing, we should soon be able to push open much wider the door that has been opened by Browall et al [2] and define invasiveness geno-types and phenotypes beneath the capsule. The second step will be to

study the evolution of invasive genotypes when they acquire capsular genes associated with less invasive capsules. By maintaining a degree of surveillance at a genomic level of resolution, we will be able to observe the evolution of such strains and their future trajectory of invasiveness.

The possibility still exists of identifying proteins conferring invasiveness that may be used as vaccine candidates, but the relative contribution of capsule versus other determinants of invasiveness is still skewed in favor of the capsule. This article reminds us that what lies beneath the capsule remains of significant biological interest.

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