Institut Pasteur
Microbes and Infection 16 (2014) 292–299
www.elsevier.com/locate/micinf

Original article

Capsular switching as a strategy to increase pneumococcal virulence in experimental otitis media model

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Received 14 May 2013; accepted 11 December 2013
Available online 20 December 2013

Abstract

We hypothesized that capsular switch event, in which pneumococcus acquires a new capsule operon by horizontal gene transfer, may result in emergence of strains with increased virulence in acute otitis media. Using serotype 6A strain from a patient with invasive pneumococcal disease and clonally distant serotype 6C strain isolated from asymptomatic carrier we created 6A:6C (6A background with 6C capsule) capsular transformants and applied whole genome macro-restriction analysis to assess conservation of the 6A chassis. Next, we assessed complement (C3) and antibodies deposition on surface of pneumococcal cells and tested capsule recipient, capsule donor and two 6A:6C transformants for virulence in chinchilla experimental otitis media model. Both 6A:6C$_{(1\text{ or }2)}$ transformants bound less C3 compared to 6C capsule-donor strain but more compared to serotype 6A capsule-recipient strain. Pneumococci were present in significantly higher proportion of ears among animals challenged with either of two 6A:6C$_{(1\text{ or }2)}$ transformants compared to chinchillas infected with 6C capsule-donor strain [$p < 0.001$] whereas a significantly decreased proportion of ears were infected with 6A:6C$_{(1\text{ or }2)}$ transformants as compared to 6A capsule-recipient strain. Our observations though limited to two serotypes demonstrate that capsular switch events can result in *Streptococcus pneumoniae* strains of enhanced virulence for respiratory tract infection.

Keywords: Pneumococcal; Capsular switching; Virulence

1. Introduction

Following the introduction of pneumococcal conjugated polysaccharide vaccines (PCVs), there has been a substantial decrease in invasive pneumococcal disease (IPD), pneumonia and otitis media due to vaccine serotypes [1–5] yet most studies report no significant change in prevalence of *Streptococcus pneumoniae* colonization among children [6,7]. The vaccine serotypes which circulated in the community prior to introduction of PCV7 have been replaced with non-vaccine serotypes (NVTs) [6,8]. It has been postulated that serotypes differ in their capacity to progress from colonization to invasive disease [5,9,10] with certain serotypes demonstrating greater invasive potential than others. The difference in 'invasive' capacity between the PCV7 serotypes and the current colonizing serotypes could explain the observed reduction in pneumococcal disease despite complete replacement in the nasopharynx [5].

Mathematical models have suggested a potential for erosion of the long term benefits of vaccination on IPD incidence [11,12]. These predictions are based on reports of a substantial increase in IPD caused by NVTs following implementation of PCVs in many geographic locations [13,14]. One of the potential mechanisms contributing to increase in disease caused by NVT could be natural

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http://dx.doi.org/10.1016/j.micinf.2013.12.002

transformation of virulent VT strains that acquired NVT capsule genes [15]. Pneumococci are genetically flexible and easily take up and incorporate new DNA into their genome. High rate of genetic recombination secures frequent horizontal transfer of genes and leads to overall high genetic diversity in *S. pneumoniae* [16,17]. An evidence of this natural plasticity of pneumococcal genome is demonstrated by presence of clonally closely related strains expressing different serotypes [18]. Among the internationally distributed epidemic strains of *S. pneumoniae*, there are several for which more than one serotype variants have been reported [19] (MLST database) [20]. These new combinations arose through "capsular switching", natural transformation events mediated by recombinational replacements within the capsular biosynthesis (*cps*) operon and its flanking regions following acquisition of DNA from other pneumococcal strains.

Selective pressure from conjugate vaccines has permitted the emergence of *S. pneumoniae* strains uncommon or rare before vaccine introduction as well as the increase in prevalence of capsule-switch strains [21]. Thus, there is a potential for strains that represent combinations of NVT capsules and genetically competent background that are evolutionary better adapted to evade host defenses to cause disease more frequently than other non-vaccine strains circulating in immunized populations. Such strains could significantly contribute to the erosion of vaccine effectiveness. One of the likely "melting pots" for new strains emergence could be children with persistent or recurrent otitis media [22].

We recently reported strains of single sequence type (ST) 1692 that expresses either 6A and 6C capsular polysaccharide types, demonstrating yet another natural capsule switch event [23] (mlst.net). Due to high homology between 6A and 6C *cps* operons, capsule switching between strains of these and other types within serogroup 6 is more common than acquisition of any other capsule type (mlst.net). Recent studies recorded an increase in carriage of serotype 6C strains subsequent to the introduction of PCV7 [6,24]. Of note, serotype 6C is not targeted in the next generation pneumococcal conjugated vaccine. Although still relatively uncommon in IPD, serotype 6C is already prevalent among pneumococcal strains isolated from otitis media in children and strains from recurrent or treatment-failed cases of AOM in particular [25–27].

We also reported a correlation between variation in binding of complement to the surface of *S. pneumoniae* and virulence of serotype 6A strains in an animal model of upper respiratory tract disease, otitis media [28]. We concluded that the differences in virulence among strains of the same serotype were due to capsule-independent attributes.

Here, we created capsule-switch variants, expressing type 6C capsule in strains originally of serotype 6A, and use capsule-donors, capsule-recipients, and newly constructed capsule-switch variants to assess how new combinations of capsule and isogenic background might affect strain potential to progress from colonization to tissue invasion and respiratory tract disease. We hypothesized that the cell surface binding of complement and the virulence of the capsule-isogenic background strain in an in vivo model of experimental otitis media will be altered. To our knowledge this is the first study demonstrating in an animal model of pneumococcal otitis media an increased virulence of capsule-recipient compared to capsule donor strains. Our findings suggest potential mechanisms whereby *S. pneumoniae* could increase its capacity to cause disease in the future.

2. Materials and methods

2.1. Bacterial isolates and growth conditions

The serotype 6A strain used as capsule recipient was isolated from case of IPD and belonged to sequence type (ST) 1390. The serotype 6C strain used as capsule donor was previously described isolate of ST1692 originally cultured from nasopharyngeal (NP) sample collected from asymptomatic carrier [29]. Both strains originated from Massachusetts. *S. pneumoniae* isolates were grown to mid log phase at 37 °C in brain-heart infusion broth supplemented with 10 μg/ml haemin and 2 μg/ml NAD (sBHI) and were used for nasopharyngeal challenge in our animal model. The strain nomenclature is shown in Table 1.

2.2. Serotyping

Pneumococcal isolates were initially serotyped at our laboratory, using the quellung reaction with Danish antisera (Statens Seruminstitut, Copenhagen, Denmark). Additional confirmation of the serotype was provided by Dr. Moon Nahm's by inhibition ELISA using monoclonal antibodies Hyp6AG1 and Hyp6AM3 that specifically reacted with 6C capsule [30].

2.3. Antibodies and complement reagents

Human complement used in the flow cytometry experiments was purchased from Sigma Chemical Company (St. Louis, MO). Heat inactivated human complement (56 °C for 30 min) was used as a control in some experiments. Fluorescein isothiocyanate (FITC)-conjugated sheep anti-human C3c and anti-human C4 (Biodesign) were used in flow cytometry assays as described previously [31]. Anti-goat IgG, anti-human IgG, and anti-mouse IgG conjugated to FITC (Sigma) were used as secondary antibodies.

2.4. Multilocus sequence typing

The genotypes of the pneumococcal strains used in this study were determined by multilocus sequence typing (MLST)

Table 1
Description of strains used in animal model.

Strain nomenclature	Capsule	Chassis/MLST	Origin
6A wt[a]	6A	1390	IPD[b]
6C wt	6C	1692	Nasopharynx
6A:6C1	6C	1390	
6A:6C2	6C	1390	
6A:6A	6A	1390	

[a] wt: wild type.
[b] IPD: invasive pneumococcal disease.