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Pia Cumagun

Juan Calix

Moon Nahm

short report

Effects of the O-acetyltransferase wcjE on the Opsonization of *Streptococcus* pneumonia in a Multiplexed Opsonophagocytic Killing Assay

Pia Cumagun[1] • Juan Calix[2] • and Moon Nahm[2,3]
[1]UAB Pathology Research Experience Program • [2]UAB Department of Microbiology • [3]UAB Department of Pathology

Abstract

Background: Although *Streptococcus pneumoniae* colonizes the nasopharynx, it can also cause pneumonia, acute otitis media, and invasive diseases such as bacteremia and meningitis (1). This Gram-positive bacterium has an extremely diverse polysaccharide capsule that allows it to evade host immune defense mechanisms (3). The genes on the capsular polysaccharide synthesis (cps) locus are responsible for the 93 different capsule types known today (3). Of particular interest in this study was the wcjE allele in the cps locus, which encodes a putative O-acetyltransferase in serotypes 9V and 11A (3, 5). In serotypes 9A and 11E, this gene is inactive (1, 3). Serotypes 9V and 11A are included in the Pneumovax 23 vaccine, whereas 9A and 11E are not included. The primary purpose of this project is to determine the role of wcjE in the non-specific killing (NSK) response of the host against *S. pneumoniae*. In addition, cross-protection against serotypes 9A and 11E in individuals vaccinated with Pneumovax 23 was examined.

Methods: The multiplexed opsonophagocytic killing assay (UAB-MOPA) protocol was followed with slight modifications (2). HL60 cells, normal rabbit complement, and adsorbed rabbit complement were used in order to observe the NSK response in the following serotypes of bacteria: 9V (JC01), 9A (JC02), 9Aα (MB01), 11A (JC03), 11E (JC04), and 11Eα (JC12). To solely investigate the NSK response of the host, no sera was added. To determine whether the vaccine against *S. pneumoniae*, Pneumovax 23, cross-protects against 9A and 11E, 10 sera samples were tested for their ability to opsonize the serotypes 9V, 9A, 11A, and 11E.

Results: Strains that had a disrupted wcjE displayed a higher NSK response than strains with a functional wcjE. Both alpha serotypes that were tested exhibited a higher NSK than strains with an inactive wcjE. In addition, the antibodies in the tested sera samples cross-protected against 9A and 11E. Serotypes 9A and 11E were preferentially killed compared to 9V and 11A.

Discussion: The functionality of wcjE shows a direct relationship with the NSK response of the host. O-acetylation of capsule in 9V and 11A is dependent on the wcjE allele and could be a possible mechanism for these serotypes to evade the host immune defense. Alpha serotypes exhibit partial O-acetylation on their capsules (3). Since 11Eα and 9Aα are intermediates of serogroup 11 and 9, respectively, it was hypothesized that the two serotypes would show an intermediate NSK percentage. Therefore, these serotypes with an intact wcjE could possibly have developed this mechanism of evading the host defense, essentially eliciting a lower NSK response. One possible cause for the preferential opsonization of 9A and 11E is the volatile nature of the O-acetate groups in vaccine PS (4).

Introduction

S.pneumoniae is a Gram-positive diplococcus that colonizes the nasopharynx. It is responsible for causing pneumonia, bacteremia, meningitis, and otitis media (1). Its most important virulence factor is its highly diverse polysaccharide capsule, allowing evasion from the host immune system (3). There are over 90 different capsule types (3). The variability is due to the genes on the capsular polysachharide synthesis (cps) locus as seen in Figure 1 (1, 3). The vaccine, Pneumovax 23, which uses purified capsules of the 23 most prevalent serotypes (i.e. 9V and 11A), has been developed to help combat *S. pneumoniae* (http://www.merck.com/).

O-acetylation of the polysaccharide capsule in serotypes 9V and 11A is dependent on wcjE, a gene located on the cps locus (3, 5). This mechanism is inactivated in 9A and 11E (1, 3). There is partial inactivation in alpha serotypes (i.e. 9Aα and 11Eα) (3). Inactivation of this gene dictates seroswitching (i.e. 11Aα 11E).

Methods and Results

The multiplexed opsonophagocytic killing assay (MOPA) was used to observe the NSK response against *Streptococcus pneumonia* serotypes 9V, 9Aα, 9A, 11A, 11Eα, and 11E. MOPA was also used in order to opsonize 9V, 9A, 11A, and 11E using 10 sera samples from people who received the Pneumovax23 vaccine.

Conclusions

Strains with a functional wcjE (JC01=9V, JC03=11A) showed a lower percentage of non-specific killing than strains with a disrupted wcjE (JC02=9A, JC04=11E). MB01 (9Aα) and JC12 (11Eα) both showed higher non-specific killing than strains with disrupted wcjE. Immunizing with serotypes 9V and 11A cross-protects against serotypes 9A and 11E. This is possibly due to volatile nature of the O-acetate group, especially during vaccine preparations (4).

Future Research

Future research may seek to compare other serotypes with a functional and non-functional wcjE to validate that the inactivation of wcjE causes a higher NSK response in the host. Serogroups 15 and 33 may be beneficial to use. Simultaneously, research may inquire as to how adsorbtion with the capsule reduces NSK. For this, the 11A phenotype may be restored with 11E in the background, and it may be determined if NSK is reduced.

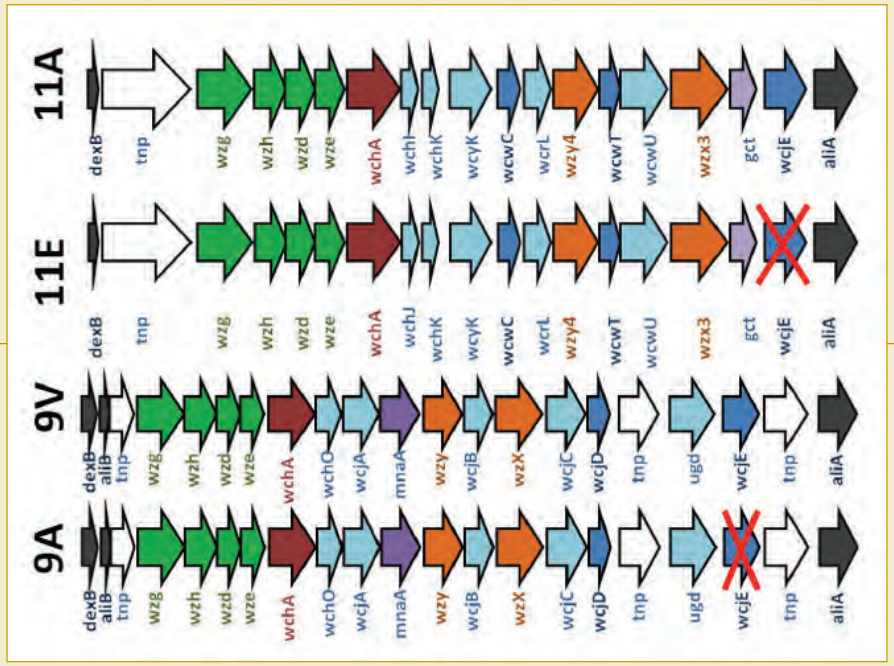


Figure 1. Cps locus. 9V (JC03) and 11A (JC01) are 2 serotypes that contain wcjE (1, 3). The only difference between 9V and 9A, as well as 11A and 11E, is the functionality of wcjE (1, 3).

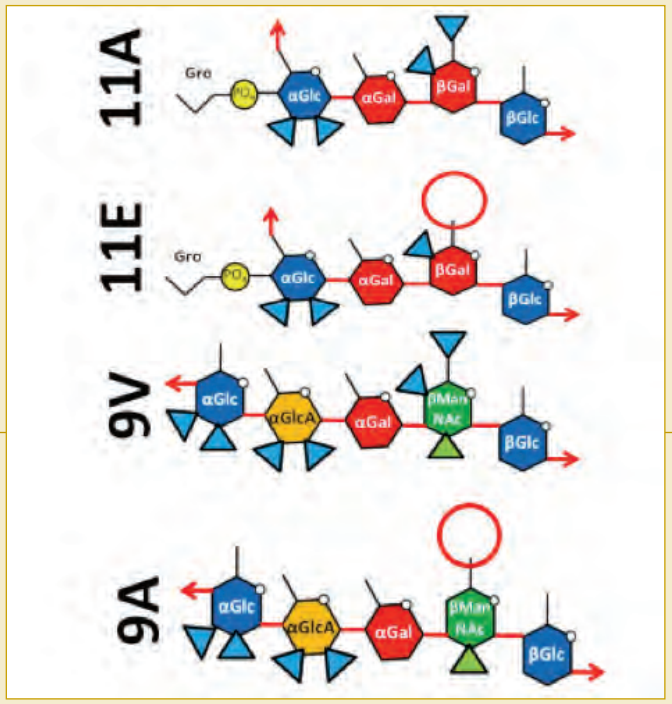


Figure 2. O-acetylation of Capsule. 9V polysaccharide capsule contains O-acetate groups (blue triangle) on the 6th codon (C6) of βManNAc, which 9A lacks (1, 5). 11A capsule also contains the βGal C6 O-acetylation, which 11E lacks (3).

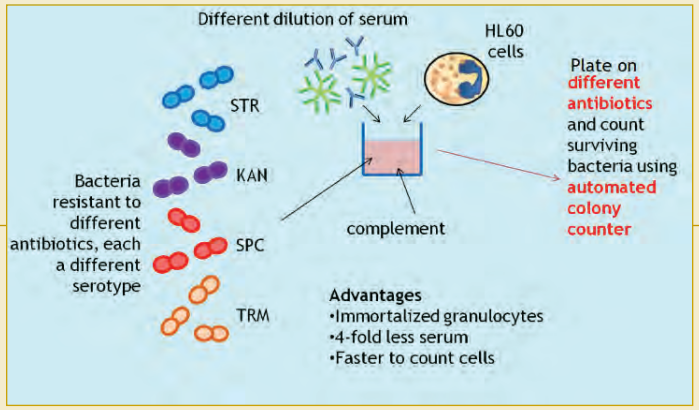
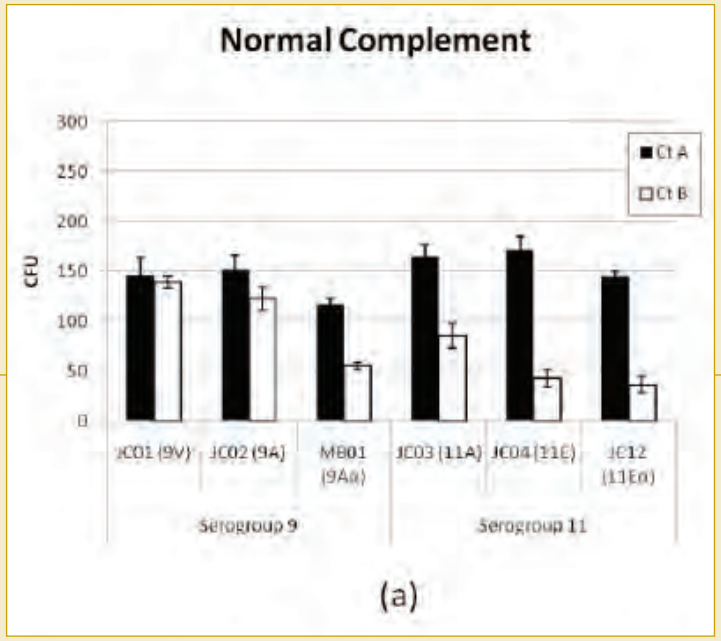


Figure 3. MOPA. The Pneumovax23 vaccine elicits antibodies to respond to the bacteria's capsule polysaccharide (2). The multiplexed opsophagocytic killing assay measures the capacity of antibodies against S. pneumoniae *and has been used to develop an effective vaccine. HL60 cells, which acted as phagocytes, and rabbit complement were used to create an environment similar to the natural environment in the host. A detailed protocol of the MOPA can be found at a www.vaccine.uab.edu (2).*



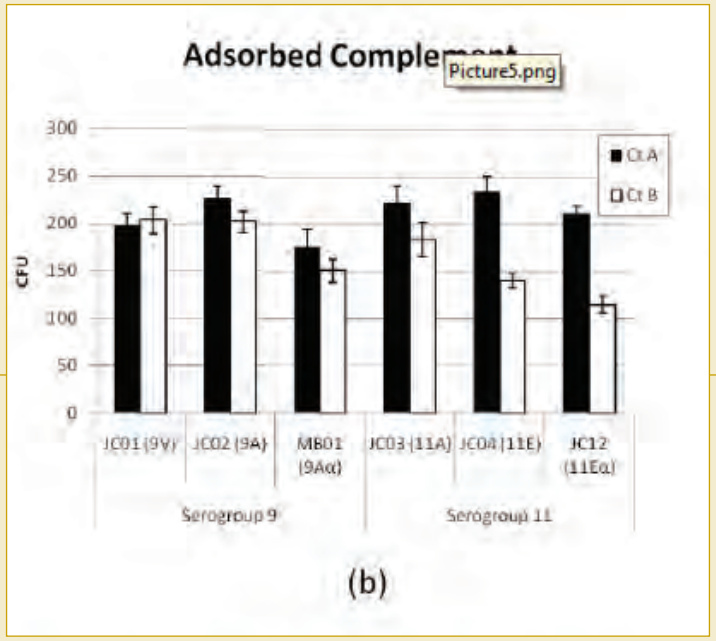


*Figure 4. Raw Data of the Normal and Adsorbed Complements. Control A (Ct A) includes the bacteria of interest, heat-inactivated complement, and HL60 cells. Control B (Ct B) is comprised of the same components with activated instead of heat-inactivated complement. The NSK was calculated using the following formula: (1-Ct B cfu/Ct A cfu) * 100. These graphs show that the NSK can be reduced by adsorbing the complement.*

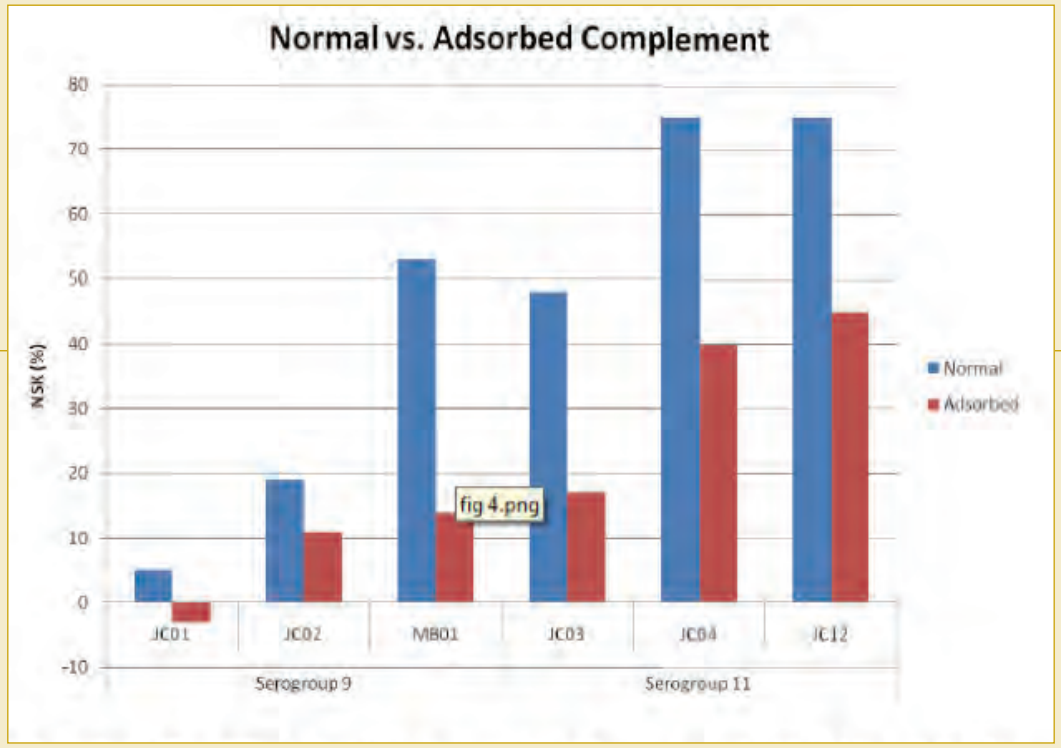


Figure 5. Non-Specific Killing Percentages Using Normal and Adsorbed Complement. Each strain was tested with normal and adsorbed complement. Adsorbed complement significantly reduced non-specific killing in all six serotypes.

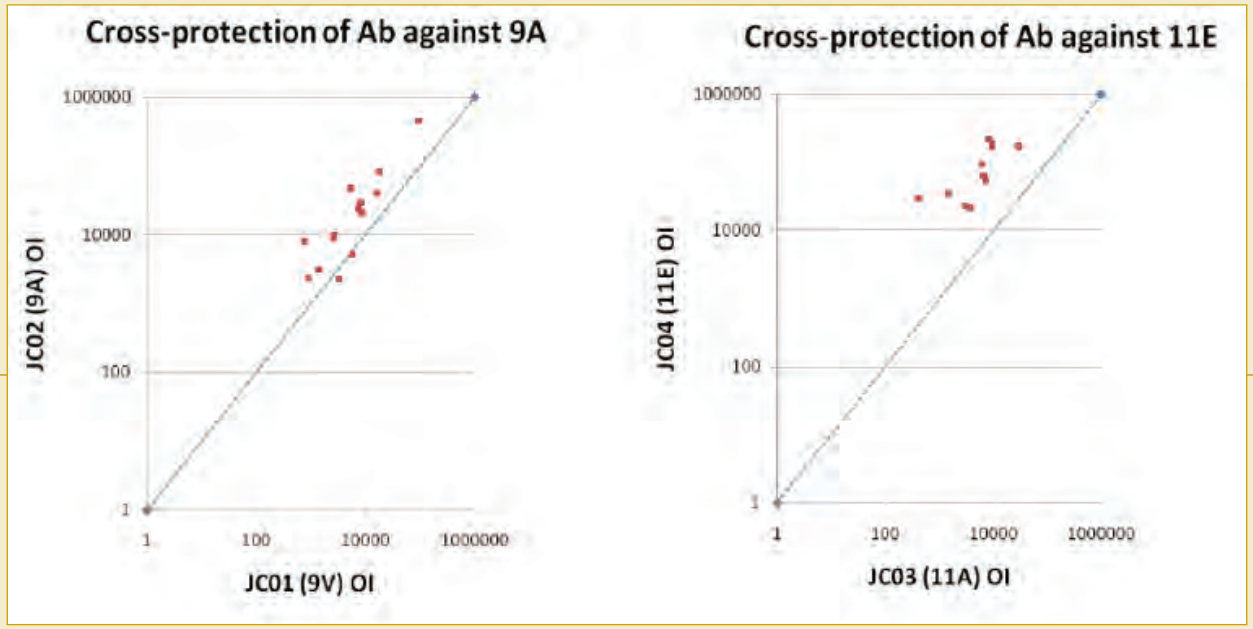


Figure 6. Cross-protection of Antibodies (Ab) against Serotypes 9A and 11E. (a) Sera displayed preferential killing of JC02. (b) Sera displayed preferential killing of JC04. The opsonizaiton (OI) index is the amount of diluted serum needed to kill 50% of the bacteria (2).

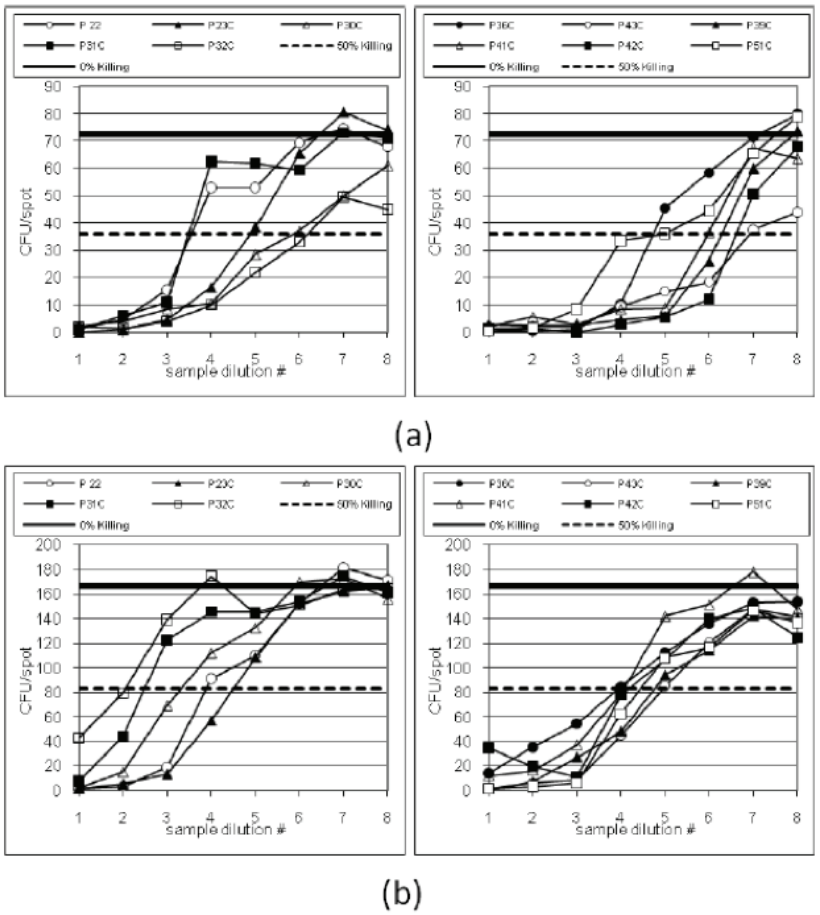


Figure 7. Examples of MOPA Data. These graphs display data from the MOPA for 9A (a) and 11A (b). The OI was determined from the data using a program called "Opsotiter 3" (2).

Acknowledgements
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