UNRESTRICTED VARIABLE LIGHT CHAIN RECOMBINATION WITH PNEUMOCOCCAL POLYSACCHARIDE SPECIFIC VARIABLE HEAVY CHAIN

Rebecca Thompson[1], Jason Mosakowski[1], Jieying Wang[1], Noor Khaskhely[1], HE Baxendale[2] and M.A.J. Westerink[1].
Department of Medicine. IIT Program. [1]University of Toledo Health Science Campus. [2]University College London.

Abstract

Background and aims: *Streptococcus pneumoniae* is a human bacterial pathogen which colonizes the nasopharynx and can result in pneumonia, meningitis and acute otitis media. The adult 23 valent pneumococcal polysaccharide (PPS) vaccine has an 80% protective efficacy in healthy young adults. However efficacy in the elderly is drastically reduced despite normal antibody levels. This phenomenon is thought to be linked to antibody structure.

Previous studies in both *Haemophilus influenza* type b polysaccharide (Hib PS) and pneumococcal polysaccharide (PPS) have suggested that the use of a specific variable light chain may be essential in maintaining polysaccharide epitope specificity. We now demonstrate that pneumococcal polysaccharide variable heavy gene usage is promiscuous in *in vitro* recombination with many variable light chains.

Methods: A PPS23F-specific variable heavy (V_H) chain was cloned into an expression cassette encoding the human IgG1 constant regions. The cassette was then paired with random variable light (V_L) chains derived from a PPS specific B cell and transfected into NSO cells. The secreted immunoglobulins were tested by ELISA and SPR for PPS binding.

Results: Though light chains were isolated from B cells specific for a variety of different PPS, when paired with a PPS23F specific V_H, the antibodies maintained specificity for PPS23F.

Conclusions: These findings suggest that the use of one specific variable light chain is not essential in retaining PPS specificity.

Introduction

Streptococcus pneumoniae is a significant cause of morbidity and mortality worldwide. The main virulence factor of *S. pneumoniae* is the capsular polysaccharide. Antibodies elicited against PPS provide protection against invasive disease. The adult 23 valent purified PPS vaccine has an 80% protective efficacy in healthy young adults. However efficacy in the elderly is drastically reduced despite normal antibody levels. This phenomenon is thought to be linked to antibody structure.

Previous data characterizing the structure-function relationship of anti-pneumococcal antibodies has been gathered primarily from the use of combinatorial libraries. Combinatorial libraries pool multiple B cells and PCR the variable immunoglobulin regions. Combinatorial libraries are used for antibody repertoire analysis and assume that the random heavy and light chain pairs formed represent the native repertoire. This is supported by data that demonstrates the *in vivo* use of similar variable heavy and light combinations.

The consistent pairing of various V_H with identical V_L, as seen in response to Hib (Reason) and PPS (Lucas, Zhou), suggests a restricted V_H-V_L pairing in nature. In response to Hib, the predominant variable light chain A2 is used in the repertoire across different individuals and within the same individual. The phenomenon responsible for this pairing limitation has yet to be elucidated.

The goal of our study was to explore the reported restricted gene usage in response to polysaccharide antigens and to determine if this observation is the result of physiological or structural limitations of antibodies that recognize polysaccharide antigens. To test these concepts one variable heavy chain specific for PPS23F was paired with several alternative PPS-specific variable light chains. Recombinant human antibodies were tested for functionality and influences on pneumococcal polysaccharide binding.

Materials and Methods

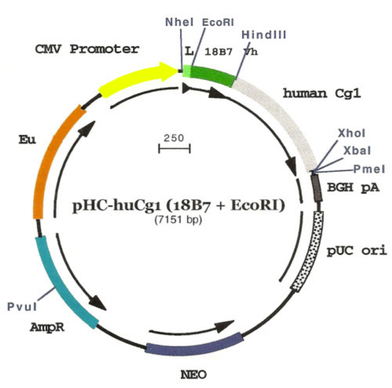
Pneumococcal polysaccharide vaccine naïve young volunteers between the ages 18-30 years participated in the IRB-approved study. Volunteers were immunized with the 23-valent PPS vaccine. Blood samples were collected pre-immunization, seven days and four weeks post-immunization.

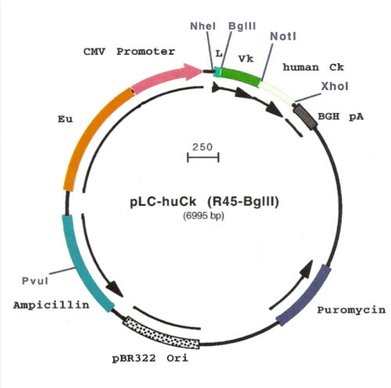
Single PPS23F positive B cells were isolated and the variable gene regions were amplified using PCR. PCR fragments were cloned, transformed and sequenced.

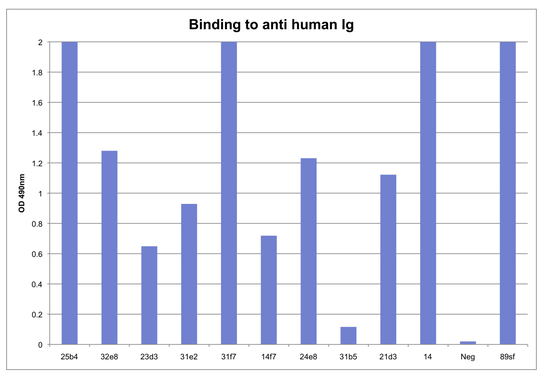
Variable light gene fragments were PCR amplified using transfer primers to add BglII and NotI restriction sites. CBE2, a PPS23F specific variable heavy chain was obtained from Baxendale *et al* and EcoRI and HindIII restriction sites were inserted. The variable chains were each ligated into the appropriate pHC-huC plasmid (McLean *et al*). pHC-huC containing variable heavy and light sequences were then transfected into NSO cells.

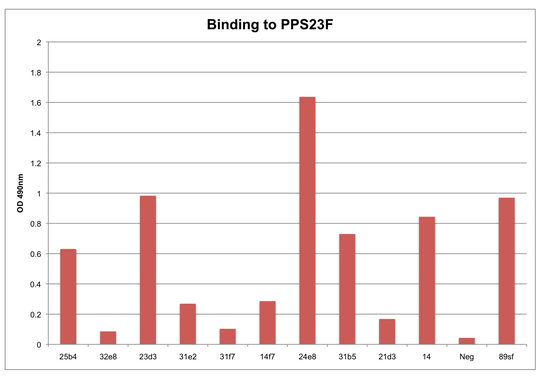
Surface plasmon resonance (SPR) measured the avidity of the antibody (Reichert 3700DC). The sensor chip with immobilized anti-human Ig antibody bound to its surface was used to capture recombinant human antibody. PPS23F was injected over the chip surface to determine binding avidity.

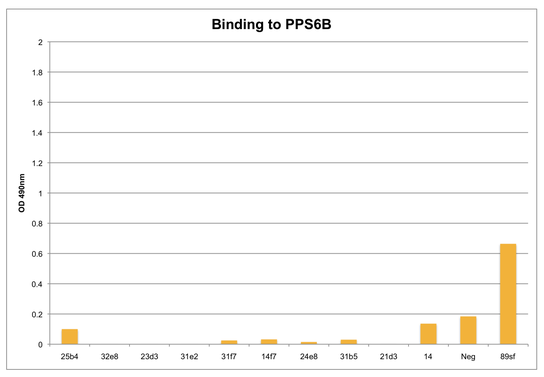
Clone	Isolating Antigen	VL	CDR3
21d3	PPS14	L2	QQYNNRPRT
14F2	PPS4	B3	QQYYSTPVT
23d3	PPS14	B3	QQYYSTPAT
24e8	PPS14	B3	QQYYSTPYT
25b4	PPS14	A27	QQYGSSPPWT
31b5	PPS23	A27	QQYDRSPLT
31F7	PPS23	L6	QQRSNWPPLLT
31e2	PPS23	A20	QKYNGAPFT
32e8	PPS23	O12	QRSSGGPIS

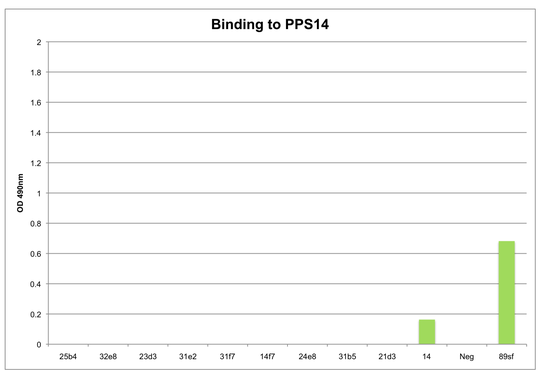


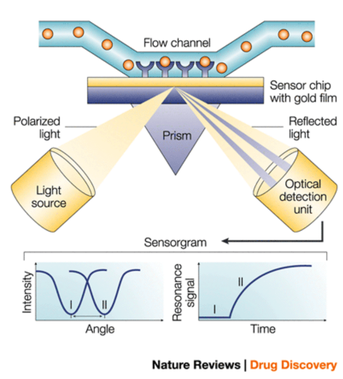


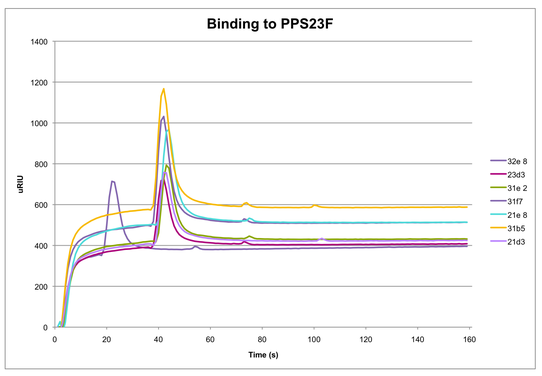












Results and Discussion

The CBE2 V_H was cloned and expressed paired with a variety of V_L chains isolated from different PPS. Analysis of these recombinant antibodies by ELISA demonstrates that these antibodies maintain their specificity for PPS23F. However, analysis with SPR demonstrates that these antibodies possess unique binding characteristics.

Although there are certain capsular polysaccharides where a specific variable light chain gene is required for epitope binding this does not seem to be the case with PPS23F. It is plausible that the variable light chain does not significantly contribute to the structural requirements of PPS23F binding. In contrast for Hib PS, an arginine in position 95a of the light chain is essential for binding. To date, we have not identified an essential amino acid sequence in the CDR3 of V_L for PPS.

In the future more clones will be studied by sequence analysis and binding avidity to PPS23F to better define the structural requirements necessary for PPS23F binding.

References

Baxendale HE, Goldblatt D. Correlation of molecular characteristics, isotype, and in vitro functional activity of human antipneumococcal monoclonal antibodies. Infect Immun. 2006 Feb;74(2):1025-31.

Lucas AH, McLean GR, Reason DC, O'Connor AP, Felton MC, Moulton KD. Molecular ontogeny of the human antibody repertoire to the *Haemophilus influenzae* type B polysaccharide: expression of canonical variable regions and their variants in vaccinated infants. Clin Immunol. 2003 Aug;108(2):119-27.

McLean GR, Nakouzi A, Casadevall A, Green NS. Human and murine immunoglobulin expression vector cassettes. Mol Immunol. 2000 Oct;37 (14):837-45.

Reason DC, O'Connor AP, Lucas AH. Structural determinants of the human idiotype HibId-1. J Mol Recognit. 2001 Nov-Dec;14(6):393-400.

Reason DC, Zhou J. Correlation of antigenic epitope and antibody gene usage in the human immune response to *Streptococcus pneumoniae* type 23F capsular polysaccharide. Clin Immunol. 2004 Apr;111(1):132-6.

Zhou J, Lottenbach KR, Barenkamp SJ, Lucas AH, Reason DC. Recurrent variable region gene usage and somatic mutation in the human antibody response to the capsular polysaccharide of *Streptococcus pneumoniae* type 23F. Infect Immun. 2002 Aug;70(8):4083-91.

Acknowledgements

This study was supported by grants AG15978 from the NIA and 1R56AI081558 from the NIAID to MAJW.