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Immune Response to Pneumococcal Polysaccharides in Elderly and Young Adults: Expression of Human Recombinant Antibody Avidity and Isotype Fine Specificity

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Abstract

Streptococcus pneumoniae is a human bacterial pathogen which colonizes the nasopharynx. Infection may result in pneumonia, meningitis and acute otitis media. Adult vaccination in the U.S. includes 23 purified pneumococcal polysaccharide capsular serotypes. This vaccine has an 80% protective efficacy in healthy young adults. Yet despite comparable antibody levels in the elderly the efficacy is drastically reduced.

Previously our lab has shown a significant difference between elderly and healthy young adults in the immunoglobulin variable gene usage in response to polysaccharide. To investigate these findings further, a single B cell expansion system, which maintains physiologic variable heavy (VH) and variable light (VL) immunoglobulin chains, was utilized. The goal is to correlate specific human VH and VL natural pairings to potentially higher or lower antibody affinities.

A single B cell secreting antibody specific to pneumococcal polysaccharide 23F was isolated by flow cytometry, expanded in culture then cloned and sequenced. To fully investigate the role of specific genes in polysaccharide binding these native variable chain pairs were cloned into a human recombinant antibody expression vector, pHC-huC. pHC-huCG1 and pHC-huCG2 possess the human gamma 1 and gamma 2 constant region isotypes respectively. These two isotypes predominate the response to pneumococcal polysaccharide. Expressed recombinant human antibodies were analyzed by Western blot, ELISA and surface plasmon resonance (SPR). Enzymatic digestion of these human recombinant antibodies into Fab and (Fab')2 fragments will enable the fine analysis of the immunoglobulin structural components responsible for antibodies of greater affinity and avidity.

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.

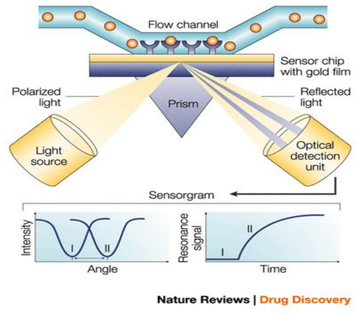
The goal of our study was to explore the reported restricted gene usage in response to polysaccharide antigens and to determine if this observation is the result of physiological or structural limitations of antibodies that recognize polysaccharide antigens. To test these concepts one variable heavy 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.

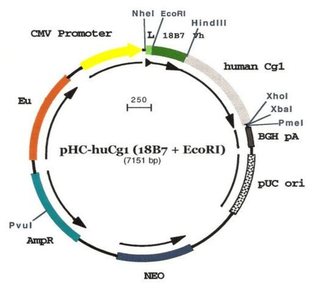
Materials and Methods

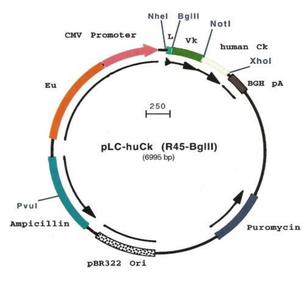
Pneumococcal polysaccharide vaccine naïve young volunteers between the ages 18-30 years participated in the IRB-approved study. Volunteers were immunized with the 23-valent PPS vaccine. Blood samples were collected pre-immunization, seven days and four weeks post-immunization. Single PPS23F positive B cells were isolated and the variable gene regions were amplified using PCR. PCR fragments were cloned, transformed and sequenced.

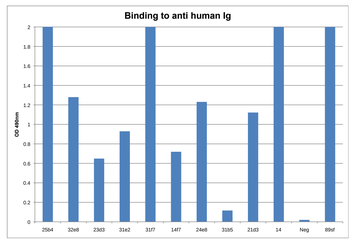
Variable light gene fragments were PCR amplified using transfer primers to add BglII and NotI restriction sites. CBE2, a PPS23F specific variable heavy chain was obtained from Baxendale *et al* and EcoRI and HindIII restriction sites were inserted. The variable chains were each ligated into the appropriate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

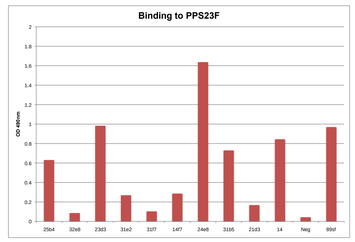
Surface plasmon resonance (SPR) measured the avidity of the antibody (Reichert 3700DC). The sensor chip with immobilized anti-human Ig antibody bound to its surface was used to capture recombinant human antibody. PPS23F was injected over the chip surface to determine binding avidity.

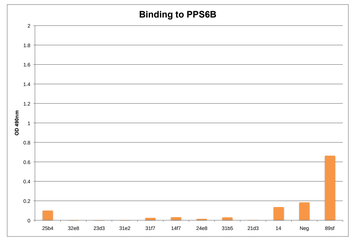


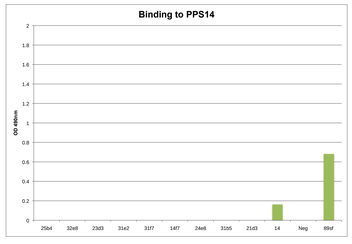


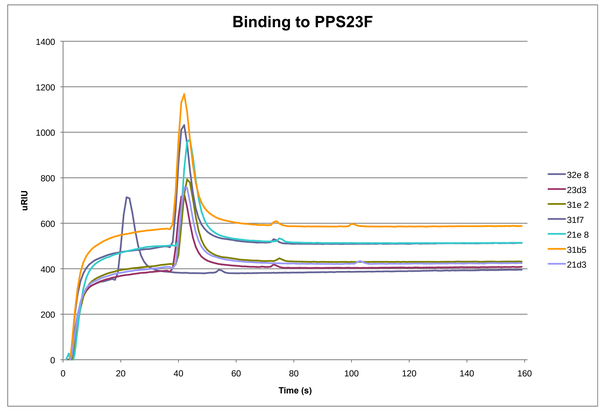












Clone	Isolating Antigen	VL	CDR3
21d3	PPS14	L2	QQYNNRPRT
14f2	PPS4	B3	QQYYSTPVT
23d3	PPS14	B3	QQYYSTPAT
24e8	PPS14	B3	QQYYSTPYT
25b4	PPS14	A27	QQYGSSPPWT
31b5	PPS23	A27	QQYDRSPLT
31f7	PPS23	L6	QQRSNWPPLLT
31e2	PPS23	A20	QKYNGAPFT
32e8	PPS23	O12	QRSSGGPIS

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.

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Acknowledgements

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