Immune Response to Pneumococcal Polysaccharides in Elderly and Young Adults: Expression of Human Recombinant Antibody Isotypes and Antibody Affinity

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Abstract

Streptococcus pneumoniae is a human bacterial pathogen which colonizes the nasopharynx resulting in pneumonia, meningitis and acute otitis media. The current vaccine administered to adults over the age of 65 in the U.S. includes 23 purified pneumococcal polysaccharide capsular serotypes. This vaccine has an 80% protective efficacy in healthy young adults however despite normal antibody levels the efficacy in the elderly is drastically reduced.

Our lab has previously shown a significant difference between elderly and young in the genes utilized in response to polysaccharide. We are further investigating the young and elderly response using a single B cell expansion system which maintains physiologic variable heavy (VH) and variable light (VL) immunoglobulin chains. Our aim over the next three years is to correlate specific human VH and VL natural pairings to potentially higher or lower antibody affinities.

B cells secreting antibodies specific to pneumococcal polysaccharide 14 or 23F were isolated by flow cytometry, expanded in single cell culture then cloned and sequenced. To fully investigate the role of specific genes in polysaccharide binding these paired variable chains were cloned into a mammalian recombinant antibody expression vector, pHC-huC. pHC-huCG1 and pHC-huCG2 possess the human gamma 1 and gamma 2 constant region isotypes respectively. The expression of the two isotypes predominate in the response to pneumococcal polysaccharide illustrates the shift in production of IgG2 to IgG1 as we age. Successfully expressed recombinant human antibodies were then analyzed by Western, 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

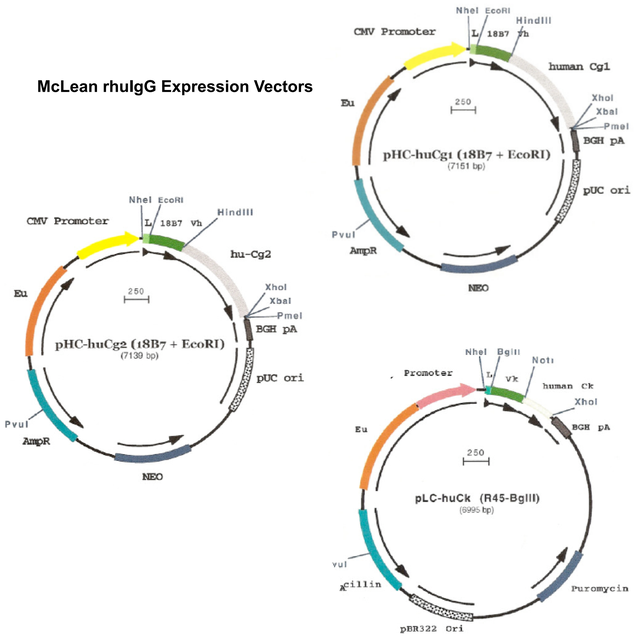
Streptococcus pneumoniae is a leading cause of morbidity and mortality in developed as well as developing countries. The organism colonizes the respiratory tract of healthy children and adults. It is estimated that *S. pneumoniae* causes 50,000 cases of bacteremia, 500,000 cases of pneumonia and 3,000 cases of meningitis per year in the United States alone, resulting in 40,000 deaths per year. Globally, pneumococcal infections have been estimated to cause 1.2 million deaths per year. Pneumococcal disease has a characteristic age distribution with the highest incidence seen at the extremes of age, the very young and the elderly. It is the most common organism isolated from elderly patients with community acquired pneumonia and accounts for at least 30% of all cases. Several reports have emphasized the importance of age in increasing the risk of acquiring pneumococcal infection.

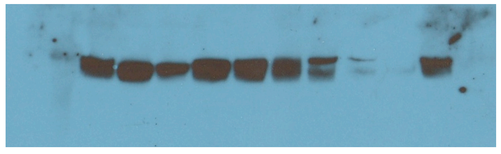
Despite the use of appropriate antibiotics and intensive care, the case fatality rate of pneumococcal bacteremia remains at 15 to 20% in children/young adults and 30 to 40% in the elderly, and this rate has not changed in the past 40 years. The currently available pneumococcal polysaccharide (PPS) vaccines are based on the observation that antibodies directed at the polysaccharide capsule protect against disease by mediating complement dependent opsonophagocytosis. Efficacy studies indicate that the currently licensed PPS vaccine is highly effective (±80%) in young adults. In contrast, several studies have noted a marked decreased efficacy in the elderly (44%). Paradoxically, studies designed to determine the post-vaccination antibody concentrations to the pneumococcal capsular polysaccharides in the elderly indicate that these levels are similar to those in younger adults.

In summary, pneumococcal vaccine studies suggest an age-related decrease in vaccine efficacy that appears to be in direct conflict with studies of post-vaccination antibody concentrations. Previous studies indicate that despite adequate IgG antibody concentrations, the elderly have a significant reduction in opsonophagocytic activity against all serotypes tested. Reduced functional activity of anti-pneumococcal PPS directed antibodies in the elderly may explain the discrepancy between antibody concentration and vaccine efficacy studies. However, the mechanisms responsible for these age-related changes in the immune response in humans remain to be elucidated.

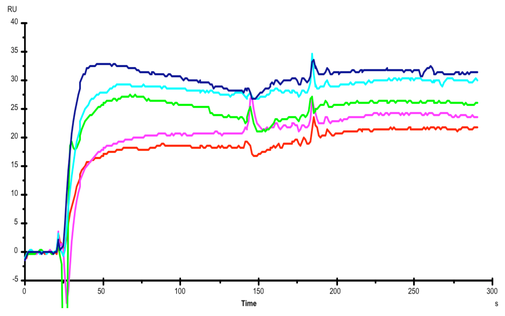
Materials and Methods

Briefly, VH and VL regions from high, intermediate and low affinity anti-PPS14 and PPS23F will be amplified from TOPO TA cloning vector by PCR using primers specific for the 5' and 3' regions of the heavy and light chains. The 5' primer for VH will be designed complementary to the 5' region with the addition of an *Eco*RI site and ribosome recognition site upstream of the start codon. The 3' primer for VH will be designed to be complementary to the J chain (3') region, with the addition of a *Hind*III site. For VL the 5' primer will encode an *Bgl*II restriction site and the 3' primer a *Not*I restriction site. PCR products will be cloned into pCR®2.1 TOPO® (Invitrogen, Carlsbad, CA) and sequence of several clones will be confirmed by sequence analysis. VH regions will be digested with *Eco*RI and *Hind*III and subcloned into either pHC-huCG1 or huCG2 expression vector. VL regions will be digested with *Bgl*II and *Not*I and subcloned into the pHC-huCK expression vector. Expression vectors will be amplified by transformation into *E. coli* Top 10 (Invitrogen, Carlsbad, CA) and vector DNA purified using an endotoxin free plasmid purification kit and sequenced (Qiagen, Valencia, CA). Expression vectors will be co-transfected into HEK293 cells and plated into 96 well plates at 10^4 cells/well. Forty-eight hours following transfection expression of IgG will be detected by ELISA. Differences in isotype binding will be analyzed by surface plasmon resonance and enzymatic digestion.





Western blot detecting expression of immunoglobulin by McLean rhuIgG vectors transfected into HEK293 cells.



BIAcore titration and K_D determination of anti-6B PPS antibody. The antibody was titrated at concentrations of 33, 47, 67, 100, 133 nM starting with the bottom curve and ascending.

Discussion

The subclass distribution of the immune response to the capsular polysaccharide of *S. pneumoniae* is clearly age-related. In response to both PPS and conjugate vaccines, children <5 years of age show a predominance of IgG1 antibodies, while adults demonstrate a predominance of IgG2. Furthermore, the IgG1:IgG2 ratio continues to decrease with advancing age resulting in a relative increase in IgG2 and further decrease in IgG1 in the elderly versus younger adults. Although several studies have tried to correlate antibody subclass with functional activity, the use of polyclonal serum containing antibodies with different VH/VL precludes meaningful data. Thus, at the present time the role of IgG subclass in functional activity against PPS has not been defined. Theoretically, based on increased complement activating ability, a classic effector function, one could argue that IgG1 may be more desirable. However, a number of studies have shown that the Fc region can influence antibody fine specificity and avidity, functions previously considered to be specific to the variable region. Moreover, the ability of IgG2 to form dimers may potentially result in more avid binding of polysaccharide antigens. These studies in fact implicate that affinity maturation can occur through (sub)class switching. Defining the relationship between IgG subclass and functional activity, as both Fv and Fc contribute to antibody avidity, is thus an essential component in studies designed to increase our understanding of the elderly immune response to PPS. Furthermore, the ability to manipulate subclass distribution in response to PPS by choice of protein carrier (ex. tetanus toxoid vs. CRM_{197}) or adjuvant illustrates the clinical relevance of these studies for future vaccine/adjuvant development aimed at the elderly population.

We will generate recombinant human antibodies of the IgG1 and IgG2 subclasses. We will use the VH and VL regions derived from one low, one intermediate and one high affinity anti-PPS14 and PPS23F antibody. The avidity (association and dissociation constants) as well as functional activity of the recombinant antibodies will be defined using SPR.

Summary

We have successfully cloned variable regions into the McLean expression vectors and tranfected into HEK293 cells. The expression of recombinant human immunoglobulin has been confirmed by Western blot. I am currently being trained on the surface plasmon resonance machine. Our lab has demonstrated the ability to calculate binding affinity using SPR technology. This will enable the comparison of affinities between the IgG1 and IgG2 isotypes while preserving the antibody variable region. In the future several other sequences will be cloned into the McLean expression vector and analyzed. π

Acknowledgements

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