Isolation and characterization of human polyreactive pneumococcal polysaccharide antibodies

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Abstract

Streptococcus pneumoniae is a human bacterial pathogen which colonizes the nasopharynx. *S. pneumoniae* is a major cause of pneumonia, meningitis and acute otitis media. The main virulence factor of *S. pneumoniae* is the capsular polysaccharide. Antibodies against the capsular polysaccharide provide protection against disease. Natural or polyreactive antibodies are believed to provide a first line of defense against pneumococcal invasion. These antibodies protect against infection and/or decrease the bacterial load if infection is already present. It is thought that human polyreactive antibodies are selected into the marginal zone B cell compartment, as demonstrated in immunoglobulin transgenic mice.

Polyreactive human pneumococcal polysaccharide (PPS) IgG antibodies have not been extensively studied. We analyzed polyreactive antibodies that bound PPS4, PPS6B, PPS14 and PPS23F. These antibodies were isolated from single PPS specific B-cells allowing for the analysis of human immunoglobulins with natively paired variable regions. Although isolated individually, these antibodies demonstrated similar characteristics. Each antibody possessed a variable light (VL) chain with a similar CDR3 length and a variable heavy (VH) chain with a significantly increased number of flexible amino acids. While these antibodies are polyreactive and structurally alike, kinetic analysis revealed unique KD values.

To analyze the role of the constant region of these antibodies and their affect on antibody avidity to PPS, these nonspecific variable regions were expressed as IgG1 or IgG2 and subjected to kinetic analysis using surface plasmon resonance. The IgG1 antibodies uniformly had a stronger avidity to PPS14 and PPS23F when compared to IgG2. To further emphasize the importance of the constant region in antibody avidity and fine specificity, analysis of the antibody's F(ab)'2 fragment binding to PPS14 and PPS23F resulted in similar KD values. These studies suggest that antigen recognition by polyreactive antibodies is determined by a conserved variable light chain CDR3 length and longer, more flexible variable heavy CDR3s when compared to pneumococcal polysaccharide-specific sequences while differences in specific avidities are modulated by antibody isotype.

Introduction

Streptococcus pneumoniae is a human bacterial pathogen which colonizes the nasopharynx and is a major cause of pneumonia, meningitis and acute otitis media. The main virulence factor of S. pneumoniae is the capsular polysaccharide. Antibodies against PPS provide protection against disease. Natural or nonspecific antibodies are believed to provide a first line of defense against pneumococcal invasion. These antibodies are part of the early phase of the immune reaction and protect against infection and/or decrease the bacterial load if infection is already present. Little is known about inherent human nonspecific PPS antibodies. It is thought that human polyreactive antibodies are selected into the marginal zone B cell compartment, as demonstrated in immunoglobulin transgenic mice.

Murine studies have identified a B cell subset responsible for the production of these antibodies. B1a cells are a distinct population characterized by the CD5 surface marker. B1a cells spontaneously secrete low avidity, polyreactive antibodies against PPSs and other antigens. Experimental evidence has suggested that the B1 cell population decreases with age possibly contributing to disease susceptibility.

We isolated PPS-binding single B cells and several B cell clones were identified that bound multiple PPSs. The goal of this study was to explore the structural and kinetic characteristics of isolated polyreactive human PPS antibodies. Furthermore, we analyzed the role of the antibody constant region by comparing avidity values for variable heavy and variable light (VH/VL) pairs expressed as IgG1, IgG2 and F(ab)'2.

Materials and Methods

Healthy young adult volunteers were vaccinated with Pneumovax®. Six days post-vaccination, blood was taken and peripheral blood leukocytes were isolated using lymphocyte separation media followed by red blood cell lysis. Next CD19+ B cells were incubated with fluorescently labeled pneumococcal polysaccharide. Using flow cytometry, PPS specific B cells were single-sorted into 96 wells plates.

After 3 weeks of growth, B cell culture supernatant was tested by ELISA to detect binding to PPS and immunoglobulin secretion. Positive B cell cultures were harvested and cells were lysed. First strand cDNA was constructed using reverse transcriptase and cellular mRNA. cDNA was used as a template for PCR.

The B cell immunoglobulin variable regions were PCRed and cloned into pCR2.1® vector and transformed into *E. coli*. Minipreps of plasmid DNA were sent for sequencing. After confirmation of variable region insertion, plasmids were used as a PCR template to add restriction sites for ligation into the expression vector. Several B cell clones were identified that bound to multiple PPS. The variable regions of these clones were ligated into recombinant human immunoglobulin expression cassettes and transfected into HEK293 cells for antibody secretion.

Polyreactive antibodies were analyzed to identify their unique characteristics using disruption ELISA, OPSA, sequence attributes and avidity calculation using SPR.

A

Clone	VH CDR3	# of Mutations	% Homology	V gene	J gene
34C10	AKDSRGSTPRAFDP	12	97	3-23	JH5b
33G8	ARDGRQQWLVRPYYYGMDV	5	99	1-18	JH6b
32E8	TKWRWQQSEFDY	29	93	3-7	JH4b
31E2	AKDSRGSTPRAFDP	36	88	3-66	JH5b
31B5	AKKAFSGYSPFDY	20	94	3-23	JH4b
21B2	ARNGGVGATDPPYYYYGMDV	5	99	3-30	JH6b
24F5	AKDRSLREYSSSWYYPFYYYGMDV	4	99	3-23	JH6b
33E2	AREEYYGSGFDP	4	99	3-48	JH5b

B

Clone	Vk CDR3	# of Mutations	% Homology	V gene	J gene
34C10	QKYNSAPFT	12	99	A20	JK3
33G8	QQANSFSLT	10	99	L5	JK4
32E8	QRSSGGPIS	35	90	O12	JK5
31E2	QKYNGAPFT	14	97	A20	JK3
31B5	QQYDRSPLT	15	98	A27	JK4
21B2	QRSSGGPIS	37	89	O12	JK5
24F5	QKYNGAPFT	15	93	A20	JK3
33E2	QQHYNTPT	14	98	B3	JK5

Figure 1. Polyreactive antibody sequencing. A) Variable heavy chain B) Variable light chain

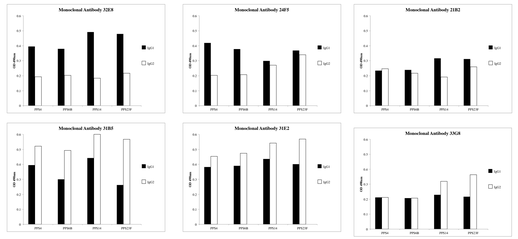

Figure 2. Polyreactive antibody binding to multiple PPS by ELISA.

Al	PPS14		PPS23F	
Clone	IgG1	IgG2	IgG1	IgG2
31B5	0.027	0.025	0.093	0.0935
32E8	0.11	0.076	0.14	0.089
33E2	0.151	0.0455	0.0454	0.0453

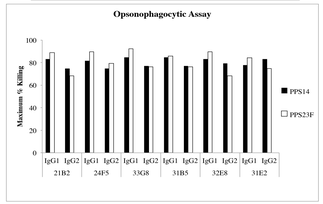

Figure 3. Polyreactive antibody functional activity.

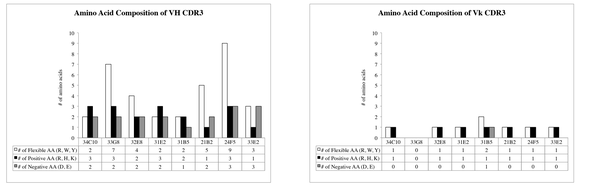

Figure 4. Number of flexible, positive and negative amino acids in CDR3 of polyreactive antibodies.

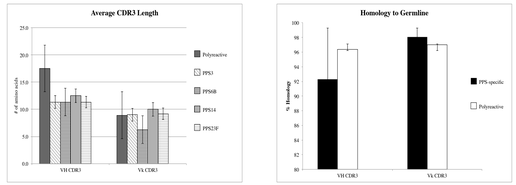

Figure 5. Comparison of polyreactive and PPS-specific antibodies.

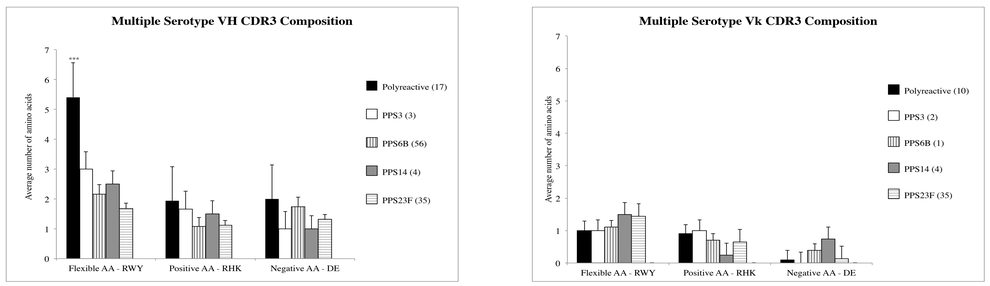

Figure 6. Average number of flexible, positive and negative amino acids in the CDR3 of polyreactive and PPS-specific antibodies.

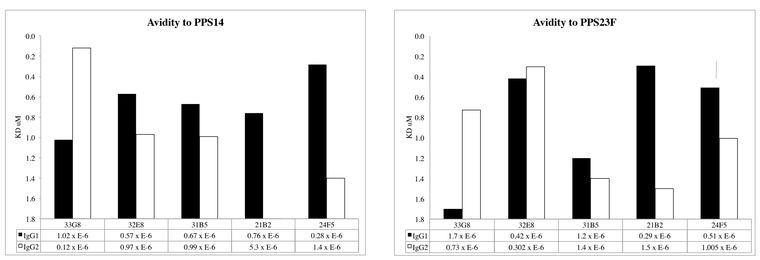

Figure 7. Comparison of polyreactive antibody avidity expressed as IgG1 or IgG2.

K_D	PPS14		PPS23F	
Clone	IgG1 F(ab')2	IgG2 F(ab')2	IgG1 F(ab')2	IgG2 F(ab')2
33G8	0.49492uM	0.5001uM	0.500uM	0.4989uM
21B2	0.5359uM	1.35362uM	0.69445uM	0.80091uM
24F5	1.07uM	1.16921uM	0.98uM	1.00uM

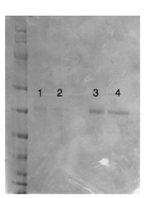

Figure 8. Avidity analysis of F(ab)'2 fragments.

Discussion

Low avidity, polyreactive antibodies are part of the body's first line of defense against pneumococcal infection. These natural antibodies recognize and bind a variety of foreign and self-antigens including protein and polysaccharide antigens. Natural antibodies have been reported for several bacterial polysaccharides however the characteristics of these antibodies remain to be elucidated. $CD5^+$ B1 cells are thought to be responsible for the production of natural antibodies in humans.

Natural antibody variable regions classically possess few mutations and are closely related to germ-line. Among the clones analyzed in our study the number of variable region mutations varied greatly. Casali *et al*. demonstrated that B1 cells could generate hypermutated antibodies. Moreover, using a variety of techniques including gene shuffling and site-directed mutagenesis, they showed that the immunoglobulin VH region provides the major structural predictor for antibody polyreactivity, mainly provided by the heavy chain CDR3. The ability of B1 cells to generate hypermutated antibodies may explain the high number of mutations in clones 32E8, 31E2 and 31B5. The amino acids arginine, tryptophan and tyrosine have been associated with antibody polyreactivity. These residues interact with a wide variety of antigens due to their ability to form molecular interactions. Pneumococcal polysaccharide-specific antibodies express fewer flexible amino acids on average when compared to polyreactive sequences. This decrease in number of flexible amino acids may diminish the flexibility of the antibody resulting in a more rigid and specific antibody.

The polyreactive antibodies we analyzed were expressed in IgG1 and IgG2 form. It has been reported that antibody isotype, thus antibody constant region, may influence antibody fine specificity and avidity. Consistent with previous reports, we also found a notable difference in binding avidity between IgG1 and IgG2 isotypes with identical VH/VL regions. The IgG1 isoform of most of our polyreactive monoclonal antibodies demonstrated a greater avidity and bactericidal activity than IgG2 isoforms expressing identical VL/VH pairs. Morelock *et al*. attributes this increased avidity of IgG1 isotype to unimpeded Fab arm movement and increased hinge flexibility. Structures that make the antigen binding site more flexible, may allow the antibody to bind multiple antigens. The IgG1 isoform possesses fewer disulfide bonds and more amino acids in the constant region resulting in greater flexibility. This increase in molecular flexibility may, in part, explain the overall increased avidity of the IgG1 isotype for these polyreactive monoclonal antibodies. Although the variable region is responsible for antigen recognition, the constant region of the antibody is necessary for stability, fine specificity and optimal avidity.

Summary

Polyreactive antibodies to PPS in our study despite low avidity by disruption ELISA and SPR were bactericidal against multiple serotypes of pneumococcus suggesting clinical significance. The VH regions expressed were mainly VH3, namely VH 3-23. Although VL conserved CDR3 length, VH CDR3 length varied greatly as did the number of mutations. Moreover, the VH CDR3 was characteristically longer than PPS-specific antibodies.

Additionally the CDR3 possessed more arginine, tyrosine and tryptophan amino acids allowing for a greater flexibility compared to PPS-specific antibodies. Each VH/VL pair was expressed as IgG1 and IgG2 isotypes. Overall for both PPS14 and PPS23F, the IgG1 isotype bound more avidity to PPS and was more functional corresponding to higher avidity suggesting a role of the constant region in antibody specificity and function. The common characteristics discovered in these human polyreactive antibodies may aid in establishing guidelines for the detection of the natural human anti-pneumococcal antibody in the repertoire.

Acknowledgements

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