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Paratope diversity in the human antibody response to *Bacillus anthracis* protective antigen

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Abstract

The active component of the licensed human anthrax vaccine (BioThrax™, or AVA) is a *Bacillus anthracis* toxin known as protective antigen (PA). Second generation anthrax vaccines currently under development are also based on a recombinant form of PA. Since the current and future anthrax vaccines are based on this toxin, it is important that the immunobiology of this protein in vaccinated humans be understood in detail. We have isolated and analyzed the PA-specific antibody repertoire from an AVA-vaccinated individual. When examined at the clonal level, we find an antibody response that is complex in terms of the combinatorial elements and immunoglobulin variable genes employed. All PA-specific antibodies had undergone somatic hypermutation and class switch recombination, both signs of affinity maturation. Although the antigenic epitopes recognized by the response were distributed throughout the PA monomer, the majority of antibodies arising in this individual following vaccination recognize determinants located on the amino-terminal (PA_{20}) sub-domain of the molecule. This latter finding may have implications for the rational design of future PA-based anthrax vaccines.

Keywords

Bacillus anthracis; protective antigen; PA; antibody repertoire; repertoire analysis; anthrax; vaccine; human immune response; human monoclonal antibody

Introduction

The currently licensed anthrax vaccine (AVA or BioThrax™; Bioport Corporation, Lansing Michigan) consists of a sterile, bacteria-free filtrate prepared from a culture of a non-encapsulated *Bacillus anthracis* strain designated V770-NP1-R. In addition to various bacterial products, the vaccine is formulated to contain aluminum hydroxide as an adjuvant, benzethonium chloride as a preservative, and formaldehyde as a stabilizer (AVA, 2002). The primary immunogenic ingredient is the cell surface recognition component of the tripartite anthrax toxin complex known as protective antigen (PA). The vaccination series consist of

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three subcutaneous injections at 0, 2, and 4 weeks, and three booster vaccinations at 6, 12, and 18 months. Annual booster immunizations are recommended (AVA, 2002).

Although the vaccine itself is poorly characterized, a substantial body of evidence demonstrates that the toxin component PA is both necessary and sufficient to produce a protective antibody response following vaccination (Leppla et al., 2002). The undefined nature of AVA, along with the extended dosing schedule and requirement for yearly boosters have driven attempts to develop a more practical vaccine that is better characterized, well tolerated, and immunogenic. A vaccine containing purified recombinant PA (rPA) is currently under development as a replacement for AVA, and is in clinical trials to determine safety and immunogenicity. Since both AVA and the next generation vaccine are based on PA, it is important that the immunobiology of the human response to PA be understood in detail.

Reported here is the isolation and molecular analysis of the PA-specific antibody repertoire derived from an AVA-vaccinated individual who was enrolled in a CDC-sponsored clinical trial designed to address changes in route of administration and immunization regimens. The antibody response in this recipient was complex in terms of variable (V) gene usage, the combinatorial elements utilized, and the specific PA epitopes recognized. All PA-specific antibodies had undergone somatic hypermutation (SHM) and class switch recombination (CSR), both signs of affinity maturation. We have also determined that the majority of individual antibodies arising in this individual following vaccination recognize antigenic epitopes located in the amino-terminal (PA_{20}) sub-domain of the PA monomer. This latter finding may have implications for toxin neutralization and the rational design of future PA-based anthrax vaccines.

Materials and Methods

Subjects

The donor analyzed in this report was recruited from individuals taking part in a larger study of the response to AVA being conducted at Baylor College of Medicine. Human subject protocols were reviewed and approved by the Institutional Review Boards at both Children's Hospital Oakland and Baylor College of Medicine.

Construction of Fab expression libraries

Fab expression libraries were constructed from MNCs enriched for PA-specific B cells in a manner similar to that previously described for polysaccharide-specific expression libraries (Reason et al., 1997; Reason and Zhou, 2006; Zhou et al., 2002; Zhou et al., 2004). PA, PA_{20}, and PA_{63} were purchased from List Biological Laboratories, Campbell, CA. PA-specific Fabs were identified using a sensitive ^{125}I-labeled PA capture assay and lysates of individual *E. coli* expression cultures. Positive isolates were re-cloned, heavy (H) and light (L) chain gene sequence determined, and PA-specific binding confirmed by ELISA. Initial sequence analysis utilized the NCBI IgBlast server (http://www.ncbi.nlm.nih.gov/igblast/) to identify candidate germline gene (Altschul et al., 1997). Subsequent analysis, alignments and translations were performed using MacVector (Accelrys Inc, Princeton, NJ). H and L chain V region gene nomenclature is as described in the IMGT database (Lefranc et al., 1999; Matsuda et al., 1998). Complementarity determining regions (CDRs) are as defined in (Kabat et al., 1991). Selected Fab clones were converted to full chain IgG1 antibodies and expressed in Chinese Hamster Ovary (CHO) cells using an in house PCI-derived bicistronic eukaryotic expression vector. Antibody was concentrated from the cell culture supernatant for use in binding assays.

Construction of PA20- and D4-GFP fusion proteins

The amino-terminal (residues 1-191) and the domain 4 carboxy-terminal (residues 587 – 735) portion of the PA monomer were cloned using PCR and expressed fused to intact green fluorescent protein (GFP). Cloning primers for the amino-terminal fragment were ATATGAATTCTATGGAAGTTAAACAGGAGAACCG (5') and ATATGGAT CCTCCTTCTACCTCTAATGAATC (3'). Cloning primers for the domain 4 region were GCATTAGAATTCGCATCACCATCACCATCACATGAATATTTTAATAAGA GATAAACG (5') and CGTATATCTAGAAGGATCCCCTATCTCATAGCCTTTTTTAGAAAAGAT (3'). Fusion proteins were expressed in *E. coli* and purified by nickel-chelate chromatography.

Domain specificity of PA-specific antibodies

The domain specificity of individual PA-specific antibodies was determined using capture assays, western blots of proteolytic fragments of PA, and western blots of PA_{20}- and D4-GFP fusion proteins. In capture assays, 96-well plates coated with light chain-specific antibody were used to capture individual PA-specific antibodies. Plates were then washed and incubated with radio-labeled PA_{83}, PA_{63}, PA_{20}, or D4-GFP. Binding was detected using PhosphorImager detection plates (Molecular Dynamics, Sunnyvale, CA). For western blots of proteolytic PA fragments, 1 μg each of PA_{83}, PA_{63}, and PA_{20} were electrophoresed using 4-12% Bis-Tris polyacrylamide gels (NuPAGE, Carlsbad, CA), electrically transferred to PVDF membranes, and probed with individual PA-specific antibodies. Binding was visualized by means of an alkaline-phosphatase conjugated goat antibody specific for human kappa or lambda light chains followed by BCIP/NBT color development. Western blots of PA_{20}- and D4-GFP fusion proteins were processed in a similar fashion.

Antigen binding and Fab concentration assays

Fab concentration was determined by a capture ELISA in which goat anti-human Fd (The Binding Site, Birmingham, UK) or goat anti-IgA (Sigma, St. Louis, MO) immobilized on a microtiter plate captures Fab which is then detected by alkaline-phosphatase labeled goat anti-human L chain (Biosource International, Camarillo, CA). This assay is standardized with a purified Fab standard whose concentration was calculated from UV absorbance at 280 nm. A binding in ELISA was determined for both Fabs and full-chain IgG1 antibodies on 96-well plates coated with 5 μg/ml PA_{83} and developed with alkaline-phosphatase conjugated goat antibody specific for human kappa or lambda light chains.

Results

Approximately 6400 individual Fab clones were screened for antigen-specific binding. Fifty-nine PA-specific Fabs were isolated, of which 35 were unique in V_H sequence, V_L sequence, or both (summarized in Table 1). These clonal isolates bound PA in an antigen-specific and concentration dependent manner (Figure 1). The 35 unique Fabs represented 11 independent V_H gene rearrangements (utilizing 8 different V_H genes) and 11 unique V_L rearrangements (utilizing 10 different V_L genes). All V regions were mutated as compared to their germline gene of origin. Both kappa and lambda light chains were utilized in the response. In concordance with serum antibody, IgG1 was the predominant heavy chain isotype, although IgG2, IgG3, and IgA isotypes were also isolated. For ease of reference, the isolates have been grouped into families based on their unique V_H gene rearrangement (Table 1).

Somatic hypermutation

All V_H genes (Table 2) and V_L genes (Table 3) were mutated as compared to their germline gene of origin. Mutations were seen in both the complementarity determining regions (CDRs)

as well as the framework regions of the V genes. Mutations were rarely observed in the light or heavy chain constant region (average of 0.3 mutations/constant region), indicating that the observed mutations are most likely generated during somatic hypermutation and are unlikely to have arisen as PCR artifacts. The majority of V_L mutations (52%) and 47% of V_H mutations were associated with the RGYW/WRCY sequence motifs recognized as "hotspots" of mutation during activation-induced cytidine deaminase (AID)-mediated hypermutation of immunoglobulin genes (Wagner and Neuberger, 1996). These frequencies are roughly twice that of the occurrence of these motifs in the germline V genes. The isolation of several clonally related members of the IGHV3-15 family illustrates the degree to which the PA-specific antibody repertoire can be diversified by somatic hypermutation, even within the progeny of a single B cell rearrangement event. The V_H members of this family had highly similar CDR3 regions, were all rearranged with J_H1, and shared several mutations as compared with the IGHV3-15 germline gene. The V_L members of this family were all rearranged with $J_\lambda3$, had a non-encoded residue inserted at the V/J junction, and shared several mutations when compared to the IGLV1-40 lambda germ line gene of origin. It is therefore likely that the members of this family derived from a single initial rearrangement event and diverged in sequence as a result of somatic hypermutation during clonal expansion. It should be noted that only those derivative clones retaining affinity for antigen (PA) would be detected using our methodology.

Class Switch Recombination

The heavy chain isotype of serum antibody directed against PA is primarily IgG1. Serum antibodies directed against protein antigens are in general dominated by the IgG1 isotype (Hammarstrom et al., 1987; Papadea and Check, 1989). Thirty-one of the 35 paratopes reported herein are also IgG1 in concordance with this fact. A single member of the IGHV3-15 family (55C7; Table 1) had undergone secondary CSR to IgA1. The sharing by this clone of the CDR3 sequence and several mutations with the IgG1 isolates of this family (Table 2) indicates this secondary CSR occurred in a IgG1 precursor, and not directly from the (presumed) IgM progenitor during the course of ongoing SHM. The alternative interpretation, that of an IgA1 precursor giving rise to the IgG1 clonal isolates is less likely, due to the relative position of the IgG1 and IgA1 constant region genes in the germline, and the mechanism responsible for CSR which results in the deletion of intervening gene sequences (Jack et al., 1988; Mills et al., 1992). The lack of sibling V_H sequences for the single IgG3 isolate (4A12; Table 1) prevents placement of this CSR in the context of ongoing SHM. The IGHV4-59 family sequences (Table 2) are notable in that, although all are mutated as compared to the germline gene of origin, the high degree of sequence similarity in the 4 members of this group indicate that CSR giving rise to IgG1, IgG2, and IgA1 isotypes occurred late in the divergence of these clones, and without additional SHM following class switch.

Sub-domain specificity

The PA molecule is an 83 kd protein and as such would be expected to present a variety of antigenic epitopes to the immune system. The domain structure of PA is understood in detail (Brossier et al., 2000; Little and Lowe, 1991; Petosa et al., 1997; Singh et al., 1991), and various functional aspects of the molecule have been ascribed to the various sub-domains of the molecule (Ahuja et al., 2001; Cunningham et al., 2002; Novak et al., 1992). Radio-labeled capture assays and western blots were utilized to determine the domain specificity of the various PA-specific paratopes described in this study. In addition to the purified proteolytic fragments PA_{63} and PA_{20}, GFP-fusion proteins containing the amino-terminal PA_{20} domain (residues 1-191) and the caboxy-terminal domain 4 (residues 587-735) were constructed, expressed, and purified for use in capture assays and Western blots. The results of these assays are shown in Table 4. As would be expected, domain specificity was consistent within those paratope families in which multiple members were available for analysis. Seven of the 11 paratope families were specific for epitopes found in the PA_{20} portion of the molecule. Two bound to

epitopes found in domain 4, and 2 bound to residues within PA_{63} but not associated with domain 4. Although the three assays used (capture assay, western blot of fusion proteins, western blot of proteolytic fragments) were not contradictory for any of the assayed paratopes, there were a few cases where they were not internally consistent. Clone 11A11, for example, bound PA_{20} in the capture assay, and the PA_{20}-GFP fusion protein in the Western blot, but did not bind the PA_{20} proteolytic fragment in the western blot. We believe these cases are indicative of an alteration of epitope structure in the different methods of fragment preparation and presentation, and highlight the necessity of utilizing multiple methodologies when assaying epitope specificity.

Discussion

The currently approved vaccine used for the prevention of anthrax infection is safe and, to the degree to which it can be tested, effective (Friedlander et al., 1999; Sever et al., 2004; Sever et al., 2002). It has several shortcomings, however. The requirement of an extended 18 month immunization regimen before full protection is achieved, the necessity of yearly booster vaccinations to maintain protective levels of serum antibody, and the perception (although unsupported) of adverse side effects associated with immunization have driven attempts to develop a new, or "second generation" vaccine formulation with improved performance characteristics (Brey, 2005; Leppla et al., 2002). These vaccines have recombinant PA as their active component, and are currently in clinical trials. Initial results suggest little improvement in immunogenicity as compared to AVA (Gorse et al., 2006). An understanding of the immunobiology of the human antibody response to PA at the molecular level may facilitate the more rational design of a vaccine candidate.

The biology of the anthrax toxin system has been extensively studied and is understood in detail (reviewed in (Brossier and Mock, 2001; Leppla, 1995)). PA is an 83 kd bi-functional protein that recognizes the widely distributed cell surface receptors TEM8 and CCMG and provides cell targeting (Bradley et al., 2001; Scobie et al., 2003). Binding is rapidly followed by furin-mediated cleavage of PA_{83} at the RKKR motif (residues 164-167) to produce an amino terminal fragment (PA_{20}), and the remainder of the PA monomer (PA_{63}) (Brossier and Mock, 2001). PA_{20} is thought to have no further role in intoxication. Cleavage allows PA_{63} to form ring-shaped heptamers on the cell surface, and also exposes binding sites for the other two members of the *B. anthracis* toxin system, Lethal Factor (LF) and Edema factor (EF). Following heptamerization and LF and/or EF binding, the entire complex is internalized by means of receptor mediated endocytosis (Brossier and Mock, 2001). Once within the acidic environment of the endocytic vacuole, the lytic components of the toxin system are actively transported across the endocytic vacuole membrane into the cytoplasm of the cells (Ren et al., 2004). Lethal factor (776 residues) is a zinc protease with specificity for several members of the mitogen-activated protein kinase (MAPKK) family (Brossier and Mock, 2001). Edema factor (767 residues) is an adenylate cyclase and provokes a substantial increase in intracellular cAMP (Brossier and Mock, 2001).

Our research utilizes repertoire cloning as a methodology for analyzing antigen-specific antibody response in humans (Lucas et al., 2001; Reason and Zhou, 2004; Zhou et al., 2002; Zhou et al., 2004). In the case of complex immunogens such as proteins, it allows us to analyze the monoclonal components of the antibody response in terms of their individual epitopes and functionality. By analyzing different individuals in a population, we can also determine the degree to which different members of the population utilize the same mechanisms in generating antibody diversity. A shortcoming of this approach is that native H and L chain pairing is lost. We have shown through direct protein sequencing and idiotypic analysis that repertoire cloning faithfully reproduces the serum repertoire of polysaccharide-specific antibodies that arise in vaccinated individuals (Reason et al., 1997; Zhou et al., 2002; Zhou et al., 2004), and that in

these systems *de novo* antigen-specific paratopes are not created by this methodology. Protein-specific antibody repertoires are more diverse and, as demonstrated in this study, even a highly enriched protein-specific B cell population contains multiple paratopes utilizing several different H and L chains. We cannot discount that combinatorial pairing in such a complex mixture might give rise to an antigen-binding Fab not present in the *in vivo* population, especially in cases where the majority of the contact residues are located on one chain. We have therefore eliminated from analysis those Fabs in which V gene pairing deviated from the majority of isolates for that family and which demonstrated reduced binding affinity for PA. This exclusion criteria is informal, however, and the data must be interpreted with this in mind. And, although our analysis was fairly extensive, it is likely that screening of additional clones from the expression library would reveal additional PA-specific Fabs. As a result of these two facts, we may have underrepresented the complexity of the response in this donor.

Combinatorial Diversity in the response to PA

In addition to the recruitment of 8 V_H and 10 V_L germline genes, a variety of previously understood mechanisms combined to diversify the initial response to PA in this individual. Three different rearrangements of the IGHV3-30 germline gene with two J region gene segments (Table 2) generate substantial diversity in the H chain CDR3 region, both in terms of amino acid sequence and in length. Likewise, two separate IGHV 3-33 rearrangements were identified based on their differing CDR3 sequences and lengths. The three IGHV3-30 rearrangements pair with different V_L genes (both kappa and lambda) as do the two IGHV3-33 rearrangements. The insertion of non-encoded residues at the V-J junction (the IGLV1-40 lambda L chains), as well as the deletion of germline encoded residues at this junction (the IGLV3-1 lambda L chain), have been demonstrated in other antigen-specific repertoires and are examples of diversifying events that take place during the initial stages of V gene rearrangement. VJ and VDJ joining, V_H/V_L pairing, V_H CDR3 generation, and the insertion and/or deletion of residues at the V/J junction are all examples of diversification that occur independent of antigenic stimulation early in B cell development.

Somatic hypermutation in the response to PA

A major source of diversity in this donor's overall response to PA was somatic hypermutation. SHM is antigen driven and therefore occurs following vaccination or infection. SHM is also believed to require the recruitment of T cells or T cell derived cytokines (Miller et al., 1995). All of the V genes utilized by these paratopes were mutated as compared to their germline gene of origin. Mutations were restricted to the V regions in both V_H and V_L sequences, and were highly correlated with the RGYW/WRCY motifs that are the established targets of AID-mediated SHM (Wagner and Neuberger, 1996). Mutations were not confined to the CDRs and occurred in the framework regions as well. It is difficult to recognize mutations that occur in the CDR3 region of the V_H gene, as there are usually no well-defined germline elements available for comparison. Our isolation of several members of the IGHV3-15 family allow us to demonstrate that SHM occurs in the V_H CDR3 region as well. The lack of mutations in the L chain constant region and the CH1 region of the H chains indicates that these mutations are physiologic in origin, and not the result of PCR artifact. In the two cases where multiple rearrangements of the same germline genes were isolated (the IGHV 3-30 and IGHV 3-33 families), there was a tendency for mutations to target the same CDR residues across the different rearrangements (Table 2), possibly indicating residues that are in contact with the antigen.

Class switch recombination

IgG1 is the principal antigen-specific antibody isotype in hyperimmune antisera raised to protein antigens such as PA. This isotype bias is reflected in the predominance of IgG1 in the

Fabs we isolated from this individual. It is of interest to note that in the IGHV4-59 family where secondary CSR did occur (Table 1), SHM was apparently restricted to that period prior to the class switch, resulting in antibodies with mutated but identical paratopes associated with IgG1, IgG2, and IgA1 isotypes. All IGKV2-30 light chain members of this family were likewise identical in sequence. It is likely that SHM in these clones terminated at the point of secondary CSR. Although more difficult to interpret, the single IgA1 member of the IGHV3-15 family (Table 2) is identical in V region sequence to the IgG1 clone 65H8, and may represent another example of SHM terminating following secondary CSR. This is in contrast to what has been reported for the response to carbohydrate antigens where CSR has been shown to occur throughout the course of ongoing SHM (Reason and Zhou, 2006;Zhou et al., 2002;Zhou et al., 2004).

Domain specificity of the component paratopes

The serum antibody response to any complex protein antigen is the summation of contributions made by the individual responding B cell clones. The analytical techniques we employ allow us to characterize both the structure and the contribution made by each of the individual paratopes to the total antibody response. We used several different assays to access the domain specificity of the paratopes we isolated. Since all assays involve some modification or possible partial denaturation of the antigen, the use of multiple assays increases the likelihood of preserving targeted epitopes. In addition, the PA_{20}-GFP fusion protein was designed to retain the furin cleavage site, thereby allowing us to detect epitopes that span that junction. Together, these techniques allowed us to unambiguously assign each paratope to a corresponding region of the toxin. Although low in resolution, epitope mapping at the domain level permits an estimation of the minimum number of epitopes recognized in the response, and suggest mechanisms by which they might function to neutralize toxicity.

The procedures we employ to generate and identify PA-specific Fabs may in some way bias our results towards paratopes that bind a particular sub-domain of the molecule, but we believe this to be unlikely. The PA used for biotinylation, cell selection, radio-iodination, and screening is a highly purified 83 kd molecule that appears on silver-stained PAGE gels to be free of any significant contamination with smaller proteolytic fragments. Residues available for biotinylation and radio-iodination are evenly distributed throughout the molecule. Individual colonies are screened by capturing ^{125}I-PA from solution to maximize the epitope integrity of the antigen. The complex distribution of H and L V genes utilized in the PA-specific response make it unlikely that an epitope bias could be introduced during the initial PCR reaction based on primer design. Taken together, these factors suggest that the epitope distribution we observe for our isolated Fabs reflects the epitope distribution present in the ongoing immune response in the vaccinated individual.

Paratopes were identified that recognized epitopes in domain 4, in PA_{63} (excluding domain 4), and in PA_{20}. As would be expected, in those families with multiple diversified isolates, domain specificity was conserved across all isolates, although relative affinity appeared to decrease in some due to their accumulated somatic mutations. Although the PA_{20} domain of the molecule constitutes less than 25% of the mass of the intact monomer, a majority (7/11) of the paratope families isolated from this individual react exclusively with antigenic epitopes associated with this amino-terminal sub-domain. If this epitope distribution is true for the majority of vaccine recipients, it may have implications for vaccine effectiveness. PA_{20} is cleaved from the remaining part of the molecule immediately after binding to the cell surface receptor, and is not thought to play any further role in intoxication. Although antibodies binding to the PA_{20} region of the molecule could conceivably block furin mediated cleavage and thus be protective, it is also possible that although these antibodies bind PA with high avidity, they are non-functional in terms of toxin neutralization. The mechanism responsible for this bias in

epitope distribution is unknown, and may include factors intrinsic to the primary amino acid sequence of the two fragments. However, it may also arise from differences in antigen processing and peptide presentation. PA_{20} most likely enters the antigen processing pathway through the route established for foreign proteins (Brodsky and Guagliardi, 1991). PA_{63}, on the other hand, directs its own entry to the cell. It is possible that these two different entry pathways result in a difference in the efficiency of peptide presentation. The bias in antibody epitope specificity towards determinants associated with PA_{20} might therefore arise as a secondary result deriving from this difference in antigen presentation.

Conclusion

Our findings in this one individual indicate that the anthrax vaccine formulation currently in use recruits a diverse variety of responding B cell of clones.Furthermore, vaccination with AVA induces clonal expansion, somatic hypermutation, and class-switch recombination in antibodies specific for the immunogenic toxin component PA. These activities are consistent with the generation of a mature and efficacious antibody response. We also find the percentage of antibodies reactive with PA_{20}-associated epitopes to be disproportionate to the size of this proteolytic fragment, raising the possibility that many of the individual antibody species that make up the serological response are not capable of neutralizing toxin function. Additional donors are currently being analyzed to determine if this epitope bias is characteristic of the PA-specific response in general.

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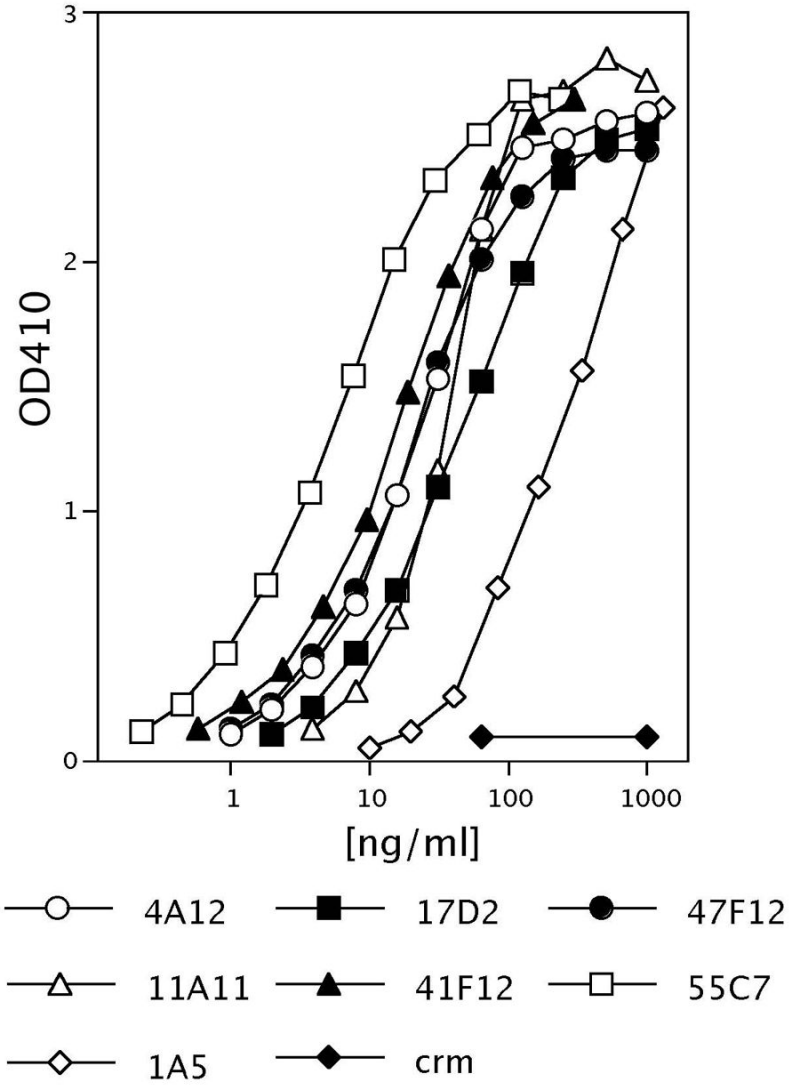

Figure 1.
Protective antigen binding by representative PA-specific human monoclonal antibodies in an ELISA assay. All antibodies bound PA in a concentration dependent manner and did not bind the mutant diphtheria toxin CRM_{197} negative control toxin at any concentration tested.

TABLE 1

Characteristics of the unique PA-specific Fab fragments

Clone	V_L	%Identity[a]	J_L	L3(aa)[b]	IGHV	%Identity[a]	Isotype	J_H	H3[b]	Family[c]
11A11	IGKV1-5	96.5	Jκ2	8	IGHV1-18	95.8	IgG1	JH4	18	H1-18
4A12	IGKV1D-39	95.6	Jκ4	9	IGHV1-46	91.7	IgG3	JH4	12	H1-46
34E3	IGLV1-40	95.0	Jλ3	12	IGHV3-15	94.1	IgG1	JH1	13	H3-15
35F7	IGLV1-40	97.6	Jλ3	12	IGHV3-15	98.5	IgG1	JH1	13	
40D10	IGLV1-40	94.7	Jλ3	12	IGHV3-15	96.3	IgG1	JH1	13	
41A3	IGLV1-40	96.1	Jλ3	12	IGHV3-15	97.0	IgG1	JH1	13	
41C5	IGLV1-40	96.5	Jλ3	12	IGHV3-15	93.7	IgG1	JH1	13	
46D1	IGLV1-40	97.3	Jλ3	12	IGHV3-15	94.1	IgG1	JH1	13	
49F1	IGLV1-40	96.8	Jλ3	12	IGHV3-15	94.1	IgG1	JH1	13	
51D6	IGLV1-40	97.3	Jλ3	12	IGHV3-15	93.7	IgG1	JH1	13	
52E7	IGLV1-40	97.5	Jλ3	12	IGHV3-15	91.5	IgG1	JH1	13	
53H4	IGLV1-40	98.8	Jλ3	12	IGHV3-15	94.1	IgG1	JH1	13	
54C7	IGLV1-40	99.1	Jλ3	12	IGHV3-15	94.8	IgG1	JH1	13	
55C7	IGLV1-40	97.6	Jλ3	12	IGHV3-15	92.6	IgA1	JH1	13	
56F6	IGLV1-40	95.0	Jλ3	12	IGHV3-15	94.1	IgG1	JH1	19	
59H10	IGLV1-40	97.6	Jλ3	12	IGHV3-15	94.5	IgG1	JH1	13	
65A8	IGLV1-40	98.8	Jλ3	12	IGHV3-15	94.1	IgG1	JH1	13	
65G8	IGLV1-40	96.5	Jλ3	12	IGHV3-15	94.5	IgG1	JH1	13	
65H8	IGLV1-40	97.6	Jλ3	12	IGHV3-15	94.8	IgG1	JH1	13	
66A5	IGLV1-40	94.7	Jλ3	12	IGHV3-15	94.8	IgG1	JH1	13	
66B2	IGLV1-40	95.9	Jλ3	12	IGHV3-15	93.7	IgG1	JH1	13	
17D2	IGKV1-9	98.4	Jκ4	9	IGHV3-30	95.1	IgG1	JH3	15	H3-30a
65A3	IGLV3-1	99.7	Jλ3	9	IGHV3-30	94.3	IgG1	JH6	17	H3-30b
47F12	IGLV1-44	96.7	Jλ3	11	IGHV3-30	94.0	IgG1	JH6	18	H3-30c
59G5	IGLV1-44	97.0	Jλ3	11	IGHV3-30	94.0	IgG1	JH6	18	
8H2	IGKV6-21	96.3	Jκ2	9	IGHV3-33	94.7	IgG1	JH4	13	H3-33a
19E1	IGKV6-21	99.1	Jκ2	9	IGHV3-33	94.7	IgG1	JH4	13	
41F12	IGLV1-47	97.3	Jλ1	11	IGHV3-33	95.1	IgG1	JH4	12	H3-33b
55F9	IGLV1-47	97.0	Jλ1	11	IGHV3-33	95.8	IgG1	JH4	12	
20C7	IGKV1-9	99.4	Jκ2	9	IGHV3-43	95.8	IgG1	JH6	17	H3-43
51C5	IGLV3-21	97.6	Jλ2	11	IGHV3-74	95.8	IgG1	JH4	14	H3-74
1A5	IGKV2-30	97.6	Jκ1	9	IGHV4-59	90.8	IgG1	JH1	11	H4-59
1B10	IGKV2-30	97.3	Jκ1	9	IGHV4-59	90.8	IgA1	JH1	11	
4B1	IGKV2-30	97.3	Jκ1	9	IGHV4-59	90.8	IgG1	JH1	11	
13E12	IGKV2-30	97.3	Jκ1	9	IGHV4-59	90.8	IgG2	JH1	11	

[a] Percent nucleotide identity over the entire V region as compared to the corresponding germline gene.

[b] Number of residues comprising the V_L and IGHV CDR3 region; aa = amino acids.

[c] Family denotes unique IGHV rearrangements

TABLE 2

H chain CDRs[a]

Clone	CDR1	CDR2	CDR3	J	Isotype
IGHV1-18	**SYGIS**	**WISAYNGNTNYAQKLQG**			
11A11	NF--T	Y-------A--------	DRGSRWFGEFPDEYYFDY	JH4	IgG1
IGHV1-46	**SYYMH**	**IINPSGGSTSYAQKFQG**			
4A12	S-FV-	M---R----N-------	VNWAYGDYDFDY	JH4	IgG3
IGHV3-15	**NAWMS**	**RIKSKTDGGTTDYAAPVKG**			
35F7	D---T	---R---------------	DVLGIVIIVGAAH	JH1	IgG1
40D10	K----	---R-S-------------	-------------	JH1	IgG1
59H10	Q-H--	-M-R-S-------------	--R----------	JH1	IgG1
46D1	Q-H--	-M-R-S-------G-----	--R----------	JH1	IgG1
41A3	Y-S-T	---R---------------	--R----------	JH1	IgG1
49F1	K---T	---RRS-------------	--------R---Q	JH1	IgG1
55C7	Y-R--	---R--E------------	-------------	JH1	IgG1
52E7	Y-R--	---N-R--E--H-------	-------------	JH1	IgG1
66A5	K---T	---R-S----I--------	--R----------	JH1	IgG1
34E3	K---T	---RRS-------------	--------R---Q	JH1	IgG1
41C5	----T	---RRS-------------	------------Q	JH1	IgG1
51D6	----T	---RRS-------------	------------Q	JH1	IgG1
53H4	Q-H--	-M-R-S-------G-----	--R----------	JH1	IgG1
54C7	-----	---R-S----I----S---	------------Q	JH1	IgA1
56F6	K---T	---RRS-------------	--------R---Q	JH1	IgG1
65A8	----T	---RRS-------------	------------Q	JH1	IgG1
65H8	-----	---R-S----I----S---	------------Q	JH1	IgG1
65G8	D---T	---R-S-------------	-T----R------	JH1	IgG1
66B2	----T	---RRS-------------	------------Q	JH1	IgG1
IGHV 3-30	**SYGMH**	**VISYDGSNKYYADSVKG**			
17D2	R----	------GD-K-V------	WDYVWESYRGKAFDI	JH3	IgG1
65A3	--A--	L------NT---------	LISYDGNTKYYADSVKG	JH6	IgG1
47F12	--T--	L-----T--N-G---T-	ARVIVPAGSNYNQYGMDV	JH6	IgG1
59G5	--T--	L-----T--N-G---T-	ARVIVPAGSNYNQYGMDV	JH6	IgG1
IGHV 3-33	**SYGMH**	**VIWYDGSNKYYADSVKG**			
8H2	DF---	L--N---K---------	WGYYYGSGSPPEY	JH4	IgG1
19E1	DF---	L--N---K---------	WGYYYGSGSPPEY	JH4	IgG1
41F12	T----	---F---------------	EDGSYHQGPFDY	JH4	IgG1
55F9	T----	---F---------------	EDGSYHQGPFDY	JH4	IgG1
IGHV 3-43	**DYTMH**	**LISWDGGSTYYADSVKG**			
20C7	-----	--T----R-F--N----	GPNRRDSFGLHYYGLDV	JH6	IgG1
IGHV 3-74	**SYWMH**	**RINSDGSSTSYADSVKG**			
51C5	R-Y--	R--N---------------	EGRPMGLGTSVGMN	JH4	IgG1
IGHV 4-59	**SYYWS**	**YIYYSGSTNYNPSLKS**			
1A5	-HF-	---SG-T---D------	GDMVTGDPGDY	JH1	IgG1
1B10	-HF-	---SG-T---D------	-----------	JH1	IgA1
4B1	-HF-	---SG-T---D------	----------H	JH1	IgG1
13E12	-HF-	---SG-T---D------	-----------	JH1	IgG2

[a]Heavy chain CDR residues and gene usage of PA-specific Fabs. Translation of germ line CDRs shown for comparison.

TABLE 3

L chain CDRs[a]

Clone	CDR1	CDR2	CDR3		J
IGKV2-30	**RSSQSLVYSDGNTYLN**	**KVSNRDS**	**MQGTHWP**		
1A5	-------S---K----	---K---	--A---L	WT	JK1
IGKV6-21	**RASQSIGSSLH**	**YASQSFS**	**HQSSSLP**		
8H2	-------RN--	-V-----	-----F-	YT	JK2
19E1	--------N--	-------	-----F-	YT	JK2
IGKV1-5	**RASQSISSWLA**	**DASSLES**	**QQYNSYS**		
11A11	---H---T---	-A---Q-	---S-Y	YT	JK2
IGKV1-9	**RASQGISSYLA**	**AASTLQS**	**QQLNSYP**		
17D2	-------R---	------G	-------	LT	JK4
20C7	----E------	-------	-------	YT	JK2
IGKV1D-39	**RASQSISSYLN**	**AASSLQS**	**QQSYSTP**		
4A12	------AR---	D----HT	--T----	LT	JK4
IGLV1-40	**TGSSSNIGAGYDVH**	**GNSNRPS**	**QSYDSSLSG**		
51D6	----------K---	-------	--------- **D**	WV	JL3
34E3	---F--V-S-S---	--T----	-----R--- **D**	WV	JL3
35F7	----------N---	-------	--------- **D**	WV	JL3
41A3	---G------S---	-------	---G----- **D**	WV	JL3
65H8	----------N---	-------	--------- **D**	WV	JL3
65A8	----------S---	-------	--------- **D**	WV	JL3
52E7	----------N---	-------	--------- **D**	WV	JL3
53H4	----------S---	-------	--------- **D**	WV	JL3
54C7	----------S---	-------	----R---- **D**	WV	JL3
46D1	----------S---	-------	-----R-G- **E**	WV	JL3
55C7	----------S---	-------	-----R-G- **E**	WV	JL3
59H10	----------S---	-------	-----R-G- **E**	WV	JL3
65G8	---G------S---	-------	---G----- **D**	WV	JL3
41C5	--D---M---S---	-------	--------- **D**	WV	JL3
56F6	---F--V-S-S---	--T----	-----R--- **D**	WV	JL3
40D10	---F--V-S-S---	--T----	-----R--- **D**	WV	JL3
66A5	---F--V-S-S---	--T----	-----R--- **D**	WV	JL3
49F1	----------S---	--T----	----R---- **D**	WV	JL3
66B2	---L------K---	-------	----N---- **D**	WV	JL3
IGLV1-44	**SGSSSNIGSNTVN**	**SNNQRPS**	**AAWDDSLNG**		
47F12	---W--T-R----	--T----	-------K-	WM	JL3
59G5	---W----R-A--	T-T----	-------K-	WV	JL3
IGLV1-47	**SGSSSNIGSNYVY**	**SNNQRPS**	**AAWDDSLSG**		
41F12	---------H--C	R-D----	-S-----T-	QV	JL1
55F9	---------H--C	R-D----	-S-----T-	QV	JL1
IGLV3-1	**SGDKLGDKYAC**	**QDSKRPS**	**QAWDSSTA**		
65A3	----------	-------	--------	T	JL3
IGLV3-21	**GGNNIGSKSVH**	**DDSDRPS**	**QVWDSSSDH**		
51C5	------R----	--R----	----NI---	VI	JL2

[a]Light chain CDR residues and gene usage of PA-specific Fabs. Translation of germ line CDRs shown for comparison.

Table 4

PA Domain Binding by PA-specific Fabs

Clone	V_L	V_H	Capture assay				Western blots		
			PA_{83}[a]	PA_{63}[a]	PA_{20}[a]	$_fD4$[b]	$_fD1$[c]	$_fD4$[b]	PrFrag[a]
11A11	IGKV1-5	IGHV1-18	+	−	+	nd	+	−	-
4A12	IGKV1D-39	IGHV1-46	+	+	−	+	−	+	PA_{63}
35F7	IGLV1-40	IGHV3-15	+	−	+/−	−	+	−	PA_{20}
40D10	IGLV1-40	IGHV3-15	+	−	+	−	+	−	PA_{20}
59H10	IGLV1-40	IGHV3-15	+	−	+	−	+	−	PA_{20}
46D1	IGLV1-40	IGHV3-15	+	−	+	−	+	−	PA_{20}
41A3	IGLV1-40	IGHV3-15	+	−	+/−	−	+	−	PA_{20}
49F1	IGLV1-40	IGHV3-15	+	−	−	−	+/−	−	-
55C7	IGLV1-40	IGHV3-15	+	−	+	−	+	−	nd
52E7	IGLV1-40	IGHV3-15	+	−	+	−	+	−	nd
66A5	IGLV1-40	IGHV3-15	+	−	+	−	+	−	PA_{20}
34E3	IGLV1-40	IGHV3-15	+	−	+	−	+	−	PA_{20}
41C5	IGLV1-40	IGHV3-15	+	−	+/−	−	+	−	PA_{20}
51D6	IGLV1-40	IGHV3-15	+	−	+	−	+	−	PA_{20}
53H4	IGLV1-40	IGHV3-15	+	−	+	−	+	−	PA_{20}
54C7	IGLV1-40	IGHV3-15	+	−	+	−	+	−	nd
56F6	IGLV1-40	IGHV3-15	+	−	+	−	+	−	PA_{20}
65A8	IGLV1-40	IGHV3-15	+	−	+	−	+	−	nd
65H8	IGLV1-40	IGHV3-15	+	−	+	nd	+	−	PA_{20}
65G8	IGLV1-40	IGHV3-15	+	−	+	−	+	−	PA_{20}
66B2	IGLV1-40	IGHV3-15	+	−	+	−	+	−	nd
17D2	IGKV1-9	IGHV3-30	+	+	−	−	−	−	PA_{63}
65A3	IGLV3-1	IGHV3-30	+	−	+/−	−	+	−	nd
47F12	IGLV1-44	IGHV3-30	+	−	+	−	+	−	PA_{20}
59G5	IGLV1-44	IGHV3-30	+	−	+	−	+	−	PA_{20}
8H2	IGKV6-21	IGHV3-33	+	−	+	−	+	−	PA_{20}
19E1	IGKV6-21	IGHV3-33	+	−	+	nd	+	−	PA_{20}
41F12	IGLV1-47	IGHV3-33	+	−	+	−	+	−	PA_{20}
55F9	IGLV1-47	IGHV3-33	+	−	+	−	+	−	PA_{20}
20C7	IGKV1-9	IGHV3-43	+	+	−	+	−	+	-
51C5	IGLV3-21	IGHV3-74	+	−	+	−	−	−	-
1A5	IGKV2-30	IGHV4-59	+	+	−	−	−	−	PA_{63}
1B10	IGKV2-30	IGHV4-59	+	+/−	−	−	−	−	PA_{63}
4B1	IGKV2-30	IGHV4-59	+	−	−	−	−	−	nd
13E12	IGKV2-30	IGHV4-59	+	+/−	−	−	−	−	nd

[a] Purified PA_{83} or purified PA_{63} or PA_{20} proteolytic fragments.

[b] Purified Domain 4-GFP fusion protein.

[c] Purified Domain 1(PA_{20})-GFP fusion protein.