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Repertoire of Human Antibodies against the Polysaccharide Capsule of *Streptococcus pneumoniae* Serotype 6B

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We examined the repertoire of antibodies to *Streptococcus pneumoniae* 6B capsular polysaccharide induced with the conventional polysaccharide vaccine in adults at the molecular level two ways. In the first, we purified from the sera of seven vaccinees antipneumococcal antibodies and determined their amino acid sequences. Their VH regions are mainly the products of VH3 family genes (candidate genes, 3-23, 3-07, 3-66, and 3-74), but the product of a VH1 family gene (candidate gene, 1-03) is occasionally used. All seven individuals have small amounts of polyclonal κ^+ antibodies (Vκ1 to Vκ4 families), although κ^+ antibodies are occasionally dominated by antibodies formed with the product of the A27 Vκ gene. In contrast, λ^+ anti-6B antibodies are dominated by the antibodies derived from one of 3 very similar Vλ2 family genes (candidate genes, 2c, 2e, and 2a2) and Cλ1 gene product. The $V\lambda2^+$ antibodies express the 8.12 idiotype, which is expressed on anti-double-stranded-DNA antibodies. In one case, Vλ is derived from a rarely expressed Vλ gene, 10a. In the second approach, we studied a human hybridoma (Dob1) producing anti-6B antibody. Its VH region sequence is closely related to those of the 3-15 VH gene (88% nucleotide homology) and JH4 (92% homology). Its VL region is homologous to the 2a2 Vλ2 gene (91%) and Jλ1/Cλ1. Taken together, the V region of human anti-6B antibodies is commonly formed by a VH3 and a Vλ2 family gene product.

Streptococcus pneumoniae is a significant pathogen, accounting for a large fraction of pneumonia, sepsis, and meningitis (12). Antibiotic treatment of pneumococcal infections has become less effective due to a recent dramatic increase in the prevalence of antibiotic-resistant strains of *S. pneumoniae* in many parts of the world (4). While pneumococcal infections could be prevented with pneumococcal vaccines, the currently available 23-valent pneumococcal vaccine, which contains the capsular polysaccharide (PS) of 23 commonly found serotypes of *S. pneumoniae*, is not protective for young children (24) and may be effective only for subpopulations of older adults (28), the two populations most susceptible to *S. pneumoniae* infections. Thus, there is a great need for an effective pneumococcal vaccine; the main approach used for improving the pneumococcal vaccine is to conjugate the PS to a carrier protein as done for *Haemophilus influenzae* type b vaccines (29, 35).

Although this PS-protein conjugate vaccine may elicit antibody responses in young children, the conjugation process can alter the antigenic epitopes, and the conjugate vaccine may elicit antibodies with altered repertoire or induce undesirable antibodies. It has been suggested that some PS (particularly those that are linear and contain phosphodiester bonds) may be mimotopes of DNA, as some anti-DNA antibodies bind the bacterial PS from *Klebsiella* species (22) and *Neisseria meningitidis* group B (16). The currently available 23-valent pneumococcal vaccine has been known to increase the antibody displaying the 8.12 idiotope (14), which is expressed on nephropathic anti-double-stranded-DNA (dsDNA) antibodies (20). The capsular PS from *S. pneumoniae* serotype 6B is, like DNA, a linear polymer with phosphodiester bonds (8). Although 6B capsular PS is poorly immunogenic, all new vaccines will contain 6B capsular PS (35) because infection by *S. pneumoniae* serotype 6B is common. Our preliminary study showed that some anti-6B antibodies frequently express λ light chains (25), which suggests that anti-6B antibodies may, like anti-dsDNA antibodies, display the 8.12 idiotype that is expressed on λ light chain.

Expression of human Vλ-Cλ genes has not been systematically studied so far, although these genes have several unique features. Unlike the case for other constant-region genes, there are seven Cλ genes, each associated with one unique Jλ gene (23). In some individuals, the region between Cλ2 and Cλ3 is amplified and may contain up to 10 Cλ genes (40). Furthermore, unlike human Vκ genes and mouse Vλ genes, human Vλ genes are grouped according to gene family. For instance, Vλ2 gene family members are located together near the Jλ1 gene whereas all members of the Vλ4 gene family are found together away from the Jλ1 gene in the 5′ direction (13). Thus, there may be limits in extrapolating our observations on the expression of murine V genes or of human Vκ genes to those of human Vλ genes. Since antibodies to 6B PS are often λ^+ (25), studies of human antibodies to 6B PS would allow studies of Vλ expression in young children and adults. We therefore investigated the V-region structure of antibodies to the capsular PS of serotype 6B at the molecular level, using 23-valent PS vaccine.

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MATERIALS AND METHODS

Antipneumococcal antisera. Healthy adult volunteers were immunized with the 23-valent PS vaccine from Lederle Laboratories (Pearl River, N.Y.) or from Pasteur Merieux (Lyon, France). Serum samples were collected from the volun-

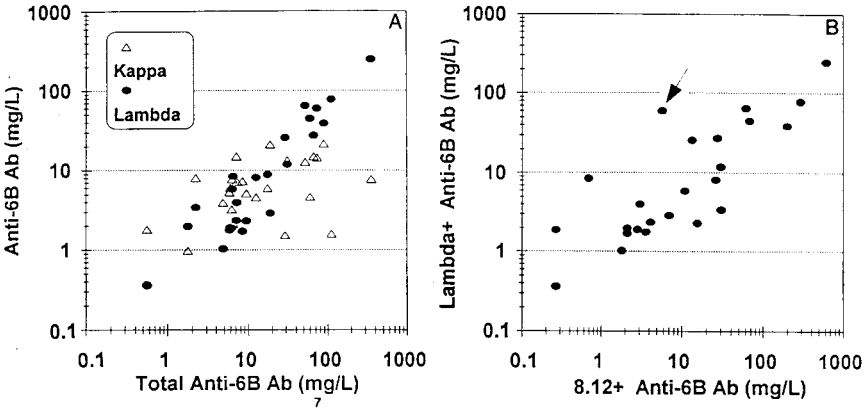

FIG. 1. Relationship between the amount of anti-6B antibodies (Ab) expressing the 8.12 idiotype and the amount of anti-6B antibodies expressing λ light chain for volunteers vaccinated with a PS vaccine (B), compared with the relationship between total anti-6B and anti-6B antibodies expressing either κ or λ chain (A). Donor P26, who was found to express antibody derived from a Vλ10 family gene, is indicated with an arrow. Panel A was reproduced with permission of the publisher (J. Infect. Dis. 174:75–82, 1996).

teers 1 month after vaccination. A serum pool (89-SF) was obtained from C. Frasch (Food and Drug Administration, Bethesda, Md.) and used as the standard in all assays. The standard contains 24.3 μg of total (27), 17.6 μg of K^+, and 6.7 μg of λ^+ anti-6B PS antibody per ml (25).

Seven donors were chosen for the sequence studies. Six donors were chosen because the antibody responses were high (upper 50th percentile) and there were only three to four antibody clones in the serum by isoelectric focusing analysis. Most individuals had three to four antibody clones, although some had more (data not shown). The seventh donor (P26) was chosen because the serum had higher levels of λ^+ anti-6B antibodies than of 8.12^+ anti-6B antibodies.

ELISA. The amount of anti-6B PS antibody was determined by sandwich-type enzyme-linked immunosorbent assays (ELISAs). Briefly, the wells of Immulon II plates (Dynatech, Chantilly, Va.) were coated at 37°C with 6B PS (10 μg/ml; American Type Culture Collection, Rockville, Md.) overnight in phosphate-buffered saline, which was prepared fresh, using water from a Milli-Q UF water purification system (Millipore, Bedford, Mass.), to minimize the background signal. The plates were washed and blocked with phosphate-buffered saline containing 1% nonfat milk (Carnation, Los Angeles, Calif.). The human serum pool (89-SF) was used as a standard. Samples were preabsorbed with 3 μg of C-PS (Statens Seruminstitut, Copenhagen, Denmark) per 20 μg of serum in a total volume of 1 ml of diluent for 30 min at room temperature. The serum samples were then added to wells, serially diluted, and incubated for 3 to 5 h at room temperature. Plates were then washed, and alkaline phosphatase-conjugated goat antibody against total human immunoglobulin (Ig), kappa or lambda chain, was added. To measure 8.12^+ anti-6B antibody, 8.12 monoclonal antibody was added to wells, and goat anti-mouse Ig antibody labeled with alkaline phosphatase was added later. 8.12 recognizes human antibodies expressing the Vλ2 family gene product. The amount of enzyme immobilized to the well was determined with *para*-nitrophenyl phosphate substrate (Sigma) in diethanolamine buffer. Optical density at 405 nm was read with a microplate reader (Cambridge Technology, Watertown, Mass.). The amount of antibody in the sample was determined by comparing the optical density of the samples to the standard, using piecewise linear interpolation.

Purification of anti-6B antibody from immune serum and determination of its amino acid sequence. Purification was performed as described for antibodies to *H. influenzae* type b PS (34), with slight modifications as described below. An Ig fraction was separated from the immune serum (50 to 200 ml) by precipitation in 50% saturated ammonium sulfate. The Ig fraction was passed over a Sepharose column conjugated with 6B PS. The 6B PS was conjugated to Sepharose following CNBr treatment. The 6B-Sepharose column saturated with anti-6B antibody was washed first with 100 mM NaCl containing 50 mM Tris (pH 7.4), next with 100 mM borate buffer (pH 8.4) containing 5 mM phosphocholine (PC) and 0.01% Tween 20, and last with 150 mM NaCl. Preliminary studies showed that 5 mM PC eluted most anti-C-PS antibodies binding the haptenic determinant PC. Anti-6B antibody (50 to 200 μg) was eluted from the column with 3.5 M $MgCl_2$ (pH 3.5), and the elution fraction containing purified protein was neutralized with 1 M Tris and dialyzed against normal saline buffered with 50 mM Tris (pH 7.5). To obtain the IgG fraction, the antibody was passed over Sepharose columns coupled with HB57 and HA1, monoclonal antibodies specific for IgM and IgA, respectively. The λ and κ fractions of the IgG antibody were obtained by depleting κ and λ antibodies with the use of anti-κ (monoclonal antibody HK-2) and anti-λ (monoclonal antibody HL-1) antibody columns, respectively. The purified antibody was assessed for antigen specificity for 6B; purity and clonality were tested by ELISA and isoelectric focusing methods as described elsewhere (34).

Amino acid sequencing. Purified antibodies were separated into the light and heavy chains in a sodium dodecyl sulfate-polyacrylamide gel, and the separated chains were electrophoretically transferred to a polyvinylidene difluoride (PVDF) membrane. The amino acid sequence was obtained from the peptide-containing membrane with an Applied Biosystems Inc. (Foster City, Calif.) model 470A gas-phase sequencer by Midwest Analytical, Inc. (St. Louis, Mo.). To obtain the sequences in the CDR2 and CDR3 regions, the PVDF membrane containing the peptide chain was treated with CNBr or BNPS-Skatole to cleave the chain at either methionine or tryptophan, respectively, before the amino acid sequence was obtained (33). To obtain the sequence in the CDR2 region of VH, the VH blot was treated with 0.02 mg of *o*-phthalaldehyde (OPA) per 1 ml of butylchloride at the appropriate sequencing cycle. OPA treatment makes all the N-terminal amino acids except proline resist the peptide degradation chemistry and reduces the sequencing noise, thereby extending the sequencing depth (3).

Nucleotide sequencing of VH and VL region cDNAs from Dob1 hybridoma. Dob1 hybridoma secreting human IgG2λ anti-6B antibody was produced by fusing K6H6/B5 cells (5) with human peripheral blood mononuclear cells, which were obtained from an individual immunized with the 23-valent PS vaccine (unpublished data). Dob1 mRNA, extracted by the method of Chomczynski and Sacchi (7), was reverse transcribed with oligo(dT) and a reverse transcriptase. cDNAs of VH and VL regions were obtained from the Dob1 cDNA by 35 cycles of PCR using primers GAGGTGCAGCTG(G/T30)TGGAGTCT and GAC(C/G)GATGGGCCCTTGGTGGA for VH and CAGTCTGCGCTGACTCA(A/G)CCG(G/C)CCTCT and AGAGGA(G/C25)GG(C/T30)GGGAACAGAG TGAC for VL (18). The PCR cycle was 3 min at 72°C for extension, 1.5 min at 95°C for melting, and 2 min for annealing. To minimize spurious PCR products, the annealing temperature of 65°C for the first cycle was decreased by 1°C per cycle for the first 15 cycles and maintained at 50°C for the remaining cycles. After confirmation of the presence of a PCR product of appropriate length by electrophoresis, the PCR product was cloned in *Escherichia coli* with a TA cloning kit (Invitrogen, San Diego, Calif.) by ligation to the pCR2.1 vector and transformation of *E. coli* with that vector. Plasmid DNA was isolated from five ampicillin-resistant, galactosidase-deficient *E. coli* colonies by using a commercial kit (Wizard *Plus* Miniprep; Promega, Madison, Wis.), and the DNA insert containing the VH (or VL) region was recovered from the plasmid. The forward and reverse sequences of the DNA insert were determined by the Sanger dideoxy method, using fluorescent terminators (DNA sequencing kit from Perkin-Elmer, Foster City, Calif.) and by measuring electrophoretically separated fluorescent bands with a model 377 DNA sequencer from Applied Biosystems. The forward and reverse sequences from several bacterial colonies were assembled into the complete VH and VL sequences. DNA sequences were compared by the clustered method, using a commercial program from DNAstar Inc. (Madison, Wis.).

RESULTS

Idiotope 8.12 is commonly expressed among anti-6B PS antibodies. Pneumococcal vaccination has been shown to increase the level of antibodies displaying the 8.12 idiotype, which is now known to be expressed by Vλ2 family gene products. Previously, we reported frequent expression of the λ light chain by anti-6B PS antibodies, especially among the high responders (Fig. 1A) (25). Consequently, we tested whether the λ^+ anti-

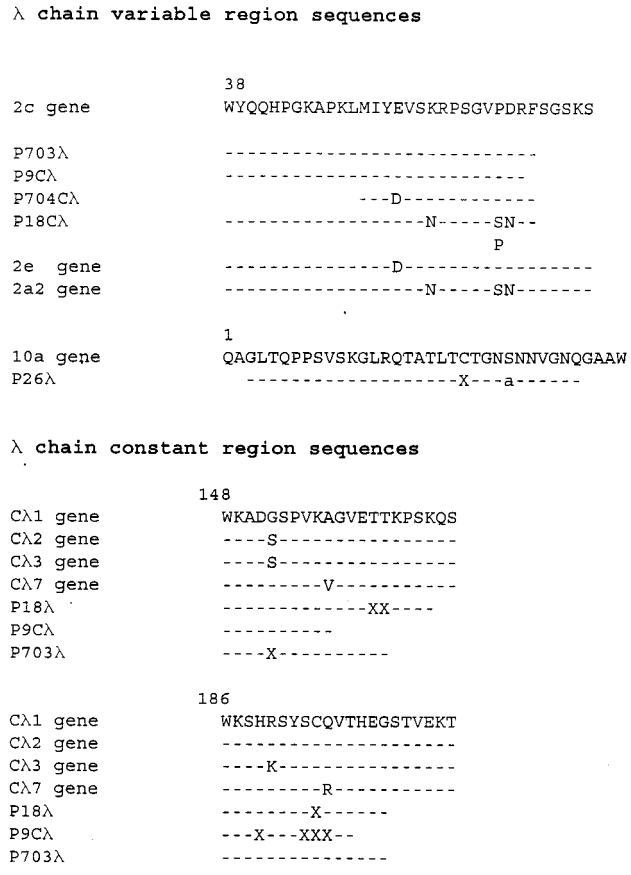

FIG. 2. Amino acid sequences of the V region of the light chain of anti-6B antibodies expressing the λ light chain. Dayhoff amino acid notation and the residue numbering system of Kabat et al. (17) are used. X indicates no residue identified; the X at position 22 of P26λ is likely an invariant cysteine not identifiable by our sequencing method. The lowercase letter denotes the recovery of a smaller than expected amount of amino acid during the Edman degradation cycle; the solid line denotes identity to the reference sequence at the top. Tryptophan at positions 38, 148, and 186 are inferred by the cleavage method.

6B antibodies express the 8.12 idiotope by measuring the concentrations of anti-6B PS antibodies expressing the 8.12 idiotope in the serum samples from 25 adults and by comparing them with the anti-6B antibodies expressing the λ light chain (Fig. 1B). Almost all of the λ^+ anti-6B PS antibodies expressed the 8.12 idiotope, with a strong correlation between the two parameters ($r = 0.81$). In only 3 of 25 samples was the 8.12 idiotope expressed in less than 10% of the λ^+ anti-6B PS antibodies. Thus, VL genes belonging to the Vλ2 family must encode most of λ^+ anti-6B PS antibodies.

Products of several similar Vλ2 genes and the Cλ1 gene are used for anti-6B PS antibodies. Initial attempts to determine the amino acid sequences of the λ-chain antibodies revealed a blocked N terminus. Since the λ light chain often has methionine at position 49, we treated the PVDF blot (with λ chain) with CNBr and determined the sequence of 14 amino acids starting at position 50 (Fig. 2). The amino acid sequences from the P9Cλ and P703λ fractions were identical and matched exactly to that of the 2c Vλ gene. The sequence of P704Cλ was identical to that of the 2e gene, which differs from the 2c gene only by one amino acid (E versus D at position 52) in this part of the V region. However, the sequences of these three samples at this region (positions 49 to 64) differed from other Vλ gene sequences by more than three amino acids. We therefore conclude that P704Cλ, P9Cλ, and P703Cλ are derived from either the 2c or 2e Vλ2 gene and not from other Vλ genes. In contrast, the sequence from sample P18Cλ matched exactly that of the 2a2 Vλ gene and differed from other Vλ gene sequences by more than three amino acids. Thus, the P18C Vλ region is likely the product of the 2a2 Vλ gene. The amino acid sequences from positions 38 to 49 obtained following Skatole digestion of the light chain matched exactly those of the 3 Vλ genes listed above and supported our conclusion.

In addition to the tryptophan at position 38 of the Vλ region, there are two tryptophan residues (at positions 148 and 186) in the Cλ region (11). Thus, Skatole digestion yields four λ-chain fragments, and because one chain is blocked, each cycle of Edman degradation identifies three amino acids (Fig. 2, bottom). Because the sequences of Cλ genes are known (23), all amino acids found in each sequencing cycle can be identified as arising from either V- or C-region fragments. The fourth cycle of Edman degradation identified both G and R, which are expected if the Cλ is derived from Cλ1 or Cλ7 gene (23). It is unlikely that the pneumococcal antibodies are derived from either the Cλ2 or Cλ3 gene because if they were S or K would have been identified at this cycle. Also, we did not observe R or V in the ninth cycle, which should have been present if the Cλ of anti-6B is derived from the Cλ7 gene. Since Cλ4 and Cλ5 genes are pseudogenes and Cλ6 has a stop codon (23), the Cλ portion of anti-6B antibodies is derived from the Cλ1 gene.

The Vλ10 gene product can be used for anti-6B antibodies. Several serum samples (e.g., P26C [indicated with an arrow in Fig. 1B]) had high levels of λ^+ anti-6B antibodies without expressing the 8.12 idiotope in proportion. This finding suggested that these samples may be the product of a Vλ gene other than Vλ2. Indeed, the λ chain of anti-6B antibodies isolated from serum sample P26C had an unblocked N terminus and the N-terminal sequence closely matched that of Vλ gene 10a, which belongs to the recently defined Vλ10 family (Fig. 2) (42). Compared with the sequence of Vλ gene 10a, our sequence lacked the first two amino acids at the N terminus and had one potential mismatch (from S to A) at the 24th cycle.

Many Vκ genes are used to form anti-6B antibodies. To obtain a more complete picture of the V region repertoire of anti-6B antibodies, we determined the sequences of anti-6B antibodies expressing the kappa chain which were prepared by affinity chromatography (Fig. 3). In two cases, we found that one antibody clone dominated the kappa response and were able to separate the clone and obtain the unique amino acid sequence of the VL region. In these two cases, the sequences of the N-terminal 25 bases of the two light-chain preparations match perfectly the A27 sequence and differ from sequences of other Vκ genes by at least two amino acids (Fig. 3). In most cases, κ^+ anti-6B antibodies from an individual often have amino acid sequences of the genes of several different Vκ gene families (data not shown), and we could not identify the dominant κ clones. While some of the Vκ sequences that we observed may have been those of contaminants, κ^+ anti-6B an-

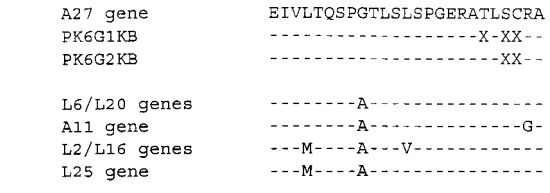

FIG. 3. Amino acid sequences of the V region of the light chain of anti-6B antibodies expressing the κ light chain. Symbols are as in Fig. 2.

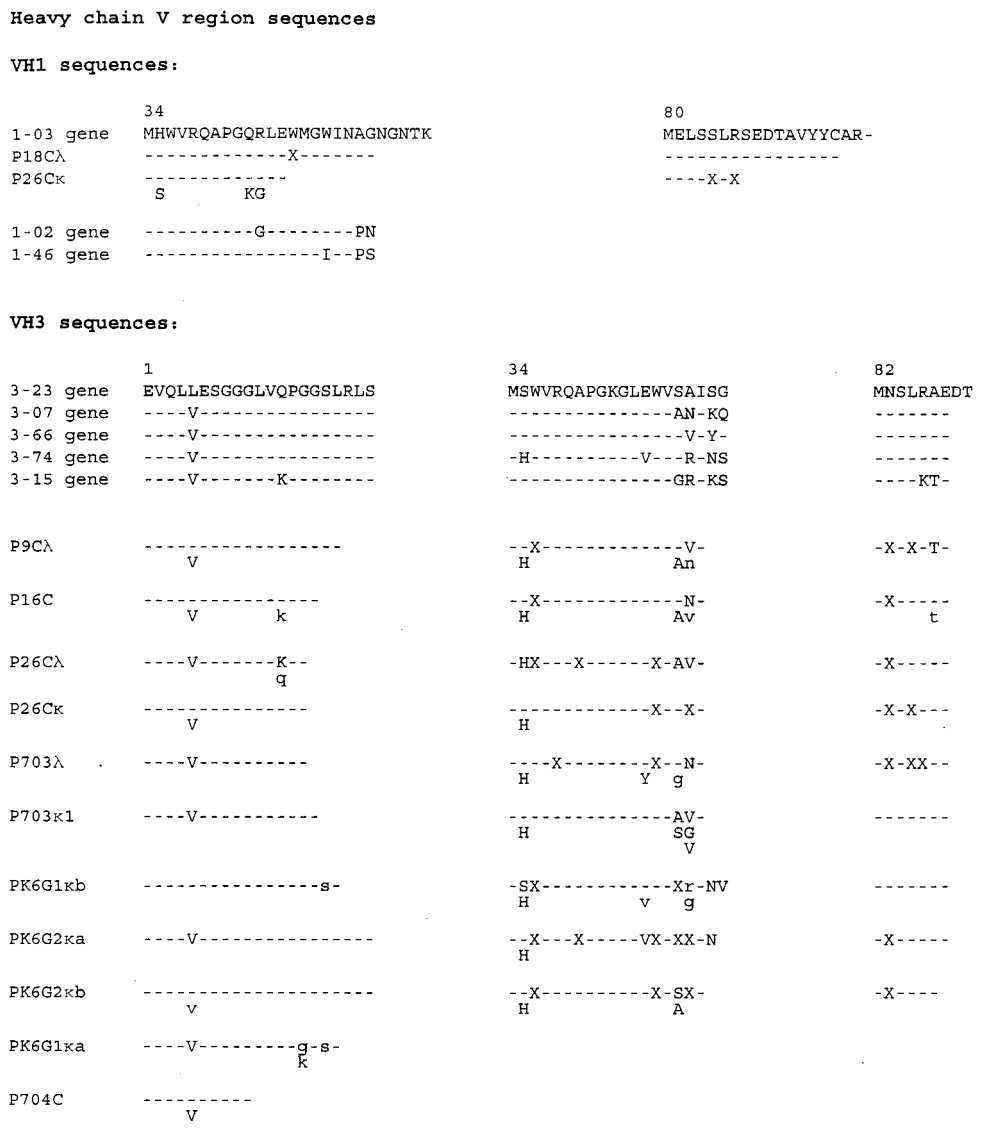

FIG. 4. Amino acid sequences of the V region of the heavy chain of anti-6B antibodies. Symbols are as in Fig. 2.

tibodies in a given individual appear to be derived from several Vκ genes belonging to different families.

Characterization of the VH region of anti-6B antibodies. The heavy-chain sequences were mostly of the VH3 subgroup (Fig. 4). However, the N-terminus of the heavy chain of the antibody P18Cλ was blocked. The PVDF membrane bearing the P18Cλ heavy chain was digested with Skatole and then treated with OPA at the fifth cycle of Edman degradation to suppress the amino acids arising from the C-region fragments. The sequence of 18 amino acids matched perfectly to that of the FR2-CDR2 region of VH1 gene 1-03 (Fig. 4) but differed from that of other VH1 genes by at least three residues. We confirmed our sequence by cleaving the heavy chain at methionine (position 34) with CNBr and treating it with OPA at the seventh cycle. Taken together, the results suggest that the VH gene 1-03 is the candidate gene for P18C VH.

Because most VH1 subgroup heavy chains have the blocked N terminus, our purified antibodies may contain, in addition to the VH3 antibodies, a significant amount of antibodies expressing VH1 gene products. To exclude the presence of VH1 gene products hidden in our antibody preparations, we cleaved all of the heavy-chain preparations studied above with Skatole and treated the fragments with OPA at the fifth cycle of Edman degradation. One sample (P26Cκ) yielded two FR2-CDR2 region sequences; one sequence corresponding to a small amount of VH1 subgroup antibody (Q and M at cycles 7 and 12, respectively), and the other sequence corresponding to a large amount of VH3 subgroup antibody (K and V at cycles 7 and 12, respectively). In all other samples, we found no VH1 sequences, only the VH3 FR2-CDR2 region sequences.

Although there are allelic variations, the human genome contains about 21 VH genes belonging to the VH3 family (8, 41). When the amino acid sequences of both the N-terminal and CDR2 regions are compared with the germ line VH sequences, four VH3 family genes (3-23, 3-07, 3-66, and 3-74) appear to be the candidate genes for anti-6B antibodies (Fig. 4). Taken together, the results suggest that most of anti-6B antibodies are clearly derived from the VH genes belonging to VH3 family, but they are occasionally derived from VH1 family gene(s) as well.

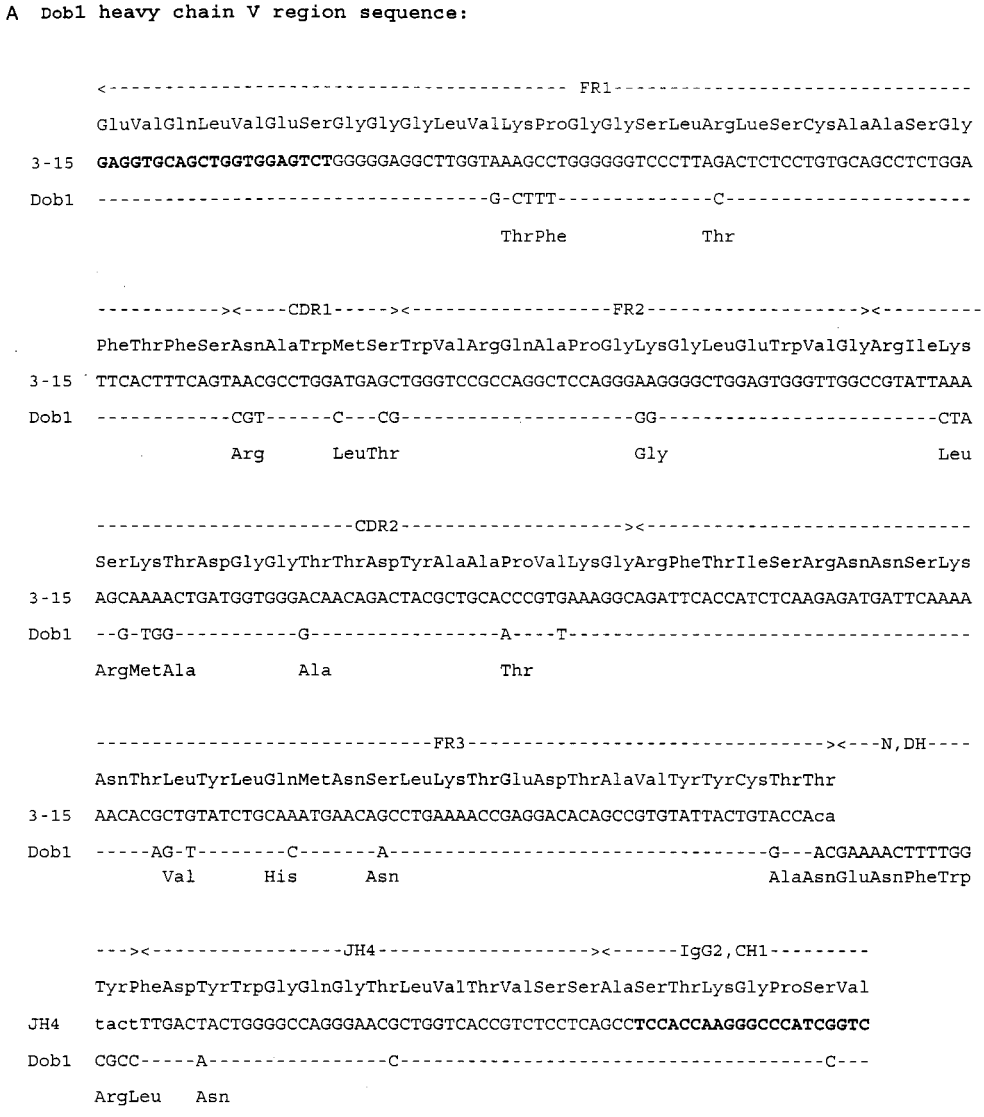

FIG. 5. cDNA sequences of VH (A) and VL (B) regions of Dob1. The first lines denote the VH and VL domains as assigned by Kabat et al. (17). The third lines denote the reference DNA sequences for the heavy and light chains, which were based on the sequences of VH gene 3-15 (DP-38) (GenBank accession no. Z12338), JH4 gene (Z14191), IgG2 gene (J00230), VL gene 2a2 (Z73664), and Jλ1 and Cλ1 genes (X51755). The second lines denote amino acids translated from the reference sequences. The fourth and fifth lines, respectively, denote cDNA sequences of Dob1 VH and VL regions and their amino acid translations. The fifth lines show only the translated amino acids of Dob1 cDNAs that are different from those of the reference sequences.

The human anti-6B hybridoma Dob1 expresses the gene products of the Vλ2 and VH3 gene families. To confirm the findings obtained with amino acid sequences of anti-6B antibodies, we produced one human-mouse hybridoma (Dob1) secreting anti-6B antibody and determined the DNA sequences of its VH and VL regions. As shown in Fig. 5, the VH and VL regions are clearly derived from genes in the VH3 and Vλ2 families. The VH region nucleotide sequence of Dob1 displayed an 88% match with VH gene 3-15 and 79% match with 3-72, whereas the match with all other VH3 genes ranged from 73 to 68% by the clustered method. The Dob1 JH region sequence best (92%) matched the JH4 sequence, followed by less than 83% match with all other JH sequences. Taken together, the results suggest that the candidate VH and JH genes for Dob1 are 3-15 and JH4. The D-JH splicing occurred 5′ to TTGA, which is a common splice site (43). The D-region sequence of Dob1 lacked obvious resemblance to any of the 27 D genes in the human genome (9). Analysis of the VL region of Dob1 showed that it best matched 2a2 (91%) and 2b2 (91%) sequences but was still similar to the 2e gene sequence (88%). Other Vλ gene sequences displayed less than 74% similarity with the Dob1 sequence. At the Jλ region, the Dob1 sequence matched best the Jλ1 sequence. There was no evidence for the extra nucleotides inserted at the VL-JL junction. There are several nucleotide sequence changes consistent with somatic mutations. For instance, position 49 is leucine in Dob1 but methionine in all Vλ2 genes. Although this particular change was not observed with purified antibody proteins, the Dob1 sequence supports our conclusion that the most common form of anti-6B antibodies is formed with a VH3 gene and a Vλ2 gene.

DISCUSSION

A major technical difficulty in studying the human antibody repertoire is that it is very difficult to obtain human hybridomas stably producing antibodies. Consequently, a practical alterna-

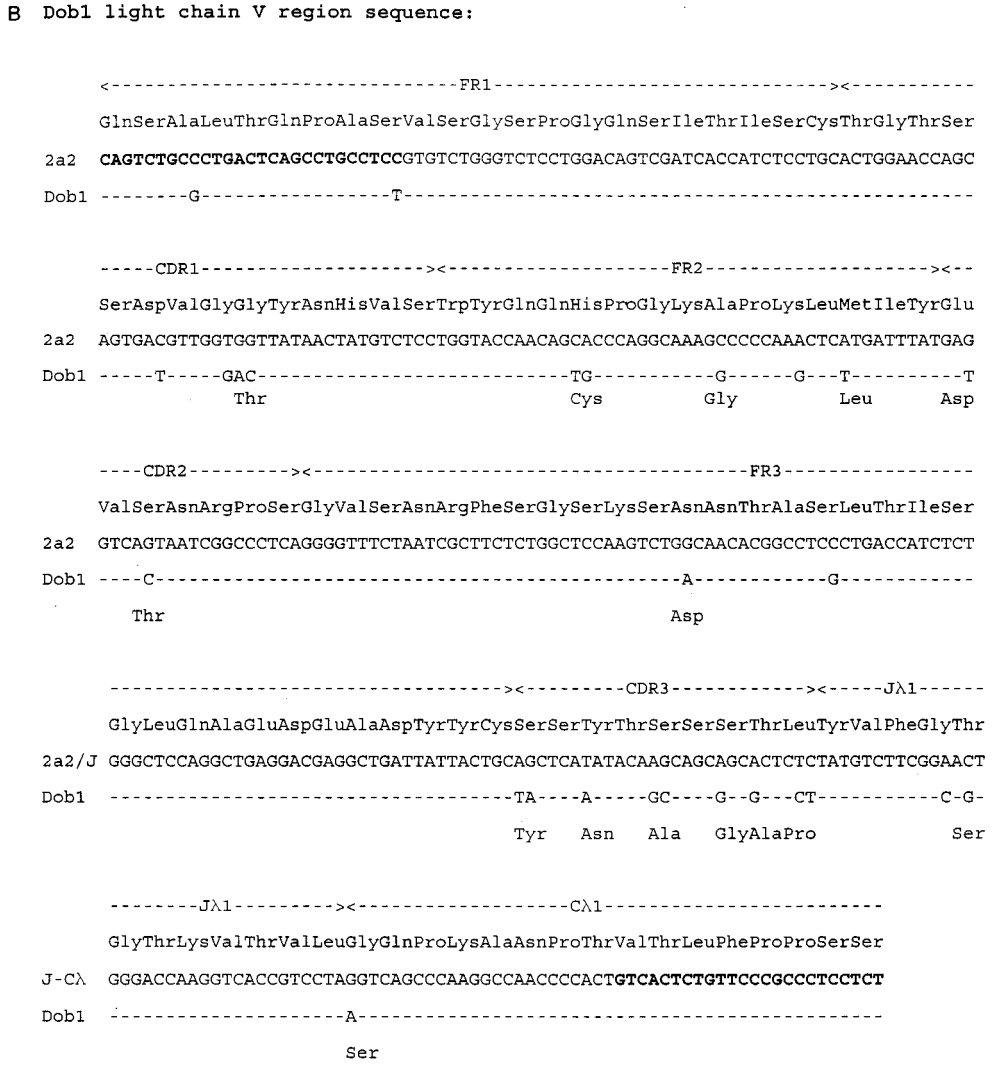

FIG. 5—*Continued.*

tive approach is to purify clonal antibodies from immune sera and determine their amino acid sequences (34). This alternative approach is limited by difficulties in obtaining the sequences of the internal part of the V region, which requires the cleavage of antibody molecules, generally at methionine or tryptophan. Purification of a specific peptide fragment from other cleavage peptides is difficult because only a limited amount of antibody protein (about 10 to 200 μg from 1 U of blood) can be obtained from immune sera. On the other hand, sequencing the mixture of cleavage peptides produces many amino acids at each sequencing cycle, and it is difficult to identify the sequence of the desired peptide fragment. Our strategy was to treat the mixture of peptide fragments, at the appropriate cycle of amino acid sequencing, with OPA, which blocks all peptides at the N termini except the one with proline at the N terminus. This is effective because both VH and VL regions of antibody regularly have methionine-proline or tryptophan-proline pairs. For instance, VH invariantly has tryptophan at position 36 and proline at position 41. We found that this simple strategy permits one to obtain the FR2-CDR2 region sequence with very small amounts of purified antibodies.

Our amino acid analysis of anti-6B antibodies shows that the antibodies generally use the products of genes in the VH3 (Table 1) and Vλ2 (Fig. 1) gene families. Because our amino acid sequences are variable and incomplete, it is hard to determine exactly what VH3 family genes are used to form anti-6B antibodies. Nevertheless, we believe that the candidate VH genes are 3-07, 3-23, 3-66, and 3-74. V3-23 gene is expressed in a large number of B cells in the normal peripheral blood (38) as well as among some anti-dsDNA antibodies (6). Examining the light chain of anti-6B antibody, we observed the expression of both Vκ and Vλ genes. Although the product of the A27 Vκ gene, a very commonly expressed gene (10), was found to dominate κ^+ anti-6B antibodies in some cases, the most common observation was that anti-6B antibodies encoded by multiple Vκ genes are expressed in small amounts in all individuals. In contrast to the κ chain, the λ chain of anti-6B antibodies is encoded by only a few, closely related Vλ2 genes. Among them, 2a2 has been found to be expressed most commonly (27% of all λ chains [15]). There are alleles among 2a2 genes, and this allelism may affect the choice of the specific Vλ2 genes. Taken together, the V-region structure of anti-6B antibody is typically derived from VH3 and Vλ2 genes.

This conclusion is further supported by the analysis of a hybridoma (Dob1) secreting anti-6B antibody. Also, detailed analysis of the Dob1 sequence strongly suggests that anti-6B

TABLE 1. Summary of V-gene usage by anti-6B antibodies[a]

Clone name	VH	Vλ	Vκ
Dob1	VH3 (DP38)	Vλ2 (2a2 or 2b2)	None
P18Cλ	VH1	Vλ2 (2a2)	
P704C	VH3	Vλ2 (2e)	
P9Cλ	VH3	Vλ2 (2c)	
P703Cλ	VH3	Vλ2 (2c)	
P703Cκ-A	VH3		Vκ1, Vκ2, Vκ3, Vκ4
P703Cκ-B	VH3		Vκ1, Vκ2, Vκ3, Vκ4
Pk6G2κ-A	VH3		Vκ3 (A27)
Pk6G1κ-B	VH3		Vκ1, Vκ2, Vκ3, Vκ4
Pk6G1κ-A	VH3		Vκ3 (A27)
Pk6G1κ-B	VH3		Vκ2 (A1 or A17), Vκ3
P16Cκ	VH3		Vκ1, Vκ2, Vκ3, Vκ4
P26Cλ	VH3/VH1	Vλ10 (10a)	

[a] κ or λ at the end of the sample name indicates either the κ or λ fraction of an antibody preparation. The suffixes "A" and "B" indicate bands of high (about 27 kDa) and low (about 26 kDa) molecular mass. When the sequence allowed identification of a strong candidate V-family gene, its name is shown in parentheses.

antibodies can be made with commonly expressed V-gene elements. The VL region of Dob1 is derived from one of the two genes belonging to Vλ2 family (2a2 or 2b2), probably the commonly used 2a2 gene (15). Its Vλ gene is directly joined to Jλ1 without any evidence for unique amino acid(s) at the N region of VL, and the VH region is comprised of V3-15 and JH4, which is expressed over half of all antibodies (31). Incidentally, the V3-15 and 2a2 Vλ combination was also observed for an antibody to *H. influenzae* type b PS, JB21 (1). Although the D region could not be associated with a specific D gene, it is joined with the JH gene at the typical site of the JH gene. Both VH and VL regions of Dob1 display evidence for somatic mutations, which were reported to be present among anti-PS antibodies (1, 30, 32). Taken together, the results indicate that a canonical antibody for 6B PS is formed with the V-gene components that are frequently expressed, and the structure does not suggest why humans have difficulty in producing anti-6B antibodies upon immunization.

Our study of anti-6B antibodies provides several pieces of information about λ-chain usage in humans. So far, the expression of human λ gene components has not been studied even though the genomic organization of human Vλ and Cλ genes is quite distinct from those of other Ig chains. First, we show for the first time an expressed Vλ10 gene product at the protein level. Previously, expression of this gene was noted only in an in vitro variant of a cell line (39). Compared to the translated sequence of the 10a Vλ gene, our amino acid sequence lacked the two N-terminal residues. The cleavage of N- or C-terminal residues has been observed for antibodies with blocked N termini (37a) or enzymes (2). Second, we observed that four anti-6B antibody clones use the Cλ1 gene only; this observation is unlikely due to random occurrence since Cλ1 is expressed in only about 10% of all λ^+ myelomas (21) or serum Igs expressing the λ light chain (37).

Our study provides a clear explanation for the observation made previously by Livneh et al. that pneumococcal vaccine elicits antibodies expressing the 8.12 idiotype (19). In fact, we found that there is a striking similarity between anti-6B antibodies and those anti-dsDNA antibodies containing VH3 and Vλ2 gene products (26, 36). Interestingly, P16C, which uses a VL that is very similar to anti-dsDNA antibody, uses a VH1 gene product although VH3 genes are most commonly expressed for anti-6B. Perhaps anti-6B antibodies cross-reactive to dsDNA have been eliminated by apoptosis during the immune response to the pneumococcal vaccines. To test if there is a censoring of the B-cell repertoire, we are currently producing hybridomas by using fusion partners expressing Bcl-2. Despite this structural similarity, we have not yet seen a strong binding of the anti-6B antibodies to dsDNA. Also, immunization with 23-valent PS vaccines does not increase the level of anti-dsDNA antibodies in adults (14). Nevertheless, new pneumococcal conjugate vaccines may need to be tested for eliciting antibodies to dsDNA.

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REFERENCES

1. **Adderson, E. E., P. G. Shackelford, A. Quinn, P. M. Wilson, M. W. Cunningham, R. A. Insel, and W. L. Carroll.** 1993. Restricted immunoglobulin VH usage and VDJ combinations in the human response to *Haemophilus influenzae* type b capsular polysaccharide. Nucleotide sequences of monospecific anti-*Haemophilus* antibodies and polyspecific antibodies cross-reacting with self antigens. J. Clin. Investig. **91:**2734–2743.
2. **Billadello, J. J., D. G. Roman, A. M. Grace, B. E. Sobel, and A. W. Strauss.** 1985. The nature of post-translational formation of MM creatine kinase isoforms. J. Biol. Chem. **260:**14988–14992.
3. **Brauer, A. W., C. L. Oman, and M. N. Margolies.** 1984. Use of o-phthalaldehyde to reduce background during automated Edman degradation. Anal. Biochem. **137:**134–142.
4. **Breiman, R. F., J. C. Butler, F. C. Tenover, J. A. Elliott, and R. R. Facklam.** 1994. Emergence of drug-resistant pneumococcal infections in the United States. JAMA **271:**1831–1835.
5. **Carroll, W. L., K. Thielemans, J. Dilley, and R. Levy.** 1986. Mouse X human heterohybridomas as fusion partners with human B cell tumors. J. Immunol. Methods **89:**61–72.
6. **Chen, P. P., M. F. Liu, C. A. Glass, S. Sinha, T. J. Kipps, and D. A. Carson.** 1989. Characterization of two immunoglobulin VH genes that are homologous to human rheumatoid factors. Arthritis Rheum. **32:**72–76.
7. **Chomczynski, P., and N. Sacchi.** 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162:**156–159.
8. **Chothia, C., A. M. Lesk, E. Gherardi, I. M. Tomlinson, G. Walter, J. D. Marks, M. B. Llewelyn, and G. Winter.** 1992. Structural repertoire of the human VH segments. J. Mol. Biol. **227:**799–817.
9. **Corbett, S. J., I. M. Tomlinson, E. L. L. Sonnhammer, D. Buck, and G. Winter.** 1997. Sequence of the human immunoglobulin diversity (D) segment locus: a systematic analysis provides no evidence for the use of DIR segments, inverted D segments, "minor" D segments of D-D recombination. J. Mol. Biol. **270:**587–597.
10. **Cox, J. P. L., I. M. Tomlinson, and G. Winter.** 1994. A directory of human germ-line Vκ segments reveals a strong bias in their usage. Eur. J. Immunol. **24:**827–836.
11. **Dariavach, P., G. Lefranc, and M.-P. Lefranc.** 1987. Human immunoglobulin C lambda 6 gene encodes the Kern+Oz-lambda chain and C lambda 4 and C lambda 5 are pseudogenes. Proc. Natl. Acad. Sci. USA **84:**9074–9078.
12. **Fedson, D. S.** 1988. Pneumococcal vaccine, p. 271–299. *In* S. A. Plotkin and E. A. Mortimer, Jr. (ed.), Vaccines. W. B. Saunders Co., Philadelphia, Pa.
13. **Frippiat, J. P., S. C. Williams, I. M. Tomlinson, G. P. Cook, D. Cherif, D. L. Paslier, J. E. Collins, I. Dunham, G. Winter, and M. P. Lefranc.** 1995. Organization of the human immunoglobulin lambda light-chain locus on chromosome 22q11.2. Hum. Mol. Genet. **4:**983–991.
14. **Grayzel, A., A. Solomon, C. Aranow, and B. Diamond.** 1991. Antibodies elicited by pneumococcal antigens bear an anti-DNA-associated idiotype. J. Clin. Investig. **87:**842–846.
15. **Ignatovich, O., I. M. Tomlinson, P. T. Jones, and G. Winter.** 1997. The creation of diversity in the human immunoglobulin Vλ repertoire. J. Mol. Biol. **268:**69–77.
16. **Kabat, E. A., K. G. Nickerson, J. Liao, L. Grossbard, E. F. Osserman, E. Glickman, L. Chess, J. B. Robbins, R. Schneerson, and Y. H. Yang.** 1986. A human monoclonal macroglobulin with specificity for a (2-8)-linked poly-N-acetyl neuraminic acid, the capsular polysaccharide of group B meningococci and Escherichia coli K1, which crossreacts with polynucleotides and with denatured DNA. J. Exp. Med. **164:**642–654.
17. **Kabat, E. A., T. T. Wu, H. M. Perry, K. S. Gottesman, and C. Foeller.** 1991. Sequences of proteins of immunological interest. Department of Health and Human Services, Bethesda, Md.
18. **Lieberman, J. M., S. S. Chiu, V. K. Wong, S. Partridge, S. J. Chang, C. Y. Chiu, L. L. Gheesling, G. M. Carlone, and J. I. Ward.** 1996. Safety and immunogenicity of a serogroups A/C *Neisseria meningitidis* oligosaccharide-

protein conjugate vaccine in young children. A randomized controlled trial. JAMA **275:**1499–1503.
19. **Livneh, A., E. Gazit, and B. Diamond.** 1994. The preferential expression of the anti-DNA associated 8.12 idiotype in lupus is not genetically controlled. Autoimmunity **18:**1–6.
20. **Livneh, A., A. Halpern, D. Perkins, A. Lazo, R. Halpern, and B. Diamond.** 1987. A monoclonal antibody to a cross-reactive idiotype on cationic human anti-DNA antibodies expressing lambda light chains: a new reagent to identify a potentially differential pathogenic subset. J. Immunol. **138:**123–127.
21. **Livneh, A., J. L. Preud'homme, A. Solomon, and B. Diamond.** 1987. Preferential expression of the systemic lupus erythematosus-associated idiotype 8.12 in sera containing monoclonal immunoglobulins. J. Immunol. **139:**3730–3733.
22. **Naparstek, Y., D. Duggan, A. Schattner, M. P. Madaio, F. Goni, B. Frangione, B. D. Stollar, E. A. Kabat, and R. S. Schwartz.** 1985. Immunochemical similarities between monoclonal antibacterial Waldenstrom's macroglobulins and monoclonal anti-DNA lupus autoantibodies. J. Exp. Med. **161:**1525–1538.
23. **Niewold, T. A., C. L. Murphy, D. T. Weiss, and A. Solomon.** 1996. Characterization of a light chain product of the human JCλ7 gene complex. J. Immunol. **157:**4474–4477.
24. **Ortqvist, A., J. Hedlund, L. Burman, E. Elbel, M. Hofer, M. Leinonen, I. Lindblad, B. Sundelof, and M. Kalin.** 1998. Randomized trial of 23-valent pneumococcal capsular polysaccharide vaccine in prevention of pneumonia in middle-aged and elderly people. Lancet **351:**399–403.
25. **Park, M. K., Y. Sun, J. V. Olander, J. W. Hoffmann, and M. H. Nahm.** 1996. The repertoire of human antibodies to the carbohydrate capsule of *Streptococcus pneumoniae* 6B. J. Infect. Dis. **174:**75–82.
26. **Paul, E., and B. Diamond.** 1993. Characterization of two human anti-DNA antibodies bearing the pathogenic idiotype 8.12. Autoimmunity **16:**13–21.
27. **Quataert, S. A., C. S. Kirch, L. J. Q. Wiedl, D. C. Phipps, S. Strohmeyer, C. O. Cimino, J. Skuse, and D. V. Madore.** 1995. Assignment of weight-based antibody units to a human antipneumococcal standard reference serum, lot 89-S. Clin. Diagn. Lab. Immunol. **2:**590–597.
28. **Robbins, J. B., R. Austrian, C. J. Lee, S. C. Rastogi, G. Schiffman, J. Henrichsen, P. H. Makela, C. V. Broome, R. R. Facklam, R. H. Tiesjema, and J. C. Parke, Jr.** 1983. Considerations for formulating the second-generation pneumococcal capsular polysaccharide vaccine with emphasis on the cross-reactive types within groups. J. Infect. Dis. **148:**1136–1159.
29. **Robbins, J. B., R. Schneerson, P. Anderson, and D. H. Smith.** 1996. Prevention of systemic infections, especially meningitis, caused by *Haemophilus influenzae* type b. JAMA **276:**1181–1185.
30. **Rubinstein, L. J., and K. E. Stein.** 1988. Murine immune response to the Neisseria meningitidis group C capsular polysaccharide. II. Specificity. J. Immunol. **141:**4357–4362.
31. **Sanz, I.** 1991. Multiple mechanisms participate in the generation of diversity of human H chain CDR3 regions. J. Immunol. **147:**1720–1729.
32. **Scott, M. G., D. L. Crimmins, D. W. McCourt, G. Chung, K. F. Schable, R. Thiebe, E. M. Quenzel, H. G. Zachau, and M. H. Nahm.** 1991. Clonal characterization of the human IgG antibody repertoire to Haemophilus influenzae type b polysaccharide. IV. The less frequently expressed VL are heterogenous. J. Immunol. **147:**4007–4013.
33. **Scott, M. G., D. L. Crimmins, D. W. McCourt, I. Zocher, R. Thiebe, H. G. Zachau, and M. H. Nahm.** 1989. Clonal characterization of the human IgG antibody repertoire to *Haemophilus influenzae* type b polysaccharide. III. A single VKII gene and one of several JK genes are joined by an invariant arginine to form the most common L chain V region. J. Immunol. **143:**4110–4116.
34. **Scott, M. G., J. J. Tarrand, D. L. Crimmins, D. W. McCourt, N. R. Siegel, C. E. Smith, and M. H. Nahm.** 1989. Clonal characterization of the human IgG antibody repertoire to *Haemophilus influenzae* type b polysaccharide. II. IgG antibodies contain VH genes from a single VH family and VL genes from at least four VL families. J. Immunol. **143:**293–298.
35. **Siber, G. R.** 1994. Pneumococcal disease: prospects for a new generation of vaccines. Science **265:**1385–1387.
36. **Sniadack, D. H., B. Schwartz, H. Lipman, J. Bogaerts, J. C. Butler, R. Dagan, G. Echaniz-Aviles, N. Lloyd-Evans, A. Fenoll, N. I. Girgis, J. Henrichsen, K. Klugman, D. Lehmann, A. K. Takala, J. Vandepitte, S. Gove, and R. F. Breiman.** 1995. Potential interventions for the prevention of childhood pneumonia: geographic and temporal differences in serotype and serogroup distribution of sterile site pneumococcal isolates from children—implications for vaccine strategies. Pediatr. Infect. Dis. J. **14:**503–510.
37. **Solomon, A.** 1986. Light chains of immunoglobulins: structural-genetic correlates. Blood **68:**603–610.
37a. **Solomon, A.** Unpublished data.
38. **Stewart, A. K., C. Huang, B. D. Stollar, and R. S. Schwartz.** 1993. High-frequency representation of a single VH gene in the expressed human B cell repertoire. J. Exp. Med. **177:**409–418.
39. **Stiernholm, N. B. J., B. Kuzniar, and N. L. Berinstein.** 1994. Identification of a new human Vlambda gene family—VlambdaX. J. Immunol. **152:**4969–4975.
40. **Taub, R. A., G. F. Hollis, P. A. Hieter, S. Korsmeyer, T. A. Waldmann, and P. Leder.** 1983. Variable amplification of immunoglobulin lambda light-chain genes in human populations. Nature **304:**172–174.
41. **Tomlinson, I. M., G. Walter, J. D. Marks, M. B. Llewelyn, and G. Winter.** 1992. The Repertoire of human germline VH sequences reveals about fifty groups of VH segments with different hypervariable loops. J. Mol. Biol. **227:**776–798.
42. **Williams, S. C., J. P. Frippiat, I. M. Tomlinson, O. Ignatovich, M.-P. Lefranc, and G. Winter.** 1996. Sequence and evolution of the human germline Vlambda, repertoire. J. Mol. Biol. **264:**220–232.
43. **Yamada, M., R. Wasserman, B. A. Reichard, S. Shane, A. J. Caton, and G. Rovera.** 1991. Preferential utilization of specific immunoglobulin heavy chain diversity and joining segments in adult human peripheral blood B lymphocytes. J. Exp. Med. **173:**395–407.

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