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Variable Region Sequences of a Protective Human Monoclonal Antibody Specific for the *Haemophilus influenzae* Type b Capsular Polysaccharide

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A hybridoma secreting a human immunoglobulin G2 κ monoclonal antibody (MAb) specific for the capsular polysaccharide of *Haemophilus influenzae* type b (Hib) was isolated. This MAb, designated CA4, was bactericidal to Hib in vitro and protected infant rats from Hib bacteremia. Nucleotide sequence analysis of CA4 variable (V) region cDNA showed that the heavy (H)-chain V region was of subgroup III and was 96% identical to the V_H germ line gene segment DP77 (V3-21). The light (L)-chain V region was of the κ subgroup III and was 94% identical to the A27 (Humkv325) germ line gene, which is commonly used by rheumatoid factors and other autoantibodies. MAb CA4 did not have rheumatoid factor activity and did not react with histones, DNA, or chromatin. These findings identify an additional V_HIII gene segment which can contribute to the anti-Hib capsular polysaccharide repertoire and demonstrate that a V_L gene commonly encoding autoantibodies can be utilized for protective immunity.

Immunity to encapsulated bacteria is mediated largely by antibodies specific for the capsular polysaccharide (PS) antigens. The lack of expression of these antibody specificities in infancy and early childhood contributes to the susceptibility of this population to developing invasive diseases caused by encapsulated bacteria. Until recently, *Haemophilus influenzae* type b (Hib) was a leading cause of bacterial meningitis in children in North America. Since the introduction of Hib polysaccharide-protein conjugate vaccines for use in infants (57), a dramatic decline in invasive Hib disease has occurred (35, 38). This reduction can be attributed to the ability of these vaccines to prime and induce a protective antibody response to the Hib PS. The mechanisms by which the conjugate vaccines induce protection, however, are poorly understood. Considerable evidence suggests that these vaccines differ in their immunogenicity (11, 14) and in the quality of the anti-Hib PS antibodies they induce (46).

The human antibody repertoire to Hib PS has come under intense scrutiny in the past few years. This interest stems from the desire to define the genetic and molecular bases of protective antipolysaccharide immunity and to develop a model system for understanding the rules governing the expression of these important antibody specificities in humans. The antibody repertoire to Hib PS is remarkable for its limited heavy (H)-chain variable (V) region gene utilization. V_H regions are restricted to the V_HIII family (3, 49, 51) and derive from either the V_H 26, V_H 9.1 (also known as V3-23 and V3-15, respectively [34]), or closely related gene segments (3, 4, 7). Light (L)-chain V region utilization is more complex since as many as eight distinct V_L region genes contribute to the antibody repertoire (2, 7, 47–49). In contrast to this capacity to generate a sizable antibody repertoire at the population level, individual repertoires frequently consist of only a few distinct antibody clonotypes (21), and particular V regions dominate. For example, anti-Hib PS antibodies having V_L regions encoded by a single κII gene constitute by far the most prevalent V_L region (29, 30, 48).

Studies using anti-idiotypic reagents of defined specificity have shown that the anti-Hib PS V_L region repertoire is dynamically controlled by factors related to age and vaccine formulation. Different Hib PS conjugate vaccines induce distinctive patterns of V region expression (16), and the relative expression of particular V_L regions differs between infants and adults. For example, infants vaccinated with Hib PS coupled to the outer membrane protein complex of group B *Neisseria meningitidis* produce little or no serum anti-Hib PS antibodies having κIII V regions, whereas κIII antibodies are readily demonstrable in vaccinated adults (28).

In an effort to define further the V regions contributing to the protective anti-Hib PS repertoire, we determined the V_H and V_L nucleotide sequences of a human Hib PS-specific monoclonal antibody (MAb) which is biologically active against Hib organisms. Our results demonstrate that this MAb utilizes a V_HIII gene not previously known to encode anti-Hib PS antibodies and a $V_κ$III gene closely resembling that encoding a variety of autoantibodies.

MATERIALS AND METHODS

Isolation of hybridoma CA4. A 41-year-old healthy adult white male received a subcutaneous injection of 40 μg of Hib PS vaccine (gift of Porter Anderson, University of Rochester). Although this individual had not been vaccinated previously with Hib PS, his prevaccination serum contained 0.25 μg of anti-Hib PS antibody per ml. In the absence of vaccination, most older children and virtually all adults have detectable serum antibody to Hib PS, which presumably reflects their exposure to Hib or to Hib PS cross-reactive antigens (43). Five days after the subject was vaccinated, heparinized peripheral blood was obtained, and mononuclear leukocytes were isolated by centrifugation through lymphocyte separation medium (Organon Teknika, Durham, N.C.). Leukocytes were fused to the

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azaserine-sensitive mouse-human heteromyeloma JL-H7 cell line (27) by using 50% polyethylene glycol. Cells were cultured in flat-bottom microtiter wells containing mouse peritoneal cells. Culture medium consisted of RPMI 1640 supplemented with 5% fetal calf serum, 50 μM 2-mercaptoethanol, 2 mM glutamine, 1 mM sodium pyruvate, 100 U of penicillin-streptomycin per ml, 0.25 μg of amphotericin B per ml, 16 μM hypoxanthine, 12 μM azaserine, and 16 μM thymidine. After 1 week, cultures were fed every other day by aspiration of half the medium and addition of fresh medium. Culture supernatants were screened for anti-Hib PS antibody activity by using a Hib PS enzyme-linked immunosorbent assay (ELISA; see below). An anti-Hib PS-secreting cell line, designated CA4, was cloned by repeated limiting dilution in the presence of mouse peritoneal cells.

Purification of MAb CA4. Tissue culture supernatant, obtained from high-density cultures of CA4 hybridoma cells, was brought to 50% saturation by the addition of solid ammonium sulfate. After the supernatant was stirred overnight, the precipitate was harvested by centrifugation, washed once with 50% saturated ammonium sulfate, and then dialyzed against 0.1 M acetate–0.15 M NaCl (pH 5.0). The immunoglobulin G (IgG) fraction was isolated by adsorption to protein G-agarose (Pierce Chemical Co., Rockford, Ill.) and then eluted with 0.1 M glycine-HCl (pH 2.8). Eluates were neutralized with 1.0 M Tris (pH 8.0), and the fractions containing protein were pooled and dialyzed against phosphate-buffered saline (PBS; pH 7.2). The purified IgG was absorbed with agarose-coupled goat antibodies specific for human λ light chains (Caltag Laboratories, South San Francisco, Calif.) to remove the low levels of λ chain-containing antibodies. The λ chain derives from the H-7 heterohybridoma fusion partner. The IgG was also absorbed with agarose-coupled antibodies specific for bovine IgG (FisherBiotech, Pittsburgh, Pa.) to eliminate any bovine IgG which may have been copurified from the culture supernatant. The purity of CA4 was assessed by isoelectric focusing and Western blotting (immunoblotting) as described previously (29). pI standards were purchased from Pharmacia Biotech, Piscataway, N.J. Protein was detected by using silver reagent as described in the manufacturer's instructions (Bio-Rad Laboratories, Hercules, Calif.).

Anti-Hib PS antibody assays. The isotypes of the CA4 MAb were assessed by ELISA (15). Microtiter wells, coated with Hib PS–poly-L-lysine, were incubated with culture supernatant or with purified CA4 and then reacted with alkaline phosphatase-coupled antibodies specific for either human IgG, IgA, IgM, κ, or λ (Caltag Laboratories), or the IgG subclasses (The Binding Site, San Diego, Calif.). Mouse MAb B12, specific for the human κIII subgroup (31), in conjunction with enzyme-conjugated anti-mouse immunoglobulin, was used to verify that CA4 expressed a κIII L chain.

A modification of a previously described radioantigen binding assay, utilizing ^{125}I-Hib PS (43), was used to quantitate CA4 anti-Hib PS activity and to evaluate antigen-binding specificity. Diluent consisted of PBS with 10% fetal calf serum. Fifty microliters of ^{125}I-Hib PS containing ~30,000 cpm (specific activity, ~45 μCi/μg) was mixed with 50 μl of diluted CA4 or reference anti-Hib PS antibodies. The mixtures were incubated for 2 h at 37°C and overnight at 4°C. After adding 100 μl of 100% saturated ammonium sulfate to the mixtures and incubating them at 4°C for 2 h, the precipitates were harvested by centrifugation (16,000 × *g*), washed once with 50% saturated ammonium sulfate, and then counted in a dry-well gamma counter. Background ^{125}I-Hib PS (precipitated in the presence of diluent alone) was subtracted from the respective means of duplicate determinations. Anti-Hib PS antibody concentration was determined by comparison with a standard curve generated by a reference anti-Hib PS antiserum provided by the Center for Biological Evaluation and Research and assigned a value of 70 μg of anti-Hib PS per ml. A modification of this assay, which evaluates binding at two different concentrations of ^{125}I-Hib PS, was used for avidity determination of CA4 (17). An inhibition assay was used to examine the reactivity of CA4 with unlabeled Hib PS and other polysaccharides. CA4, at a concentration of 200 ng/ml, was mixed with an equal volume of polysaccharide at various concentrations. After incubation of the mixture for 2 h at 37°C and overnight at 4°C, the Hib PS-binding activity was determined by using the radioantigen binding assay described above. The final concentration of CA4 used in this assay, i.e., 100 ng/ml, gives half-maximal binding of ^{125}I-Hib PS in the radioantigen binding assay. The percent inhibition of ^{125}I-Hib PS binding was calculated as follows: 100 × [1 − (mean cpm bound by CA4 in the presence of polysaccharide/mean cpm bound by CA4 in the presence of diluent alone)].

Bactericidal and rat protection assays. MAb CA4 was tested for complement-mediated bactericidal activity in vitro as described previously (5). Briefly, ~3 × 10^3 log-phase Hib (strain Eagan) organisms per ml were reacted with various concentrations of MAb CA4 or, as controls, with purified polyclonal human IgG1 and IgG2 anti-Hib PS antibodies (5) in the presence of 20% agammaglobulinemic serum as a source of complement. After incubation at 37°C for 60 min in a shaking water bath, serial dilutions were plated on chocolate agar plates. Hib colonies were scored after ~24 h of incubation at 37°C.

An infant rat assay was used to evaluate in vivo protection (5). Five- to 7-day-old rats received a subcutaneous injection of either purified IgG1 or IgG2 anti-Hib PS antibodies, purified MAb CA4, or, as a negative control, a human IgG2 myeloma protein (a gift of Hans L. Spiegelberg, University of California, San Diego, La Jolla). The next day, the rats received an intraperitoneal injection of ~100 log-phase Hib (strain Eagan) organisms. Eighteen to 24 h later, blood was taken and assayed quantitatively on chocolate agar for the presence of Hib organisms.

Autoantibody assays. CA4 was evaluated by an ELISA for rheumatoid factor activity and for binding to histones, chromatin, and denatured DNA as described previously (44). CA4 reactivity with native DNA was evaluated by an immunofluorescence assay with *Crithidia lucilea* (6).

Sequencing of CA4 V regions. RNA was extracted from CA4 hybridoma cells by a microadaptation of the guanidine thiocyanate procedure (42). cDNA was synthesized by using either the 3′ PCR primers (see below) or oligo(dT)$_{18}$. cDNA was amplified by PCR, ligated into a plasmid vector, and transfected into host bacteria, and individual clones were isolated on antibiotic-containing plates. The H chain was independently cloned and sequenced twice by using two different sets of PCR primers and two different cloning vectors. The CA4 L chain was independently cloned and sequenced three times by using three different sets of PCR primers and three different cloning vectors. For both the H and L chains, the 5′ PCR primers were designed to hybridize either to the leader sequence or to the first few codons of framework 1. The 3′ PCR primers were complementary to either sequences of the constant (C) region immediately distal to the V-C junction, to the 3′ end of C_κ, or to the 3′ end of the γ-2 hinge region (26). All primers introduced restriction enzyme sites to facilitate ligation into the cloning vectors. Double-stranded DNA was sequenced by a modification (55) of the original chain termination method (45), using Sequenase (United States Biochemicals, Cleveland, Ohio).

Nucleotide sequence accession numbers. The CA4 V_H and V_L nucleotide sequences are available from EMBO or GenBank under accession numbers U06787 and U06788, respectively.

RESULTS

Isolation and characterization of MAb CA4 and analysis of antigen-binding specificity. A heterohybridoma secreting a Hib PS-reactive human IgG MAb, designated CA4, was isolated from a fusion between a mouse-human heterohybridoma cell line and peripheral blood lymphocytes from an adult immunized with Hib PS. After several rounds of cloning by limiting dilutions, CA4 hybridoma cells were cultured to high density, and the CA4 MAb was isolated from tissue culture supernatant as described in Materials and Methods. Figure 1 shows the results of an isoelectric focusing analysis of the purified MAb. A single spectrotype consisting of several tightly linked bands corresponding to a pI of 7.7 to 7.9 is revealed both by silver staining (Fig. 1A) and by reactivity with ^{125}I-Hib PS (Fig. 1B).

In a radioantigen binding assay, CA4 gave concentration-dependent binding to ^{125}I-Hib PS. The avidity of this binding was calculated to be 2.3×10^9 M^{-1} as determined by comparing relative ^{125}I-Hib PS binding at two different concentrations of radiolabeled Hib PS (data not shown) (17). ^{125}I-Hib PS binding by CA4 was completely inhibited by native Hib PS, a polymer of 3-β-D-ribose-(1-1)-ribitol-5 phosphate, but was not inhibited by high concentrations of either *Escherichia coli* K100 PS [3-β-D-ribose-(1-2)-ribitol-5 phosphate], which is structurally related to the Hib PS, or by the PSs of *N. meningitidis*, groups A and B, or by *Streptococcus pneumoniae* type 6 and 14 PSs (Fig. 2). CA4 also reacted with Hib PS in an ELISA format. Analysis of CA4 binding by an ELISA using isotype-specific antibodies showed CA4 to be an IgG2 κIII antibody (data not shown). When tested in an ELISA against a panel of autoantigens, CA4 (at a concentration of 5 μg/ml) was unreactive with IgG from several species including humans and was unreactive with histones, chromatin, or denatured DNA (data not shown). In addition, CA4 (at a concentration of 25 μg/ml) was unreactive with nuclear antigens as determined by an immunofluorescence assay with *C. lucilea* (data not shown).

MAb CA4 is functionally active against Hib. CA4 was evaluated for its biological activity against Hib organisms in vitro and in vivo. Figure 3 shows the results of a complement-dependent Hib bactericidal assay. CA4 gave a bactericidal dose-response activity similar to that of polyclonal human IgG1 and IgG2 anti-Hib PS antibodies. No killing of Hib organisms was observed with complement alone, i.e., agammaglobulinemic serum, or with CA4 in the presence of heat-inactivated agammaglobulinemic serum. CA4 was as effective as the control IgG1 and IgG2 anti-Hib PS antibodies in preventing bacteremia in infant rats (Table 1). At a dose of 0.1 μg/rat, the IgG1 polyclonal antibodies were more effective than either CA4 or the IgG2 polyclonal antibodies in modulating the level of Hib bacteremia.

CA4 V region sequences. The V_H cDNA sequence and its formal translation product are shown in Fig. 4. The V_H region is a member of the V_HIII family, a result consistent with previous studies of isolated serum antibodies (49, 51) and of hybridoma cDNAs (3), which show that human anti-Hib PS V_H regions are biased towards the V_HIII family. A comparison of the CA V_H sequence with sequences contained in the GenBank and EMBO databases identified a human V_HIII gene segment known as DP77 as the germ line gene (also known as V3-21 [34]) most closely related to the CA4 V_H segment. DP77 was isolated from a genomic library prepared with peripheral blood leukocytes from a single adult donor (56). The CA4 V_H nucleotide sequence is 96% identical to the DP77 nucleotide sequence (comparing coding residues 1 to 94). CA4 differs from DP77 at 11 bases, resulting in eight amino acid substitutions, three of which lie in complementary determining region 1 (CDR1) and CDR2. Genetic polymorphism and the possibility of somatic mutation make definitive assignment of DP77 as the parental gene of the CA4 V_H region problematic. The differences between the CA4 V_H gene sequence and that of DP77 may be somatically generated or the CA4 V_H region may arise from a germ line gene closely related to, yet distinct from, DP77. However, the nucleotide sequence

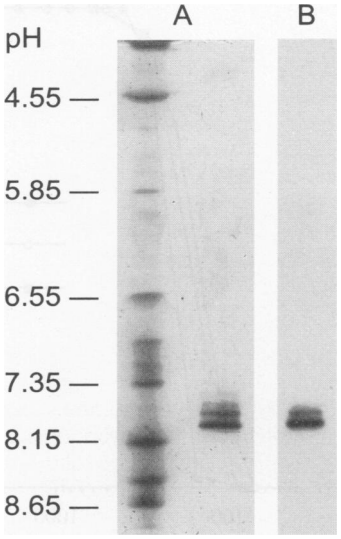

FIG. 1. Isoelectric focusing (IEF) analysis of purified MAb CA4. (A) Silver-stained IEF gel of CA4. pI standards are in the left lane. (B) Autoradiogram of CA4 IEF Western blot reacted with ^{125}I-Hib PS.

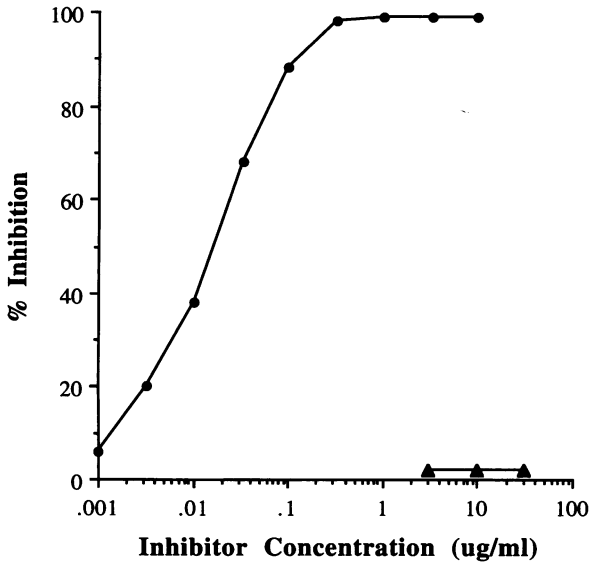

FIG. 2. Ability of Hib PS (●) and the PSs of *E. coli* K100, *N. meningitidis* groups A and B, and *S. pneumoniae* types 6 and 14 (▲) to inhibit the reaction between CA4 and ^{125}I-Hib PS.

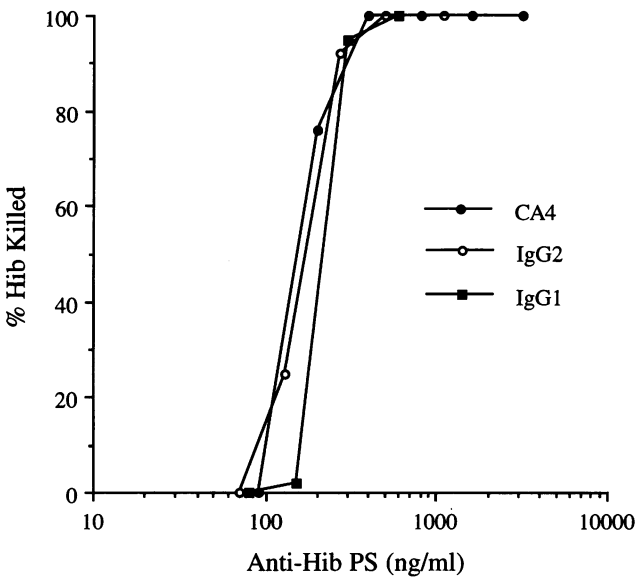

FIG. 3. Hib complement-dependent bactericidal assay of MAb CA4 and of positive control human polyclonal IgG1 and IgG2 anti-Hib PS antibodies.

of the CA4 V_H segment is sufficiently different from V_H 26 and V_H 9.1 (88 and 78% homology, respectively), the two V_H gene segments which encode all previously described anti-Hib PS MAbs, to eliminate these two genes as candidates encoding the CA4 V_H region. The CDR3 of the CA4 V_H may be created by the fusion of DXP'1 (20) and the DA4 germ line elements (20) (Fig. 4). CA4 uses the J_H4b segment in germ line configuration (58) (Fig. 4).

Figure 5 shows the CA4 V_κ cDNA sequence and its translated amino acid sequence. The V_κ region is a member of the κIII subgroup, and comparison with all known V_κ nucleotide sequences indicates that the A27 (53) (also known as Humkv325 [8, 41]) and A11 (also known as Humkv305 [8]) V_κ gene segments are the closest relatives to the CA4 sequence, being 94 and 93% identical to the CA4 V_κ sequence, respectively. The A27 gene product can be distinguished from the A11 gene product by the presence of a glycine residue at position 9 (47). Since CA4 contains a glycine in this position, it is likely that A27 is the germ line gene encoding the CA4 κIII V region. The CA4 nucleotide sequence differs from the A27 sequence at 15 bases (Fig. 5). Ten of these 15 differences result in eight amino acid substitutions, five of which are in CDR1 and CDR2. The CA4 J region derives from the J_κ1 germ line element and differs from the germ line sequence at five bases, two of which result in two amino acid substitutions. In CA4, a proline occurs at the V-J junction (position 95a), which is in contrast to the majority of the anti-Hib PS MAbs sequenced to date, which contain an arginine at this position (2, 48).

TABLE 1. Ability of MAb CA4 to protect infant rats from Hib bacteremia

Antibody	Dose (μg/rat)[a]	No. of bacteremic rats/total no.	Geometric mean CFU/ml of blood[b]
IgG2 myeloma	1.0	10/10	40,000
IgG2 polyclonal anti-Hib PS	1.0	2/5	4
	0.5	1/5	2
	0.1	6/6	25,000
IgG1 polyclonal anti-Hib PS	1.0	0/6	1
	0.5	4/6	24
	0.1	6/6	1,660
MAb CA4	1.0	1/6	1
	0.5	4/6	20
	0.1	6/6	27,500

[a] The concentration of the IgG2 myeloma protein was determined by absorbance, using an optical density at 280 nm of 1.5 for a 1.0-mg/ml solution. Concentration of the anti-Hib PS antibodies was determined by using the radioantigen binding assay.

[b] For calculation purposes, animals having sterile blood cultures were assigned a value of 1.0 CFU/ml.

DISCUSSION

Previous studies have shown that 2 V_HIII gene segments, V_H 26 (V3-23) and V_H 9.1 (V3-15), or close relatives, encode human anti-Hib PS antibodies. Of 15 hybridoma cDNAs analyzed to date, 9 use V_H 9.1 and 6 use V_H 26 (1, 3, 4, 7). The gene segment encoding the CA4 V_H is a member of the V_HIII family but is only distantly related to either V_H 26 or V_H 9.1, indicating that an additional V_H can contribute to the anti-Hib PS repertoire. The CA4 V_H cDNA nucleotide sequence is 96% identical to that of DP77, a gene isolated from an adult genomic library (56). Thus, DP77 or a close relative is likely to be the parental germ line gene from which the CA4 V_H region is derived. Similar to V_H 26 and V_H 9.1, DP77 can be expressed in early development since a gene identical to DP77, known as E54 3.2, has been isolated from a human cDNA library prepared with a liver from a 13-week fetus (10). The CDR3 of CA4 appears to be created by a D-D fusion between the DXP'1 and DA4 segments in combination with the J_H4b segment. Of the 15 anti-Hib PS V_H cDNAs sequenced previously (3, 4, 7), J_H4 is used in about half, and the CDR3 regions vary in length by up to 34 bp. The CDR3 of CA4 is 39 bp long, and therefore, it represents the longest anti-Hib PS H-chain CDR3 described to date.

Two germ line V_κIII gene segments, L16 (also known as Humkv328 [8]) and A27 (also known as Humkv325 [8, 41]), contribute to the anti-Hib PS repertoire. The L16 gene is used by an anti-Hib PS hybridoma (7), and the usage of the A27 gene has been inferred from partial amino acid sequences obtained from clonally purified serum anti-Hib PS antibodies (47). The CA4 κIII V region derives from the A27 gene or a closely related gene and appears to have undergone somatic mutation since five of the eight amino acid differences between the CA4 translation sequence and the A27 translation sequence occur in CDR1 and CDR2. The CA4 V_κIII region has recombined with the J_κ1 segment, which is used by other anti-Hib PS antibodies in combination with the κII-A2 V gene segments (48). The occurrence in CA4 of a proline residue at the V-J junctional residue 95a is notable since the majority of both κ and λ anti-Hib PS antibodies contain an arginine residue at this location (2, 48). It has been suggested that this residue plays an important role in the binding of the negatively charged Hib PS, although this requirement apparently is not stringent, since a proline residue occurs at position 95a not only in the CA4 V_κ but also in the κI V region used by the anti-Hib PS hybridoma ED6.1 (2).

The human V region antibody repertoire to Hib PS is assembled from a few V_HIII gene segments and as many as eight V_L segments. The use of multiple D and J segments in combination with nontemplated nucleotide additions provides additional diversity in the third CDRs. Somatic hypermutation

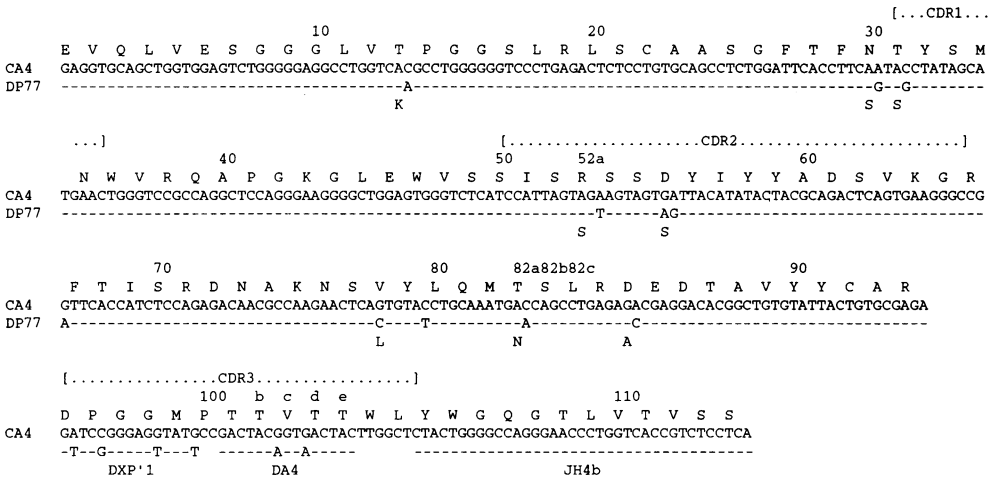

FIG. 4. Nucleotide sequence and translated amino acid sequence of the CA4 V_H region. The germ line nucleotide sequences of the V_HIII gene segment DP77 (56) (also known as V3-21 [34]), DXP'1, DA4, and J_H4b are shown for comparison. Dashes indicate identity with the CA4 sequence. Only those portions of the D segments that align with the CA4 sequence are shown. The amino acid residues of the DP77 translation product that differ from those of CA4 are shown below the DP77 nucleotide sequence. The numbering of residues and the locations of the CDRs are as described in reference 22.

appears to play a variable role. The V_H 26 and V_H 9.1 gene segments may be expressed in near-germ line configuration or may be somatically mutated (3, 4, 7). Anti-Hib PS V_L regions fall into two categories: those such as the V_κII-A2 region which are expressed in germ line or near-germ line configuration (7, 48) and those which are somatically mutated variants and which derive from a number of V_L groups (2, 47). The CA4 MAb represents an example of an anti-Hib PS antibody that appears to have V_H and V_L regions which have mutated from their germ line counterparts. In some instances, correlations have been made between the usage of particular V_L regions, including the κIII subgroup, and cross-reactivity with the *E. coli* K100 capsular polysaccharide, which is structurally related to Hib PS (2, 47). However, CA4 does not react with K100, and therefore, this property cannot be considered a universal fine-specificity marker of anti-Hib PS antibodies with κIII V regions.

Despite the capacity to generate a relatively large number of different V_H and V_L combinations, the serum anti-Hib PS population expressed in individuals frequently is oligoclonal (21) and dominated by only a few V regions. For example, the κII-A2 V region, which is thought to be expressed in germ line or near-germ line configuration, is by far the most commonly expressed V_L region, being present in most individuals and constituting on average more than half of the serum anti-Hib PS antibody in vaccinated adults (29, 30). In contrast, the less frequently expressed V_L regions are somatically mutated variants (2, 47). These findings, when taken with the observation that κII-A2 anti-Hib PS antibodies expressing the HibId-1 idiotype appear to be selectively induced in adults following

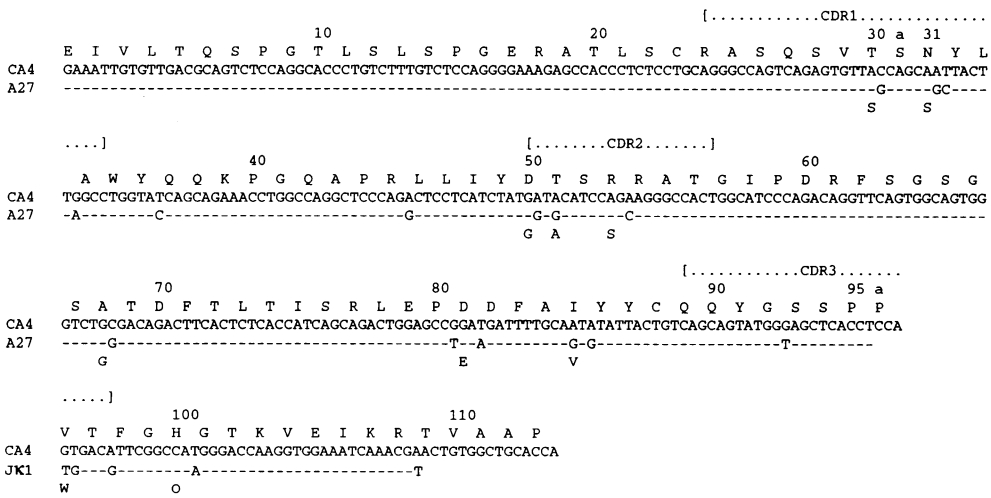

FIG. 5. Nucleotide sequence and translated amino acid sequence of the CA4 V_κ region. The nucleotide sequences of the A27 germ line gene segment (53), also known as Humkv325 (8, 41), and the J_κ1 segment are shown for comparison. Dashes indicate identity with the CA4 sequence. The amino acid residues in the A27 and the J_κ1 translation products that differ from those of CA4 are shown below the respective nucleotide sequence. The numbering of residues and the location of the CDRs are as described in reference 22.

vaccination (29), suggest that the unmutated A2 V region, when paired with an appropriate V_H region, creates a high-affinity anti-Hib PS paratope, whereas other V_L regions, such as the CA4 A27 V region, may require mutations to generate high-affinity anti-Hib PS combining sites. Anti-Hib PS antibodies with κIII V regions are of particular interest in this regard since their expression is temporally controlled. Following vaccination with Hib PS-outer membrane protein complex, κIII antibodies are undetectable in 2-month-old infants, are variably represented in 18-month-old children, but are quite prevalent in adults (28). $V_κ$III antibodies such as CA4 may arise in the primary B-cell pool in near-germ line configuration with low affinity for Hib PS. Perhaps over time, exposure to either Hib, to Hib PS cross-reactive antigens, or possibly to self antigens (see below) generates a memory population that has undergone hypermutation and has acquired higher affinity for Hib PS. Consistent with this suggestion is a recent study showing that virgin B cells (surface IgM^+ and IgD^+) present in adult peripheral blood express the A27 gene product in germ line configuration whereas memory B cells (surface IgM^- and IgD^-) express A27 in mutated form (25). The CA4 MAb probably derives from a memory B-cell population; it is of the IgG isotype, and although it was isolated from an adult who had received only a single vaccination with Hib PS, this individual (as do virtually all adults [43]) appeared primed since his serum contained anti-Hib PS antibody prior to vaccination.

A growing body of evidence suggests that protective antibodies specific for foreign antigens utilize the same or similar germ line V_H and V_L segments as those used by antibodies reactive with self antigens. The A27 (Humkv325) gene segment is used by CA4, which is biologically active against Hib, by a human neutralizing anticytomegalovirus antibody (MAb EV1-15) (36) and by a human neutralizing anti-human immunodeficiency virus gp120 antibody (MAb F105) (32). A27 is perhaps best known for its occurrence in autoantibodies. The A27 V segment is predominant in rheumatoid factors (9, 13), is utilized by antibodies reactive with low-density lipoprotein (39), intermediate filaments (39), and DNA (12), and is also prevalent in B cells infiltrating the salivary glands of patients with Sjogren's syndrome (24). Moreover, A27 is expressed in high frequency in B-cell malignancies such as chronic lymphocytic leukemia (23) and in small lymphocytic non-Hodgkin's lymphoma (40). Particular V_H gene segments are also commonly used by both antipathogen and antiself antibodies (19, 37). For example, the V_H 26 and the V_H 9.1 genes encoding the majority of anti-Hib PS antibodies are used by self-reactive antibodies (7, 37). For the most part, the sharing of V segments by antibodies specific for foreign and self antigens does not confer shared antigen-binding specificity. The CA4 MAb does not have rheumatoid factor activity or DNA-binding activity despite its usage of the A27 $V_κ$ region, and the majority of anti-Hib PS MAbs having autoantibody-like V_H regions do not have self reactivity, although two anti-Hib PS MAbs do show weak reactivity with double-stranded and single-stranded DNA (4). The lack of autoreactivity by CA4 and other antipathogen antibodies using the A27 gene segment parallels the findings of Martin and colleagues (33). They showed that pairing different V_H regions (differing primarily in the third CDR) with a single V_L region, consisting of a rearranged A27 (Humkv325) V segment derived from a rheumatoid factor-producing lymphocytic leukemia cell line, did not generate recombinant antibodies with rheumatoid factor activity. Presently, it is not clear why particular V genes are recurrently utilized to generate both antiself and antinonself specificities or why some of these same V genes are overrepresented in the human peripheral repertoire (18, 54). These patterns may reflect genetic mechanisms that limit or bias V region utilization (37), or they may arise from a functional relationship between paratopes reactive with foreign and self antigens such that the antibody repertoire to foreign antigens derives at least in part from a generative B-cell pool that has mutated from its original self-reactivity (18, 33).

In conclusion, the sequence analysis of the CA4 MAb provides evidence for the use of an additional V_HIII gene segment in the human antibody repertoire to Hib PS and confirms previous partial protein sequence data that the A27 gene is utilized by some κIII anti-Hib PS antibodies. A recent report shows that the CA4 Fab fragment, unlike the majority of V_HIII antibodies, has little or no binding to staphylococcal protein A (52), and therefore, the V region sequences of CA4 may offer further utility in mapping the sites responsible for protein A binding to V_HIII domains. Since the CA4 MAb can be purified to homogeneity from culture supernatants and since the V region sequences of CA4 are known, CA4 represents a good candidate for crystallization studies. In addition, CA4 appears to be a high-affinity protective antibody against Hib, and therefore, CA4 might be considered as an alternative prophylaxis to hyperimmune polyclonal globulin (50) for the treatment of immunodeficient individuals or others at high risk for developing invasive Hib disease.

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