Diversity of Immunoglobulin Light Chain Usage in the Human Immune Response to *Haemophilus influenzae* Type b Capsular Polysaccharide

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ABSTRACT. The response to the capsular polysaccharide of *Haemophilus influenzae* type b (Hib PS) has been used to determine the molecular basis of antibody gene diversity in humans. In contrast to the relatively restricted nature of anti-Hib PS heavy-chain variable region gene expression, a variety of light-chain variable region genes may encode this antibody (Ab) response. Light-chain variable region gene usage appears to determine the expression of certain Ab idiotypes and fine antigen specificity. To further define the role of light-chain variable region gene usage in important anti-Hib PS Ab subgroups, we have cloned and sequenced a number of immunoglobulin light-chain variable region genes (IgVL) from human monoclonal IgA anti-Hib PS Ab generated in response to Hib PS-protein conjugate vaccines. Three of these Ab are encoded by unusual variable segments. One κ -Ab is encoded by the "predominant" V κ II A2 germline gene but, in contrast to a previously reported A2-encoded IgVL sequence, differs from the A2 germline sequence. The IgVL sequence of a second Ab is the only sequence of a κ -Ab that cross-reacts with the structurally related antigen *Escherichia coli* K100 polysaccharide reported to date. This IgVL is encoded by a V κ III-segment most closely homologous to the Humhv328/L16 germline gene, whereas previous reports suggested V κ III-encoded anti-Hib PS Ab might be exclusively encoded by the germline gene Humhv325/A27. A λ -Ab, also cross-reactive with K100 polysaccharide, is encoded by a V λ III family member, in contrast to our previous report of four λ -Ab with similar antigen specificity that appeared to be encoded by a single V λ VII-gene family member. Each of the three Ab reported here contain an arginine codon at the VJ joint, indicating an important role in antigen binding. Our data indicate, in marked contrast to the restricted nature of the heavy-chain anti-Hib PS repertoire, a much greater diversity in light-chain variable gene segment usage. (*Pediatr Res* 33: 307-311, 1993)

Abbreviations

Hib PS, *Haemophilus influenzae* type b capsular polysaccharide

IgVL, Ig light-chain variable region gene

Ab, antibody

K100 PS, *Escherichia coli* K100 capsular polysaccharide

PCR, polymerase chain reaction

V, light-chain Ig variable segment

J, light-chain Ig joining segment

FR, framework region

CDR, complementarity-determining region

We have used the human immune response to Hib PS as a model to examine the molecular basis of antibody diversity and antibody-antigen interaction (1, 2). Previous studies have shown heavy-chain variable region gene usage in this immune response to be restricted, with preferential use of both certain VH3 heavy-chain variable segments and VDJ joints (unpublished manuscript). In contrast, at least nine V κ and V λ segments may encode anti-Hib PS Ab (2-5). However, two important subgroups of anti-Hib PS Ab have been identified. First, the immune response of most subjects immunized with Hib PS vaccine is quantitatively dominated by Ab expressing a cross-reactive idotype, Hibid-1, which is associated with a V κ II gene family member, A2 (2, 4, 6). We, and others have previously shown that these A2-encoded Ab appear to be unmutated, even if antigen is presented as a protein-conjugated polysaccharide (2, 4). The complete nucleotide sequence of one such VH segment is completely identical to the A2 germline sequence (2), and partial amino acid sequences of several other A2-encoded Ab are also identical or nearly identical to the translated A2 gene sequence (4). The absence of somatic mutation and the apparent restriction of important anti-Hib PS light chains to single germline elements would place great dependence for effective immunity on the germline endowment of the individual. Absence or polymorphism of these important germline elements may result in the lack of expression of these Ab in an individual's anti-Hib PS repertoire. The lack of somatic mutation would make it impossible for less effective VL germline genes to acquire increased avidity through amino acid replacement substitutions.

A second portion of the anti-Hib PS Ab response cross-reacts with the structurally similar antigen K100 PS (3, 7). It has been postulated that this cross-reactivity may contribute to the acquisition of "natural" anti-Hib PS Ab by young children (7, 8). Anti-Hib PS Ab that cross-react with K100 PS may be encoded by V κ I, V κ III, and V λ genes (2, 3). We previously suggested the cross-reactivity of λ -anti-Hib PS Ab was mediated by a single V λ VII germline gene (2). To date, no nucleic acid or amino acid sequence of a κ -Ab cross-reacting with K100 PS has been reported.

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To further examine the role of light-chain variable region gene usage in this immune response, we have obtained nucleotide sequences of three additional IgVL genes encoding monoclonal anti-Hib PS Ab. Each of these IgVL genes is atypical. These findings suggest that the human IgVL response to Hib PS may be more complex than previously noted.

MATERIALS AND METHODS

Generation of hybridoma cell lines secreting human monoclonal anti-Hib PS Ab has been previously described (2, 6). Briefly, healthy volunteers were immunized with either Hib PS conjugated to diphtheria toxoid (Hib PS-D) (Connaught Laboratories, Swiftwater, PA) or Hib oligosaccharide conjugated to *Corynebacterium diphtheriae* CRM¹⁹⁷ protein (Lederle Laboratories, Wayne, NJ). Seven d after immunization, peripheral blood lymphocytes were harvested and fused to a nonsecreting murine myeloma cell line, yielding heterohybridoma cell lines secreting human MAb specific for Hib PS. Specific anti-Hib PS binding was documented by Ab binding to ¹²⁵I-labeled antigen in an RIA (9) and by inhibition of binding by 1.25 µg/mL of soluble Hib PS in an ELISA (10). Cross-reactive idiotype expression was measured by the ability of the antiidiotypic Ab Hibid-1 to inhibit Hib PS binding as previously described (6). Cross-reactivity with *E. coli* K100 PS was assayed by inhibition of binding to Hib PS-polylysine with 100 µg/mL of soluble K100 PS in an ELISA as described (2). IgVL segments were amplified by PCR, using amplification primers and PCR conditions as reported earlier (2), and directly cloned into pCR1000 phagemid vectors (Invitrogen, San Diego, CA). Sequencing of IgVL genes was performed by the dideoxy technique (9). Two to four clones were obtained and sequenced for each IgVL gene.

The most closely homologous germline and rearranged variable segments were identified by comparison of anti-Hib PS IgVL genes to sequences entered into the Genbank database.

RESULTS

Hybridoma cell lines. Three hybridoma cell lines, obtained from unrelated subjects, were selected for study to examine the structural basis for certain serologic characteristics (expression of a V κ II/A2-associated idiotype and K100 PS cross-reactivity) (Table 1). All subjects had detectable anti-Hib PS Ab in preimmunization sera and demonstrated substantial increases in Ab concentration 1 mo after immunization. Thus, these monoclonal anti-Hib PS Ab are representative of a secondary immune response.

IgVL genes. The Ann2 V κ segment is 98% homologous (five bp different) to the A2 germline gene at both the nucleic acid and amino acid level and uses a J κ 1 segment that differs from the germline sequence by a single nucleotide (Figs. 1 and 2) (4, 15). The Ann2 nucleic acid sequence differs from our previously reported RC3 V κ segment, which was 100% homologous to A2,

and the Ann2 translated amino acid sequence differs from the previously reported partial amino acid sequences of five A2-encoded anti-Hib PS Ab by one or two residues (2, 4). The Ann2 VJ joint has an arginine residue at position 95a, as do all A2-encoded anti-Hib PS Ab and most other anti-Hib PS Ab described to date (2–4).

LD21 is a κ -Ab using a V κ III segment 91.5% homologous to the germline gene Humhv328/L16 and a J κ 2 segment closely homologous to the published germline sequence (Fig. 3) (13, 15). The variable segment shares 94.8% identity with Humhv328/L16 in FR and 81.9% in CDR. The translated amino acid sequence of LD21 is 84% homologous to the Humhv328/L16 germline gene, 91.4% in FR and 62.5% in CDR. The LD21 VJ joint also has an arginine residue at position 95.

The CB20 IgVL gene is encoded by a V λ III gene segment 70.5% homologous to the germline gene V λ III.1 (81.2% in FR regions and 42.3% in CDR), and, in common with all λ -anti-Hib PS Ab sequenced to date, uses a J λ 2/3 segment (Fig. 4) (2, 14, 15). All these J λ segments substitute the codon "GTG" for the germline "GTA" at position 92, a silent change likely to reflect a shared genetic polymorphism. The translated CB20 amino acid sequence is 75.4% homologous to the previously reported V λ III.1 gene, 83.1% in FR and 55.1% in CDR. The VJ joint of CB20, like the two κ -Ab, has an arginine codon at position 95.

DISCUSSION

To further examine the role of light-chain usage in important subgroups of anti-Hib PS Ab, we have obtained IgVL nucleotide sequences of three human MAb specific for Hib PS.

Reported sequences of A2-encoded anti-Hib PS Ab are remarkable in that they have shown little or no evidence of polymorphism or somatic mutation (2, 3). We previously found the nucleic acid sequence of the A2-encoded IgA Ab RC3 V κ -segment to be identical to the reported A2 germline gene (2). Furthermore, only one of five partial amino acid sequences of IgG anti-Hib PS Ab is reported to differ (at a single residue) from the translated amino acid sequence of A2 (4). In contrast, the Ann2 nucleotide sequence differs at five positions from that of A2. These differences may result from three processes: 1) the A2 gene may be polymorphic among individuals, 2) Ann2 may be encoded by another germline gene closely related to A2 or 3) somatic mutation of the rearranged A2 gene segment may have occurred. Neither polymorphism of the A2 gene nor the existence of another germline gene more homologous to Ann2 than A2 has been noted (4, 16). Although four of five base differences occur in CDR, only one of these differences results in an amino acid substitution. This pattern is not typical of antigen-driven somatic mutation. However, we have recently shown that at least a portion of the heavy-chain anti-Hib PS Ab response is mutated, and these IgVH genes also did not exhibit significantly elevated ratios of nucleic acid differences resulting in amino acid replace-

Table 1. Hybridoma cell lines*

Hybridoma	Isotype	Vaccine	Anti-Hib PS Ab		Hibid-1 expression	K100 PS	Homologous germline genes	
			Pre	Post			IgVH	IgVL
Ann2	IgA1/ κ	Hib OC	1.7	110	+	–	VH3 VH26 (12)	V κ II A2 (4)
LD21	IgA2/ κ	Hib PS-D	0.7	58	–	+	NK†	V κ III Humhv328/L16 (13)
CB20	IgA2/ λ	Hib OC	21.0	75	–	+	VH3 LSG6.1‡	V λ III III.1 (14)

* Columns show name of hybridoma cell line, heavy-chain isotype and light-chain constant region usage, vaccine formulation (Hib PS-D is *Haemophilus influenzae* type b polysaccharide conjugated to diphtheria toxoid; Hib OC is *Haemophilus influenzae* type b oligosaccharide conjugated to CRM¹⁹⁷ protein), subject's serum anti-Hib PS Ab concentration immediately before and 1 mo postimmunization, expression of the V κ II/A2-associated cross-reactive idiotype Hibid-1, cross-reactivity of Ab with K100 PS, and closest germline VH and V κ / λ genes identified. Numbers in parentheses are reference numbers.

† NK, not known.

‡ Unpublished manuscript.

It has been suggested that Ab V λ segments may be divided

into families and subfamilies on the basis of “invariant” amino acids and nucleic acid sequence homology, with homology from subgroup to subgroup <75% and within subgroups >76% (14). The CB20 V λ segment appears to be a member of the V λ III gene family, lacking only an invariant valine (substituted for by Ile) at position 47. The most homologous germline sequence to the CB20 V λ segment described to date is the V λ IIIC gene V λ III.1. However these gene segments share only 75.4% identity (14). Although homology between V λ III.1 and CB20 is considerably greater in FR than in CDR regions (81.2 *versus* 42.3%), the ratio of nucleic acid differences that result in amino acid replacements to silent changes is low (<2:1), suggesting differences between these segments are not a result of somatic mutation. Rather, the CB20 gene is likely to be encoded by another V λ III germline gene. The CB20 V λ segment is 78.6% homologous to the V λ IIIB cDNA cML13 and 77.3% homologous to the V λ IIIC cDNA cML23; thus, its subfamily assignment is not clear (14). This overlap of subfamilies has not been generally observed. For example, in a large series of V λ genes described by Combriato and Klobbeck (14), the homology of V λ IIIC subfamily members ranged from 91.8 to 98.8%, and two V λ IIIA members were

Fig. 3. Nucleotide sequence of the human anti-Hib PS Ab LD21 Ab IgVL gene. Shown for comparison are the nucleotide sequences of the germline V_{κ} III gene Humhv328/L16 (13) and the germline J κ 2 segment (15). Also shown is the translated amino acid sequence of LD21. This sequence has been submitted to the Genbank database.

Fig. 4. Nucleotide sequence of the human anti-Hib PS Ab CB20 Ab IgVL gene. Shown for comparison are the nucleotide sequences of the V λ III gene III.1 (14) and the germline J λ 2/3 segment (15). Also shown is the translated amino acid sequence of CB20. This sequence has been submitted to the Genbank database.

Anti-Hib PS Ab cross-reactive with K100 PS express V λ , V κ I, or V κ III segments, but not the "predominant" V κ II/A2 segment (2, 3). Consistent with these findings, the Ann2 Ab, encoded by the A2 germline gene, does not bind K100 PS. We previously obtained sequences of four V λ -expressing anti-Hib PS Ab which cross-react with K100 PS. All of these segments appeared to derive from a single germline V λ VII gene (2). CB20, however, is encoded by a V λ III gene family member, suggesting that a more heterogeneous group of V λ genes may encode K100 PS cross-

CDR-3 of each of these three hybridoma IgVL genes are notable for the presence of an arginine codon at position 95 or 96. The 5' proximal nucleotides of the J κ 1 gene are "TGG" and the 3' proximal nucleotides of A2 are "CC." Thus, the codon "AGG" at the Ann2 VJ must have been created by the uncommon event of an "N" addition (of at least a single "G"), as previously described in the A2-encoded anti-Hib PS IgVL gene RC3 (2).

The LD21 V κ segment is less similar to a known germline gene. The arginine codon may, therefore, be encoded by an unknown germline gene or may result from either somatic mutation or "N" addition. The Arg-95 codon of CB20 may be encoded by the V λ segment or by "N" addition. Ten of 11 anti-Hib PS IgVL genes we have identified to date feature an arginine residue at the VJ joint, implying that this amino acid is very important in antigen binding.

We have obtained nucleotide sequences of IgVL genes from three monoclonal human anti-Hib PS Ab representative of serum antibodies with important serologic or antigen-binding characteristics. Each of these genes uses IgVL gene segments that have not previously been described, suggesting that the diversity of the total anti-Hib PS Ab repertoire and that of important subgroups of Ab may be greater than appreciated. It has been hypothesized that poor response to Hib PS vaccine or infection might result from the absence of one or more of the germline Ig gene segments critical to this immune response (4). The identification of an increasingly large number of IgVL gene segments that may be used to encode anti-Hib PS Ab suggests that this postulate is relevant only to the much more restricted heavy-chain variable region gene usage.

These new Ab clonotypes may be quantitatively minor constituents of the serum anti-Hib PS Ab response, inasmuch as partial amino acid sequences of somewhat larger numbers of Ab have not previously detected similar IgVL usage. Interpretation of these data is confounded by the use of different vaccine formulations, the selection of Ab for study at different time points in the immune response, and the selection of Ab of a particular heavy-chain isotype. Additional studies are required to examine the expression of IgVL segments in response to different vaccine formulations and to define the structural basis of antigen specificity.

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