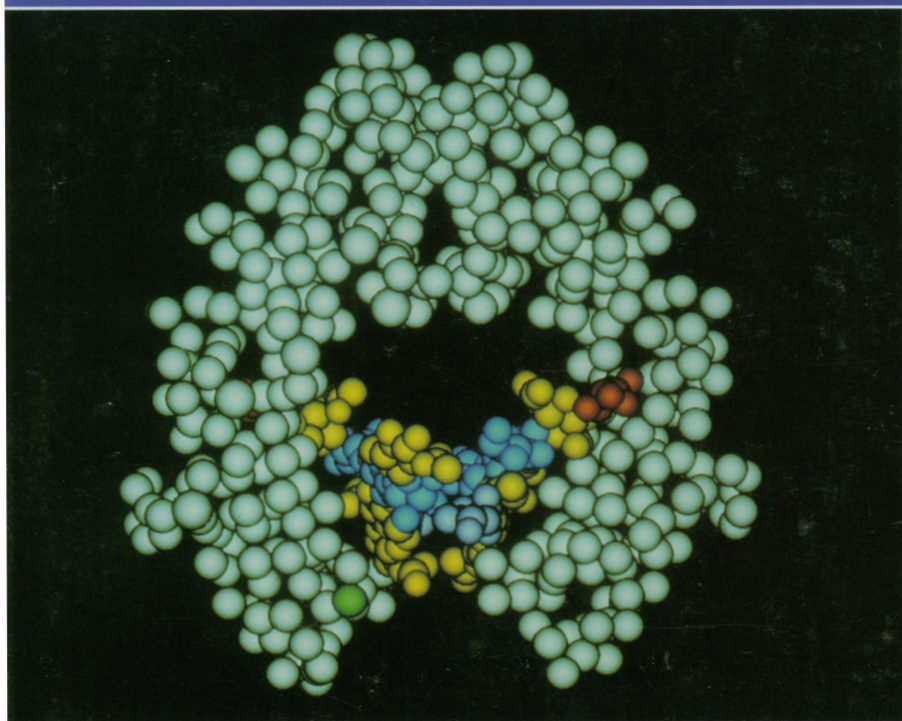


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Abbreviations (material in parentheses for explanation only)		
A, ampere(s)	g, gram(s)	No.(s), number(s)
Å, angstrom (10^{-8} cm)	g, unit of gravity	NP-40, Nonidet P-40
AA, arachidonic acid	GFR, glomerular filtration rate	NS, not significant
ACTH, adrenocorticotropin	GSH, glutathione, reduced	OD, optical density
AIDS, acquired immunodeficiency syndrome	h, hour	1,25(OH) ₂ D, 1,25-dihydroxyvitamin D
apo, apolipoprotein (also apo A, apo B, etc.)	HBSS, Hanks' balanced salt solution	25(OH)D ₃ , 25-hydroxyvitamin D ₃
ATP, adenosine triphosphate (also ADP, AMP, CTP, UDP, etc.)	HDL, high density lipoprotein	osM, osmolar
BCG, Calmette-Guérin bacillus	Hepes, <i>N</i> -2-hydroxyethylpiperazine- <i>N'</i> -2- ethane sulfonic acid	osmol, osmole(s)
bp, basepair	HIV, human immunodeficiency virus	% (with numeral), percent
BP, blood pressure	HLA, human histocompatibility leukocyte antigens	<i>P</i> , probability
BSA, bovine serum albumin	HPLC, high performance liquid chromatography	PAGE, polyacrylamide gel electrophoresis
BTU, British thermal unit	HSA, human serum albumin	PBL, peripheral blood lymphocyte
C, complement	Id, idiotype	PBMC, peripheral blood mononuclear cell
°C, degree Celsius	ID ₅₀ , 50% infective dose	PBS, phosphate-buffered saline
cAMP, cyclic AMP	IFN, interferon	PCO ₂ , partial pressure of CO ₂
cDNA, complementary DNA	Ig, immunoglobulin	PD, potential difference
CFA, complete Freund's adjuvant	IL, interleukin (e.g., IL-2)	PG, prostaglandin (also PGE, PGF, etc.)
CFU, colony-forming unit	IU, international unit(s)	PHA, phytohemagglutinin
Ci, curie(s)	°K, degree kelvin	pl, isoelectric point
CoA, coenzyme A	kb, kilobase	PMN, polymorphonuclear leukocyte
cpm, counts per minute	kcal, kilocalorie(s)	PMSF, phenylmethylsulfonyl fluoride
cps, counts per second	<i>K_d</i> , dissociation constant	PO ₂ , partial pressure of O ₂
Con A, concanavalin A	<i>K_i</i> , inhibition constant	psi, pounds per square inch
CSA, colony-stimulating activity	<i>K_m</i> , Michaelis constant	PTH, parathyroid hormone
<i>d</i> , density	KRB, Krebs-Ringer bicarbonate buffer	PWM, pokeweed mitogen
D, dalton	LD ₅₀ , 50% lethal dose	<i>r</i> , correlation coefficient
kD, kilodalton	LDL, low density lipoprotein	RA, rheumatoid arthritis
DEAE, diethylaminoethyl	liter(s), liter(s)	<i>R_f</i> , retardation factor
DME, Dulbecco's modified Eagle's medium	μl (not λ), microliter(s)	RIA, radioimmunoassay
DMSO, dimethylsulfoxide	ml, milliliter(s)	rpm, revolutions per minute
DNA, deoxyribonucleic acid	LPS, lipopolysaccharide	RNA, ribonucleic acid
DNP, dinitrophenyl	m, meter(s)	RQ, respiratory quotient
DTT, dithiothreitol	cm ³ (not cc), cubic centimeter(s)	s, second(s)
dyn · s · cm ⁻⁵ , units of peripheral vascular resistance	μm or 10 ⁻⁶ m (not μ), micrometer(s) (micron)	SD, standard deviation
EBV, Epstein-Barr virus	M, molar	SDS, sodium dodecyl sulfate
ED ₅₀ , 50% effective dose	mAb, monoclonal antibody	SE, standard error
EDTA, ethylenediaminetetraacetate	MEM, minimum essential medium	SEM, standard error of the mean
EGTA, ethyleneglycol-bis(β-aminoethyl ether)- <i>N,N'</i> -tetraacetic acid	μeq, microequivalent(s)	SOD, superoxide dismutase
ELISA, enzyme-linked immunosorbent assay	meq, milliequivalent(s)	sp act, specific activity
E/T ratio, effector/target ratio	MHC, major histocompatibility complex	<i>t</i> _{1/2} , half-life
eV, electron volt(s)	mmHg, millimeters of mercury	TCA, trichloroacetic acid
°F, degree Fahrenheit	mo, month	TLC, thin-layer chromatography
FACS®, registered trademark of Becton Dickinson and Company for a fluorescence activated cell sorter	mol, mole(s)	Tris, tris(hydroxymethyl)-aminomethane
FCS, fetal calf serum	mol wt, molecular weight	<i>t</i> test, Student's <i>t</i> test
FFA, free fatty acids	<i>M_r</i> , relative molecular mass	TX, thromboxane
FITC, fluorescein isothiocyanate	mRNA, messenger RNA	U, unit(s)
FMLP, formylmethionylleucylphenylalanine	<i>n</i> , number in study, group	V, volt(s)
	N, normal (concentration)	VLDL, very low density lipoprotein
	NAD, nicotinamide adenine dinucleotide (also NADH, NADP, etc.)	<i>V_{max}</i> , maximum velocity
	ND, not determined	vol, volume(s)
	NE, norepinephrine	vWf, von Willebrand factor
		W, watt(s) (voltampere)
		kW, kilowatt(s)
		wk, week
		wt, weight
		yr, year

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Cover picture: The Fc portion of immunoglobulin G. A computer-generated model demonstrating the three-dimensional configuration of the amino acid backbone (light spheres) and the opposing biantennary oligosaccharide structures. The blue spheres represent mannose, the yellow spheres *N*-acetylglucosamine, the red spheres galactose, and the gray/blue spheres fucose. The terminal galactose is absent from one of the arms of the left hand oligosaccharide moiety exposing amino acid residues that become potentially available for immune interaction. See related article in this issue by Axford et al., pp. 1021–1031.

Immunoglobulin light chain variable region gene sequences for human antibodies to Haemophilus influenzae type b capsular polysaccharide are dominated by a limited number of V kappa and V lambda segments and VJ combinations.

E E Adderson, ... , P M Wilson, W L Carroll

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Research Article

The immune repertoire to Haemophilus influenzae type b capsular polysaccharide (Hib PS) appears to be dominated by certain light chain variable region genes (IgVL). In order to examine the molecular basis underlying light chain bias, IgVL genes have been cloned from a panel of heterohybridomas secreting human anti-Hib PS (antibody) (anti-Hib PS Ab). One hybridoma, representative of the predominant serum clonotype of anti-Hib PS Ab in older children and adults following immunization or Hib infection, uses a V kappa II segment identical to the germline gene A2, and a JK3 segment. A second kappa hybridoma uses a member of the V kappa I family and a JK4 segment. Four lambda antibodies, all cross-reactive with the structurally related antigen Escherichia coli K100 PS, use V lambda VII segments which are 96-98% homologous to one another, and may originate from a single germline gene. Two additional lambda antibodies, not K100-cross-reactive, are encoded by members of the V lambda II family. All lambda antibodies use highly homologous J lambda 2 or J lambda 3 segments. The VJ joints of all lambda antibodies and the V kappa II-encoded antibody are notable for the presence of an arginine codon, suggesting an important role in antigen binding. Although more complex than heavy chain variable region gene usage, a significant portion of serum anti-Hib PS Ab is [...]

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Immunoglobulin Light Chain Variable Region Gene Sequences for Human Antibodies to *Haemophilus influenzae* Type b Capsular Polysaccharide Are Dominated by a Limited Number of V_κ and V_λ Segments and VJ Combinations

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Abstract

The immune repertoire to *Haemophilus influenzae* type b capsular polysaccharide (Hib PS) appears to be dominated by certain light chain variable region genes (IgV_L). In order to examine the molecular basis underlying light chain bias, IgV_L genes have been cloned from a panel of heterohybridomas secreting human anti-Hib PS (antibody) (anti-Hib PS Ab). One hybridoma, representative of the predominant serum clonotype of anti-Hib PS Ab in older children and adults following immunization or Hib infection, uses a V_κII segment identical to the germline gene A2, and a JK3 segment. A second kappa hybridoma uses a member of the V_κI family and a JK4 segment. Four lambda antibodies, all cross-reactive with the structurally related antigen *Escherichia coli* K100 PS, use V_λVII segments which are 96–98% homologous to one another, and may originate from a single germline gene. Two additional lambda antibodies, not K100-cross-reactive, are encoded by members of the V_λII family. All lambda antibodies use highly homologous J_λ2 or J_λ3 segments. The VJ joints of all lambda antibodies and the V_κII-encoded antibody are notable for the presence of an arginine codon, suggesting an important role in antigen binding. Although more complex than heavy chain variable region gene usage, a significant portion of serum anti-Hib PS Ab is likely to be encoded by a limited number of V_κ and V_λ segments and VJ combinations, which may be selectively expressed during development, or following antigen exposure. (*J. Clin. Invest.* 1992; 89:729–738.) Key words: B-cell repertoire • somatic mutation • gene rearrangement • carbohydrate antigen

Introduction

The development of the antibody repertoire involves the recombination of individual members of two or three groups of germline segments, nucleotide insertion or deletion at these junctions, and somatic mutation of rearranged genes (1–4). The individual contributions of these molecular mechanisms

to the generation of the immune repertoire against a specific antigen has been extensively studied in murine systems, but it is uncertain whether these findings can be extrapolated to humans. The chromosomal organization of the human heavy and light chain variable region segments within the immunoglobulin loci differ from those of mice (5–7). Furthermore, the response of highly inbred laboratory animal strains, and the use of simple haptens in most studies, may not be comparable with the human response to clinically relevant antigens, such as those presented by microbial pathogens.

The immune response to *Haemophilus influenzae* type b capsular polysaccharide (Hib PS)¹ is an excellent model to examine the immune repertoire to a functionally significant antigen. Hib is a major bacterial pathogen in young children and antibody directed against the capsular polysaccharide, a repetitive polymer of polyribosylribitol phosphate, protects against invasive infection (8). Similar to the response to other polysaccharide antigens, the immune response to Hib PS is age-dependent. Children under 5 yr have lower antibody responses and are at greatest risk of infection. Additionally, there appear to be variations in individual and ethnic susceptibilities to invasive Hib disease (9–11). Analysis of the immune response to Hib PS will contribute to our understanding of the development of the human antibody repertoire, and provide insight into age-related and possible genetic predisposition to infection caused by Hib.

Previous studies employing partial amino acid sequencing of anti-Hib PS serum antibody purified to clonality have shown that light chains of anti-Hib PS antibody are encoded by V_λ, and at least three V_κ gene families (12). Although highly informative, these studies have inherent technologic limitations. To obtain sufficient antibody for analysis, only individuals with antibody concentrations much higher than those elicited in young infants could be studied (12). Amino acid sequences were unobtainable on V_κ-encoded antibodies, and certain regions of V_κ-encoded antibodies (12, 13). Obtaining nucleic acid sequences of variable region genes encoding anti-Hib PS Abs would permit precise comparison of variable region genes obtained from different individuals, allowing classification of these genes into germline families and subfamilies, analysis of D- and J-segment usage, and assessment of the role of somatic mutation in the clonal evolution of this immune response. Additionally, this information could be applied to examination of the germline and expressed immune repertoire

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1. Abbreviations used in this paper: Ars, anti-p-azophenylarsonate; Hib, *Haemophilus influenzae* type b; Hib PS, Hib capsular polysaccharide; IgV_L, light chain variable region gene; PCR, polymerase chain reaction; SSC, standard saline citrate.

of individuals of various ages, and populations at high risk for invasive Hib infection.

Our previous work, and that of others, indicated that the heavy chains of anti-Hib PS Abs are encoded by a very restricted number of V_H III gene segments, in combination with a variety of D and J_H segments (12, 14). We have extended our studies to examine light chain variable region gene (IgV_L) usage in the human anti-Hib PS response. A major component of serum anti-Hib PS Ab expresses a cross-reactive idiotype associated with the use of a V_L II gene (15, 16). Antibodies expressing this light chain account for the majority of anti-Hib PS antibody in older infants and adults following invasive Hib disease or immunization. Most lambda-encoded anti-Hib antibodies and some kappa-encoded (V_L I and V_L III) anti-Hib PS Abs cross-react with the capsular polysaccharide of *E. coli* K100 (12, 17). Naturally acquired antibody of the majority of children expresses K100 PS cross-reactivity, and thus, colonization with this organism may provide protective immunity before exposure to Hib (18). Our results indicate that two important anti-Hib PS light chains, one associated with V_L II expression and the other with lambda antibodies cross-reactive with K100 PS, are encoded by two germline elements that may be expressed with little or no somatic mutation. Moreover, a nongermline-encoded arginine at the VJ joint is seen in the majority of anti-Hib PS Ab light chains, a result suggesting an essential role in antigen recognition.

Methods

Hybridomas. Heterohybridomas secreting human monoclonal anti-Hib PS antibody were obtained, as previously described (14). Volunteers were immunized with either plain Hib-PS vaccine (Praxis Biologicals, Rochester, NY) or Hib-PS diphtheria toxoid conjugate vaccine (Hib-PS-D) (Connaught Laboratories, Swiftwater, PA). 7 d following immunization, peripheral blood lymphocytes were harvested and fused to the nonsecreting mouse myeloma SP2/O-Ag14. Anti-Hib PS Ab was detected by binding to Hib PS in an ELISA, as previously described (14). Specificity was documented by antibody binding to ^{125} I-labeled antigen (19), and by inhibition of binding by 1.25 μ g/ml of soluble Hib PS in an ELISA (20). Cross-reactivity with *E. coli* K100 PS was assayed by inhibition of binding to Hib PS-poly-L-lysine with 100 μ g/ml of soluble K100 PS in an ELISA. An antibody concentration was selected that gave half-maximum absorbance in the ELISA. Greater than 15% inhibition was considered positive. The *E. coli* K100 PS was prepared from *E. coli* strain Easter, by the same method used to prepare Hib PS (19). The generation of hybridoma line 16M3C8 was described previously (21).

Cloning and sequencing of IgV_L genes. Total RNA was prepared from 10^6 hybridoma cells using the guanidine isothiocyanate method (22). 2 to 8 μ g of total RNA served as template for first-strand cDNA synthesis, as previously described (14). 10 pmol of a consensus antisense primer corresponding to conserved sequences within the human kappa or lambda constant region (23) (C_κ codons 181-172: 5'-CAGCGTCAAGCTTCTGCTGTGGCTGTAGGT 3', C_λ codons 171-181: 5'-CAGGCTCAGGAAGCTTCTGGCCGCTACTTGT 3') was used to initiate cDNA synthesis by avian myeloblastosis virus (AMV) reverse transcriptase (RT; Life Sciences, St. Petersburg, FL). One half (10 μ l) of the cDNA reaction was directly diluted into polymerase chain reactions (PCR), (24).

PCR conditions were as previously described, except that 70 pmol of an internal constant region consensus primer (C_κ codons 125-117: 5'-CAACGGATCCTCTGATGGCGGGAAGAT 3', C_λ codons 125-117: 5'-TGGGGATCCAGCTCCTCAGAGGAGGG 3') and 70 pmol of a degenerate 3' kappa or lambda leader sequence primer (kappa: 5'-GGGAATTCATGGACATG(GA)(GA)(GA)(GAT)(TC)CC-

(ACT)(GAC)G(TC)(GT)CA(GC)CTT 3', lambda 5'-GGGAATTCA-TG(GA)CCTG(GC)(AT)C(TC)CCTCTC(TC)(TC)CT(GC)(AT)-(TC)C 3') were used to amplify rearranged variable region genes (14, 25).

PCR products were isolated from low melting-point agarose gels (FMC Bioproducts, Rockland, ME). Artificial restriction enzymes sites in each primer allowed directional cloning into M13mp18 and M13mp19 phage vectors (Boehringer Mannheim Corp., Indianapolis, IN). Sequencing of V_L genes was performed by the dideoxy technique (26). Two to five positive clones were obtained and sequenced for each hybridoma gene.

The 16M3C8 light chain was generated by amplification of first-strand cDNA, with primers corresponding to amino acid positions -7 of the leader, to position 6 of the lambda light chain (5'-CTGCACAGGGTCCTGGGCCGAGCTCGTGGTGACTCA 3'), and the C_λ constant region (antisense, 5'-GCATTCTAGACTATTATGAACATTCTGTAGGGGC3'). Blunt-ended PCR products were cloned into SmaI cut M13 mp18 and M13mp19 and sequencing was also performed by the dideoxy method.

Southern blot analysis of genomic DNA. Peripheral blood was obtained from 10 unrelated healthy adult volunteers, from L.S. (the donor of hybridoma LSF2), and from a parent of L.S. Lymphocytes were separated on a Ficoll-Hypaque gradient, and genomic DNA was prepared by Proteinase K digestion (27). 10 μ g of each subject's genomic DNA was digested with BamHI (4 U/ μ g DNA) (Boehringer Mannheim Corp.) at 37°C for 16 h, according to manufacturer's specifications. Digested DNA was separated by electrophoresis in a 0.8% agarose gel using TAE buffer (0.04 M Tris-acetate/0.001 M EDTA), and was transferred to Duralon UV membranes (Stratagene Inc., La Jolla, CA), using a positive pressure blotting apparatus (Posiblitter; Stratagene, Inc.). To examine germline representation of anti-Hib PS Ig genes, genomic DNA was hybridized with a 320-bp EcoRI/SmaI V_λ segment from hybridoma LSF2 that lacked the associated J_λ and C_λ regions. Probe was radiolabeled to high specific activity using T7 DNA polymerase (28). Prehybridization and hybridizations were carried out in 5 $\times$ standard saline citrate (SSC), 5 $\times$ Denhardt's solution, and 200 μ g/ml sheared and denatured salmon sperm DNA (Boehringer Mannheim Corp.) at 42°C overnight. Filters were washed with 2 $\times$ SSC, 0.2% SDS at room temperature twice, then with 0.2 $\times$ SSC, 0.2% SDS at 55 to 60°C twice, and exposed to X-OMAT AR film (Eastman Kodak Co., Rochester, NY) for 2-7 d.

In order to further localize germline candidates within V region families, an oligonucleotide probe corresponding to conserved sequences within the CDR-1 region (sense oligonucleotide: 5'-GGCTC-CAGCACTGGAGATGTCACCAGT 3', antisense oligonucleotide: 5'-GTAGGGATAATGACCACTGGTGACATCTCC 3') was hybridized to genomic DNA using the method described by Hellman (29). 15 μ g of BamHI-digested human genomic DNA was separated by electrophoresis in 0.8% agarose gel using TBE buffer (0.09 M Tris-borate/0.002 M EDTA). Agarose gels were dried under vacuum and hybridized in 5 $\times$ saline/sodium phosphate/EDTA (SSPE), 0.1% SDS, and 10⁶ μ g/ml of sheared and denatured salmon sperm DNA with 2 $\times$ 10⁶ cpm of probe/ml of hybridization solution at 60°C for 16 h (30, 31). Hybridization conditions were previously adjusted to minimize nonspecific binding. Gels were washed with 6 $\times$ SSC at room temperature for 20 min twice, followed by a 4-h room temperature wash. The gel was finally washed in 6 $\times$ SSC for 3 min at 64°C, and exposed to X-OMAT AR film for 4 d.

Results

Hybridomas

Eight hybridoma cell lines from seven individuals were initially selected from 20 cell lines. All individuals demonstrated substantial increases in serum anti-Hib PS Ab 1 mo after vaccination (Table I). Hybridomas were selected to exemplify serologic characteristics (kappa or lambda expression, and K100 PS

Table 1. Summary of Hybridoma Cell Lines*

Hybridoma	Isotype	Age	Vaccine Form	K100 Inh of mAb	Serum Anti-Hib PS Ab			
					Total Ab		λK100 Inh	
					Pre	Post	Pre	Post
RC3	IgA/k	Adult	PS-D	—	1.1	300	11	11
ED6.1	IgG ₂ /k	4 yr	PS-D	—	0.8	22	0	0
SB5/D6	IgA ₁ /λ	Adult	PS-D	+	2.0	688	30	27
RAY4	IgA ₂ /λ	Adult	PS-D	+	1.9	28	0	42
LSF2	IgA ₁ /λ	Adult	PS	+	ND	138	ND	29
JB32	IgA ₁ /λ	11 yr	PS	+	0.3	125	19	33
JB21	IgG ₂ /λ	11 yr	PS	—	0.3	125	19	33
16M3C8	IgG ₂ /λ	Adult [‡]	PS	—	ND	ND	ND	ND

* Columns show name of hybridoma cell line, isotype of anti-Hib PS Ab secreted, age of subject at immunization, type of vaccine (PS is plain polysaccharide, PS-D is Hib PS covalently linked to diphtheria toxoid), presence (+) or absence (—) of inhibition of binding of anti-Hib PS Ab by *E. coli* K100 PS, concentration of total serum anti-Hib PS Ab pre- and 1 mo post-immunization, and percent inhibition of binding of serum lambda antibodies to Hib PS by *E. coli* K100 PS in pre- and 1 mo post-immunization sera. [‡] Pool of 3 adult donors. ND, not done.

cross-reactivity) and to represent the response to “T-independent” plain polysaccharide, or “T-dependent” polysaccharide-protein conjugate forms of antigen. (Table I).

Four lambda-expressing anti-Hib PS Ab, obtained from four different subjects, cross-reacted with K100 PS. One of these individuals, RAY4, had no detectable serum cross-reactivity before immunization. The Abs JB32 and JB21 were obtained from the same individual, and differed in heavy chain isotype and K100 cross-reactivity.

IgV_L genes. IgV_L nucleic acid sequences were obtained from two kappa and six lambda hybridomas. Candidate germline IgV_L sequences were sought by comparing hybridoma sequences to those registered with EMBL and GenBank (32).

Four lambda-expressing hybridomas, derived from four unrelated subjects, use V_λ segments which are 96–98% homologous to one another, and are members of the germline V_λVII family of which a single member, 4A, has been previously described (33) (Fig. 1). Notably, each of these four V_λVII-encoded hybridomas cross-reacts with K100 PS. The high degree of homology between these segments suggests that these genes may be encoded by a single, or group of, closely related germline elements. The four hybridoma genes share a number of nucleic acid differences (maximum nucleic acid sequence homology 89%) from the only previously sequenced V_λVII germline gene 4A, implying that these genes may be derived from another, as yet undescribed, V_λVII germline element.

The homology of the translated amino acid sequences of these 4 V_λVII sequences parallels the nucleic acid homology. The hybridoma V_λVII genes are 90 to 97% homologous at the amino acid level (Fig. 2). Framework regions are 94 to 100% homologous, whereas CDR regions are 72 to 96% homologous. Whether these minor differences reflect genetic polymorphisms, or somatic mutation, cannot be resolved from these data.

JB21 and 16M3C8, the lambda-expressing hybridomas which do not cross-react with K100 PS, are encoded by a V_λII gene family member (34), (Fig. 3). JB21 is 91% homologous to the germline V_λ2.1 gene (99% homologous in framework regions and 74% homologous in CDRs). 16M3C8 is 89% homologous to V_λ2.1 (95% in framework regions and 77% in CDRs). JB21 shares 87% amino acid homology with the translated V_λ2.1 germline sequence (99% in framework regions and 60% in CDR regions), and 16M3C8 shares 82% amino acid homology (92% in framework regions and 60% in CDRs) (Fig. 4).

Germline V _λ VII	CAG	ACT	GTG	GTG	ACT	CAG	GAG	CCC	TCA	CTG	ACT	GTG	TCC	CCA	GGA	GGG	ACA	GTC	ACT	CTC	ACC
RAY4	...	G...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
SB5/D6	...	G...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
LSF2	...	G...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
JB32	...	G...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

Germline V _λ VII	TGT	GCT	TCC	AGC	ACT	GGA	CTC	ACC	AGT	GGT	TAC	TAT	CCA	AAC	TGG	TTC	CAG	CAG	AAA	CGT	GGA
RAY4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
SB5/D6	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
LSF2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
JB32	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

Germline V _λ VII	CAA	GCA	CCC	AGG	GCA	CTG	ATT	TAT	AGT	ACA	AGC	AAC	AAA	CAC	TCC	TGG	ACC	CCT	GCC	CGG	TTC	TCA
RAY4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
SB5/D6	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
LSF2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
JB32	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

Germline V _λ VII	GGC	TCC	CTC	CTT	GGG	GGC	AAA	GCT	GCC	CTG	ACA	CTG	TCA	GGT	GTG	CAG	CCT	GAG	GAC	GAG	GCT	GAG
RAY4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
SB5/D6	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
LSF2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
JB32	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

Germline V _λ III	TAT	TAC	TGC	CTG	CTC	TAC	TAT	GGT	GGT	GCT	CAG	GTA	TTC	GGC	GGA	GGG	ACC	AAG	CTG	ACC	GTC	CTA
RAY4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
SB5/D6	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
LSF2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
JB32	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

Figure 1. Nucleotide sequences of the V_λ and J_λ genes from four hybridoma cell lines (RAY4, SB5/D6, LSF2, JB32), and from the V_λVII germline gene, 4A (33). Numbering is according to Kabat (23). These sequence data are available from EMBL/GenBank Data Libraries under accession numbers M80917 (RAY4), M80918 (SB5/D6), M80915 (LSF2), and M80919 (JB32).

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Figure 2. Translated amino acid sequences of V_λ and J_λ from four hybridoma cell lines (RAY4, SB5/D6, LSF2, JB32), and from the V_λVII germline gene, 4A (33). (See Fig. 1.)

These two V_λII-encoded gene segments are 91% homologous to one another, and have in common a number of nucleic acid differences from the V_λ2.1 germline gene (especially in CDRs). Whether they derive from another germline gene, or whether these differences reflect mutation, is unknown.

All four of the V_λVII-encoded antibodies are also encoded by V_HIII segments closely homologous to the germline gene 9.1 (5). Similar V_H segments also encode Ab utilizing other V_λ and V_κ gene segments (Table II) (manuscript in preparation). It is uncertain whether pairing of 9.1-like V_H and V_λVII-encoded heavy and light chains contributes to binding specificity or avidity. JB21 and RC3, also encoded by 9.1-like V_H gene segments, but paired with V_λII- and V_κII-encoded light chains, respectively, do not cross-react with K100 PS.

All six of the lambda light chains are encoded by a common J_λ segment closely homologous to both J_λ2 and J_λ3 germline genes (Fig. 1) (23). J_λ2 is associated with the use of the (Kern⁻Oz⁻)C_λ segment 2, and J_λ3 with the (Kern⁻Oz⁺)C_λ segment 3 (37). Each of these J_λ segments substitutes the codon GTG for the reported germline sequence GTA at amino acid position 97. Interestingly, this change does not result in an amino acid replacement, and is therefore not of importance in antigen binding. This minor sequence difference may reflect a shared genetic polymorphism, rather than somatic mutation.

The hybridoma RC3 uses a V_λII segment identical to the germline V_λII gene A2, and a J_λ3 segment closely homologous to the germline sequence (Fig. 5) (13). The second kappa hybridoma, ED6.1, is encoded by a member of the V_λI family

germline V12.1	CAG	TCT	GCC	CTG	ACT	CAG	CCT	GCC	TCC	GTG	TCT	GGG	TCT	CCT	GGA	CAG	TCG	ATC	ACC	20
JB21	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	-T-
16M3C8	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	-T-

germline V12.1	ATC	TCC	TGC	ACT	GGA	ACC	AGC	AGT	GAT	GTT	GGG	AGT	TAT	AAC	CTT	GTC	TCC	TGG	TAC	30
JB21	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
16M3C8	...	...	...	G-	...	-G-	...	...	...	...	...	...	...	...	...	...	...	...	...	...

germline V12.1	CAA	CAG	CAC	CCA	GGC	AAA	GCC	CCC	AAA	CTC	ATG	ATT	TAT	GAG	GGC	AGT	AAG	CGG	CCC	40
JB21	...	-A	...	...	...	...	...	...	...	...	...	...	...	...	...	-T-	-C-	-TT	...	...
16M3C8	...	-A	...	...	...	...	...	...	...	...	-A	C-	...	...	...	-T-	TA-	-TT	...	...

germline V12.1	TCA	GGG	GTT	TCT	AAT	CGC	TTC	TCT	GGC	TCC	AAG	TCT	GGC	AAC	ACG	GCC	TCC	CTG	ACA	50
JB21	...	...	...	...	G-	...	...	...	...	...	...	...	...	...	...	...	...	...	-C-	...
16M3C8	...	-A	...	...	...	...	...	...	...	...	...	-A	...	...	...	...	...	...	-C-	...

germline V12.1	ATC	TCT	GGG	CTC	CAG	GCT	GAG	GAC	GAG	GCT	GAT	TAT	TAC	TGC	TGC	TCA	TAT	GCA	GGT	60
JB21	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	ACC	...
16M3C8	G-	...	...	...	-A	C-	...	...	...	...	...	...	...	...	...	...	...	AGG	A-	...

germline V12.1	AGT	AGC	ACT	TTA	*GTA	TTC	GGC	GGA	GGG	ACC	AAG	CTG	ACC	GTG	CTA	germline J12/3	70
JB21	-C	-C-	GTC	CGG	-G	...	...	...	...	...	-A	...	...	...	...	...	...
16M3C8	GAC	-C-	...	CGG	-G	...	...	-G	...	...	...	...	...	...	...	...	...

Figure 3. Nucleotide sequences of V_λ and J_λ genes from two hybridoma cell lines (JB21 and 16M3C8). Also shown is a germline gene V_λ2.1 (34), and germline J_λ2/3 gene (23). An asterisk denotes the beginning of the J_λ segment. Numbering is according to Kabat (23). These sequence data are available from EMBL/GenBank Data Libraries under accession numbers M80916 (JB21) and M80921 (16M3C8).

germline V λ 2.1	Q	S	A	L	T	Q	P	A	S	V	S	G	S	P	G	Q	S	I	T	I	S	C
JB21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16M3C8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-

germline V λ 2.1	T	G	T	S	S	D	V	G	S	Y	N	L	V	S	W	Y	Q	Q	H	P	G	K
JB21	-	-	-	-	-	-	-	-	A	-	-	Y	-	-	-	-	-	-	-	-	-	-
16M3C8	A	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

germline V λ 2.1	A	P	K	L	M	I	Y	E	G	S	K	R	P	S	G	V	S	N	R	F	S	G
JB21	-	-	-	-	-	-	-	D	V	T	N	-	-	-	-	-	-	D	-	-	-	-
16M3C8	-	-	-	-	I	L	-	D	V	Y	I	-	-	-	-	-	-	-	-	-	-	-

germline V λ 2.1	S	K	S	G	N	T	A	S	L	T	I	S	G	L	Q	A	E	D	E	A	D	Y
JB21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16M3C8	-	-	-	-	-	-	-	-	-	-	V	-	-	-	-	P	-	-	-	-	-	-

germline V λ 2.1	Y	C	C	S	Y	A	G	S	S	T	L	*V	F	G	G	G	T	K	L	T	V	L
JB21	-	-	S	-	-	T	T	-	T	V	R	-	-	-	-	-	-	-	-	-	-	-
16M3C8	-	-	S	-	-	R	S	D	T	-	R	-	-	-	-	-	-	-	-	-	-	-

Figure 4. Translated amino acid sequences of V λ and J λ genes from two hybridoma cell lines (JB21 and 16M3C8). Shown for comparison is the translated sequence of the V λ germline gene V λ 2.1, and J λ 2/3 germline gene. An asterisk denotes the beginning of the J λ segment. (See Fig. 3.)

(36), and uses a J λ 4 segment differing from the germline sequence by a single base (Fig. 6). The ED6.1 sequence is identical to the partial amino acid sequence reported previously for 2 kappa antibodies purified from serum (12).

VJ joints of seven of these eight hybridoma V λ genes are notable for the presence of an arginine codon (Fig. 7). Comparison of germline sequences indicates that in the RC3 V λ gene, the codon CGA could only have arisen by the addition of a non-germline-encoded G at the V λ J λ joint. In the V λ VII-encoded antibodies, the relevant germline V λ segment is unknown. The arginine codon may be germline-encoded, or result from either mutation or N sequence addition. The VJ splice junction in all lambda antibodies is identical, implying this V λ /J λ combination is a necessary prerequisite for generation of the arginine. Since the CDR-3 region encodes the third hypervariable region, the frequent finding of an arginine residue at this location, and particularly its presence in both kappa

and lambda anti-Hib PS Ab clonotypes, implies this residue is of considerable importance in antigen binding.

Southern blots/analysis of germline V λ VII genes

The available repertoire of V λ VII family members was examined by hybridization of human genomic DNA with the V λ VII probe generated from LSF2. 10 prominent bands, as well as an additional 5 fainter bands, were demonstrated (Fig. 8). Notably, polymorphism such as the presence or absence of a 5.0-kb fragment, and varying intensity of the 3.5-kb fragment, was demonstrated even within this relatively small sample. The human lambda variable region locus is believed to contain about 60 members, of which 10 are members of the V λ VII family (33, 38). The five less prominent bands may represent members of other V λ families sharing significant homology with the V λ VII family. To determine if the V λ VII-encoded antibodies derive from a single germline gene, an oligonucleotide probe corresponding to conserved V λ VII CDR-1 sequence was used to identify a single 3.7-kb genomic DNA fragment. Although more than one V gene may be present in a single restriction fragment, both the repeated isolation of highly homologous V λ VII genes, and the single prominent band detected with the oligonucleotide probe, suggests that a single V λ gene may be responsible for the generation of lambda anti-Hib PS Ab that cross-reacts with K100 PS (Fig. 9). Less prominent higher molecular weight bands are also present, but hybridize weakly to the oligomer after subtraction of nonspecific background binding by high molecular weight DNA.

Discussion

To examine the molecular basis of the immune response to Hib PS, a panel of hybridomas secreting human monoclonal anti-Hib PS Ab has been developed. Our previous studies showed exclusive use of members of the V λ III gene family in this im-

Table II. Summary of V λ H and V λ L Gene Segments*

Hybridoma	V λ H Segment	V λ L Segment
SB5/D6	V λ III 9.1 (5)	V λ VII 4A (33)
RAY4	V λ III 9.1	V λ VII 4A
JB32	V λ III 9.1	V λ VII 4A
JB21	V λ III 9.1	V λ II 2.1 (34)
RC3	V λ III 9.1	V λ II A2 (13)
LSF2	V λ III 9.1	V λ VII 4A
16M3C8	V λ III VH26 (35)	V λ II 2.1
ED6.1	N.K.	V λ I clone KC-1 (36)

* Columns show name of hybridoma cell line and most closely homologous known germline V λ H and V λ L segments. Reference for germline shown in parentheses. NK, Not known.

[illegible]

	D	I	Q	M	T	Q	S	P	S	¹⁰ L	S	A	S	V	G	D	R	
ED6.1	GAC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	GTA	GGA	GAC	AGA
	V	T	I	P	C	R	A	S	Q	S	I	S	S	Y	L	N	W	Y
ED6.1	GTC	²⁰ ACC	ATC	CCT	TGC	CGG	GCA	AGT	CAG	AGC	ATT	AGC	AGC	TAT	TTG	AAT	TGG	TAT
	Q	H	K	P	G	K	A	P	V	L	L	I	L	D	T	S	N	L
ED6.1	CAG	CAC	AAA	⁴⁰ CCA	GGG	AAA	GCC	CCT	GTG	CTC	CTC	ATC	TTA	⁵⁰ GAT	ACA	TCC	AAT	^{CDR-2} TTA
	Q	S	G	V	P	S	R	F	S	G	S	G	S	G	T	D	F	T
ED6.1	CAA	AGT	GGG	GTC	CCA	⁶⁰ TCA	AGG	TTC	AGT	GGC	AGT	GGA	TCT	GGG	⁷⁰ ACA	GAT	TTC	ACT
	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	Q
ED6.1	CTC	ACC	ATC	AGC	AGT	CTG	CAA	⁸⁰ CCT	GAA	GAT	TTT	GCA	ACT	TAC	TAC	TGT	CAA	⁹⁰ CAG
	S	Y	T	L	P	L	T	F	G	G	G	T	K	V	E	I	K	R
ED6.1	AGT	TAC	^{CDR-3} ACT	CTC	CCG	CTC	ACT	TTC	GGC	GGA	GGG	ACC	AAG	GTG	GAG	ATC	AAA	CGA

	V _κ		V _κ or 'N'	J _κ												
germline A2	L	P		W T F G P G T K V D I K R												
HIB RC3	-	-	R	F - - - - - - - - - - - - -												germline J _κ 3
HIB ED6.1	T	L	P	L - - - G - - - - - - - - -												
				L - - - G - - - - - - - - -												germline J _κ 4

	V _λ		V _λ or 'N'	J _λ										
HIB RAY4	G	A	R	V F G G G T K L T V L										
HIB SB5/D6	-	-	-	- - - - - - - - - - - - -										
HIB LSF2	-	-	-	- - - - - - - - - - - - -										
HIB JB32	A	D	-	- - - - - - - - - - - - -										
HIB JB21	T	V	-	- - - - - - - - - - - - -										
HIB 16M3C8	T	T	-	- - - - - - - - - - - - -										

Figure 7. Translated amino acid sequences of VJ joints from seven hybridoma cell lines (RC3, ED6.1, RAY4, SB5/D6, LSF2, JB21, and 16M3C8). Also shown are translated amino acid sequences of the germline V_κ gene A2, and germline J_κ3, J_κ4, and J_κ2/3 genes. (See Figs. 2, 4, and 6.)

mune response, and the apparent restriction to two or three germline V_H genes. In contrast, V_L gene usage is more complex, with antibody encoded by a number of V_λ and V_κ families (12). Analysis of light chain variable region genes obtained from cell lines selected to represent various characteristics of serum antibodies showed that despite diversity in the variety of light chain variable region gene segments utilized, most serum anti-Hib PS Abs may be encoded by a limited number of V_κ and V_λ germline genes.

The light chain distribution of anti-Hib PS Ab is characterized by a predominance of kappa light chains, although some individuals predominantly or exclusively express lambda light chains (39, 40). Scott et al. have shown that V_κII light chains are the most commonly expressed light chains among clonally purified serum anti-Hib PS antibodies from adults with high responses to immunization. Limited amino acid sequences of a number of these serum antibodies showed that greater than 60% of subjects immunized with plain Hib PS produce antibody encoded by a V_κII family member, and further, that a number of these antibodies show identical amino acid homology with the translated amino acid sequence of the germline V_κII gene A2 (12, 13). Lucas et al. have described an anti-idiotypic monoclonal antibody (Hibid-1) that identifies anti-Hib PS antibodies expressing this light chain (16). On average, 60%

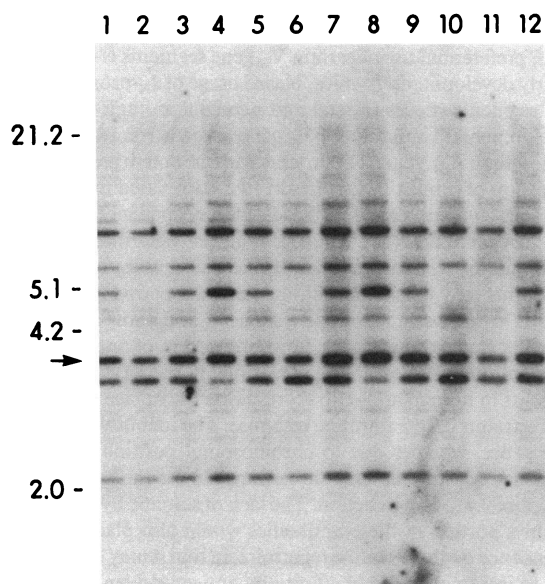


Figure 8. Southern blot analysis for V_λVII family members in BamHI-digested human genomic DNA. Lane 12 contains genomic DNA from the subject from whom hybridoma LSF2 was obtained. Lane 9 contains genomic DNA from a parent of LS. The remaining 10 donors are unrelated. An arrow indicates the 3.7-kb fragment which is identified by the oligonucleotide probe illustrated in Fig. 9.

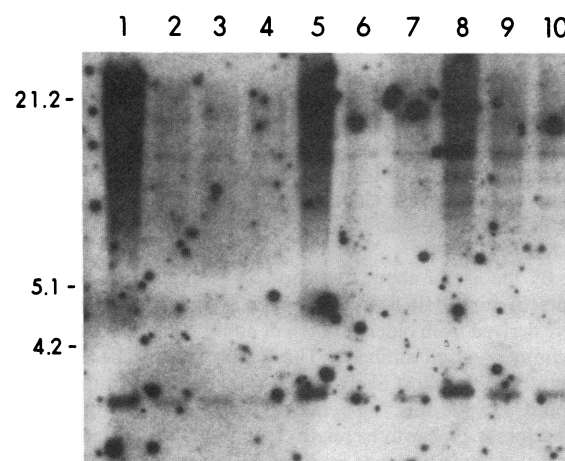


Figure 9. Dried gel hybridization of BamHI-digested human genomic DNA with an oligonucleotide probe corresponding to conserved V_λVII hybridoma CDR-1 sequences. Genomic DNA in lanes 1, 4, 5, 6, 7, 9 and 10 of Fig. 8 corresponds to that in lanes 2, 7, 4, 8, 10, 5 and 9 of Fig. 9, respectively.

of the total anti-Hib PS antibody of most children and adults immunized with either Hib PS or Hib PS-conjugate vaccine expresses this idiotype (15, 16). Taken together, this suggests the most common clonotype of anti-Hib PS Ab is encoded by a single V_HII gene. The V_H locus contains approximately 50 functional genes, of which 40% are members of the V_HII family (41). The V_HII A2 germline gene is known to be present in a single copy (13). Our findings definitively demonstrate that at least a portion of V_HII-encoded antibodies are encoded by unmutated germline elements. Our subject R.C. was immunized with PS-protein conjugate vaccine, whereas Scott's subjects were immunized with the plain PS vaccine. Thus it is possible that the unmutated V_HII A2 segment is utilized irrespective of antigen presentation in a T-independent or T-dependent form.

Lambda variable region gene usage appears similarly restricted. The majority of lambda-expressing anti-Hib PS Ab in adults with high antibody responses to vaccine cross-reacts with *E. coli* K100 PS (17). The capsular polysaccharides of Hib and *E. coli* K100 are structurally similar and gastrointestinal colonization with *E. coli* K100 induces antibody to Hib PS in humans (42). "Naturally occurring" anti-Hib PS Ab in children commonly cross-reacts with K100 PS, whereas Ab of individuals immunized with Hib PS or responding to Hib infection is less frequently cross-reactive (18). The kappa/lambda ratio of natural anti-Hib PS Ab is lower in infants than in older children, and in the vaccine-induced antibody of adults (39). Further, Hibid-1 (the CRI associated with expression of the V_HII light chain) is expressed less frequently and comprises a smaller proportion of total anti-Hib PS Ab in preimmune compared with postimmunization sera. Expression of Hibid-1 following immunization is similar in adults and children. These results imply that cross-reactive antigens may induce the natural antibody of children. K100 PS-cross-reactive antibody may provide protective immunity in young children upon first exposure to Hib. These cross-reactive antibodies appear to be subsequently replaced or dominated by antibody specific for Hib PS when individuals are exposed to this antigen by immunization, colonization, or infection. We demonstrate that cross reactivity of lambda antibodies may be mediated through a single light chain germline gene. The mechanism for the clonal dominance of the V_HII antibodies is unclear; the avidity for Hib PS of these K100 PS-cross-reactive antibodies from adults is similar to that of Hib specific PS antibodies (P. Shackelford, unpublished observation).

Lambda antibodies expressing the V_HII gene did not cross-react with *E. coli* K100 PS. Whether all noncross-reactive antibodies are encoded by closely related members of the V_HII gene family will require further characterization of additional antibodies.

By the age of about 5 yr most individuals have acquired protective anti-Hib PS Ab. All of the hybridomas in this study were derived from older children or adults, who had detectable preimmunization antibody. The B cells immortalized by fusion, therefore, most likely represent a secondary immune response. Despite this, V_H gene segments obtained from these hybridomas appear to be relatively unmutated (14), and it appears that some V_L genes are similarly conserved. The RC3 V_L gene segment is identical to the A2 germline gene, and although the pertinent V_HVII germline gene is not known, the paucity of differences in V_HVII segments from four different subjects suggests these gene segments are relatively unmutated. J_K and J_L segments are also highly homologous to germline ele-

ments. Thus, at least a portion of the anti-Hib PS response represents the use of unmutated germline elements.

VJ joints of the majority of rearranged V_L genes also appear to be an additional source of restriction in the anti-Hib PS immune response. Although there is variability in the use of J_L segments, V_HII-encoded V_L genes are characterized by the presence of an arginine at amino acid position 95a (13). In the RC3 V_L gene and at least one of the antibodies (B-G2a) described by Scott, this appears to have arisen by N sequence addition. In four other kappa antibodies described by Scott, alternative joining may have generated this invariant arginine (13). All of the lambda V_L genes in the present study are similarly characterized by an invariant arginine-96. The germline V_LVII segment is not known; therefore the mechanism used to generate this codon is uncertain. The invariant presence of this residue in many anti-Hib PS Abs supports the previous suggestion that it may be of great importance in antigen binding (13). This also is supported by studies of the murine anti-*p*-azophenylarsenate (Ars) response. Anti-Ars light chains also contain an invariant arginine at position 96, encoded by intracodonic splicing of a single V_L (V_L10-Ars) and a single J_L (J_L1) segment (43). This arginine is essential for Ars binding, and the substitution of tyrosine for arginine has been shown to abolish binding (44). The presence of arginine residues clustered within the CDR's of the heavy chains of murine anti-DNA antibodies correlates with increased affinity, and the arginine requirement in the anti-Hib PS response may indicate its participation in the recognition of the similar phosphate-ester linkage of the repeating carbohydrate moiety (45). Thus, although diversity exists in the use of a variety of V and J segments, certain VJ combinations, which produce arginine codons by junctional flexibility or N segment addition, may be strongly selected.

Does the restricted nature of V_H and V_L segment usage in the human anti-Hib PS response explain the age-related acquisition of immunity to this organism? In both mice and humans, preferential use of certain V_H gene segments is observed in early development. In mice, biased usage of J_H-proximal V_H gene segments occurs in fetal and neonatal animals (46, 47). Developmental regulation of V_L gene usage is less extensively studied than V_H genes. Teale and Morris noted preferential expression of certain V_L families in LPS-stimulated murine fetal B cells compared to adult B cells, with no evidence for position-dependent bias (48). Kaushik et al. also described nonstochastic use of V_L families in adult mice, suggesting molecular constraints may persist beyond the neonatal period (49). Two or three V_H gene segments, and a single V_L and a single V_L gene segments, appear to encode a large proportion of anti-Hib PS Ab in immune subjects. The use of highly conserved V gene segments in human anti-Hib PS response may place considerable restraints on the immune response. The inability to utilize these genes, whether due to chromosomal position or other molecular constraints, may be responsible for the age-related susceptibility to Hib infection. The lack of somatic hypermutation in a portion of these antibodies would also place greater dependence on the germline repertoire, in that it may be impossible to improve antibody affinity by antigen-driven selection of favorable mutations. The absence or polymorphism of these genes also may contribute to individual and ethnic susceptibility to invasive Hib disease. In addition, the repertoire differences detected in some individuals (such as the absence of kappa antibody, or the absence of the V_HII-associated CRI) may be explained by the use of other minor light chains. An individ-

ual missing the A2 germline gene has been described, although the functional implications of such a deletion are not known (13).

Expression of certain V_H gene family members in mice is correlated with B cell subpopulations (50). An alternative explanation for the poor response to plain Hib PS may be a maturational delay in recruitment of a critical B cell population that selectively expresses important variable region gene segments. We and others note that an arginine is expressed at the VJ joint in the great majority of anti-Hib PS light chains. In some circumstances N segment addition is required to generate this codon. The addition of these extra nucleotides is infrequent in light chain recombination, suggesting that these B cell precursors may be relatively infrequent (51). Moreover, N segment addition also appears to be developmentally regulated. Neonatal murine B cells lack N sequences, and their presence in heavy chain recombination increases with time (52). In addition, N sequence addition may also correlate with certain B cell subsets. N segments appear to be rare in Ly1-B cell-derived populations (53).

In contrast to plain polysaccharide vaccines, most young infants respond well to the newer Hib-PS protein conjugate vaccines (54). It is presently unknown if this antigen formulation recruits an alternative antibody repertoire, or if, by an uncertain mechanism, developmental restriction of a "dormant" B cell subset is overcome. To address this question, V gene expression in infants must be examined.

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