A Defective V_κ A2 Allele in Navajos Which May Play a Role in Increased Susceptibility to *Haemophilus influenzae* Type b Disease

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Abstract

The antibody response to *H. influenzae* type b (Hib) is pauciclonal, and is dominated by antibodies using the V_κA2 gene. Navajos have a 5–10-fold increased incidence of Hib disease compared with control populations. We hypothesized that a polymorphism in one of the genes in this oligoclonal response may lead to increased disease susceptibility. Since the predominant A2+ anti-Hib antibodies have high avidity for Hib and can be unmutated, the A2 V_κ gene was analyzed. Over half of the Navajos studied, but only one control individual, had a new allele of A2, termed A2b, with three changes from the published A2 germline sequence. One of the changes was in the recombination signal sequence, suggesting that the A2b allele might not undergo V-J rearrangement very frequently. This possibility was confirmed by analyzing the relative frequency of non-productive A2 rearrangements in A2a/b heterozygous Navajos. Many fewer A2b rearrangements were observed, showing that the A2b allele is defective in its ability to undergo rearrangement. The prevalence of this allele in Navajos may play a role in their increased susceptibility to invasive Hib disease. If so, it would underscore the importance of the germline Ig repertoire for protective antibody responses to pathogenic bacteria in unimmunized children. (*J. Clin. Invest.* 1996. 97: 2277–2282.) Key words: immunoglobulin variable region • gene rearrangement • B-lymphocytes • antibody diversity • immunoglobulin genes

Introduction

Haemophilus influenza type b (Hib)[1] is the major cause of bacterial meningitis in infants (1). The antibody response to *Haemophilus influenza* type b (Hib) has been well characterized, and is very pauciclonal (2, 3). All antibodies sequenced so far are derived from one of four V_HIII genes (4–7). To date, 9 dif-

ferent light chain genes, both lambda and kappa, have been observed in the anti-Hib response (3, 7–13). However, by far the most predominant are antibodies using one specific V_κII gene, A2 (3, 7). Most individuals respond to Hib vaccination with a response containing significant amounts of antibodies encoded by the A2 V_κ gene (A2+ antibodies). On average, the A2+ antibodies comprise 60% of the total antibody response, although the range is quite large (3). The A2+ antibodies have been shown to have the highest avidity of the anti-Hib antibodies (13). There is a direct correlation between bactericidal activity of Hib immune serum and avidity for Hib (14). Also, many of the sequenced A2+ anti-Hib antibodies are unmutated, at least at the light chain level, whereas all other sequenced anti-Hib antibodies have apparently undergone somatic mutation (3–5, 7–11). Together, these data suggest that an effective antibody response may require either a significant proportion of A2+ antibodies, which may be unmutated, or non-A2 antibodies which must have undergone somatic mutation and affinity maturation as is likely to happen in the response to conjugate vaccines.

There is strong evidence for a genetic component in the disease susceptibility to Hib. Eskimos from southwest Alaska, Navajos and Apaches have a 5–10-fold higher incidence of Hib infection compared to North America Caucasians (15–18). These three populations are genetically very closely related. The Navajo, Apache, and Southwest Alaskan Eskimos are the only Native Americans who are descended from the Na-dene group, which migrated from Asia $\sim$ 12,000 years ago (19–21). Before the recent advent of conjugate vaccines, it was documented that 2.5% of Southwest Alaskan Eskimo children under 12 mo of age develop Hib disease, and overall the disease was fivefold more prevalent than in control North American populations (16). The ability to respond to Hib immunization with a robust antibody response has also been shown to have a genetic component, and may play an important role in the genetic susceptibility to disease (22–24). The ability of Navajos to respond to Hib polysaccharide vaccination is > 10 fold lower than age matched white children, correlating with their high rate of Hib disease. These Native American populations do respond to the conjugate vaccines, although multiple immunizations are required to obtain titers comparable to control groups (22).

The response of unimmunized infants to a bacterial infection is likely to be restricted initially to germline antibodies. Since the antibody response to Hib is pauciclonal, we hypothesized that some of the genetic component of increased Hib susceptibility in these Native Americans might be due to a polymorphism of one of the gene segments used in the Hib antibody response. We proposed that polymorphism of the A2 V_κ gene would be most likely to impair the response since it is associated with the only unmutated yet high avidity antibody in the anti-Hib response. Thus, in the absence of a functional A2 gene, the anti-Hib response would utilize other light chain

1. *Abbreviations used in this paper:* CDR, complementarity determining region; Hib, *Haemophilus influenzae* type b; RSS, recombination signal sequence.

V genes which may only form effective anti-Hib binders after somatic mutation. In agreement with this hypothesis, we show here that Navajos, but not control individuals, have an allele of A2 which appears to be defective in its ability to undergo V-J rearrangement.

Methods

DNA samples. Control individuals were randomly obtained from the donor pool of the GCRC at The Scripps Research Institute. They consisted of 8 Caucasians, 3 Asians, and 4 Hispanics. PBMC were obtained by Ficoll-Hypaque purification. DNA was made as previously described (25). Buffy coat white blood cells were obtained from healthy unrelated Navajos as part of an ongoing gene linkage study in Athabascan-speaking Native Americans. All but two of the Navajos identified themselves as being 100% Navajo. One was 25–50% Apache (also high risk for Hib (23)) and one was 12% Hopi. The DNA was made by standard procedures (26).

PCR, cloning, and sequencing. Unrearranged genes were amplified with primers flanking the A2 and A18 coding regions. AF76 (5′-ATGGCTCGAGCCACACCACTGCA) or AF100 (5′-GCTCCTC-GAGAAGACATATCTACC) were the 5′ primers and were located 5′ of the promoter region. Both were used in combination with AF68 (5′-CTGAGCGGCCGCCCAGACAAGCAGTGCAAG), which was in the 3′ flanking region. PCR conditions were 25–30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 2 min, followed by 5 min extension at 72°C. Rearranged kappa genes were amplified with AF90 (5′-CATTAAGCTTTCACATAACCTTGCAC) and AF80 (5′-ACG-TTTGAATTCCACCTTGGTCCC). AF90 is in the A2/A18 leader intron, and AF80 is in the J_{κ} region. AF80 is identical with $J_{\kappa}1$ and 4, except for the changes to create the restriction site. It has one base pair change from $J_{\kappa}2$, and even though that change is 10 bp from the 3′ end of the primer, it does not amplify $J_{\kappa}2$ well. It also has one base pair change from $J_{\kappa}3$, an infrequently used J_{κ}. PCR conditions for amplifying rearranged sequences were 40 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1.5 min, followed by 5 min at 72°C. For the A2-specific PCR a primer AF103 (5′-TATTTAAGCTTGGGGTTCATT-TAAAGAG), was used which is in the only region of more than one nucleotide mismatch between A18 and A2. PCR conditions were 5 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 1.2 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1.2 min. A secondary nested PCR was required for specificity, and was done with AF90 and AF80 for 30 cycles. One fifth of the primary PCR was added to the secondary PCR as template. All amplifications were done with a hot start technique using paraffin wax. Amplified DNA was digested with NotI and XhoI, size selected on an agarose gel, purified with Qiaex Gel Extraction Kit (Qiaex, Chatsworth, CA), and ligated into precut pBluescript KS (Stratagene, San Diego, CA). The plasmids were electroporated into XL1 Blue (Stratagene, San Diego, CA), miniprepped and sequenced as previously described (27). For full length sequences, internal sequencing primers were used as well as T3 and T7 primers.

Typing of A2 haplotype. Cloned PCR products were sequenced with T7 primer, and the 6 A2/A18 polymorphic sites that are within the 3′ terminal 300-bp of the PCR product were evaluated. Usually 2–3 separate PCR reactions were done per individual. Individuals were called homozygous if 2–3 PCR reactions gave only one A2 allele. The small quantities of available DNA precluded Southern blot analysis of genomic DNA.

Results

A new allele of A2 in Navajos. The kappa locus consists of two copies of most genes due to a duplication of most of the locus. Thus, all but eight V_{κ} genes have a copy which is closely related in sequence although very distant in the locus (28). The dupli-

cate of the distal A2 gene is the pseudogene A18, which differs from A2 by only 15 bp of the 1.24 kb which is sequenced from each (7, 29). A18 is a pseudogene since it has a termination codon in place of the conserved cysteine in FR3 (29). Since the two genes are so similar in sequence, we obtained A18 sequences whenever we analyzed A2 sequences. DNA from PBMC was amplified with primers flanking the A2/A18 gene pair. We observed two alleles of A2 and one allele of A18 which have not previously been described in addition to the published alleles of A2 and A18 (7), which we now term A2a and A18a. One new A2 allele, A2c, and one new A18 allele, A18b, were present in all populations analyzed; these alleles are described in another study (30). The second new A2 allele, A2b, was present in 15 of the 28 Navajo DNA samples which we tested but in only one of the 15 control DNA samples. The one control who was heterozygous for A2b was a Hispanic individual originally from Mexico; whether this individual has any Navajo, Apache or other genetically related ancestry is not known.

The A2b allele has three changes in sequence from A2a (Fig. 1). There is a one bp change in FR2 at position 43 which results in a serine to proline change. Many other $V_{\kappa}II$ genes have the Ser in that position, and thus this change is clearly not deleterious to light chain function. Although a non-conservative substitution, the location of this amino acid is not near the complementarity determining region (CDR)3, and thus is not too likely to affect antigen binding. The second change is a C →A change near a pyrimidine rich region of the promoter 14 bp upstream of the decamer (octamer). The third change is a one bp change in the sixth nucleotide of the heptamer of the recombination signal sequence (RSS). The A2a gene has a consensus heptamer. It is well documented that changes from the consensus RSS will decrease the efficiency of rearrangement (31). Changes in the first three nucleotides of the heptamer are extremely deleterious, whereas changes in the other four bases are more variable in effect. Therefore this change in the A2b allele could be potentially crippling, depending upon the effect of this particular substitution on the RSS.

Initially, all alleles were fully sequenced. For typing purposes, individuals were then analyzed for the six polymorphic positions in the 3′ portion of the PCR product. 12/28 Navajos were heterozygous for the A2b allele, and 3/28 were homozygous for A2b. Of the three, one was likely to be hemizygous for
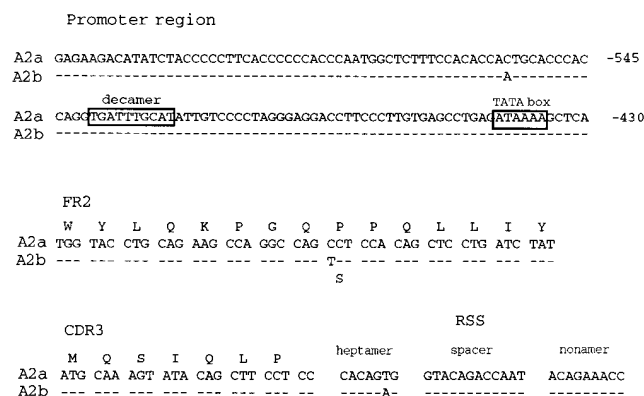
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      Promoter region

A2a  GAGAAGACATATCTACCCCCTTCACCCCCCACCCAATGGCTCTTTCCACACCACTGCACCCAC  -545
A2b  -----------------------------------------------------A---------

          decamer                                     TATA box
A2a  CAGG[TGATTTGCAT]ATTGTCCCCTAGGGAGGACCTTCCCTTGTGAGCCTGAC[ATAAAA]GCTCA  -430
A2b  -----------------------------------------------------------------


      FR2
      W   Y   L   Q   K   P   G   Q   P   P   Q   L   L   I   Y
A2a  TGG TAC CTG CAG AAG CCA GGC CAG CCT CCA CAG CTC CTG ATC TAT
A2b  --- --- --- --- --- --- --- --- T-- --- --- --- --- --- ---
                                      S


      CDR3                                         RSS
      M   Q   S   I   Q   L   P     heptamer    spacer        nonamer
A2a  ATG CAA AGT ATA CAG CTT CCT CC  CACAGTG   GTACAGACCAAT  ACAGAAACC
A2b  --- --- --- --- --- --- --- --  -----A-   ------------  ---------
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Figure 1. The full sequence of A2b is available from GenBank under accession number U41643. It is the same as A2a in all positions except the three described here.

A2b, since in three separate PCR reactions, we obtained 24 A18 sequences but only 5 A2b sequences. Lack of sufficient DNA precluded analysis by Southern blot to confirm hemizygosity.

It should be noted that we have not shown that these are, in fact, true alleles, as opposed to a gene duplication. The main argument in favor of these being alleles is that we obtained 289 A2 sequences (alleles a, b, and c) and 282 A18 sequences (alleles a and b) from the 28 Navajos. Of the 16 Navajos with the A2b gene, we obtained 155 A2 sequences and 172 A18 sequences. If there were a gene duplication of A2a to generate A2b, we would have expected an increased ratio of A2:A18 sequences. Also no individual was observed with all three A2 alleles.

Many fewer A2b than A2a rearrangements. Because of the change in the RSS, we predicted that the A2b gene would not undergo V-J rearrangement efficiently. Since many of the Navajos tested were A2a/A2b heterozygotes, we could directly test the efficiency of rearrangement in vivo by determining the relative frequency of rearrangement of the two alleles. Rearranged sequences were amplified with a J region primer and a primer in the leader intron of the A2/A18 gene pair. The leader primer matches all alleles of A18 and A2 exactly except for the changes to create the restriction site for cloning, and thus equally amplifies all A2 and A18 alleles. Since A2a and A2b have one nucleotide difference in FR2, we distinguish between the two alleles by sequencing. A2c alleles share that FR2 nucleotide change with A2b, precluding analysis of rearranged sequences in A2b/A2c Navajo heterozygotes. Controls analyzed for rearrangements were mainly A2a homozygotes, but some A2a/A2c heterozygotes were also used. All rearranged genes were typed by analysis of the 5 polymorphic sites at the 3′ end of the V gene coding region. Table I shows that 15 out of 17 A2 rearrangements in the Navajo DNA use A2a, while only 2 use A2b.

Table I. Rearranged Sequences Using A18, A2a, and A2b V_{κ} Genes

Donor	A2 genotype	A2a,c	A2b	A18a,b
Navajo				
HC	(a/b)	2	1	20
KB	(a/b)	4		21
No. 9	(a/b)	3		35
No. 21	(a/b)	5	1	17
No. 23	(a/b)	1		5
Total		15	2	98
Control				
No. 1	(a/a)	22		50
No. 5	(a/a)	4		16
No. 6	(a/a)	2		7
No. 2	(a/c)	4		20
No. 4	(a/c)	1		7
Total		29		100

DNA from PBMC of controls or Navajos were amplified with a V_{κ} primer which amplifies all alleles of A2 and A18 equally, and a J_{κ} primer. Amplified DNA containing these VJ rearrangements were cloned and sequenced. The A18 or A2 allele used in each rearrangement was identified by the sequence variations in each allele.

Table II. Rearranged A2a and A2b Sequences from A2a/A2b Heterozygous Navajos

	A2a	A2b
A2-specific PCR	43	2
A2 + A18 PCR*	15	2
Total sequences	58	4

An A2-specific PCR was performed which equally amplifies rearrangements using all alleles of VkA2, but which does not amplify A18 rearrangements. This data is added to that derived in Table I (*) to give the total number of A2a and A2b rearrangements from heterozygous Navajos.

Many of the rearranged sequences were from A18. Since A18 is located 450 kb from the J_{κ} cluster, whereas A2 is 1600 kb upstream of J_{κ} and is also in the opposite transcriptional orientation from the J segments, this is not totally unexpected (this issue is further discussed below). We therefore set up an A2-specific PCR protocol. There was only one region of the 1.2 kb sequence which had more than a one base pair mismatch between A2 and A18, so we used that region for a primer, even though it was not an optimal location for a primer due to its AT-rich composition. A secondary nested PCR was necessary to obtain specificity. From this type of PCR, we obtained an additional 45 A2 sequences from A2a/b heterozygous Navajos, and 43 of those were A2a (Table II). Thus, in total we had 58 A2a rearranged alleles and 4 rearranged A2b alleles. This demonstrates that the A2b allele is extremely underrepresented in the peripheral repertoire.

Relative rate of V-J rearrangement of the A2 alleles. Since the A2b allele had a nonconsensus RSS, it seemed likely that the lower representation of A2b could be due to an intrinsically lower rate of V-J rearrangement. Once a productive rearrangement occurs, that immature B cell can undergo clonal expansion in the periphery, if stimulated by antigen. Hence, analysis of productive rearrangements cannot reveal the initial frequency of rearrangement of a given gene. However, nonproductive rearrangements are unable to be skewed in this way, and thus more accurately reflect the intrinsic rate of rearrangement. Therefore, in order to determine the relative rates of rearrangement of the two alleles, we analyzed the sequences described above to determine the number which were out-of-frame (Table III). 70% of the A2a sequences were in-frame, which is expected since it is a functional gene. Surprisingly, 3 out of 4 A2b rearrangements were out-of-frame. With such a small sample size, it is not possible to say if this is significant or not. Nonetheless, of the non-productive A2 rearrangements, 25 were A2a sequences and 3 were A2b sequences. Thus, the

Table III. Out-of-frame Rearrangements Show Relative Rates of Rearrangement of A2a vs. A2b Alleles

	A2a	A2b	A2a:A2b
Navajo (A18 + A2 PCR)	4	2	
Navajo (A18 -specific PCR)	21	1	
Total Navajo A2 sequences	25	3	8.3

All of the A2 sequences from Tables I and II in which the V-J rearrangements were out-of-frame are listed.

Table IV. Out-of-frame Rearrangements Show Relative Rates of A18 vs. A2 Rearrangements

	A18	A2a	A2b	A18:A2
Control	71	12		5.9
Navajo	70	4	2	11.7

All of the sequences from Tables I and II in which the V-J rearrangements were out-of-frame are listed.

A2b allele has only 12% of the rearrangement potential of A2a. A2b is impaired, but not totally defective, in the ability to undergo V-J rearrangement.

The J-distal A2 gene rearranges less frequently than its J-proximal A18 duplicate. The available cDNA data to date show that the J_κ-distal copy genes are significantly under-represented (31, 32, and our unpublished data). Since the A2 gene used in the anti-Hib response is in the distal half of the kappa locus, it is likely that this gene rearranges much less frequently than the V_κ genes in the proximal half of the locus. Therefore, it was of interest to determine the relative rates of V-J rearrangement of the A18 gene, which is in the J_κ-proximal copy, and the A2 gene, which is in the distal copy, in the control population. To determine this, analysis of out-of-frame A2 and A18 rearrangements was done on the control samples (Table IV). There were 71 out-of-frame A18 rearrangements and 12 out-of-frame A2 rearrangements, indicating that the distal A2 gene rearranges about six times less frequently than the J-proximal A18. Considering the very low representation of distal copy genes in general in the expressed cDNA repertoire, this is actually a surprisingly high ratio.

Discussion

Although a variety of somatically mutated antibodies can bind to Hib, and can apparently be effective in clearing bacteria, only one type of anti-Hib antibody has been identified which can bind Hib without somatic mutation, at least at the level of the light chain. Since Hib primarily strikes infants and young children, we suggested that unmutated antibodies might be the main defense against bacterial infection in unimmunized children. We therefore analyzed Navajo DNA to determine if a polymorphism of the A2 gene might be present which might explain their increased susceptibility to Hib disease, and we found such a polymorphism. Over half of the Navajos studied had a new allele of A2 which had three changes from the published A2 sequence. The change which we felt was most likely to be deleterious was in the heptamer of the RSS, which is changed from the consensus CACAGTG to CACAG_AG_. Such a change could reduce the frequency with which the A2b gene undergoes rearrangement. The effect of non-consensus RSS on V-J rearrangement rates was studied by Hesse et al. using recombination substrates where single base changes could be made in consensus RSS in the plasmid substrate, and the rate of rearrangement of the substrate was then determined after transfection of the substrate into pre-B cells (32). Their work showed that changes in the first three nucleotides in the heptamer uniformly reduced the rearrangement frequency to ~ 1% of control levels. Single nucleotide changes in the other four nucleotides of the heptamer were more vari-

able, reducing the frequency by 13–94% as compared to the consensus RSS. At position 6, where we observed the change to an A in A2b, Hesse et al. observed that a change to a C decreased the recombination frequency to 46–87% of one with the consensus RSS. A recent comprehensive compilation of 453 actual TCR and Ig RSS showed that 11% of heptamers have a C at that position (33). However, the same compilation did not reveal any sequences with this new A2b heptamer, and only 1% of the sequences had an A at that position, all with other changes in the RSS. This suggested that a change to an A might be more deleterious than the studied change to a C. This A2b heptamer was seen 4 times in an analysis of the germline sequences of the VλII family (34). Importantly, none of these four germline genes have ever been seen in cDNA or antibodies, despite the fact that their germline sequence looks to be functional, again suggesting that this specific change would be very deleterious. Recently, another study was done with recombination substrates, and one substrate contained the A2b heptamer, but with a different nonamer (35). Recombination of this plasmid was reduced to 39.6% of that seen in the recombination substrate with the consensus RSS. Hence, we predicted that this particular heptamer sequence might be responsible for the apparent nonfunctionality of these VλII genes and of the Navajo A2b allele. By analyzing the number of nonfunctional rearrangements of A2a vs. A2b in heterozygotes, we were able to demonstrate that the rearrangement frequency of A2b in vivo was only ~ 12% of that of A2a. Thus, the A2b allele is significantly impaired in its ability to undergo V-J rearrangement.

Although we propose that this impaired rearrangement is primarily due to the variation in the RSS, there is also a change in the A2b promoter just upstream of the octamer near a pyrimidine-rich region. Kappa promoters contain a variable number of elements in addition to the required TATA box and octamer/decamer which comprise the minimal essential promoter elements. The pentadecamer element and at least three different pyrimidine-rich elements have been reported to synergize with the octamer for optimal kappa transcription under some, but not all, experimental conditions (36–39). Several proteins binding to this region have been described (40–42). However, not all of these elements are present in V_κII promoters, and thus the regions of the A2 promoter which are important for optimal transcription are not known.

Defects in the promoter region at sites where nuclear factors bind would be expected to affect the level of transcription of rearranged kappa genes. In addition, germline transcription of many Ig and TCR gene segments has been well documented to precede V(D)J rearrangement. Thus, a defective promoter region could also prevent, or reduce the efficiency of, V(D)J rearrangement. In fact, a human Vλ gene has been found with a defective octamer sequence, and it makes greatly reduced levels of germline transcripts and undergoes V-J rearrangement very poorly (43). Thus this change in the A2b promoter could potentially affect both the rate of rearrangement as well as the expression of rearranged genes. However, the importance of the region 5′ of the octamer in kappa promoters for germline transcription, and thus the effect of this one nucleotide change, is unknown.

A defective promoter could also explain why we observed a lower rate of rearrangement of this allele in vivo than was observed with the recombination substrate having the same altered heptamer (35). Alternatively, the apparently lower re-

combination rate may be due to the low numbers of A2b rearrangements which we observed. Finally, it is also possible that the A2b heptamer change is more deleterious in the context of the A2 RSS, with its non-consensus nonamer as well as its individual spacer sequence, than in the context of a consensus RSS. It is of interest, however, that most of the A2b rearrangements are out-of-frame. If the only defect in the A2b allele were in the rate of rearrangement, one would expect that rearrangements using it would have the same increased frequency of productive rearrangements as A2a due to clonal selection for B cells with functional receptors. Because of the very small sample size, the predominance of out-of-frame rearrangements could be coincidental. Attempts to obtain more A2b rearrangements by amplifying A2b/b homozygotes were unsuccessful, presumably due to the very low frequency of rearrangement of the A2b allele. However, if the promoter were defective because of this change or any other changes in the A2b promoter further upstream from the region which we sequenced, it may be that A2b is in fact a fully defective allele.

43% (12/28) of the Navajos which we studied were heterozygous for A2b, and only 11% (3/28) were homozygous. If most of the Navajos then had at least one copy of the A2a allele, why is this not sufficient to provide protection against Hib? One possibility is that the increased incidence of Hib disease might be restricted to the 11% of Navajos who are homozygous (or hemizygous) for the A2b allele. Although the rate of invasive Hib disease in Navajos under 5 yr of age was 5–10-fold higher than that of the general U.S. population, the absolute frequency of affected individuals was well under the estimated percentage of A2b homozygotes (15, 22).

Alternatively, A2b/A2a heterozygotes might also be at increased risk of invasive Hib disease. As described below, it is likely that the A2+ anti-Hib antibodies are made very infrequently in A2a homozygous individuals, and the frequency may be so low that some heterozygotes may not have any appropriate A2 anti-Hib precursors when they become infected. The reason that it might be particularly hard to make the highest affinity unmutated antibody to Hib is because the V_κ gene used in this antibody is 1.5 Mb from the J_κ segments and it must rearrange by inversion (44, 45). In addition, the light chain has an unusually long CDR and importantly, it appears to require a specific amino acid, arginine, at the V_κ-J_κ junction which can only be added via N region addition (7). It has been observed in studies of cDNA that the kappa genes in the distal half of the locus, all of which rearrange by inversion, and all of which are over 1 Mb from the J_κ region, are used infrequently (46, and our unpublished data). In 117 cDNA kappa sequences from cord blood, we did not observe a single rearranged A2 gene (G. Lugo and A. J. Feeney, unpublished data). Since the expressed repertoire which is measured in cDNA does not necessarily reflect the rate of rearrangement, we compared the frequency of out-of-frame rearrangements in the 131 sequences which we had from our control individuals. There were about six times more out-of-frame A18 rearrangements than out-of-frame A2 rearrangements, demonstrating that rearrangements to the distal locus are in fact disfavored at a mechanistic level.

In addition to the rate of rearrangement, the characteristic junction in the A2 anti-Hib antibodies is very rare. Since A18 is a pseudogene, analysis of all of the A18 sequences, in frame or out-of-frame, should reveal the unbiased extent of junctional diversity. Although CDR3 lengths of 10 amino acids are seen in 7% of our rearranged A18 sequences, we did not see a single example of an A18 rearrangement with a CDR3 of 10 amino acids and an arginine at the junction in over 200 sequences.

We have shown that a new A2 allele which is significantly impaired in its ability to undergo V-J recombination is present in half of the population with increased risk of Hib disease, but in only one individual from the control population. Since the A2 gene is the only gene which apparently can be used in its unmutated form to make a Hib-binding antibody, we suggest that this polymorphism may play a major role in the greatly increased disease susceptibility in Navajos. Although there are likely to be several different reasons for susceptibility to Hib disease in general (47, 48) we suggest that individuals would be much more likely to develop Hib disease if they came from a population which had a high frequency of a defective A2 allele. This hypothesis predicts that the frequency of this defective A2b allele would be increased in individuals who had Hib disease, and that individuals with two copies of the defective allele would be very susceptible to Hib disease. If this hypothesis is correct, it would underscore the critical importance of the germline repertoire for effective protective immunity to pathogenic bacteria in unimmunized individuals, even if other relatively higher affinity antibodies can be generated after appropriate immunization protocols.

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