Genetic Basis of Human Complement C4A Deficiency

Detection of a Point Mutation Leading to Nonexpression

Giovanna Barba, Christian Rittner, and Peter M. Schneider

Institute for Legal Medicine, Johannes Gutenberg University, D-6500 Mainz, Germany

Abstract

The fourth component of the human complement system (C4) is coded for by two genes, C4A and C4B, located within the MHC. Null alleles of C4 (C4Q0) are defined by the absence of C4 protein in plasma. These null alleles are due either to large gene deletions or to nonexpression of the respective genes. In a previous study, evidence was obtained for nonexpressed defective genes at the C4A locus, and for gene conversion at the C4B locus. To further characterize the molecular basis of these nonexpressed C4A genes, we selected nine pairs of PCR primers from flanking genomic intron sequences to amplify all 41 exons from individuals with a defective C4A gene. The amplified products were subjected to single-stranded conformation polymorphism (SSCP) analysis to detect possible mutations. PCR products exhibiting a variation in the SSCP pattern were sequenced directly. In 10 of 12 individuals studied, we detected a 2-bp insertion in exon 29 leading to nonexpression due to the creation of a termination codon, which was observed in linkage to the haplotype HLA-B60-DR6 in seven cases. In one of the other two individuals without this mutation, evidence was obtained for gene conversion to the C4B isotype. The genetic basis of C4A nonexpression in the second individual is not yet known and will be subject to further analysis. (*J. Clin. Invest.* 1993. 91:1681–1686.) Key words: genetic deficiency • complement system • insertional mutation • single-stranded conformation polymorphism • major histocompatibility complex

Introduction

The fourth component of the human complement system (C4)[1] is coded for by two genes, C4A and C4B, located within the MHC class III region on the short arm of chromosome 6 (1). The two C4 isotypes differ in their amino acid sequences by < 1%. The two C4 genes are tandemly arranged together with two genes for the cytochrome P_{450} steroid 21-hydroxylase (CYP21A and B), each located 3' to the C4A and C4B genes, respectively (2, 3). The C4A gene is usually ~ 22 kb long,

1. *Abbreviations used in this paper:* C2 and C4, second and fourth components of complement; C4A and C4B, isotypes A and B of the fourth component of complement; C4Q0, C4 null allele; SSCP, single-stranded conformation polymorphism.

whereas the C4B gene is polymorphic in size with either 22 or 16 kb. This size variation is due to the presence of a 7-kb intron located ~ 2.5 kb from the 5' end of the C4 genes (4, 5).

At the protein level C4 is highly polymorphic, with > 40 variants including null alleles (C4Q0) at both loci (6). Null alleles are defined by the absence of C4 protein in plasma and are present in the normal population at frequencies of 0.1–0.3 (7). Complete deficiency of C4 is a rare event and is correlated with severe immune complex disease (8). Partial deficiency of one of the two isotypes (C4A or C4B) is more common and is also associated with an increased susceptibility to autoimmune or immune complex disease (e.g., in patients with systemic lupus erythematosus [9, 10], subacute sclerosing panencephalitis [11], or primary biliary cirrhosis [12]).

Genetic variation is also evident at the genomic level, as demonstrated by the detection of gene deletions and duplications. C4 null alleles are due either to large deletions comprising an entire C4 gene and the flanking 21-hydroxylase gene, or to nonexpression of the respective gene (13, 14). In a previous study on the molecular basis of C4 null alleles, evidence was obtained for defective genes at the C4A locus, and for gene conversion at the C4B locus as demonstrated by the presence of C4A-specific sequences (15).

To further characterize the molecular basis of the defective C4A genes, we developed a strategy based on PCR amplification and genomic DNA sequence analysis. We selected nine pairs of primers from the published C4A genomic sequence (5) to amplify all 41 exons from individuals homozygous for C4AQ0. All individuals studied carried a nonexpressed C4A gene on one haplotype and a C4A gene deletion on the other haplotype. The amplified DNA fragments were subjected to single-stranded conformation polymorphism (SSCP) analysis to detect possible mutations. Finally, direct DNA sequencing of the PCR product exhibiting a variation in the SSCP pattern was used to define the type of mutation. Using this approach we detected a 2-bp insertion in exon 29 leading to nonexpression. However, this mutation was only found in 10 of 12 haplotypes studied.

Methods

Selection of individuals and genetic typing. 12 individuals with homozygous C4A deficiency were selected. All individuals had been haplotyped for MHC class I, II, and III gene products in family studies by our laboratory as well as by other collaborating laboratories (6, 15, 16) according to the following methods: HLA-A, -B, and -C, and -DR antigens were assigned by the microlymphocytotoxicity assay (17); C4 typing was carried out by high voltage agarose gel electrophoresis in a discontinuous buffer system after desialation of the samples with neuraminidase, followed by immunofixation or hemolytic overlay (18); C4Q0 alleles were confirmed by C4 α-chain typing using SDS-PAGE (19); restriction fragment length polymorphism analysis was carried out by Southern blot with TaqI–digested genomic DNA and hybridization to C4- and CYP21-specific probes as described (14).

Primer pairs	Sequence (5'-3')	C4 exons amplified	Size
			bp
5'	CCACAACTCTGGGCCTGAGGCCA	1–4	1,323
2	CACAGGCAGAGGTCACATCAGTG		
3	GACCTCTGCCTGTGACCTACTTC	5–9	1,228
4	GTTAGCTCAGAGGTCAGAGGCAA		
5	TCCCTCTCTTCTTGGGCTCTGTC	10–13	1,129
6	TCGGCACAAGTGCCATCTCTCCT		
7	GAAGCTCTCCCACTCTGACCGCC	14–17	1,324
8	CAAGCGCCGCCACCTGTGCCCTA		
9	GCGCTTGGAAAGGCAGAACGGTC	18–22	1,386
10	ACTTAGGAAACCAATGGCTGGAG		
11	ACAGCAGGACAGGCTGCCAACTT	23–28	1,569
12	CCTCAGGCTCAGTGCCAGAGCGC		
13	CTTCCTGTTTACTTGTGGTCTCC	29–31	1,021
14	ACCCTCTCCTCCACCAGTGCCTG		
15	CCTCACATGTCCCACGTCCTCTC	32–35	827
16	ATCGAGTCCCTTCCTGCCTCATC		
17	AACAGGCCAAGGAAACCCAGTAC	36–41	1,735
18	GCAACACACACTGACACTTCGCT		

PCR amplification. Nine pairs of oligonucleotide primers were chosen according to the published C4A genomic sequence from flanking intron sequences (5) as shown in Table I and Fig. 1. With these primers all 41 exons of both C4A and C4B genes can be amplified. The PCR was performed in a thermocycler (Perkin-Elmer Corp., Norwalk, CT) with 100 ng of genomic DNA, 25 pmol of each primer, 200 µM dNTP, and 2 U Taq polymerase (Boehringer, Mannheim, FRG) using a "touchdown" protocol (20). The first 10 cycles were carried out at decreasing annealing temperatures in 1°C steps for two cycles each from 69 to 64°C, followed by 20 cycles using the following conditions: 1 min 94°C; 2 min 64°C; 5 min 72°C. For primers 3/4, a different touchdown program was carried out beginning with 12 cycles from 64 to 58°C. Isotype-specific PCR was performed to amplify only the C4A allele using a C4A-specific primer located in the C4d region and including the isotype-specific Chido-4 sequence as well as a second primer close to the possible mutation site: A-down, 5'-GACCCCTGTCCA-GTGTTAGAC-3'; 13N, 5'-TTTCAATCCACAGGGCTGGGGCC-3' using a touchdown protocol from 67 to 62°C.

SSCP analysis. PCR products were purified by adsorption to glass milk (Geneclean; BIO 101, La Jolla, CA) and digested with appropriate restriction enzymes to obtain small fragments with a size range between 700 and 50 bp. The samples were denatured with formamide by heating at 80°C for 3 min and kept on ice until loading. The mixture was separated either in a 0.75-mm-thick MDE gel in 0.6× TBE buffer (AT Biochem, Malvern, PA) or in a standard polyacrylamide gel (5% total acrylamide, 1% crosslinker, 5% glycerol) polymerized on Gel-Bond PAG film (Pharmacia LKB, Freiburg, FRG) at room temperature, applying 150 V and 5 mA for 2.5–20 h (21, 22). Using these conditions, single-stranded DNA as well as heteroduplex fragments can be analyzed. The DNA was visualized by silver staining as described with minor modifications (23).

Detection of C4 isotype-specific sequences. Restriction fragment analysis of PCR products was carried out as published by Braun et al. (15). Briefly, the amplified DNA using PCR primers L3/L4 was purified by absorption to glass milk (see above) and digested with 2 U Nla IV (New England Biolabs, Schwalbach, FRG). The digested DNA was electrophoresed in a 1.5% agarose gel and visualized by ethidiumbromide staining.

Genomic DNA sequencing. For direct sequencing of PCR-amplified products, the amplified DNA fragments were purified by adsorption to glass milk (see above). The sequencing reaction was carried out on double-stranded DNA using the T_7 Sequencing Kit (Pharmacia LKB) and α-[^{35}S]dATP (DuPont NEN, Dreiech, FRG). Isotype-specific PCR fragments were sequenced by cycle sequencing with Taq DNA polymerase using the "fmol" DNA sequencing system (Promega Corp., Madison, WI).

Results

5 of the 12 individuals with homozygous C4A deficiency and one nonexpressed C4A gene selected for this study had been examined in a previous study for the presence of defective C4A genes using PCR amplification and subsequent restriction fragment analysis (15). The other seven individuals were selected from a C4 reference typing workshop (6) and from a recent disease association study (16). In these two studies, C4 typing had been carried out at the protein and DNA level in addition to HLA typing to establish complete MHC haplotypes. These seven samples were tested as described above for the presence of nonexpressed C4A genes using restriction analysis with the enzyme Nla IV, which detects the isotype-specific Chido −4/+4 site of the C4d region (amino acids 1101–1106 located in exon 26) (24, 25; see also Fig. 1). In six of these samples, a defective C4A gene was detected (not shown), whereas in one sample (typed C4AQ0,Q0,B1,2), only the C4B-specific sequence (i.e., Chido +4) was found, as indicated by the absence of the Nla IV restriction site (Fig. 2). Thus, the C4A deficiency can be explained for this gene by conversion from C4A to C4B.

To characterize the C4AQ0 nonexpressed alleles in the remaining 11 individuals, we selected 18 oligonucleotide primers from the published genomic C4A sequence that allow the amplification of all C4 exons as shown in Fig. 1. The nine amplified genomic fragments range in size from 0.8 to 1.7 kb, and contain three to six exons each (Table I). The resulting PCR products from a normal individual without the C4 null allele (*A*) and an individual with a nonexpressed C4A gene on one haplotype and a C4A deletion on the other haplotype (*B*) are
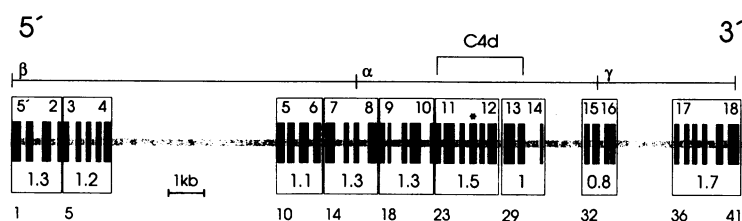

Figure 1. Map of the C4 gene structure and location of PCR primers for exon amplification. (*a*) C4 protein subunit organization and location of the isotype-specific C4d region; (*b*) location of PCR primer pairs; (*c*) sizes of amplified fragments (in kilobases); (*d*) numbers of the first exon of each amplified fragment. The asterisk indicates the location of the isotype-specific Chido −/+4 sequence in exon 26.
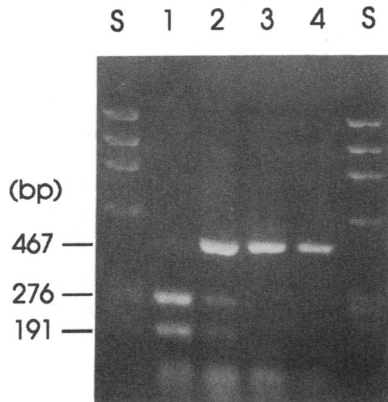
Figure 2. NlaIV restriction fragment analysis of PCR-amplified DNA for the identification of the C4 genotype. The amplified 926-bp fragment covers the C4 isotype-specific sequences: after NlaIV digestion, a single 467-bp fragment is characteristic of the C4B isotype (Chido +4) and the 276- and 191-bp fragments are characteristic of the C4A isotype (Chido −4). The genotypes studied are as follows: lane *1*, C4A genotype control C4A3,2 BQ0,Q0 with C4B to C4A gene conversion; lane *2*, C4AQ0,Q0 B1,2 with one nonexpressed C4A gene and one C4A gene deletion; lane *3*, C4AQ0,Q0 B2,1 with one C4A gene deletion and a gene conversion from C4A to C4B; lane *4*, C4B genotype control C4AQ0,Q0 B1,1 with both C4A genes deleted; *S*, ΦX 174-HaeIII-digested DNA as size marker.

represented in Fig. 3. Since both the C4A and C4B genes are amplified simultaneously in this experiment, any structural deviation in the defective gene should be visible as an additional fragment in individual B. No obvious difference in the size of the amplified exons can be seen, providing further evidence that the nondeleted C4A null gene is structurally intact.

The analysis of the nine amplified fragments by SSCP gel electrophoresis did not reveal any differences between the DNA from three control individuals and three unrelated individuals with nonexpressed C4A null alleles for primers 5'/2, 3/4, 5/6, 7/8, 9/10, 15/16, and 17/18 as shown in Fig. 4 *A* for primers 15/16. Characteristic SSCP and heteroduplex polymorphisms were obtained using primers 11/12 that amplify the central portion of the C4d region, since these exons 25–28 contain well-defined isotype-specific sequence differences coding for the Rodgers (C4A) and Chido (C4B) blood groups of the C4 molecule (26) (not shown; manuscript in preparation).

Finally, using primers 13/14 and restriction enzyme digestion with Hinf I, we detected an additional fragment of ∼ 250 bp in two of the three samples with nonexpressed C4A null alleles (Fig. 4 *B*, lanes *3* and *4*), which was not found in any of the control individuals. Therefore, this PCR fragment covering exons 29–31 was selected for detailed DNA sequence analysis.

Genomic DNA sequence analysis of this fragment using PCR primer 13 as sequencing primer revealed a mixed C4A/C4B sequence containing a 2-bp shift in the reading frame (not shown). Since the origin of this possible insertion was not visible with this sequencing reaction, it had to be very close to the sequencing primer annealing site. Therefore, we synthesized primer 13N, which anneals to the opposite coding strand and extends the sequencing reaction into the direction of the possible insertion site. Thus we detected a 2-bp insertion at nucleotide position 5877 (numbers according to reference 5) in exon 29 as demonstrated in Fig. 5. The sequence of the noncoding strand of a control individual typed C4A3B1 (*A*) is shown in comparison to that of an individual with the defective C4A gene and normal C4B1 alleles (*B*). A mixed DNA sequence pattern can be observed beginning at the C in position 5877 and extending upstream, resulting in a two-nucleotide reading frame shift. This observation could be confirmed after isotype-specific amplification with the primer A-down, which only anneals to the C4A-specific DNA sequence coding for the Ch −4 sequence in exon 26 (see Fig. 1), together with primer 14, and by sequencing both strands in this region with primers 13 and 13N (not shown).

The genomic DNA sequence of exon 29, intron 29, and the beginning of exon 30, as well as the translated amino acid sequence is illustrated in Fig. 6. In the mutationally altered C4A sequence, the last correctly translated codon would be amino acid position 1214. Beginning with position 1215, a sequence of 68 amino acid residues follows to the end of exon 29, which is completely different from the normal C4 sequence. Assuming a regular splicing of the relatively short intron 29, two more codons are found in exon 30. The next triplet in this sequence represents the stop codon TGA (Z in Fig. 6), which terminates the translation of this C4A transcript.

Our initial results using SSCP analysis (Fig. 4) provided
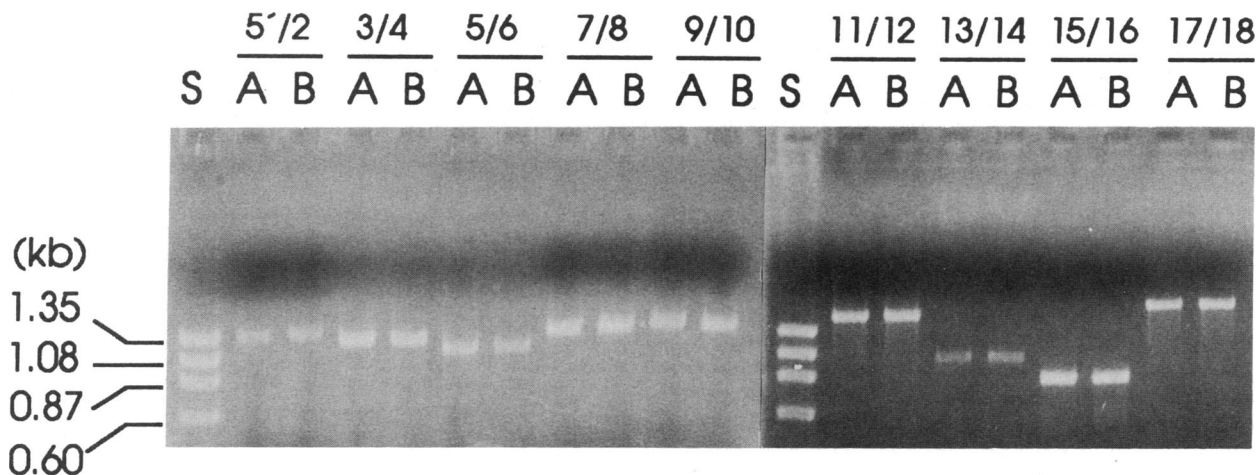
Figure 3. Exon-specific PCR amplification of DNA from one control individual (*A*, C4A3B1) and one individual with a nonexpressed C4A gene (*B*, C4AQ0B1). The respective primer pairs for each fragment (see Fig. 1 and Table I) are indicated above; *S*, ΦX 174-HaeIII-digested DNA as size marker.

A 1 2 3 4 5 6

B 1 2 3 4 5 6

250 bp ←
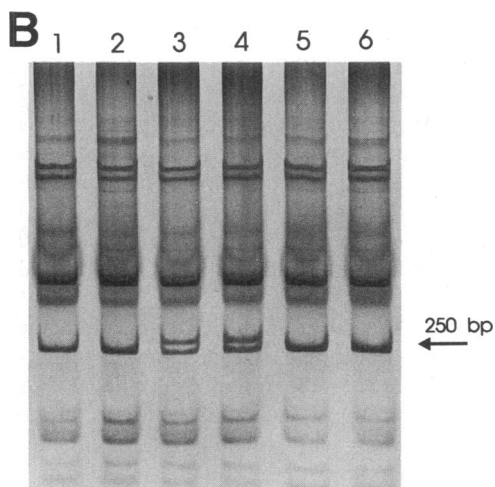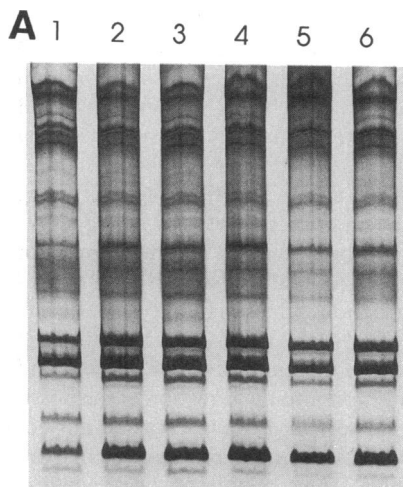
Figure 4. PCR-SSCP and heteroduplex analysis of selected exons of the C4 genes for the detection of sequence variations. (*A*) 0.8-kb fragment with exons 32–35 amplified with primers 15/16 and digested with PvuII. Electrophoresis was carried out in a 14-cm-long 0.5× MDE gel for 2.5 h at 150 V. (*B*) 1-kb fragment with exons 29–31 amplified with primers 13/14 and digested with HinfI. Electrophoresis was carried out in a 14-cm-long 5% polyacrylamide gel for 2.5 h at 150 V. The samples studied are as follows: lane *1*, C4A genotype control C4A3,2 BQ0,Q0 with C4B to C4A gene conversion; lane *2*, C4 genotype control C4A3B1; lane *3*, C4AQ0,Q0 B1,1 with one defective C4A gene and one C4A gene deletion (with a 2-bp insertion in exon 29); lane *4*, C4AQ0,Q0 B1,2 with one defective C4A gene and one C4A gene deletion (with a 2-bp insertion in exon 29); lane *5*, C4AQ0,Q0 B1,2 with one defective C4A gene and one C4A gene deletion (without a 2-bp insertion in exon 29); lane *6*, C4B genotype control C4AQ0,Q0 B1,1 with both C4A genes deleted.

evidence that the mutation leading to nonexpression of the defective C4A gene is not present in all haplotypes with C4A null genes. The individual in lane *5* of (Fig. 4 *B*) does not exhibit the additional 250-bp fragment observed in the two other C4A-deficient individuals in lanes *3* and *4*. Therefore, we studied a total of 11 individuals carrying haplotypes with non-expressed C4A null alleles using amplification of exons 29–31 and subsequent SSCP electrophoresis for the presence of this mutation. The results are summarized in Table II. Only one
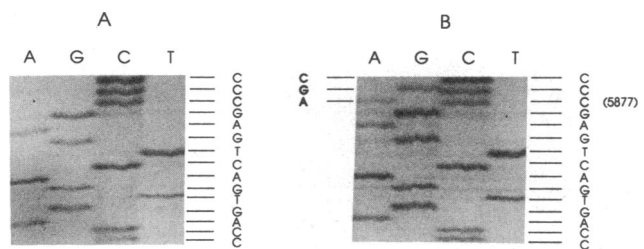
Figure 5. DNA sequence of C4 exon 29 (noncoding strand) obtained by PCR amplification with primers 13/13N and sequencing primer 13N. (*A*) Control sample C4A3B1; (*B*) C4AQ0,Q0 B1,1 with one defective C4A gene and one C4A gene deletion: the normal sequence is shown at the right side and the mutationally altered sequence with the 2-bp insertion is shown at the left side of *B* (5877 is the nucleotide position of the insertion).

haplotype was identified that did not carry the 2-bp insertion. 8 of the 10 C4A genes with the insertion were linked to HLA-B60, and, with a single exception, to HLA-DR6. The two other haplotypes had HLA-B8 and either DR3 or DR6. The adjacent C4B genes of these haplotypes expressed either the B1 or the B2 allotype. All C4B genes were short, i.e., without the 6.5-kb intron, as demonstrated by the detection of a 5.4-kb TaqI restriction fragment. The haplotype not carrying the mutation was HLA-B35, DR3.

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       (exon 29)
  5867 CTG TAC TGG GGC TCA GTC ACT GGT TCT CAG AGC ..  (normal C4
  1210  L   Y   W   G   S   V   T   G   S   Q   S   ..   sequence)


        2 bp insertion:  *  *
  5867 CTG TAC TGG GGC TCT CAG TCA CTG GTT CTC AGA GCA ATG CCG
  1210  L   Y   W   G   S   Q   S   L   V   L   R   A   M   P

       TGT CGC CCA CCC CGG CTC CTC GCA ACC CAT CCG ACC CCA TGC
        C   R   P   P   R   L   L   A   T   H   P   T   P   C

       CCC AGG CCC CAG CCC TGT GGA TTG AAA CCA CAG CCT ACG CCC
        P   R   P   Q   P   C   G   L   K   P   Q   P   T   P

       TGC TGC ACC TCC TGC TTC ACG AGG GCA AAG CAG AGA TGG CAG
        C   C   T   S   C   F   T   R   A   K   Q   R   W   Q

       ACC AGG CTG CGG CCT GGC TCA CCC GTC AGG GCA GCT TCC AAG
        T   R   L   R   P   G   S   P   V   R   A   A   S   K

       GGG GAT TCC GCA GTA CCC AA  GTAGGGGCCGTCCCCGGGCTCTGGGGG
        G   D   S   A   V   P   K  (intron 29 ................

  9071 GGGTGGGTAGTCCTCAGACCAAGGGCTTGCTTGAGTCCTGGCTCAACCTCCCTAG
       ....................................................... )


       (exon 30)
  9126 G ACA CGG TGA ...
         T   R   Z  (=stop)
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Figure 6. DNA and translated amino acid sequences of exon 29 around the insertion site and location of the stop codon in exon 30 (underlined; *Z*) in comparison to the respective sequence of exon 29 of a normal C4 gene; the changed amino acid sequence after the insertion site is printed in boldface. The GT/AG splice signal of intron 29 is also printed in boldface. The nucleotide and amino acid positions are indicated at the left, and the inserted nucleotides are marked by asterisks.

Table II. SSCP Analysis of C4A Gene Exon 29 of HLA Haplotypes with Silent C4A Null Genes

HLA−				Allotypes		Taq I fragment					No.
							CYP		CYP	Ex. 29	
A	C	B	DR	C4A	C4B	C4A	21A	C4B	21B	Ins.	
								(kb)			
2 (32)	w3	60	6	Q0	1	7.0	3.2	5.4	3.7	+	(3)
2 (24)	w3	60	6	Q0	2	7.0	3.2	5.4	3.7	+	(4)
2	w3	60	3	Q0	2	7.0	3.2	5.4	3.7	+	(1)
1	w7	8	6	Q0	2	7.0	3.2	5.4	3.7	+	(1)
2		8	3	Q0	1	7.0	3.2	5.4	3.7	+	(1)
19		35	3	Q0	2	7.0	3.2	5.4	3.7	−	(1)

Discussion

The strategy of exon-specific PCR amplification using oligonucleotide primers from flanking intron sequences, screening for possible mutation sites by SSCP analysis, and subsequent direct genomic sequencing of specific target fragments represents a rapid and powerful approach for the detection of sequence variation in well-defined genetic systems. Genomic cloning in lambda or cosmid vectors, as well as extensive DNA sequencing, is avoided, and a large number of samples can be studied simultaneously. However, the analysis of SSCP and heteroduplex DNA fragments may not detect all existing mutations. The resolution depends strongly on the sizes of the fragments to be analyzed, the composition of the gel system, and the electrophoretic conditions. According to methodological studies regarding SSCP and heteroduplex analysis, the possibility of detecting a point mutation decreases with increasing fragment size (21, 27). The insertion detected in the nonexpressed C4A gene represents a major conformational alteration of the respective fragment that facilitated its detection.

From previous studies it was known that only a proportion of C4A null alleles are due to gene deletions. The majority of these C4A gene deletions were found among the Caucasoid population within the extended haplotype HLA-A1-Cw7-B8-C2C-B fS-C4AQ0-B1-DR3 (10, 14). The molecular basis of C4A null alleles not due to gene deletions remained unclear. It could be established, however, that these genes are dysfunctional, since they carry C4A-specific DNA sequences without expressing any C4A gene product. Conversion of the C4A gene to C4B as described for the gene conversion from C4B to C4A in the HLA-B44-C4A3-BQ0 haplotype was not detected in a previous study (15). Only a single HLA haplotype has been described with a conversion from C4A to C4B in the HLA haplotype B13-C4AQ0-B6,1-DR7 (28). In this study, we identified one individual with conversion from C4A to C4B, possibly leading to homoduplication and -expression of the C4B2 allotype on the haplotype HLA-A3-Cw4-B35-C4AQ0-C4B2-DR4 (Fig. 2, Table II).

The observed 2-bp insertion in exon 29 of the defective C4A genes is in linkage with HLA-B60 for 8 of the 10 haplotypes studied. Of these, seven also have the HLA-DR6 allele. The two remaining haplotypes have the HLA-B8 allele, one with DR3 and one with DR6. All haplotypes have a short C4B gene as indicated by the presence of a 5.4-kb TaqI fragment, but show a variation regarding the C4B allotype, which may be

either B1 or B2. Thus it is very likely that all these haplotypes might have arisen from a single mutational event in an ancestral HLA-B60, DR6 haplotype. Since C4B1 and B2 are found in almost equal numbers in this study (four and six, respectively), it might be speculated that the C4B allotypic variation was introduced shortly after the mutational event, either by an independent mutation changing the amino acid sequence of the adjacent C4B gene or by a recombination between the defective C4A gene and the C4B gene. The sequence differences explaining the C4B1/B2 variation resulting in a net charge difference of the gene product with C4B1 being more basic than C4B2 have not yet been defined. The sequence differences described so far originate from the C4d region (exons 23–30), but do not account for the observed electrophoretic migration difference between the B1 and B2 variants (29, 30). Therefore, subtypes of B2 may exist that have arisen from independent mutational events resulting in a net charge of the protein characteristic for the B2 allotype.

Insertional mutagenesis as a cause of genetic defects has already been observed in a number of genes (e.g., in alpha-2-antiplasmin deficiency [31], beta-thalassemia [32], and cystic fibrosis [33]). A recent survey on this type of mutational event with insertions smaller than 10 bp by Cooper and Krawczak (34) showed that two mechanisms seem to be involved: (a) slipped mispairing mediated by direct repeats or runs of identical bases, and (b) misincorporation of bases due to the formation of secondary structure intermediates induced by palindromic sequences or symmetric elements. The insertion detected in the C4A pseudogenes belongs to the first category, since the original sequence of the insertion site CTC is changed to CTCTC by addition of a CT or a TC dinucleotide (Figs. 5 and 6). A similar insertion was observed in beta-thalassemia, where a TGTG sequence (at residue 148 − Leu) was changed to TGT-GTG by insertion of a TG dinucleotide (32). No functional gene product can be expected from the C4A mutationally altered sequence, since the reading frame shift causes a complete change of the amino acid sequence in the last third of the α-chain, followed by a termination codon at the beginning of exon 30 (Fig. 6). The resulting truncated C4 translation product is probably not stable and therefore degraded rapidly intracellularly. Thus the 2-bp insertion is sufficient to explain the nonexpression of these defective C4A genes. However, we cannot completely exclude the possibility that other mutations are also present in these genes that were not detected by SSCP analysis. Regarding the single haplotype with HLA-B35, which

does not have the described 2-bp insertion (Table II), investigations are currently being carried out to identify the unknown genetic defect also causing C4A nonexpression.

Similar observations regarding the heterogeneity of the genetic basis of deficiency as well as the finding of strong MHC haplotype linkage have been described recently for complement C2 deficiency (35). The authors identified a 28-bp deletion spanning the splice junction of exon/intron 6 of the C2 gene as the cause for deficiency. This mutation is linked to the haplotype HLA-A25-B18-C2Q0-B fS-C4A4-C4B2-DR2, whereas C2 deficiency outside this extended haplotype has a different and as yet unknown genetic basis. Our findings demonstrate that a majority of the nonexpressed C4A genes among the Caucasian population have arisen from a single insertional mutation linked to the HLA haplotype B60-DR6, which was then spread by recombination to haplotypes with different HLA-B and/or DR alleles. However, this mutation was not found in all haplotypes with a defective C4A gene studied, providing evidence for heterogeneity in the genetic basis of C4A deficiency.

Acknowledgments

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