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Multiple levels of analysis of an IGHG4 gene deletion

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Summary. Human immunoglobulin heavy chain constant region (IGHC) genes constitute a typical multigene family, usually comprising eleven genes on the telomere of chromosome 14 (14q32). In this region, deleted and duplicated haplotypes have been reported to exist with considerable frequency. Their origin is the result of either unequal crossing-over or looping out excision. In this paper, we report the characterization of a new type of deletion, involving the IGHG4 gene, in a subject who also carries a larger deletion of a previously described type on the second chromosome. Employment of several methods (polymerase chain reaction, standard Southern blot, pulsed field gel electrophoresis, serological techniques) to analyze these deleted haplotypes has resulted in a level of accuracy in their characterization that has not been achieved in previous cases. The site of recombination responsible for the IGHG4 deletion was restricted to a 2.5-kb region 3′ of the G4 gene; this rules out any possible involvement of the S regions in the recombination process. The usefulness of the various techniques in the characterization of the deletions is also discussed, together with possible future applications in the field.

Introduction

Multigene families evolve through duplication, deletion and mutations leading to functional diversification. This is also true for the human immunoglobulin constant region genes (IGHC locus) located on the long arm of chromosome 14 (q32.3) (Kirsch et al. 1982). Eleven IGHC genes have been mapped into three main clusters in the following order: M-D, G3-G1-EP1-A1, GP, G2-G4-E-A2 (Ravetch et al. 1981; Ellison and Hood 1982; Flanagan and Rabbitts 1982); the overall locus length is about 350 kb, with stretches of 60 kb and 80–100 kb separating D from G3 and A1 from G2, respectively (Bottaro et al. 1989c; Hofker et al. 1989).

The common descent of all IGHC genes through subsequent duplications is displayed by the various levels of homology within the locus, concerning nucleotide sequence, domain structure and overall cluster organization. Occasional unequal crossing-over events have led to aberrant duplicated and deleted haplotypes (Table 1). The letter may reach a significant frequency in some populations (Bottaro et al. 1989a).

Table 1. IGHC deletions and duplications

Genes involved	Type of rearrangement	Reference
G1-EP-A1-GP-G2-G4	Deletion	Lefranc et al. 1982 Lefranc et al. 1983
EP-A1-GP	Deletion	Lefranc et al. 1983
EP-A1-GP-G2-G4	Deletion	Migone et al. 1984 Chaabani et al. 1985
A1-GP-G2-G4-E	Deletion	Migone et al. 1984 Bottaro et al. 1989b
G2	Duplication	Bech-Hansen and Cox 1986
	Deletion	Bottaro et al. 1989b
GP-G2-G4-E-A2	Deletion	Bottaro et al. 1989b Hendriks et al. 1989
G1	Deletion	Smith et al. 1989
G1-EP-A1-GP-G2	Deletion	Smith et al. 1989

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Deletion characterization has been performed in previous cases by the standard Southern technique. However, this presents at least two limitations: first, it cannot resolve rearrangements, such as gene conversions or intragenic crossovers, involving restricted regions, since CH gene-specific DNA probes are not available, except for the G3 gene (Huck et al. 1986); secondly, it does not provide any long-range information about the deletion boundaries and the structure of the rearranged region.

Recently developed techniques, however, enable these problems to be overcome. The polymerase chain reaction (PCR) technique (Saiki et al. 1988) permits the selective amplification of DNA regions and their analysis to the single nucleotide level. On the other hand, pulsed field electrophoresis (PFGE; Schwartz and Cantor 1984) resolves DNA fragments up to several million basepairs, allowing the long-range analysis of DNA rearrangements.

We report here the characterization at three levels of resolution (i.e., PCR, standard Southern and PFGE), combined with serological tests, of a new type of IGHC gene deletion involving the G4 gene, discovered in a compound heterozygous subject. This multilevel approach allows the characterization of the deleted haplotype with a very high level of accuracy, and gives insights into the breakpoint location and the deletion mechanism.

Materials and methods

The proband (SPA), a healthy 55-year-old woman with isolated IgG4 deficiency was identified among 22000 blood donors serologically screened for the lack of IgG2 and/or IgG4 (Bottaro et al. 1989a). DNA samples from the proband, her husband (SPA-h), daughter (SPA-d) and niece (SPA-n) were prepared from peripheral blood leukocytes by standard phenol/chloroform extraction.

PCR analysis

Amplification involved the hinge exons of the five gamma genes, using primer oligonucleotides (24-mers) synthesized on the basis of the 3′ and 5′ flanking sequences, which are almost identical in all gamma genes. Nucleotide differences clustered in the hinge exons were exploited to construct oligonucleotide probes for the G1, G2 and G4 genes (20-mers). The rationale and oligonucleotide sequences for the PCR amplification are shown in Fig. 1.

DNA (1 μg) from SPA, from subjects carrying deletions of G2 and G4 (DEM and CRU) or of G2 alone (MOD and TIM) (Bottaro et al. 1989b) and from normal controls was subjected to 35 amplification cycles of denaturation (93°C, 1 min), annealing (50°C, 40 s) and synthesis (63°C, 40 s) using *Taq*I DNA polymerase (New England Biolabs), and slot-blotted onto nitrocellulose filters. Oligonucleotide probes terminally labelled with T4 polynucleotide kinase (New England Biolabs) were used for hybridization. Washing (2 × 2 min room temperature, 2 × 2 min 55°C) was performed in 6 × SSC (20 × SSC is 3 *M* NaCl, 0.3 *M* Na_3 citrate).

Autoradiograph exposure on Kodak XAR5 films was carried out for 4–12 h at room temperature.

Southern blot analysis

DNA (8 μg) from the proband, her relatives and normal controls were digested with the restriction endonucleases *Bam*HI, *Hin*dIII and *Pst*I (Boehringer-Mannheim) following the manufacturer's instructions. Electrophoresis (0.8% agarose gel, 2 V/cm, 20 h), transfer onto nitrocellulose filters and blot hybridization were performed by standard techniques. The following probes were used:

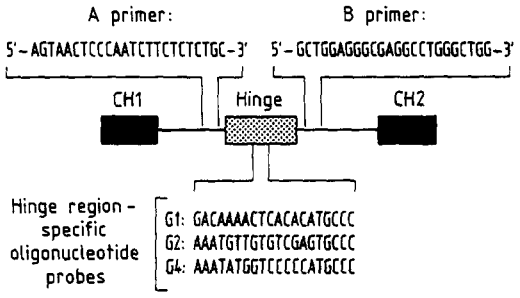

Fig. 1. Rationale of PCR amplification of the hinge exons *(dotted block)* of the IGHG genes. Oligonucleotide primers *A* and *B* were constructed on the basis of the 5′ and 3′ flanking sequences of the hinge exons; they are identical for all IGHG genes, except for a single-base substitution in the fourth position of the IGHG4 B sequence (this substitution does not significantly inhibit the amplification of the IGHG4 hinge exon, as shown in Fig. 2). The length of the amplification products is 91 bp (for the G2 and G4 hinge exons), 100 bp (G1) and 106 bp (GP and G3). The *G1, G2* and *G4 oligonucleotides* were constructed from the hinge exon sequences, which are different for each IGHG gene

G probe: a 8.0-kb *Hin*dIII fragment containing the entire IGHG1 gene and its 3′ flanking sequence, cloned into pBR322 (clone pHγ1, a kind gift from T. Honjo). It recognizes all five gamma genes (Takahashi et al. 1982).

PG probe: a 0.9-kb *Bam* HI fragment, from a region 2 kb 5′ of SG4, cloned into a pGEM plasmid from the recombinant phage Ch4A-Igγ4-2 (also a gift from T. Honjo). On *Hin*dIII-digested DNA, this probe recognizes multiple polymorphic fragments from the four functional gamma genes, plus a fifth series of fragments probably deriving from the D gene 5′ region (Bottaro et al. 1989d, and our unpublished observations).

A probe: a pBR322 cloned 1.95-kb *Pst*I fragment (pHα2-1.95, kind gift from N. Migone) containing the entire A2 gene from the recombinant phage Ch4A-H-Igα25 (Hisajima et al. 1983). It detects both A genes (Migone et al. 1984).

E probe: a 2.6-kb *Sac*I fragment containing the E gene and its 3′ flanking regions, electroeluted from the phage clone λTOU-εα (kindly provided by M. P. Lefranc; Lefranc and Rabbitts 1984). On *Bam*HI blots, it detects the two E genes in the IGHC region plus a third E-like pseudogene on chromosome 9 (Max et al. 1982).

AT probe: a 1.0-kb *Bam*HI/*Eco*RI fragment in pBR322 (clone pHα2-8, a gift from N. Migone), deriving from the recombinant phage Ch4A-H-Igα2-25 (Hisajima et al. 1983). It contains a region mapping 3.5 kb 3′ of the IGHA2 gene and detects homologous regions 3′ of both IGHA genes (Bottaro et al. 1989d).

PFGE analysis

DNA samples were prepared from peripheral blood leukocytes after red cell lysis (Baas et al. 1984), following the protocols of D. P. Barlow and H. Lehrach (Poustka et al. 1987). Digestion was performed with the *Mlu*I restriction endonuclease (Boehringer-Mannheim), and electrophoresis was run in a LKB Pulsaphor apparatus, using the hexagonal electrode array, for 40 h at 170 V, 45 min pulse time in 0.5 × TBE buffer (1 × TBE is 0.089 *M* TRIS, 0.089 *M* H_3BO_3, 2 m*M* EDTA, pH 8.3). Phage lambda multimers were used as molecular weight markers.

DNA was transfered onto Genescreen nylon filters (NEN) in denaturant solution, and prehybridized and hybridized according to Church and Gilbert (1984). The probe used was the G probe described above.

Table 2. Monoclonal antibodies used in capture ELISA tests

Clone	Specificity	Epitope location	Reference
MH16-1	IgG	CH2	Vlug et al. 1989
MH16-2	IgG	CH2	Vlug et al. 1989
MH165-1	IgG1, 2, 3	CH3	Vlug et al. 1989
2D4	IgG1, 2, 3	CH3	Vlug et al. 1989
HP6002	IgG2	CH2	Reimer et al. 1984 Jefferis et al. 1985
HP6014	IgG2	CH1	Reimer et al. 1984 Jefferis et al. 1985
HP6016	nG4m(b)	CH2	Reimer et al. 1984 Jefferis et al. 1985
MH164-1	IgG4	CH2	Jefferis et al. 1985 Vlug et al. 1989
MH164-4	IgG4	CH3	Vlug et al. 1989

Capture ELISA tests

Capture ELISA tests using a battery of monoclonal antibodies (MoAbs, shown in Table 2) were set up to assess the possible presence of hybrid IgG2/IgG4 molecules in the serum of proband SPA. They were performed by adapting published protocols (Vlug et al. 1989). Briefly, microtiter plates were coated with one MoAb, incubated with the proband's serum, washed and incubated with a different MoAb conjugated to horse radish peroxidase. Plates were then washed, revealed and read at 414 nm.

Results

PCR analysis

Autoradiograms of amplified DNA hybridized with the G1, G2 and G4 probes are shown in Fig. 2. Normal controls are positive for all oligonucleotide probes, whereas subjects lacking G2 and G4 or only G2 are negative for the corresponding signal. Thus, the oligonucleotide probes used are locus-specific. Proband SPA displays a hybridization pattern different from that of all other subjects, being positive for G2 and G1 and negative for G4. Therefore, the lack of IgG4 in her serum could be attributed to deletion of the G4 gene on both chromosomes.

Southern blot analysis

Southern blot analysis of the family group with the *Bam*HI restriction enzyme and the G probe (Fig. 3) confirmed that proband SPA lacked the bands 9.0–9.4 kb known to represent the G4 gene. The 9.7-kb GP band and the 22-kb G2 band had a half-dose intensity, indicating that only a single copy of these genes is present in proband SPA. In the daughter, the G4 band was also less intense, whereas the other bands were normal. A comparison of the restriction patterns indicated that she had inherited from her father the haplotype constituted by bands of 22, 11.5, 10.7, 9.7, and 9.0 kb (for the G2, G1, G3, GP and G4 genes, respectively). In the niece,

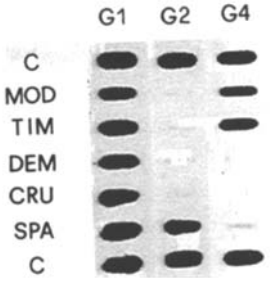

Fig. 2. Slot blot analysis of PCR-amplificated IGHG genes hinge exons from normal subjects (*C*), subjects with homozygous deletion of the G2 gene (*TIM* and *MOD*) or of the G2 and G4 genes (*DEM* and *CRU*), and proband *SPA*. The probes were the *G1, G2* and *G4* hinge exon-specific oligonucleotides described in Fig. 1. Note the different hybridization pattern of SPA in comparison with the other subjects. Film exposure time was three times longer for the *G4* probe than for *G1* and *G2*; this is probably because of the single-base mismatch of the 3′ primer oligonucleotide with the corresponding sequence of the G4 gene (see legend to Fig. 1). This mismatch probably lowers the efficiency of the amplification

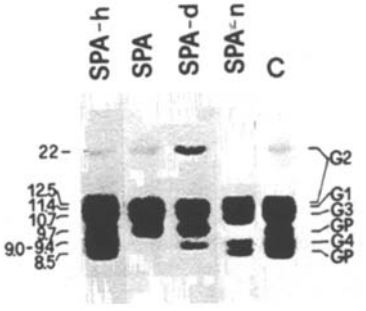

Fig. 3. Southern blot analysis of the IGHG genes (*Bam*HI enzyme, G probe). Note the lack of the *G4 bands* (9.0-kb, 9.2-kb and 9.4-kb allelic fragments, not resolvable in this gel) in proband *SPA*, and the half-intensity of the *G2* and *GP bands*. A half-intense *G4 band* is visible in *SPA-d*, whereas *SPA-n* shows lower intensity of *GP, G2* and *G4 bands*

the bands relative to G2 (12.5 kb), G4 (9.4 kb) and GP (8.5 kb) are all less intense than in controls. This pattern of segregation suggests that SPA carries a deletion encompassing the G4 gene on one chromosome and a larger deletion, including the GP, G2 and G4 genes, on the other.

Analysis with the PG4 probe (Fig. 4), which recognizes a region located 5′ of all G genes except GP, was in agreement with our previous results: SPA showed the absence of the bands relative to the PG4 region, and had a half-dose PG2 band. The PG1 and PG3 region bands, and the "X" region band, which probably maps 5′ of the D gene (A. Bottaro and A. O. Carbonara, unpublished observation) were normal. Restriction patterns in the daughter and niece confirmed the segregation of the two deletions.

The *Hin*dIII/G probe Southern pattern in proband SPA surprisingly revealed the absence of the 6.0-kb band known to represent the G2 gene, whereas the 6.3-kb G4 band was present, although reduced in intensity (Fig. 5). Comparison of the restriction maps of the G2 and G4 genes explains this finding. The two maps differ in the 5′ region in the position of the *Bam*HI site (nearer

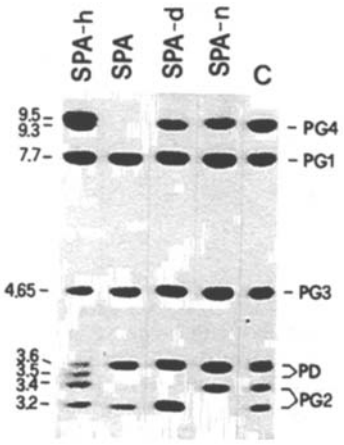

Fig. 4. *Hin*dIII enzyme, PG probe. Subject *SPA* lacks the *PG4 bands,* and has reduced intensity of the *PG2 band.* Half-doses are also visible for the *SPA-d PG4 band* and for the *SPA-n PG2* and *PG4 bands*

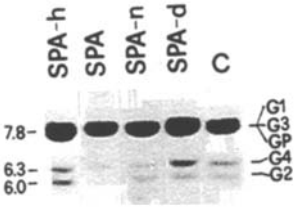

Fig. 5. *Hin*dIII enzyme, G probe. Note the lack of the *G2-specific band* and the presence of a half-intense *G4 band* in proband *SPA.* The apparent contrast between this restriction pattern and those shown in Figs. 3 and 4 is explained in the text

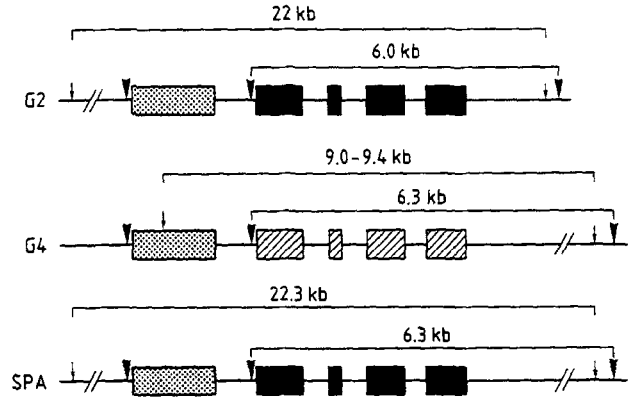

Fig. 6. Comparison of the *Bam*HI and *Hin*dIII restriction maps for the normal IGHG2 (*G2*) and IGHG4 (*G4*) genes and of the SPA IGHG2/IGHG4 hybrid fragment (*SPA*). *Black* and *striped boxes* represent the exons of the G2 and G4 genes, respectively; *dotted blocks* are the switch regions. The *zig-zag line* 3′ of *G4* and *SPA* represents the 300-bp insertion discussed in the text. Distances are not to scale; the length of relevant fragments in indicated. On standard Southern blots, it is not possible to distinguish between the 22-kb and 22.3-kb bands. *Arrows Hin*dIII, *arrowheads Bam*HI

in G4 than in G2) (Takahashi et al. 1982), and in the 3′ region because of a 300-bp insertion/duplication that has occurred within 2 kb 3′ of the G4 gene, as clearly shown by the *Mbo*I restriction maps (Johnson et al. 1986). There-

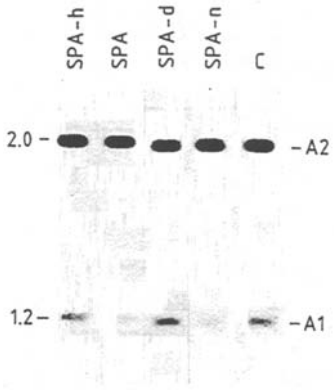

Fig. 7. *Pst*I enzyme, A probe. The *IGHA2 specific band* (2.0 kb), has a constant intensity in all lanes, whereas half-intense *IGHA1 bands* (1.2 kb) are present in proband *SPA* and her niece *(SPA-n)* compared with controls and other family members

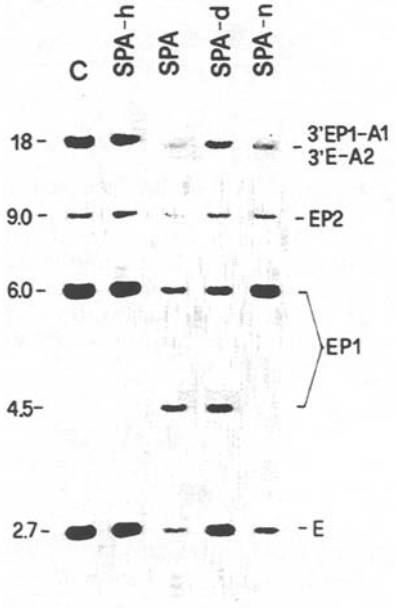

Fig. 8. *Bam*HI enzyme, E probe. The E probe detects the three E-like genes [EP1 and E are in the IGHC region, EP2 is a processed pseudogene on chromosome 9 (Battey et al. 1982)], and longer fragments (about 18 kb) comprising the EP1 and E gene 3′ flanking sequences plus the A1 and A2 genes, respectively. A RFLP for the EP1 gene (6.0 and 4.3 kb) is recognizable in *SPA* and *SPA-d.* Note the lower intensities of the *E gene band* in *SPA* and *SPA-n*

fore, a deletion of the G4 gene arising from unequal recombination downstream of the 5′ *Bam*HI restriction site and 5′ of the 300-bp insertion would lead to a hybrid G2/G4 band with the observed restriction pattern (Fig. 6).

To assess the boundaries of the larger deletion, we analyzed the restriction patterns of the A genes (*Pst*I enzyme, A probe, Fig. 7) and for the E genes (*Bam*HI, E probe, Fig. 8); in proband SPA and her niece, half-intense bands for the A1 and E genes were found, whereas her daughter was normal. Analysis of the restriction fragment length polymorphism (RFLP) alleles for a region 3′ of A2 showed that SPA was homozygous for the 3.5-kb allele (Fig. 9).

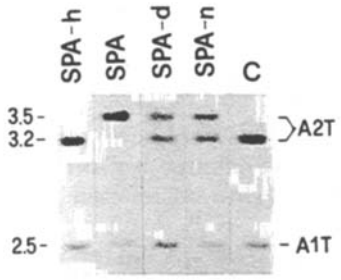

Fig. 9. *Bam*HI enzyme, AT probe. *SPA* is homozygous for the A2T 3.5-kb allele, *SPA-d* and *SPA-n* are heterozygous for the 3.2-kb and 3.5-kb alleles. The monomorphic 2.5-kb *A1T band* is fainter in *SPA* and *SPA-n*

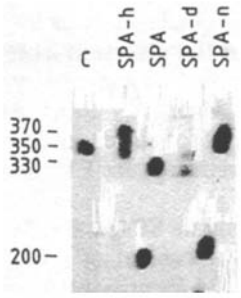

Fig. 10. Pulsed field gel analysis of the family of *SPA*. The *Mlu*I enzyme yields allelic 350-kb and 370-kb fragments containing the entire IGHC region. *SPA* shows two shorter fragments of 330 and 200 kb. The 330-kb fragment is present in *SPA-d* and the 200-kb fragment in *SPA-n*. This confirms that two deletions, one of 20 kb (including the G4 gene) and one of 150 kb (involving A1-GP-G2-G4-E), are present in the *SPA* family

Together, these results indicate that proband SPA is a compound heterozygous carrier for two different types of deletion. One encompasses only the G4 gene and the other involves the A1-GP-G2-G4-E group of genes, a type of deletion common in the Italian population (Migone et al. 1984; Bottaro et al. 1989a).

PFGE analysis

In genomic DNA digested with the *Mlu*I enzyme, the entire IGHC region can be detected as a single 350-kb hybridization band (Hofker et al. 1989). However, in the Italian population an allelic 370-kb band is also detectable at high frequency (A. Bottaro, U. Cariota, M. De Marchi, and A. O. Carbonara, submitted). In the family of SPA, the husband is a heterozygous carrier of these two alleles (Fig. 10), whereas the proband shows two unusual bands of about 330 and 200 kb, clearly corresponding to the G4 and the A1-GP-G2-G4-E deletions. The segregation of the deleted haplotypes in SPA-d and SPA-n is consistent with the data described above.

The extent of the deletions, i.e., 20 and 150 kb, are in agreement with published map data regarding the distances between the involved genes. This suggests that the process producing the deletions is simple and does not involve complex genetic rearrangements (insertions, inversions or others).

Serological

Apart from the total lack of IgG4 (< 1 μg/ml), other subclasses in the serum of SPA are present at normal levels (except for a slight decrease in IgG2) in the semiquantitative hemagglutination inhibition assay (HIA). The same hold true for her relatives; indeed, we do not see the dosage effect found in subjects with a heterozygous GP-G2-G4-E-A2 deletion, some of whom have a marked decrease in serum levels of IgG2 and/or IgA2 (Hendriks et al. 1989). However, this may partially be a result of the relatively low resolution of the HIA test that we have used.

Table 3. Detection of IgG2 and IgG4 constant regions by capture ELISA tests

	MoAb		Test specificity		Test sera[a]			
	Coat	Conjugate	Subclass	CH region	Normal	IgG2 deficient	IgG4 deficient	SPA
1.	MH16-2	MH16-1	IgG	CH2	>2.0	>2.0	>2.0	>2.0
2.	MH165-1	MH16-1	IgG	CH2-CH3	>2.0	>2.0	>2.0	>2.0
3.	HP6014	HP6016	IgG2	CH1-CH2	>2.0	–	>2.0	>2.0
4.	HP6014	2D4	IgG2	CH1-CH2-CH3	>2.0	–	>2.0	>2.0
5.	MH165-1	HP6014	IgG2	CH1-CH2-CH3	>2.0	–	1.62	0.88
6.	MH165-1	HP6016	IgG2	CH2-CH3	0.93	–	0.60	0.21
7.	HP6002	MH16-1	IgG2	CH2	0.50	–	0.40	0.40
8.	MH164-1	MH16-1	IgG4	CH2	1.26	1.29	–	–
9.	MH164-4	MH16-1	IgG4	CH2-CH3	1.19	1.10	–	–
10.	MH164-4	MH164-1	IgG4	CH2-CH3	1.73	1.26	–	–
11.	HP6014	MH164-4	IgG2/G4[b]	CH1-CH2/CH3	–	–	–	–
12.	MH164-4	HP6014	IgG2/G4[b]	CH1-CH2/CH3	–	–	–	–
13.	MH164-4	HP6016	IgG2/G4[b]	CH2/CH3	–	–	–	–
14.	MH165-1	MH164-1	IgG1, 2, 3/G4[b]	CH2/CH3	–	–	–	–

[a] Optical densities measured at 414 nm, – = < 0.10
[b] Hybrid molecules

Since the breakpoint of the G4 gene deletion was located between the G2 hinge exon and the insertion 2 kb 3′ of G4, a hybrid G2/G4 gene might have originated by recombination. This possibility was tested by using a panel of monoclonal antibodies specific for different domains of the IgG2 and IgG4 molecules in capture ELISA tests (Table 3).

No IgG4 determinants are present in SPA's serum (tests 8–10), whereas all determinants typical of IgG2 are present (tests 3–7). No serologically identifiable hybrid IgG2/IgG4 molecule is present in her serum (tests 11–14). The lower reactivity of the MoAbs directed against the IgG2 CH3 determinants in SPA's serum suggest a possible conformational modification in this region, but this may also be a result of the lower serum levels of IgG2 or partial degradation of the IgG2 molecules. In our opinion, the most likely conclusion is that the unequal recombination site in the G4 deletion is located outside the structural gene, in the region between the gene itself and the G4-specific 300-bp insertion mentioned above.

Discussion

Several types of IGHC gene deletions leading to selective Ig deficiencies, usually of multiple isotypes, have been characterized (Table 1 and discussion in Bottaro et al .1989a). Such deletions seem to be common in the Mediterranean region, but they have also recently been found in Northern European subjects (Hendriks et al. 1989; Smith et al. 1989). Although accounting for a very restricted portion of the deficiencies, they constitute the only cause of Ig isotype deficiency directly demonstrated so far.

The mechanism that produced these deletions is debated; both unequal crossing-over and looping-out models have been proposed (Lefranc et al. 1982, 1983; Migone et al. 1984; Carbonara et al. 1986). The former is suggested by the finding that all recombinations seem to have occurred between regions of high homology, the latter takes into consideration the well-known recombinogenic nature of the IGHC region, and may involve the switch recombination regions and/or the switch-specific recombinases in the deletion process. The only breakpoint region directly analyzed until now does not unequivocally suggest any particular mechanism (Keyeux et al. 1989). Indeed, it is represented by a highly homologous region shared by different IGHC genes, and behaves like a general recombination hot-spot when cloned into phage vectors; however, the region also clearly displays switch-like sequences.

The deletions described here present several interesting features. A single gene deletion including the G4 gene has not previously been reported. As suggested for the previous types, this deletion may have arisen by unequal recombination (Carbonara et al. 1986); it is the fifth type of deletion characterized in the Italian population. Thanks to some peculiarities in the restriction maps of the G2 and G4 genes, the breakpoint site could be approximately located within 2 kb 3′ from the structural gene. This rules out any possible involvement of the switch region in the origin of the deletions. Moreover, this region displays a high degree of sequence homology with the corresponding regions 3′ of the other IGHG genes (Johnson et al. 1986). It therefore seems likely that the mechanism producing the deletion is promoted by bare sequence homology with no correlation with switch-like recombination.

The identification of a restricted area as the recombination region for the G4 gene deletion is a prerequiste for the cloning and sequencing of the breakpoint, in the hope that the combination of data regarding several different deletion types will clarify the molecular mechanism and the structural constraints underlying the deletion process.

The larger deletion reported here (i.e, including the A1-GP-G2-G4-E genes) is the most common in the Italian population (Bottaro et al. 1989a). It has been suggested that the high frequency of deletions found in Mediterranean countries is the result of genetic drift and a high level of inbreeding (Lefranc et al. 1983). However, this deletion is different from those previously reported, since it is associated with the A2T 3.5 kb allele. This is the third haplotype found in association with this type of deletion, suggesting that a similar deletion process may have taken place several times independently. If this were the case, i.e., if the deletion process is relatively frequent, we would expect, in the absence of strong selection against the deletion carriers (homozygotes for deletions are usually healthy), that the frequency of the deletions should be similar in other populations.

Other relevant points arising from this report concern the technical approaches to the characterization of the deletions. Both PCR and PFGE have proved highly reliable for the identification of the deletions. In particular, PFGE blots allow the easy identification of heterozygous carriers; this is not always straightforward when performed on the basis of band intensity ratios. A PFGE screening program is presently under way in our lab and is yielding very encouraging results. On the other hand, the PCR technique will soon be fully automated, and will permit the search for homozygous deletions in very large samples of subjects. The simultaneous use of different primers and analysis with different specific oligonucleotides will probably result not only in the identification of other cases of deletion, but also in the detection and characterization of more complex and subtle types of rearrangement, such as gene conversions, which have been shown to be common in the IGHC region (Lefranc et al. 1986). In our opinion, application of either of these two methods to other population samples is essential for the clarification of the problems concerning the frequency and origin of the deletions discussed in this paper.

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