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Genetics

Multiple gene deletions within the human immunoglobulin heavy-chain cluster

(antibody isotype/unequal cross-over/immunodeficiency)

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ABSTRACT **Two subjects, of 11,000 healthy individuals screened, were found to be missing three and four immunoglobulin isotypes, respectively (IgA1, IgG2, and IgG4; IgA1, IgG2, IgG4, and IgE), and have been analyzed at the DNA level by means of Southern blotting and Ig heavy-chain-specific probes. A broad deletion within the heavy-chain constant region (C) gene cluster was found on chromosome 14 of both probands. Two different haplotypes are described: the first has lost the $C_{\alpha 1}$, $C_{\psi\gamma}$, $C_{\gamma 2}$, $C_{\gamma 4}$, and C_{ε} genes; the second lacks the $C_{\psi\varepsilon}$, $C_{\alpha 1}$, $C_{\psi\gamma}$, $C_{\gamma 2}$, and $C_{\gamma 4}$ genes. These findings confirm the reciprocal order of the Ig heavy-chain genes as derived by molecular cloning. The inclusion of the $C_{\psi\gamma}$ gene within the deleted regions confirms its location between $C_{\alpha 1}$ and $C_{\gamma 2}$. From the observed frequency of the homozygous genotype, 1%–3% of healthy subjects from our population are expected to be heterozygous for multiple heavy-chain gene deletions. Cross-over between mispaired homologous regions seems to be the favored mechanism of multiple Ig gene deletions and duplications, and, generally, in the evolution of the human Ig heavy-chain gene family.**

Nine immunoglobulin heavy-chain isotypes ($C_{\alpha 1}$, $C_{\alpha 2}$, C_{δ}, C_{ε}, $C_{\gamma 1}$, $C_{\gamma 2}$, $C_{\gamma 3}$, $C_{\gamma 4}$, and C_{μ}) are normally detectable in human serum on the basis of different antigenic and structural features of the constant domains (C). Specific effector functions (e.g., complement fixation, binding to mast cells, placental transfer, etc.) are attributes of distinct isotypes. Nevertheless, the sequence homology among the nine chains clearly implies a common evolutionary origin from a single ancestral domain, by means of subsequent gene duplication (1, 2). All the genes responsible for the synthesis of the nine heavy chains, plus two nonfunctional genes (the $C_{\psi\gamma}$ and $C_{\psi\varepsilon 1}$ pseudogenes), have been cloned (3–8) and mapped within the so-called immunoglobulin heavy-chain gene cluster (IgCH) on the long arm of chromosome 14, at 14q32.3 (9–11).

Selective deficiencies of Ig subclasses have occasionally been found, even in apparently healthy individuals (12–14). Two questions are raised by these findings: the first deals with the molecular basis of the lack of Ig chains, and the second is related to how and to what extent the lost effector functions are compensated. Of particular interest in this respect are those exceptional cases showing multiple heavy-chain deficiencies. In this paper, we describe two such subjects, who carry a combination of Ig deficits not previously reported: one lacks IgA1, IgG2, IgG4, and IgE; the other lacks IgA1, IgG2, and IgG4. The present work is focused on the molecular basis of the defect. Using restriction endonucleases and Ig gene-specific DNA probes, we show that a large DNA deletion accounts for the observed absence of heavy chains. Unequal cross-overs are suggested as the probable mechanism of origin of the deletion; their role in the evolution of the human IgCH cluster is discussed. The immunological and clinical aspects will be presented in detail elsewhere.

MATERIALS AND METHODS

The two unrelated probands were identified while screening for the absence of serum IgA1 and/or IgA2, which was carried out by means of an immunoenzymatic assay with specific rabbit antisera. A total of 11,000 individuals from different Italian regions were examined. Proband Saf is a 51-yr-old woman from Calabria, in the south of Italy; proband Fro is an 80-yr-old woman from a Sardinian village. Both subjects were apparently healthy. Family analysis was extended to the available first-degree relatives (Fig. 1). In neither family was consanguineity found.

Immunogenetic Analysis. All deficient sera were tested for the *G1m*, *G2m*, *G3m*, and *A2m* allotypic markers by the classical hemagglutination inhibition test (15). Quantification of IgA1 and IgA2 was carried out by radioimmunoassay with specific anti-subclass antisera. IgG subclasses were measured by hemagglutination inhibition at scalar dilutions of the serum. IgE level was determined by radiobinding inhibition with commercial kits (PRISTA, Pharmacia).

DNA Analysis. High-molecular-weight DNA was extracted from the buffy-coat obtained from 30 ml of blood as described (16). Eight micrograms of DNA from each sample was digested overnight with restriction endonucleases (*Sac* I, *Hin*dIII, *Bam*HI, *Pst* I; Boehringer Mannheim), size fractionated in 0.8% agarose gel electrophoresis, and transferred to nitrocellulose filters according to Southern (17). Filters were hybridized with nick-translated [^{32}P]DNA probes and washed in stringent conditions as described (18).

The DNA probes used were the following: (*i*) the switch probe, which consists of the entire Charon 4A-H24 recombinant phage, carrying the C_{μ} switch region and the C_{μ} gene within a 12-kilobase (kb) human DNA insert (19). It has been shown that multiple DNA fragments obtained after *Sac* I digestion (or its isoschizomer *Sst* I) hybridize with this probe because of sequence homology with the μ switch region (18). Some of these restriction fragments show length polymorphism (RFLP) and map to specific regions within the IgCH cluster; a few others probably map outside the IgCH region

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Abbreviations: IgCH, immunoglobulin heavy-chain cluster; RFLP, restriction fragment length polymorphism; kb, kilobase(s); C, constant region of heavy chain.

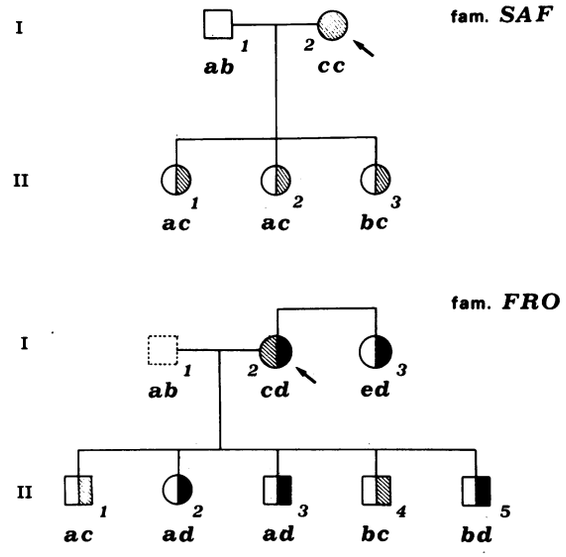

FIG. 1. Pedigrees of the families Saf and Fro. The probands are indicated by arrows. The segregation of the IgCH haplotypes is shown in italics below each symbol. □, Normal IgCH haplotype; ▨, haplotype carrying deletion of $C_{\alpha 1}$, $C_{\psi\gamma}$, $C_{\gamma 2}$, $C_{\gamma 4}$, and C_ε genes; ■, haplotype with deletion of $C_{\psi\varepsilon 1}$, $C_{\alpha 1}$, $C_{\psi\gamma}$, $C_{\gamma 2}$, and $C_{\gamma 4}$ genes. In family Fro, the individual I-1 was not examined.

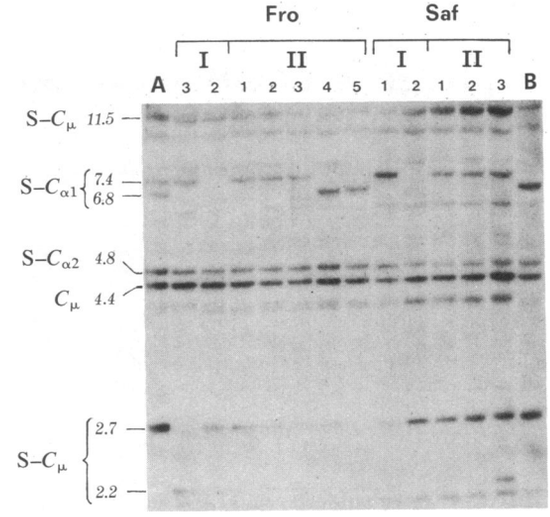

FIG. 2. Southern blotting hybridization of the switch probe and the C_μ gene to families Fro and Saf DNAs, digested with *Sac* I restriction enzyme. Lanes A and B are normal control DNAs. Family samples are coded as follows: Roman and Arabic numbers refer to the generation and individual, respectively, as shown in Fig. 1. On the left, the length in kb of the most relevant Ig switch-containing fragments are indicated. The DNA segment carrying the C_μ gene is also reported.

(18). (*ii*) The α-chain probe is represented by a 1.95-kb *Pst* I fragment, isolated through electroelution from the recombinant phage Charon 4A-HIg.α25 (7). It contains almost the entire $C_{\alpha 2}$ gene plus a short 5′-flanking sequence. On *Pst* I-digested human DNA, this probe shows two hybridizing fragments of 2.0 and 1.2 kb, which correspond to the $C_{\alpha 2}$ and $C_{\alpha 1}$ genes, respectively (8). (*iii*) The γ-chain probe consists of an 8.0-kb *Hin*dIII segment containing the $C_{\gamma 1}$ gene, cloned into pBR322 (a kind gift from T. Honjo). This probe hybridizes with all five C_γ genes—i.e., the four active ones and a pseudogene (6, 8). (*iv*) The ε-chain probe is represented by a 1.2-kb *Bam*HI/*Pst* I fragment containing the functional C_ε gene, cloned in M13 (ε1.2BP25; a gift from T. Rabbitts). This probe can also detect two pseudogenes, one of which ($C_{\psi\varepsilon 1}$) maps within the IgCH region (3). Recombinant DNA was handled in accordance with the recommended National Institutes of Health guidelines.

RESULTS

The first approach to direct genomic analysis was carried out by means of the switch probe. The hybridization of the switch probe to *Sac* I-digested DNAs from both Fro and Saf families is shown in Fig. 2. It appears that the fragments containing the C_μ gene, the C_μ switch region, and the $C_{\alpha 2}$ switch region are normally represented, with regard to both size and intensity, in the probands as well as in their relatives. Nevertheless, a striking defect is evident in the former: none of the allelic fragments known to be associated to the $C_{\alpha 1}$ switch region appears, and no new variant is detected, even after prolonged film exposure. In the siblings of Fro and Saf, a $C_{\alpha 1}$ switch-containing fragment of 7.4 or 6.8 kb is present, but it gives a half-intensity hybridization signal compared with normal homozygotes. These data suggest the existence of a DNA deletion involving the $C_{\alpha 1}$ switch region on both chromosomes of the probands. The DNA polymorphisms at the C_μ and $C_{\alpha 1}$ switch loci allowed us to establish the segregation of the IgCH haplotypes in both families (Fig. 1; Table 1). The results are in agreement with the *Gm* typing, with the advantage of being more informative.

To determine whether the $C_{\alpha 1}$ gene was included in the deletion, the α-chain probe was hybridized to the DNA samples of the two families after digestion with the restriction enzyme *Pst* I (Fig. 3). Normally, as in the control shown in the figure, two bands of different intensity appear. The first one, of 2.0 kb, is the direct germ-line counterpart of the sequence used as probe and corresponds to the $C_{\alpha 2}$ gene. The weaker band, of 1.2 kb, identifies the homologous $C_{\alpha 1}$ gene. In both probands, the latter fragment was absent, while the $C_{\alpha 2}$-containing band appeared to be of identical size and intensity as in controls. Thus, a DNA deletion including at least the $C_{\alpha 1}$ gene and the associated switch region should be present on both chromosomes of the probands. All the siblings indeed showed a decreased intensity of the 1.2-kb band, as expected in hemizygous genotypes.

DNA integrity at the C_γ gene regions was then controlled by the hybridization of *Hin*dIII- and *Bam*HI-digested DNAs to the γ probe (Fig. 4). This probe can hybridize with all five C_γ genes, due to conserved sequence homology. A typical *Hin*dIII pattern, shown by the sample Saf I-1 in Fig. 4*a*, consists of two faint bands and one strong band. On the basis of the restriction maps of the recombinant C_γ gene clones de-

Table 1. Serologic (Gm, Am) and DNA (S–C_μ, S–$C_{\alpha 1}$, S–$C_{\alpha 2}$) markers of haplotypes segregating in families Saf and Fro

	Chromosome	G1, 2, 3m	A2m	S–C_μ	S–$C_{\alpha 1}$	S–$C_{\alpha 2}$
		Family Saf				
I-1	a	*f*; *n*; *b*	1	2.7	7.4	4.8
	b	*f*; *n*; *b*	1	2.2	7.4	4.8
I-2	c	*f*; ..; *b*	1	2.7	—	4.8
	d	*f*; ..; *b*	1	2.7	—	4.8
		Family Fro				
I-1	a	*f*; *n*; *b*	1	2.7	7.4	4.8
	b	*za*; ..; *g*	1	2.2	6.8	4.8
I-2	c	*f*; ..; *b*	1	2.7	—	4.8
	d	*za*; ..; *g*	1	2.2	—	4.8

The four parents are coded according to Fig. 2. The Gm and Am markers detect variants on $C_{\gamma 1}$, $C_{\gamma 2}$, $C_{\gamma 3}$, and $C_{\alpha 2}$ heavy chains. The DNA switch polymorphic loci reported are associated with the C_μ, $C_{\alpha 1}$, and $C_{\alpha 2}$ switch (S) regions, respectively (18). The symbol *b* stands for the antigen complex b^0, b^1, b^3, b^5.

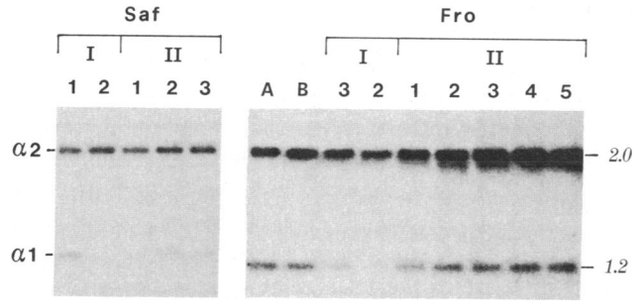

FIG. 3. Southern hybridization of the α-chain probe to DNAs from families Saf and Fro, digested with *Pst* I. Family samples are coded as reported in Fig. 2. Lanes A and B are normal control DNAs. The length in kb of the $C_{\alpha 1}$- and $C_{\alpha 2}$-containing segments (α1 and α2) is shown.

rived from two different human DNA libraries (5, 6, 8), the $C_{\gamma 2}$ gene is consistently carried on the shortest *Hin*dIII fragment, of ≈6.0 kb; the $C_{\gamma 4}$ then follows within a 6.3-kb fragment; the $C_{\psi\gamma}$, $C_{\gamma 1}$, and $C_{\gamma 3}$ genes migrate with longer fragments of similar size—i.e., 7.0–8.0 kb. As shown in Fig. 4*a*, the two probands have lost the two lighter bands of 6.0 and 6.3 kb, while they maintain a 7.9-kb broad band. The lack of γ2 and γ4 heavy chains in their sera, together with normal levels of γ1 and γ3, provides confirmatory evidence that the lighter bands do contain the $C_{\gamma 2}$ and $C_{\gamma 4}$ genes and that the latter are absent in the probands.

Are the remaining $C_{\gamma 1}$ and $C_{\gamma 3}$ genes present on both chromosomes? Is $C_{\psi\gamma}$ included in the deleted region? To answer these questions, the same γ-chain probe was hybridized to

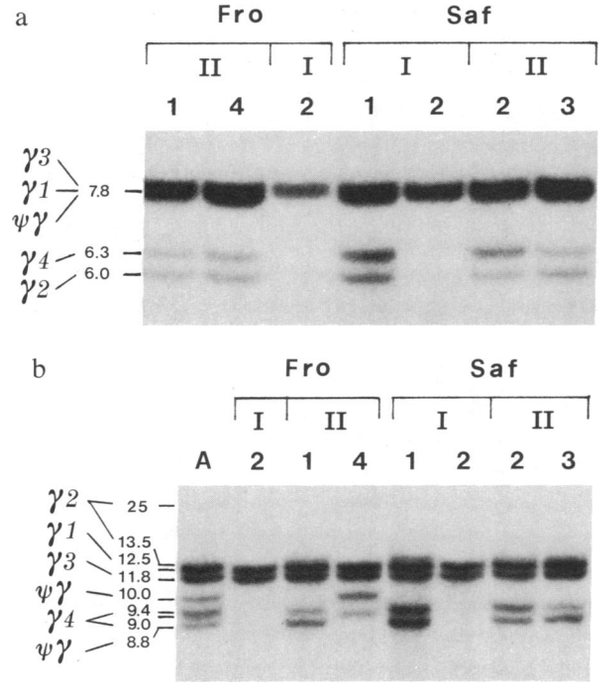

FIG. 4. Southern hybridization of the γ-chain probe to DNAs from families Fro and Saf, digested with *Hin*dIII (*a*) and *Bam*HI (*b*). The length in kb of the C_γ gene-containing fragments is indicated. The samples are coded as reported in Fig. 2. (*a*) In the third lane (Fro, I-2), less DNA was loaded onto the slot of the gel; after correction for the concentration in another experiment, the intensity appeared similar to that shown by sample Saf I-2. Sample Saf I-1 can be considered a normal control. (*b*) An unrelated control DNA (lane A) has been added for comparison. The faint 25-kb band is present only in lane A and in Fro II-4; the two bands appeared more evident after a longer exposure of the film.

*Bam*HI-digested DNAs of the two probands' relatives (Fig. 4*b*). Recent data from Linsley *et al.* (20) have shown that a γ-chain probe can reveal RFLPs after *Bam*HI digestion. In a single sample, five to a maximum of eight bands are observed. Two such typical cases are shown in Fig. 4*b* (lanes 1 and 5). It has recently been shown that the complexity of these patterns is generated by three polymorphic and by two nonpolymorphic loci (20). More recently, on the basis of segregation analysis and linkage disequilibrium data, Bech-Hansen *et al.* (21) have assigned each *Bam*HI fragment to a given C_γ locus, as follows: 10.0 and 8.8 kb, to $C_{\psi\gamma}$; 9.4 and 9.0 kb, to $C_{\gamma 4}$; 25 and 13.5 kb, to $C_{\gamma 2}$; the two invariant bands of 12.5 and 11.8 kb, to $C_{\gamma 1}$ and $C_{\gamma 3}$, respectively.

As shown in Fig. 4*b*, only two *Bam*HI bands are present in the two probands; the fragment sizes correspond to the invariant segments, and the intensity of hybridization is analogous to the control DNAs. These data strongly indicate that (*i*) the invariant segments do contain the $C_{\gamma 1}$ and $C_{\gamma 3}$ genes; (*ii*) these two genes are present in normal doses in the probands; and (*iii*) the $C_{\gamma 2}$-, $C_{\gamma 4}$-, and $C_{\psi\gamma}$-related segments are deleted on both chromosomes of these two subjects. The analysis of the polymorphic bands in siblings who have received different paternal IgCH haplotypes makes it possible to define the chromosomal association of the *Bam*HI variants. The three $Gm^{f;n;b}$ haplotypes are linked to *Bam*HI fragments of 13.5, 9.4, and 8.8 kb, while the $Gm^{za;\cdots;g}$ haplotype is linked to the 25-, 10.0-, and 9.0-kb fragments. Finally, the difference in intensity among these single-dose bands suggests that the $C_{\psi\gamma}$-related regions have stronger homology to $C_{\gamma 1}$ and $C_{\gamma 3}$ gene segments than to $C_{\gamma 2}$ and $C_{\gamma 4}$, in agreement with the analysis at the nucleotide level (6).

To define more precisely the limits of the deleted area, the ε-chain probe was used on *Bam*HI-digested DNAs (Fig. 5). This enzyme allows the recognition of the functional C_ε (2.7 kb) and the two $C_{\psi\varepsilon}$ genes—i.e., $C_{\psi\varepsilon 1}$ (6.0 kb) and $C_{\psi\varepsilon 2}$ (9.0 kb). The $C_{\psi\varepsilon 1}$ gene is a partially deleted gene located between the $C_{\gamma 1}$ and $C_{\alpha 1}$ genes (4, 7, 8), while the $C_{\psi\varepsilon 2}$ is a processed gene mapped on chromosome 9 (22). The two probands behaved differently after probing with the C_ε gene. Proband Saf showed only two bands, with the 2.7-kb fragment lacking (Fig. 5*a*, lane 1); therefore, the deletion should have included the functional C_ε gene in both chromosomes.

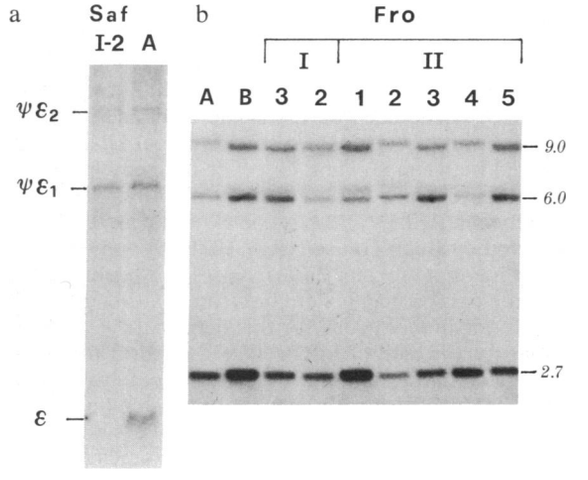

FIG. 5. Southern blotting hybridization of the ε-chain probe to DNAs from Saf I-2 and Fro family members, digested with *Bam*HI. Lanes A and B are normal control DNAs. (*a*) Only the proband of the Saf family is shown; all three siblings exhibited a half-intensity 2.7-kb band (not shown). (*b*) Two normal controls differ in the amount of DNA loaded onto the gel. Even among the family members, the quantity of DNA loaded is not identical. The intensity of the 9.0-kb band can be used either as an internal reference or for choosing the most appropriate control for comparison.

This is in agreement with the absence of IgE in her serum. On the contrary, proband Fro, who indeed possesses serum IgE, apparently exhibited all three bands (Fig. 5*b*, lane 4). Nevertheless, on closer inspection, the intensity of the 2.7- and 6.0-kb bands, compared with the 9.0-kb band as an internal reference, appeared lighter than in controls. This finding is consistent with a deletion of one copy of $C_{\psi\varepsilon 1}$ and C_ε genes. To test whether the suspected deletions were in *cis* or in *trans*, we analyzed the segregation of *Bam*HI RFLPs in the siblings whose IgCH haplotypes had been previously established (see Table 1). None of the siblings was similar either to their mother (the proband) or to normal controls; the anomaly was restricted again to the incriminated fragments of 2.7 and 6.0 kb, although in a single individual only one band at a time, either the 2.7- or the 6.0-kb band, according to the maternal haplotype, possessed a half-intensity signal. These data strongly suggest that the deletions of the $C_{\psi\varepsilon 1}$ and C_ε genes are on different chromosomes.

In conclusion, the above data indicate that five Ig heavy-chain genes are absent in both chromosomes of the two probands. Saf has apparently lost $C_{\alpha 1}$, $C_{\psi\gamma}$, $C_{\gamma 2}$, $C_{\gamma 4}$, and C_ε genes in both IgCH haplotypes, while Fro carries two distinct deletions: in one chromosome the same genes lacking in Saf are absent; in the other, the deletion includes $C_{\psi\varepsilon 1}$, $C_{\alpha 1}$, $C_{\psi\gamma}$, $C_{\gamma 2}$, and $C_{\gamma 4}$.

DISCUSSION

Complete serum deficiency of IgA1, IgG2, and IgG4 in one subject, and IgA1, IgG2, IgG4, and IgE in another one, are here shown to derive from large DNA deletions spanning the respective IgCH genes on both chromosomes 14. Analysis of the probands' families allowed us to identify two types of deleted haplotypes: in one, the $C_{\alpha 1}$, $C_{\psi\gamma}$, $C_{\gamma 2}$, $C_{\gamma 4}$, and C_ε genes are absent; in the other, the $C_{\psi\varepsilon 1}$, $C_{\alpha 1}$, $C_{\psi\gamma}$, $C_{\gamma 2}$, and $C_{\gamma 4}$ genes are lacking.

Selective absence of single IgG subclasses have been rarely identified by immunological testing for allo- and isotype determinants (12–14). Until now, the only cases of multiple IgCH gene deletions were found in apparently healthy individuals from highly inbred communities of Tunisian Berbers (23–25). The two types of Ig gene deletion observed were different from those reported here.

The relevance of our findings concerns three main points. First, the IgCH gene order, as appears in the deleted haplotypes, confirms the one obtained by DNA cloning (3–8) and by linkage analysis with both DNA and allotypic markers (18, 21). Of particular interest is the evidence that the $C_{\psi\gamma}$ gene, as yet not precisely localized by direct DNA mapping, is situated between $C_{\alpha 1}$ and $C_{\gamma 2}$. The assignment of the $C_{\psi\gamma}$ gene to this region is in agreement with the linkage disequilibria between RFLPs and C_γ gene allotypic markers, recently reported by Bech-Hansen *et al.* (21). Second, the C_ε gene deletion has not, to our knowledge, been reported before. One of the two probands here described, although apparently healthy, is homozygous for this defect; the way she compensates for the lack of IgE-mediated effector functions deserves special investigation. Finally, the pattern of gene deletions observed fits nicely with that expected on the basis of cross-over between mispaired homologous regions.

Altogether, of the four deleted chromosomes found in our two probands, three look alike; they share identical G1m, G3m, and A2m allotypes, and the same *Sac* I restriction fragments at the polymorphic Ig switch regions (Table 1). Whether they originated by independent events could only be assessed by gene cloning and DNA sequencing at the chromosomal breakpoints. The fourth deleted chromosome is different from the above in at least three polymorphic loci. Our two probands were found among 11,000 randomly selected healthy subjects, mainly blood donors, of Italian origin, screened for IgA1 and/or IgA2 deficiency. In the family of proband Saf, who was apparently homozygous for the same deletion, a common ancestor cannot be excluded, although consanguineity was not proven. Thus, at least three, if not all four, of the haplotypes should represent a random sample from the population pool. Within this limitation, and assuming Hardy–Weinberg equilibrium, it can, therefore, be estimated that in the Caucasian population, subjects heterozygous for IgCH deletions including the $C_{\alpha 1}$ gene should be on the order of 1%–3%.

The structure of the IgCH gene cluster may give some clues to the mechanism of origin of such deletions. In man, unlike the mouse (26), the C_ε, C_α, and C_γ genes are organized in two highly homologous sets, separated by an unknown length of DNA (8). It is likely that they were derived from a fairly recent duplication of a large DNA region, which contained a C_ε–C_α ancestral unit linked to one or two C_γ genes (8). Indeed, restriction enzyme mapping has revealed surprising homologies between the two duplicated sets even in noncoding regions (3–8). It is, therefore, likely that unequal pairing between intergenic segments, followed by cross-over, might give rise to multiple gene deletions as the ones here described (Fig. 6). Of the two other reported haplotypes with multiple IgCH deletions (24, 25), one is consistent with an unequal cross-over downstream from the $C_{\gamma 3}$ and $C_{\gamma 4}$ genes; on the basis of the location of the $C_{\psi\gamma}$ gene discussed above, the second can be explained as the product of a recombination downstream from $C_{\gamma 1}$ and $C_{\psi\gamma}$. It must be noted that all of these cases, although different from one another, involve mispairing of highly homologous regions ($C_{\gamma 1}/C_{\gamma 4}$; $C_{\psi\varepsilon 1}/C_\varepsilon$; $C_{\alpha 1}/C_{\alpha 2}$; $C_{\gamma 3}/C_{\gamma 4}$; $C_{\gamma 1}/C_{\psi\gamma}$). Repetitive sequences within the switch regions, which are known sites of rearrangements both *in vivo* and *in vitro* (27), might have a major role both in mispairing and in recombination.

The reciprocal products of unequal cross-over—i.e., gene duplications—should be expected at a similar or even higher frequency. Indeed, unusual haplotypes suggestive of $C_{\gamma 1}$ or $C_{\gamma 3}$ gene duplication have been occasionally observed (14, 28). The lack of specific reagents for polymorphic markers on $C_{\alpha 1}$ and $C_{\gamma 4}$ gene products and the presence of a single allotype on $C_{\gamma 2}$ and C_ε have been the major obstacle to the identification of multiple gene duplications. These shortcomings of classical immunogenetics can be overcome by direct analysis at the DNA level. By means of restriction endonucleases and a C_μ switch probe, multiple RFLPs have been described and mapped within the IgCH cluster (18); 7 of 92 (7.6%) randomly selected Caucasians appeared heterozygous for DNA duplications, which in all cases included the $C_{\alpha 1}$ gene region. This frequency fits nicely with the hypothesis of a single mechanism at the origin of both deletions and duplications.

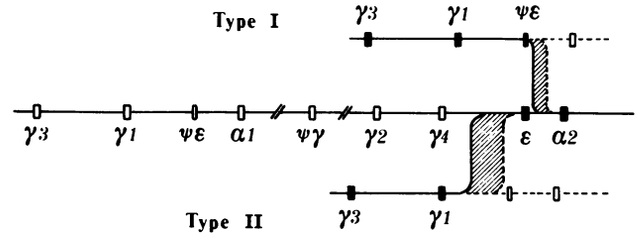

FIG. 6. Unequal homologous cross-overs that could account for the deletions of *C* region genes observed in Saf (Type I) and Fro (Types I and II). The two shaded areas represent mispaired regions were cross-over should have occurred [i.e., between the two units $C_{\psi\varepsilon}$–$C_{\alpha 1}$ and C_ε–$C_{\alpha 2}$ (Type I); and between $C_{\gamma 1}$–$C_{\psi\varepsilon}$ and $C_{\gamma 4}$–C_ε (Type II)]. The IgCH genes remaining on the recombinant deleted haplotypes are indicated by solid boxes. The C_γ, C_ε, and C_α genes are depicted according to the reported order and reciprocal distance (8), except for $C_{\psi\gamma}$, whose distance from $C_{\alpha 1}$ and $C_{\gamma 2}$ is not known.

Polymorphic variants due to difference in gene copy number are even more common within the family of the Igλ light-chain genes, in both man and mouse (29, 30). Again, the strong homology among the family members in both coding and noncoding flanking regions is apparently responsible for gene amplification and deletion. The relative frequency of deleted and duplicated Ig haplotypes is also influenced by their fitness, which in the evolutionary process will stabilize the number of isotypes typical of each species.

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