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A Multigene Deletion within the Immunoglobulin Heavy-Chain Region

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SUMMARY

The immunoglobulin heavy-chain genes are located in a cluster on chromosome 14. The simultaneous absence of the human IgG1, IgG2, IgG4, and IgA1 subclasses was previously reported in a healthy Tunisian Berber and was later shown to be due to a multigene deletion. We now describe a serological and molecular study of a different deletion observed in a healthy Tunisian.

Blot hybridization analysis of the proband's DNA using gamma, epsilon, alpha, and mu switch probes showed that the deletion involves a large region of the immunoglobulin heavy-chain gene cluster: Cψε, Cα1, Cψγ, Cγ2, and Cγ4. Incidentally, we showed that the restriction enzyme *Eco*RI alone can be used with the alpha probe to differentiate A2m types. The deletion described, present in a person homozygous for GM-Am haplotypes ($Gm^{1,17;..;5,14,11,13,10}$ $A2m^{2}$), is consistent with previous location, by association analysis, of Cψγ between Cα1 and Cγ2. There is evidence to suggest that deletions may be more common in the Mediterranean region than in North American Caucasians.

INTRODUCTION

Humans express five types or classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM. Four IgG subclasses (IgG1, IgG2, IgG3, IgG4) and two IgA subclasses (IgA1, IgA2) are known. These classes and subclasses of immunoglobulins

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are defined by the biological activities, chemical properties, and isotypic antigenic determinants of their heavy chains: α1, α2, δ, ε, γ1, γ2, γ3, γ4, and μ. The inheritance of human immunoglobulin (Ig) genes has been studied using serologically detectable genetic markers, called allotypes. These allotypic markers are located on the constant region of Ig heavy and light chains and are called Gm, Am, Em, and Km for markers of γ, α, and ε heavy chains and κ light chains, respectively [1, 2]. Recent molecular studies have revealed that human γ, ε, and α heavy-chain constant region (C_H) genes are organized in two blocks of genes: Cγ3-Cγ1-Cψε1-Cα1 and Cγ2-Cγ4-Cε-Cα2 [3]. Two other known human C_H pseudogenes, in addition to Cψε1, are Cψε2 and Cψγ. The pseudo γ gene (Cψγ) is located in the C_H cluster between Cα1 and Cγ2 [4]. A second pseudo ε gene, Cψε2, is located on chromosome 9 [5]. The first extensive deletion within the immunoglobulin heavy-chain locus was suggested by serological studies that revealed a simultaneous absence of IgG1, IgG2, IgG4, and IgA1 in a healthy Tunisian Berber [6, 7], confirmed using specific DNA probes [8]. Here, we report a different multigene deletion in a healthy Tunisian. DNA probes were used to define the extent of the deletion.

MATERIALS AND METHODS

The proband (T17) is a healthy 35-year-old male ascertained during a study of DNA polymorphisms of 32 random Tunisians.

High molecular weight DNA was extracted from leukocytes of blood samples (10–30 ml) collected in EDTA (D. Hoar, personal communication, 1983). Two or 3 μg of DNA from each sample was digested for 18–24 hrs at 37°C with the appropriate restriction enzyme (*Bam*HI, *Hin*dIII, *Sst*I, *Eco*RI, or *Pst*I). Resulting restriction fragments were separated on 0.5%–0.7% agarose gels by electrophoreses at 0.5–2 V/cm for 30–72 hrs and were transferred to Biodyne® (Pall) [9]. Filters were hybridized to nick-translated ^{32}P-labeled DNA probes, then washed in 2 × SSC (SSC: 1.5 × 10^{-4} M NaCl, 1.5 × 10^{-5} M sodium citrate) and 0.1 × SSC, 0.1% SDS successively at 56°C–60°C as described [4]. Hybridizing bands were revealed by autoradiography. Fragment sizes were determined by reference to bacteriophage λ DNA digested with *Hin*dIII.

The Cγ gene probe, 24BRH, was a 2.0-kilobase (kb) *Hin*dIII-*Eco*RI fragment of clone 24B provided by Ellison and Hood [10]. This probe contains the Cγ4 coding sequences and has been shown to cross-hybridize extensively with other Cγ genes [11, 12]. The 2.2-kb *Sst*I fragment from the switch region of Cμ was derived from phage clone h18, originally isolated by P. Early and provided by R. Wall. The 5.6-kb *Sst*I-*Bam*HI genomic fragment containing coding and 3′ flanking sequences of Cα1 and the 2.6-kb *Bam*HI fragment containing the coding region of Cε were derived from clones $Hu\alpha_1$ [13] and Huε [14], respectively, and were provided by P. Leder.

Serum of the proband T17, had been tested for immunoglobulin allotypic markers: G1m (1, 2, 3, and 17), G2m (23), G3m (5, 6, 10, 11, 13, 15, 16, 21, 24, and 28), and A2m (1 and 2) by a hemagglutination inhibition technique [15], as reported [16].

Plasma immunoglobulin levels were determined by laser nephelometry (IgG, A, and M) or by radioimmunoassay (IgE, Phadebos-test, Pharmacia, Montreal, Quebec, Canada).

RESULTS

Cγ Gene Analysis

*Bam*HI-digested DNA samples from 32 random Arabo-Berber Tunisians were analyzed to obtain population frequencies of restriction fragment length

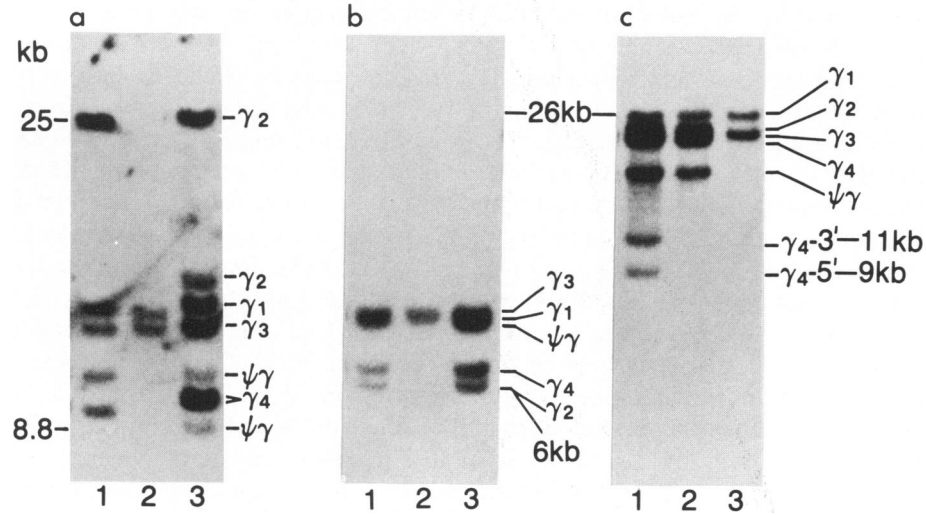

FIG. 1.—Cγ DNA fragment patterns. DNA was digested with (*a*) *Bam*HI, (*b*) *Hin*dIII, and (*c*) *Eco*RI; the Cγ4 probe was used for blot hybridization analysis. (*a*) *Lanes 1* and *3*—controls, *lane 2*—proband T17; (*b*) *lanes 1* and *3*—controls, *lane 2*—proband T17; (*c*) *lanes 1* and *2*—controls, *lane 3*—proband T17. A polymorphism involving the 11- and 9-kb bands is shown in controls. NOTE ADDED IN PROOF: Slight shortening of γ_1 fragment in proband, figure 1*a*, *lane 2*, has been shown to be a genetic variation (Helal et al., manuscript in preparation).

polymorphisms (RFLPs) detected with the Cγ4 gene probe (results to be presented in detail elsewhere). Human Cγ genes are sufficiently homologous that the Cγ4 gene probe detects all five Cγ genes (γ1, γ2, γ3, γ4, and ψγ) (fig. 1*a*). Normal individuals homozygous in the Cγ-gene cluster show five *Bam*HI fragments (e.g., lane 1) where Cγ1 has been assigned to the 12.5-kb fragment, Cγ2 to two polymorphic fragments, 25 or 13.5 kb (shown in the heterozygote, lane 3), Cγ3 to the 11.8-kb fragment, Cγ4 to two polymorphic fragments, 9.4 or 9.0 kb, and Cψγ to two polymorphic fragments, 10 or 8.8 kb [4, 10]. One of the 32 random Tunisian samples, from the proband T17, was unique, having only two fragments: an 11.8-kb fragment corresponding to Cγ3 and a 12.5-kb fragment corresponding to Cγ1 (lane 2). This result indicated the deletion of three Cγ genes: Cγ2, Cγ4, and Cψγ.

Blot hybridization using the Cγ4 probe on DNA digested with *Hin*dIII (fig. 1*b*) or *Eco*RI (fig. 1*c*) confirmed the multigene Cγ deletion. In *Hin*dIII digests of DNA from the proband, the 6.3-kb and 6.0-kb fragments, assigned to Cγ4 and Cγ2, respectively [3, 17], were absent (fig. 1*b*, lane 2). Furthermore, the broad 7.8-kb fragment was markedly lighter in intensity than in normal samples, suggesting the deletion of an additional Cγ-containing fragment, presumably that containing Cψγ. The proband's DNA, digested with *Eco*RI (fig. 1*c*, lane 3), was missing the 17.6-kb Cψγ, 23.5-kb Cγ2, and polymorphic 11- and 9-kb Cγ4 fragments observed in normal individuals (lanes 1 and 2).

Cα and Cε Gene Analysis

To further delineate the extent of the deletion in the proband, the Cα1 probe was hybridized to *Pst*I-digested DNA, an analysis that can distinguish Cα1- and

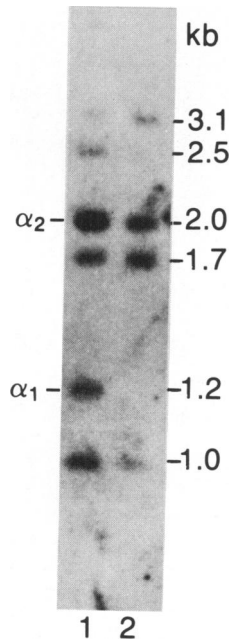

Fig. 2.—Cα DNA fragment pattern. DNA was digested with *Pst*I; the 5.6-kb Cα1 probe was used for blot hybridization analysis. *Lane 1*—normal control, *lane 2*—proband T17.

Cα2-containing fragments [18]. DNA from the proband had the 2.0-kb Cα2 band but was missing the 1.2-kb Cα1 band (fig. 2, lane 2). The decreased intensity of the 1.0-kb fragments (containing fragments of both Cα1 and Cα2) and the missing 2.5-kb band indicate that the deletion includes the Cαl coding region but does not include Cα2. The α1 probe used includes some 3′ flanking region, which presumably accounts for the additional bands not observed previously [18]. From the pattern observed for the proband's DNA, we conclude that the absent 2.5-kb fragment must be associated with the Cα1 region and the observed 1.7-kb fragment with Cα2.

Because the homology in the 5′ flanking or switch regions, the μ switch probe provides information on μ, α1, and α2 genes. Analysis with this probe on the proband's DNA digested with *Sst*I (fig. 3, lane 2) showed an absence of bands in the region designated as A [17, 19], indicating an absence of the α1 switch fragment in the proband. The C region, showing polymorphic fragments of α2 switch, are present at normal intensity in the proband; the fragment present is rare in Caucasians and of unknown frequency in Tunisians. The E region, representing μ switch, indicated a normal double dose of the 2.2-kb fragment. The 1-kb fragment in the F region, not yet specifically designated, is reduced in intensity in the proband.

In *Eco*RI-digested DNA from normal individuals of type A2m(1), the Cψε1 and adjacent Cα1 genes are contained in the same 25-kb fragment, while Cε and adjacent Cα2 are contained in the same 30-kb fragment [14], as shown in figure 4. The 10-kb *Eco*RI fragment in figure 4 (lanes 6 and 7) represents Cψε2 on chromosome 9 [5]. Individuals of type A2m(2) have an *Eco*RI restriction site in

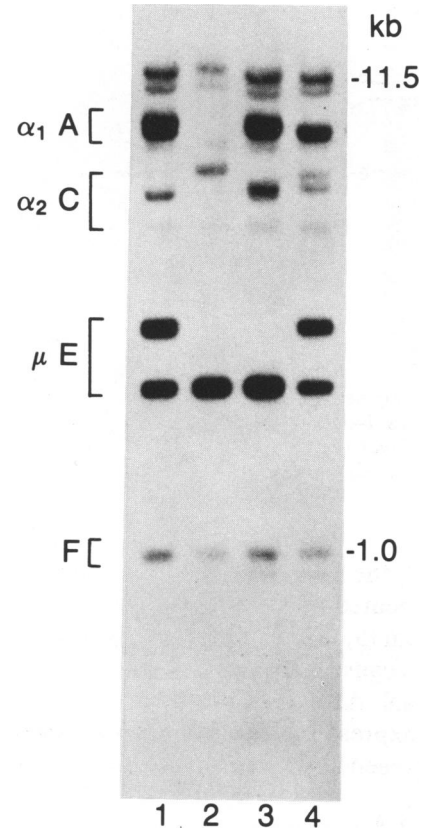

Fig. 3.—DNA fragment patterns, following digestion of DNA with *Sst*I, using the μ switch probe for blot hybridization analysis. *Lanes 1, 3,* and *4*—controls, showing genetic variation in the *C, E,* and *F* regions as designated [17]. *Lane 2*—proband T17.

the first exon of their Cα2 gene, at the site of one of the amino acid substitutions [18], resulting in homozygotes with a 15-kb Cε-Cα2 fragment and a 25-kb Cψε1-Cα1 fragment (fig. 4, lane 3). The proband's DNA digested with *Eco*RI and probed with the Cα1 probe showed the 15-kb band expected in an A2m(2) homozygote, and the Cψε fragment, but no 25-kb Cψε1-Cα fragment (fig. 4, lane 5). After removal of the hybridized Cα1 probe, the *Eco*RI-digested DNA was rehybridized with the Cε probe. The 15-kb fragment was again present, while the 25-kb fragment was absent (fig. 4, lane 7). The 10-kb band contains Cψε2. The importance of having the appropriate control, from an A2m(2) homozygote, should be noted. Furthermore, digestion with one enzyme, *Eco*RI, is as effective as two enzymes [18] for identification of A2m types (see below). This method of analysis could provide an effective replacement for A2m typing.

Results of Gm and Am Typing

Gm typing showed that the proband is homozygous for a normal $GM^{1,17;..;5,14,11,13,10}$ (or $Gm^{a,z;..;b1,b4,b0,b3,b5p;}$) haplotype. This serological anal-

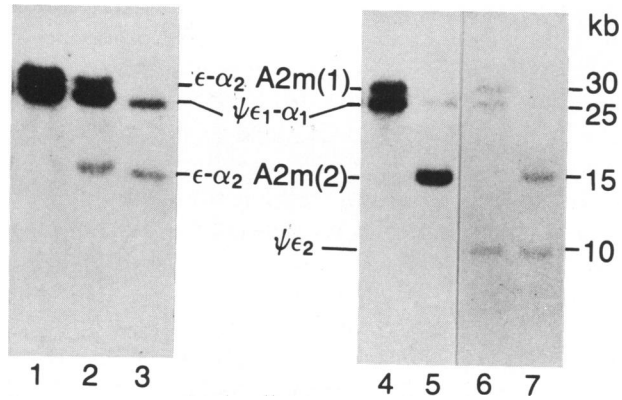

Fig. 4.—Cα and Cε DNA fragment patterns. DNA was digested with *Eco*RI, using for blot hybrization a Cα1 probe (*lanes 1–5*) and Cε probe (*lanes 6* and *7*). *Lanes 6* and *7* represent reprobing of the same gel as in *lanes 4* and *5*. *Lanes 1, 2,* and *3*—normal controls, standard typed by conventional A2m typing as 1-1, 1-2, and 2-2, respectively; *lanes 4* and *6*—normal control, *lanes 5* and *7*—proband T17.

ysis is in agreement with the DNA analysis, as alleles coding for G1m (1) and G1m (17) allotypes are located on the Cγ1 gene (12.5-kb *Bam*HI fragment) and the alleles coding for G3m (5, 14, 11, 13, 10) are located on the Cγ3 gene (11.8-kb *Bam*HI fragment). Because both Cγ3 and Cγ1 are present, the GM type of the proband is not unusual. A2m typing showed the proband to be homozygous for $A2m^2$ and therefore expressing Cα2. This result is compatible with the DNA studies indicating the presence of Cα2.

Quantitation of Ig Classes

The proband had normal concentrations, as expected, of IgM: 0.64 g/l (normal 0.5–1.5 g/l) and of IgE: 105 μg/l (normal > 41 μg/l). The concentration of IgG was below normal at 5.6 g/l (normal 7–10 g/l), compatible with a 14%–26% reduction of total IgG if the contribution from subclasses IgG2 and IgG4 is absent. The concentration of IgA was also below normal at 0.68 g/l (normal 2–3 g/l); this concentration is somewhat greater than might be expected, but is compatible with loss of the IgA1 contribution, which constitutes about 85% of total plasma IgA. (Normal values stated here are mean ± 2 standard deviations in the testing laboratory.)

DISCUSSION

This investigation has identified a deletion in the immunoglobulin heavy-chain region that includes Cψε, Cα1, Cψγ, Cγ2, and Cγ4. Analysis of DNA restriction fragments allowed us to identify specific gene deletions that could not be detected using serological markers. The absence of Cψγ is compatible with its previous localization between Cα1 and Cγ2, using analysis of the degree of association among Cγ gene DNA polymorphisms, Gm markers, and Cμ switch polymorphisms [4].

This multigene deletion presents another type of large deletion within the

immunoglobulin heavy-chain region and has not previously been identified in the homozygous state. The first type was identified in a healthy Tunisian Berber, who showed a simultaneous absence of three IgG subclasses (IgG1, IgG2, and IgG4) and one IgA subclass (IgA1) [6, 7]. DNA studies in this case indicated the absence of Cγ1, Cψε1, Cα1, Cγ2, and Cγ4 [8]. The deletion described here does not include Cγ1. The proband has a reduction of plasma IgG and IgA concentrations, as expected, probably without compensation from nondeleted subclasses. There is no evidence for a deletion of any of the 5′ portion of the IgE gene.

A deletion that appears to be similar to the one described here has been found in a Sardinian [19]. The Sardinian proband was heterozygous for a deletion similar to the one we have described and for a third type of deletion involving α1, ψγ, γ2, γ4, and ε. The latter deletion was also found in the homozygous state in one southern Italian. Whether or not the deleted region described here is identical to that in the Sardinian remains to be established. However, it is not likely to be exactly the same deletion as the Gm and Am types differ: $Gm^{1,17}$, $A2m^{1}$ in the Sardinian proband and $(Gm^{1,17;..;5,14,11,13,10})$ $A2m^{2}$ in the Tunisian proband described here. One possibility, although not highly likely, is that the same deletion occurred on two different haplotypes.

In our study of DNA fragments in Tunisians, one out of 32 normal individuals studied showed homozygosity for the large gene deletion. There was no evidence for heterozygosity for a deletion in any of the other individuals. Heterozygosity for a deletion should have been recognizable by decreased intensity of certain DNA fragments. DNA studies can detect all gene deletions corresponding to the probes used, while serological studies are limited to those homozygous for gene deletions for which serological markers are available, that is, γ1, γ3, γ2, and α2. The frequency of deletions in the Tunisian population, both Berber and Arabo-Berber, may be relatively high compared to the frequency in Caucasian populations. The detection of deletions in the homozygous state is probably due to the increased frequency of consanguinity in these populations. The occurrence of deletions at a relatively high frequency in both Italian and Tunisian populations suggests the possibility of a particularly high frequency of these deletions in the Mediterranean region. In these populations, at least four different multigene deletions have now been observed. The presence of one deletion occurring a very long time ago could have led to subsequent different deletions through unequal crossing over. The highly homologous regions within the Ig heavy-chain cluster are conducive to aberrant recombination events. This explanation is supported by the finding of similar deletions asssociated with different Gm-Am haplotypes.

Further studies to compare the various deletions and identify the specific breakpoints should help to answer these questions of origin and mechanism.

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