

The additional  $C_\lambda$  genes described in CL and CzII may be ancestral genes that segregated away from the progenitors of inbred strains, or they may represent a recent amplification of  $C_\lambda$  in some wild mouse populations. If the CL and CzII mice carry ancestral conserved genes, evolutionary drift of the DNA sequence would probably generate weaker hybridization bands than are observed. Thus, the strength of hybridization of inbred mouse  $C_{\lambda 1}$  and  $C_{\lambda 2}$  cDNA probes to CL and CzII  $C_\lambda$  genes seems to support recent amplification. There is evidence that duplication of  $C_\lambda$  genes has already occurred fairly recently in the mice from which inbred strains are derived, as indicated by the homology of the two  $C_\lambda$  units<sup>4,6,8</sup>.

It is generally accepted that a single  $C_\kappa$  gene is adequate for expression of a large repertoire of immunoglobulin  $\kappa$  light

chains in mice, so the role of multiple  $C_\lambda$  genes is not obvious. If  $J_\lambda$  amplification occurs concurrent with  $C_\lambda$  amplification, immunoglobulin  $\lambda$  diversity might ultimately be influenced. It may be that amplification of  $C_\lambda$  genes is associated with amplification of  $V_\lambda$  genes as well, but as the location of mouse  $V_\lambda$  genes relative to  $C_\lambda$  genes remains unknown, this possibility cannot yet be assessed.

We are currently preparing genomic clones from CzII mice to elucidate further the nature of the  $C_\lambda$  amplification at the DNA level. In addition, experiments at the RNA and protein levels are in progress to ascertain whether the additional  $C_\lambda$  genes are expressed.

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## Inherited deletion of immunoglobulin heavy chain constant region genes in normal human individuals

Marie-Paule Lefranc\*, G. Lefranc\*† & T. H. Rabbitts

Laboratory of Molecular Biology, The MRC Centre, Hills Road, Cambridge CB2 2QH, UK

The existence of specific probes for human genes makes it feasible to study genetic abnormalities, both inherited and acquired, at the level of the genome. In this respect, the antibody genes of man are of particular interest as they represent a multigene family expressed in many leukaemias and immunodeficiency diseases. Furthermore, selective deficiency of immunoglobulins has been described in healthy individuals<sup>1</sup>. Normally, human adults express five types of immunoglobulin—IgM, IgD, IgG, IgE and IgA (defined by the class of heavy chain constant region). Subclasses are also known in IgG (IgG1, IgG2, IgG3 and IgG4) and IgA (IgA1 and IgA2) in which the immunoglobulins contain  $\gamma 1$ ,  $\gamma 2$ ,  $\gamma 3$  or  $\gamma 4$  and  $\alpha 1$  or  $\alpha 2$   $C_H$  regions, respectively. Recently, a healthy Tunisian person was described who showed abnormal patterns of immunoglobulin expression<sup>2</sup>. The serum immunoglobulin of this individual, designated TAK3, was confined to IgM, IgD, IgG3, IgE and IgA2. We have now used cloned  $C_H$ -gene probes to study the DNA of TAK3 as well as two brothers, also Tunisian but apparently unrelated to the individual TAK3, and who show a similar immunoglobulin abnormality. We found that in these cases there seems to have been a large chromosomal deletion which includes three  $\gamma$  genes, an  $\alpha$  gene and a pseudo- $\epsilon$  gene. This deletion accounts for the simultaneous absence of certain H-chain subclasses. These results illustrate that the human immunoglobulin gene locus is capable of undergoing rapid change, which is particularly apparent within small populations in which consanguinity is common.

The patterns of hybridization with  $C_H$ -gene probes of the DNA from TAK3, and the brothers TOU and EZZ were studied by Southern filter hybridization, and compared with DNA from individuals with normal immunoglobulin expression (both of Tunisian and European origins) plus a person, TAK14 (the daughter of TAK3), who is heterozygous for the immunoglobulin abnormality. The state of the  $\gamma$  genes in TAK3 and

TOU was examined using a  $\gamma 3$  probe (clone p3.6RH4.2, which contains a 3.6-kilobase (kb) *EcoRI*–*HindIII* fragment encompassing the whole  $\gamma 3$  gene coding region<sup>3</sup>) and a  $\gamma 4$  probe (clone pBRH4.1, which contains an analogous fragment encompassing the  $\gamma 4$  gene<sup>3</sup>). These probes cross-hybridize with the genes for the various  $\gamma$  subclasses. A high degree of restriction fragment polymorphism has been observed in the  $\gamma$  genes; both the  $\gamma 4$  probe (Fig. 1a) and the  $\gamma 3$  probe (Fig. 1b) detect between five and seven hybridizing *Bam*HI fragments as exemplified by genomic DNAs from different individuals including a placental DNA obtained in Cambridge (Fig. 1b, lane 4) and two other Tunisian DNAs, designated LAT and BOU (Fig. 1a, lanes 1, 4). Common 9-, 11- and 12-kb fragments were present in all these DNAs, and in one DNA an additional 23-kb band was detected (Fig. 1a, lane 4) which we also observed in other Tunisian DNAs (data not shown).

When we analysed hybridization of the  $\gamma 4$  probe with *Bam*HI-digested DNA from TAK3, we observed only two bands (8 and 10.5 kb; Fig. 1a, lane 3) whereas hybridization with the  $\gamma 3$  probe showed only the 10.5-kb fragment (Fig. 1b, lane 2). Presumably, this 10.5-kb *Bam*HI fragment detected in TAK3 DNA represents the  $\gamma 3$  gene (because only the IgG3 subclass is expressed in TAK3). The unusual size of this fragment may result from an altered restriction site which occurred during the deletion event. The origin of the second, more weakly hybridizing component could be the pseudo- $\gamma$  gene<sup>3,4</sup> or even a partially deleted  $\gamma$  gene. Neither of the bands observed in TAK3 corresponds with the fragments detected in any of the other DNAs except TAK14 DNA (Fig. 1a, lane 2; b, lane 3) which is heterozygous for the immunoglobulin defect found in TAK3. The heterozygosity of TAK14 is demonstrated by the presence of a normal pattern of five bands plus the two unusual bands detected in TAK3 DNA. These data suggest that the simultaneous absence of  $\gamma 1$ ,  $\gamma 2$  and  $\gamma 4$  subclasses in the homozygous individual TAK3 is due to an extensive deletion of the region of chromosome 14 which carries the  $\gamma$  genes<sup>5–7</sup>. The hybridization of the  $\gamma 3$  probe to *Bam*HI-digested TOU DNA is also shown in Fig. 1b (lane 1). Interestingly, a similar  $\gamma$  deletion has occurred in TOU to that which occurred in TAK3, except that we could only detect the 10.5-kb hybridizing band in TOU DNA. These results with  $\gamma$ -gene probes show that two individuals displaying abnormal patterns of  $\gamma$ -globulin expression seem to do so because of inherited deletions of the respective  $\gamma$  genes.

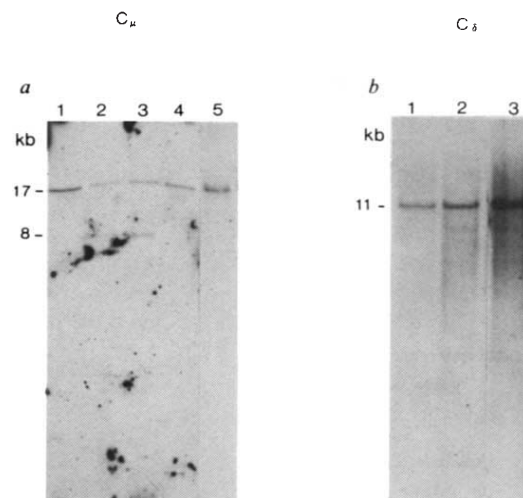
A further investigation of the  $C_H$ -gene pattern in the TAK, TOU and EZZ DNAs was made using  $\mu$ - and  $\delta$ -specific probes (Fig. 2) plus  $\epsilon$ - and  $\alpha$ -specific probes (Fig. 3), and the latter two gave further evidence for gene deletions. The probes used

\* Permanent address: Laboratoire d'Immunogénétique, Université des Sciences et Techniques du Languedoc, 34060 Montpellier, France.

† Faculté de Pharmacie, Monastir, Tunisia.

in Fig. 2 were C75p1.2 (a 1.2-kb *EcoRI* fragment of a human clone, carrying the  $C_\mu$  coding regions, cloned into pACYC184<sup>8</sup>) and M13RP2 (a 1.5-kb *EcoRI*-*PstI* fragment of a human clone containing part of the  $\delta$  gene cloned into M13mp8). All five DNA samples analysed with the  $\mu$  probe possessed 17-kb hybridizing *Bam*HI fragments (Fig. 2a) but in addition TAK14 and TAK3 showed a hybridizing band of 8 kb. The occurrence of this latter band in TAK3 (who is thought to be homozygous for the  $C_H$  locus<sup>2</sup>) is surprising; the most obvious conclusion must be that a duplication of  $\mu$  has occurred in TAK DNA. No such putative duplication has occurred in the homozygous individuals EZZ or TOU, where we find only the 17-kb *Bam*HI restriction fragment (Fig. 2a, lane 5). A completely normal pattern of hybridization between TAK and TOU DNAs was found when we used the  $\delta$  probe (Fig. 2b), as each of these DNAs display a 11-kb hybridizing *Bam*HI fragment found in 9 out of 10 control DNAs analysed (latter data not shown).

The  $\epsilon$  and  $\alpha$  genes in the various human DNAs were analysed using as hybridization probe for  $\epsilon$ , the  $\epsilon$ 1.2BP25 clone (a 2.1-kb *Bam*HI-*PstI* fragment, derived from the clone  $\epsilon$ 1.2, containing the entire human  $\epsilon$  coding region<sup>9</sup>) and as an  $\alpha$  probe the clone  $\alpha$ 2XP8 (a 600-base *XhoI*-*PstI* fragment of an  $\alpha$ -gene cloned in M13mp8<sup>10</sup>). The restriction pattern most frequently seen for  $\epsilon$ -gene hybridization in human DNA (Fig. 3) is the presence of three *Bam*HI fragments (2.7, 6 and 9 kb)<sup>9,11,12</sup>. This pattern is exemplified in Fig. 3a, lane 3, by the DNA sample LAT. A frequently observed polymorphism is the presence of a fourth, 7.5-kb band<sup>9</sup>, which is present in DNA sample BOU (Fig. 3a, lane 5). It has recently been demonstrated that the 2.7-kb fragment carries the active  $\epsilon$  gene<sup>9,11,12</sup> whereas the 6- and 9-kb fragments represent pseudogenes called  $\psi\epsilon$ 1 and  $\psi\epsilon$ 2, respectively<sup>10,11</sup>. When we examined the patterns of hybridization of the  $\epsilon$  probe to both TAK and TOU DNAs we again

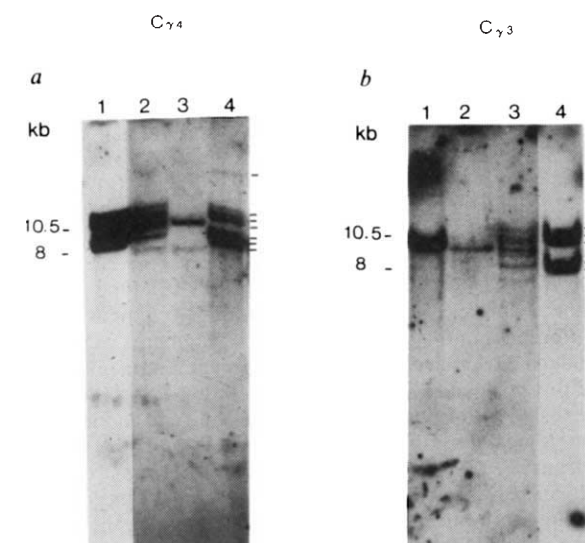


**Fig. 2** Southern hybridization of  $C_\mu$  and  $C_\delta$  probes to genomic DNA from various sources. The DNA digests were: a, probe:  $\mu$ ; slot 1, LAT; slot 2, TAK14; slot 3, TAK3; slot 4, placenta; slot 5, EZZ. b, Probe:  $\delta$ ; slot 1, TAK3; slot 2, TAK14; slot 3, TOU. **Methods:** Genomic DNA was digested to completion with *Bam*HI, fractionated and blotted from 0.8% agarose gels onto cellulose nitrate and hybridized (as in Fig. 1) to nick-translated C75p1.2 (a) and M13RP2 (b) probes. Phage  $\lambda$  DNA cut with *Hind*III was used as molecular weight markers.

found evidence for gene deletion (Fig. 3a, lanes 1, 4). Both TAK3 and TOU DNAs show the presence of both the active  $\epsilon$  gene and  $\psi\epsilon$ 2 but no  $\psi\epsilon$ 1. This observation is consistent with the deletion of the latter from these genomes because this gene is present in other DNAs (see, for example, LAT and BOU, lanes 3 and 5) including TAK14 who is heterozygous for the abnormality (Fig. 3a, lane 2).

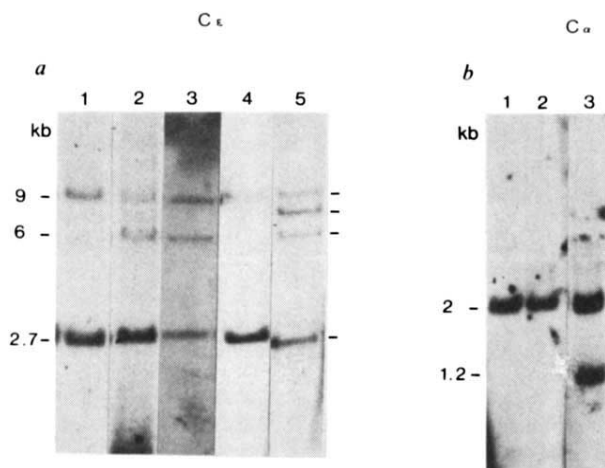
The  $\alpha$ -gene hybridization pattern observed using the  $\alpha$ 2XP8 clone with TAK and TOU is shown in Fig. 3b. In this experiment we used a sample designated TAK92 (a non-blood relative of TAK3) as the control DNA: hybridization of the  $\alpha$  probe with TAK92 (Fig. 3b, lane 3) shows two hybridization components (2 and 1.2 kb) in common with European DNAs tested simultaneously (latter data not shown). Partial nucleotide sequencing data<sup>10</sup> indicate that the 2- and 1.2-kb fragments contain  $\alpha$ 2 and  $\alpha$ 1 coding sequences, respectively. The  $\alpha$ -gene hybridization to DNA from TOU and TAK3 (Fig. 3b, lanes 1, 2) showed, in both cases, that the smaller of the hybridization components was absent, consistent with the presence of the  $\alpha$ 2 gene and deletion of the  $\alpha$ 1 gene from the genome of TAK3 and TOU individuals.

The Southern hybridization results presented here show that Tunisian individuals who exhibit simultaneous absence of IgG1, IgG2, IgG4 and IgA1 subclasses carry deletions for three  $\gamma$  genes (presumably  $\gamma$ 1,  $\gamma$ 2 and  $\gamma$ 4), a pseudo- $\epsilon$  gene ( $\psi\epsilon$ 1) and an  $\alpha$  gene presumed to be  $\alpha$ 1. These gene deletions account for the lack of specific immunoglobulin expression in these individuals. The precise location of the gene deletion in these people need not necessarily be identical but it does seem likely that switch or S-sequences are the key to the deletion event. The S-sequences are groups of short tandemly repeated units which are probably involved in the mechanism of the class switch and have been implicated in the formation of a mouse myeloma deletion mutant<sup>13</sup>. A likely hypothesis to explain the deletions described here is that illegitimate recombination has occurred within the germ line between non-adjacent S-sequences with resulting deletion of the intervening  $C_H$  genes. Presumably, such deletions occasionally occur in all human populations but would not normally be detected because they do not seem to be harmful (the person designated TAK3, for instance, is a healthy 75-yr-old woman). However, in populations where frequent consanguinity occurs the probability of



**Fig. 3** Southern hybridization of  $C_\gamma$ 3 and  $C_\gamma$ 4 probes to DNAs from individuals showing serum immunoglobulin abnormalities. a, Probe:  $\gamma$ 4; slot 1, LAT; slot 2, TAK14; slot 3, TAK3; slot 4, BOU. b, Probe:  $\gamma$ 3; slot 1, TOU; slot 2, TAK3; slot 3, TAK14; slot 4, placental DNA.

**Methods:** DNA was digested to completion with *Bam*HI, the digests were fractionated on 0.8% agarose gel, and the DNA was transferred to cellulose nitrate filters<sup>14</sup>. LAT and BOU individuals were described previously<sup>15</sup>. The hybridization was carried out for 2 days at 65°C using 6×SSC, 0.1% SDS, 0.2% Ficoll 400, 0.2% bovine serum albumin (BSA), 0.2% polyvinylpyrrolidone (PVP) and 50  $\mu$ g ml<sup>-1</sup> sonicated, denatured, salmon sperm DNA<sup>16,17</sup>, followed by washes in 1×SSC, 0.1% SDS at 65°C before autoradiography at -70°C with prefogged X-ray film<sup>18</sup>. Probes were nick-translated to a specific activity of  $\sim 10^8$  c.p.m. per  $\mu$ g. Size estimations (shown on the left of each panel) were made using  $\lambda$  cut with *Hind*III and the lines on the right of each panel signify location of restriction fragments discussed in the text.



**Fig. 3** Southern blot hybridization of  $C_\epsilon$  and  $C_\alpha$  probes to genomic DNA digested with *Bam*HI (a) or *Pst*I (b). The DNA digests were: a, Probe:  $\epsilon$ ; slot 1, TAK3; slot 2, TAK14; slot 3, LAT; slot 4, TOU; slot 5, BOU. b, Probe:  $\alpha$ ; slot 1, TOU; slot 2, TAK3; slot 3, TAK92.

**Methods:** Complete digests were separated on 0.8% agarose (a) or 1.4% agarose (b), blotted and hybridized (as in Fig. 1) with  $C_\epsilon$  probe (a) or  $C_\alpha$  probe (b). The size of fragments (given on the left of each panel) were estimated relative to  $\lambda$  phage DNA cut with *Hind*III. The lines on the right of a mark the positions of fragments discussed in the text.

homozygosity increases and such individuals are readily detected during random immunoglobulin haplotype screening.

Finally, the consistent deletion pattern of  $C_H$  genes in TAK, TOU and EZZ allows a link between the absence of  $C_H$  genes and the overall ordering of the human  $C_H$  genes described in the accompanying paper<sup>10</sup>. Two groups of cosmid clones have been described which seem to encompass  $\gamma 3$ – $\gamma 1$ – $\psi \epsilon 1$ – $\alpha 1$  (region A) and  $\gamma 2$ – $\gamma 4$ – $\epsilon$ – $\alpha 2$  (region B)<sup>10</sup>. As it seems that the deletions described here include  $\gamma 1$ ,  $\gamma 2$ ,  $\gamma 4$ ,  $\psi \epsilon 1$  and  $\alpha 1$  genes, the most probable order for the groups of cosmid clones is 5' region A–region B 3' as the deletions presumably start downstream of  $\gamma 3$  (region A) and end upstream of the active  $\epsilon$  gene (region B). We are now using gene cloning studies to clarify these issues as well as the nature and localization of the points of deletion within the  $C_H$  genes.

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## Activation of the T24 bladder carcinoma transforming gene is linked to a single amino acid change

Elizabeth Taparowsky, Yolande Suard,  
Ottavio Fasano, Kenji Shimizu,  
Mitchell Goldfarb & Michael Wigler

Cold Spring Harbor Laboratories, Cold Spring Harbor,  
New York 11724, USA

Several different transforming genes have been observed in the DNA of a variety of tumours and tumour cell lines of human and rodent origin by the ability of these genes to induce morphological transformation in NIH 3T3 cells<sup>1–5</sup>. The transforming gene found in a human bladder carcinoma cell line, T24, is *H-ras-1*, the human homologue of the Harvey sarcoma virus oncogene (*v-H-ras*)<sup>6–9</sup>. In the present study we have compared the *H-ras-1* genes cloned from T24 and normal human DNA. The *H-ras-1* gene cloned from T24 DNA induces transformation in NIH 3T3 cells, while the same gene cloned from normal cellular DNA does not. The functionally significant difference between the transforming and normal genes appears to be a single base mutation, which produces an amino acid change in the sequence of the proteins that the genes encode.

We had previously isolated transforming sequences from NIH 3T3 cells that had been transformed with DNA from T24 bladder carcinoma cells<sup>10</sup>. These sequences had undergone some rearrangement during gene transfer into NIH 3T3 cells. As a result of passage in NIH 3T3 cells, the transforming gene might have undergone less readily detectable secondary changes altering its biological activity. Therefore, in order to properly compare the transforming sequences of T24 to their normal counterparts, we directly cloned the *H-ras-1* sequences from  $\lambda$  libraries constructed from T24 DNA and human placental DNA. From the T24 library we obtained seven independent isolates, all of which contained a 6.2-kilobase (kb) *Bam*HI *H-ras-1* fragment with an identical restriction endonuclease pattern. From the placental library, we obtained clones of two types: in three (P3 type), *H-ras-1* sequences were carried on a 6.7-kb *Bam*HI fragment; in the four others (P1 type), *H-ras-1* sequences were carried on a 8.3-kb *Bam*HI fragment (see Fig. 1). Differences in the size of the *Bam*HI fragments were expected due to restriction endonuclease fragment length polymorphism about this gene in the human population<sup>10</sup>.

We tested all of our isolates for the ability to induce foci of morphologically transformed NIH 3T3 cells on DNA mediated gene transfer. DNA from all T24 isolates of *H-ras-1* efficiently transformed NIH 3T3 cells, while DNA from all placental isolates failed to induce foci. This difference must be the result of differences in DNA sequence. To facilitate our search for these differences, we tested the transforming activity of chimaeric genes. The restriction endonuclease *Xba*I cleaves the *Bam*HI fragment containing the *H-ras-1* gene into one small and one large fragment (see Fig. 1). The large and small fragments were purified from each of the three cloned genes (types T24, P1 and P3), mixed and ligated in various combinations and finally cleaved with *Bam*HI. All (and only) molecules containing the small T24 *Bam*HI/*Xba*I fragment were capable of inducing transformed foci in NIH 3T3 cells (see Table 1). To further limit the area of our search we used restriction endonuclease *Mst*II, which cleaves the small *Bam*HI/*Xba*I fragment once. The 1.7-kb *Bam*HI/*Mst*II fragment and the 0.24-kb *Mst*II/*Xba*I fragment from the T24 or P3 *H-ras-1* genes, and the large *Xba*I/*Bam*HI fragment from the P3 gene were purified, mixed and ligated in various combinations, and then cleaved with *Bam*HI. All molecules containing the 0.24-kb *Mst*II/*Xba*I fragment from T24 induced transformed foci in