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Cloning of human immunoglobulin ε chain genes: Evidence for multiple C_ε genes

(joining region probe/partial nucleotide sequence/gene order/switch region)

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ABSTRACT **An active human ε chain gene was cloned from a phage library containing partial *Eco*RI* digests of IgE-producing myeloma DNA, using the human J_H (joining) gene fragment as a probe. The ε chain gene clone was identified by partial nucleotide sequence determination. The germ-line constant region gene of the ε chain (C_ε gene) was cloned from a human fetal liver DNA library, using the cloned ε chain gene as a probe. Comparative studies on the human and mouse germ-line ε chain genes revealed that the switch (S) sequence is more conserved than the coding sequence. Restriction endonuclease *Bam*HI digestion of human DNA produced three C_ε fragments of 3.0, 6.5, and 9.2 kilobases, which were named $C_\varepsilon 1$, $C_\varepsilon 2$, and $C_\varepsilon 3$ genes, respectively. We found the three C_ε gene fragments in all of the human DNA preparations from eleven individuals. The C_ε gene expressed in the myeloma was identified as the $C_\varepsilon 1$ gene. Because the $C_\varepsilon 2$ gene is deleted from the myeloma DNA, the order of the C_ε genes is likely to be 5′-$C_\varepsilon 2$-$C_\varepsilon 1$-$C_\varepsilon 3$-3′, assuming that all the C_ε genes are on chromosome 14. The germ-line $C_\varepsilon 3$ gene was also cloned from the myeloma DNA. Characterization of the $C_\varepsilon 3$ gene revealed that it does not have the S region, suggesting that it might be a pseudogene.**

Immunoglobulin heavy (H) chains are classified into five major classes, μ, γ, α, δ, and ε. The γ chains are further divided into four subclasses in mouse and man. Recent studies on the molecular cloning of H chain constant region (C_H) genes of mouse revealed that they are organized in the order of 5′-C_μ-C_δ-$C_\gamma 3$-$C_\gamma 1$-$C_\gamma 2b$-$C_\gamma 2a$-C_ε-C_α-3′ within a 200-kilobase (kb) region (1–6). Comparative studies on the mouse C_γ genes revealed that they had evolved through gene duplications and intervening sequence-mediated domain transfer events (7, 8).

Studies on human C_H genes are interesting from the evolutionary as well as clinical point of view. Cloning and characterization of the C_ε gene is important above all because IgE serves as the mediator of allergic reactions (9). Although the human C_μ (10, 11), C_δ (12), C_γ (13), and C_α (11) genes have been cloned by using mouse probes, the amino acid sequence homology of the human and mouse ε chains is not high enough to expect cross-hybridization of the nucleotide sequences (2). We have taken advantage of the fact that a given active C_H gene is linked with a completed V_H (variable) gene that contains a J_H (joining) segment (14–19). Thus, any genomic C_H genes can be isolated, using the J_H segment as a probe, from DNAs of myelomas or cell lines that produce the particular H chains. This strategy has been successfully employed for cloning the mouse ε chain gene from an IgE-producing hybridoma (2).

In this paper, we report the cloning of an active human ε chain gene from a phage library of IgE myeloma DNA by using a human J_H gene probe. Using the C_ε fragment of the cloned ε chain gene, we have identified at least three germ-line C_ε genes in human DNA. Two out of three C_ε genes were cloned and characterized. We also propose an order for the three C_ε genes.

MATERIALS AND METHODS

Isolation of Recombinant Phages. Bacteriophages λ Charon 4A (20) and λgt*WES*·λB (21) were used as EK2 vectors and propagated in *Escherichia coli* DP50 SupF (20). Cloning experiments were carried out under P3–EK2 conditions as described by the National Institutes of Health. High molecular weight DNA was purified from the IgE-producing myeloma cell line 266B1 (22) and partially digested with restriction endonuclease *Eco*RI* after methylation of the *Eco*RI sites (23). Fragments of 15–20 kb were isolated from the partial digests and ligated with Charon 4A outer fragments with T4 ligase as described (24). The recombinant DNA was packaged *in vitro* into coat proteins (25) and phages were screened as described (26). A Charon 4A library containing partial *Hae* III/*Alu* I digests of human fetal liver DNA (27) was a generous gift of T. Maniatis (Harvard Univ.).

Other Procedures. Southern blot hybridization of restriction endonuclease-digested DNAs was performed as described (28). The mild washing conditions were 150 mM NaCl/15 mM sodium citrate/0.1% $NaDodSO_4$ for 5 min at 48°C. The stringent washing conditions were 15 mM NaCl/1.5 mM sodium citrate/0.1% $NaDodSO_4$ for 90 min at 65°C with three changes of the washing buffer. Filters were washed under the stringent conditions unless specified. DNA sequence analysis was carried out according to the method of Maxam and Gilbert (29) with slight modifications (30). High molecular weight DNAs were extracted from a human placenta and human lymphocytes of individual blood donors as described (31, 32).

RESULTS AND DISCUSSION

Rearrangement of the J_H Segment in DNA of the IgE-Producing Myeloma. The total cellular DNAs of human IgE myeloma 266B1 (22) and a human placenta were digested with several restriction enzymes and rearrangement around the J_H segment was examined by Southern blot hybridization as shown in Fig. 1. A 1.7-kb *Hha* I fragment of a germ-line C_μ gene clone Ch4A·H·Igμ-24 (10) was used as J_H probe (fragment b in Fig. 2). *Eco*RI and *Bam*HI digestions of the placenta DNA produced 22-kb and 17-kb bands, respectively, hybridizing to the J_H probe. Similarly, *Eco*RI and *Bam*HI digestions of the myeloma DNA yielded 35-kb and 3.6-kb fragments, respectively. Although the amount of the 266B1 DNA applied on the gel was

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Abbreviations: C and V, constant and variable regions of immunoglobulin chains; H chain, heavy chain; *J* and *S*, joining and switch region genes; kb, kilobase(s); bp, base pair(s).

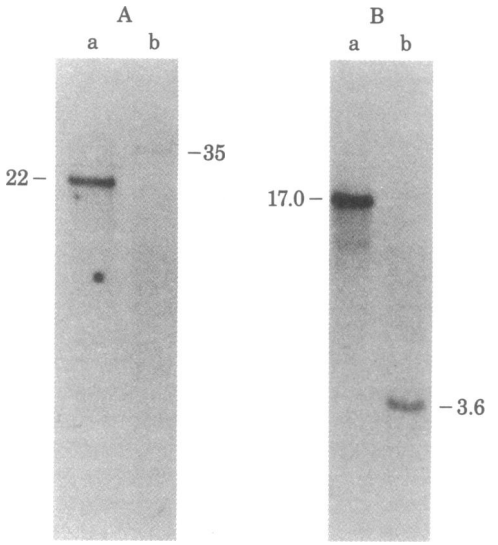

FIG. 1. Rearrangement of the J_H gene in the IgE-producing myeloma DNA. DNAs of human placenta and IgE myeloma were digested with *Eco*RI (*A*) or *Bam*HI (*B*), electrophoresed in a 0.7% agarose gel, and blotted onto a nitrocellulose filter. The filter was hybridized with the J_H probe (fragment b in Fig. 2). Numbers indicate sizes of hybridized bands in kb. DNAs used are as follows: lanes a, 2 μg of placenta DNA; lanes b, 4 μg of myeloma DNA.

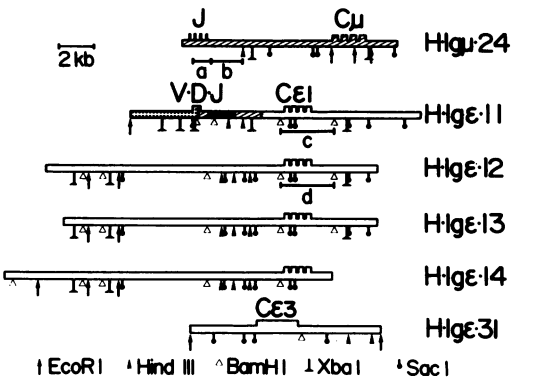

FIG. 2. Restriction maps of the cloned C_ε genes. The approximate locations of exons were determined by R-loop analysis (unpublished data) and shown by wider rectangles. The direction of transcription of the $C_\varepsilon 1$ gene is from left to right. H·Igε-11 is the expressed ε chain gene in the myeloma and is composed of at least four germ-line segments, namely V_H (stippled rectangle), J_H with the C_μ flanking sequence (hatched rectangle), a segment of unknown origin (filled rectangle), and C_ε (open rectangle). The presence of a *D* (diversity) segment is suspected but not confirmed. H·Igε-12, H·Igε-13, and H·Igε-14 are the germ-line $C_\varepsilon 1$ gene and H·Igε-31 is the germ-line $C_\varepsilon 3$ gene. The restriction sites of H·Igμ-24 (10) are shown for comparison. Horizontal bars a, b, c, and d indicate the fragments used as probes in this study (see text). The locations of J_H genes were tentatively assigned on the basis of the rearrangements of J_H in other cloned H chain genes and the number of J_H segments is not final (unpublished data).

twice as much as that of the placenta DNA, the myeloma DNA yielded considerably fainter J_H bands than the placenta DNA did. These results indicate that the myeloma DNA contains a single copy or less of the J_H gene segment, which is rearranged.

Nonproductive rearrangements and deletion of the J_H segment take place frequently in inactive chromosomes of myeloma cells and lymphocytes (33, 34). A single copy of the J_H gene segment in the 266B1 myeloma cell may be attributable to the deletion of the J_H segment from the homologous chromosome or the loss of the homologous 14th chromosome. Karyotype analysis of the 266B1 cell revealed a reduced number of group D chromosomes (22), of which the C_H gene-bearing chromosome 14 is one (35).

Cloning of the Rearranged ε Chain Gene. Because an active C_H gene is associated with the J_H gene at its 5′ side (14–19), the single J_H gene segment in the 266B1 DNA is expected to be linked to the C_ε gene. A Charon 4A phage library containing partial *Eco*RI* digests of the myeloma DNA was screened by using the human J_H probe. Out of about 9×10^6 phages 19 positive clones were obtained. Restriction fragments of all the positive clones were indistinguishable from each other, indicating that they are the descendants of a common parental recombinant. The positive clone was named Ch4A·H·Igε-11, the insert of which was called H·Igε-11.

The restriction map of H·Igε-11 was constructed by digestions with various combinations of restriction enzymes as shown in Fig. 2. To locate the V_H gene in H·Igε-11, Southern blots of the digested Ch4A·H·Igε-11 DNA were hybridized with the 5′ and 3′ J_H gene probes, the 1.0-kb and 1.7-kb *Hha* I fragments of Ch4A·H·Igμ-24, which are indicated by fragments a and b, respectively, in Fig. 2. Both probes hybridized with the 3.0-kb *Xba* I fragment, but the 5′ and 3′ J_H probes hybridized with the 1.0-kb and 3.6-kb *Bam*HI fragments, respectively, as shown in Fig. 3 (lanes b and c). From these results, the V_H gene was tentatively located as shown in Fig. 2. The restriction map of the cloned DNA H·Igε-11 coincides with that deduced from Southern hybridization of the myeloma DNA, using the J_H probe.

Disagreement of the restriction maps of the region 3′ to the J_H segment between H·Igε-11 and H·Igμ-24 and the weak hybridization of the 3′ J_H gene probe to the myeloma DNA (Fig. 1) suggests that an ≈1-kb segment of unknown origin, carrying one *Bam*HI and one *Hin*dIII cleavage site, might be inserted into the J_H segment of the 266B1 DNA. Alternatively, such a difference may represent a polymorphism of the J_H flanking sequence among human individuals.

To ascertain the presence of the C_ε gene in the cloned seg-

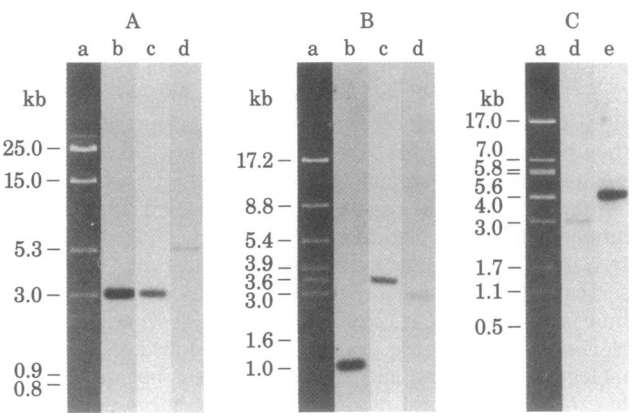

FIG. 3. Southern blot hybridization of Ch4A·H·Igε-11 and Ch4A·H·Igε-12 DNAs with human J_H, mouse C_ε, and mouse S_ε (switch) probes. Ch4A·H·Igε-11 DNA was digested with *Xba* I (*A*) or *Bam*HI (*B*), and Ch4A·H·Igε-12 DNA was digested with *Bam*HI (*C*). Fragments were separated in 0.5% agarose gels and Southern blot filters were hybridized with nick-translated probes indicated. Lanes a show ethidium bromide stain of restriction fragments. The other lanes show autoradiograms of Southern blots. The probe used in each lane is as follows: lanes b, fragment a in Fig. 2 (5′ J_H probe); lanes c, fragment b in Fig. 2 (3′ J_H probe); lanes d, 0.3-kb *Hin*dIII/*Hha* I fragment of M·Igε-7 DNA, which contains the C_H3 domain sequence of the mouse C_ε gene; lane e, the 3.0-kb *Bam*HI fragment of M·Igε-7 DNA (the mouse S_ε probe). Filters used for the mouse C_ε probe (lanes d) were washed under the mild washing conditions. The other filters were washed under the stringent conditions.

ment, a Southern blot of the digested Ch4A·H·Igε-11 DNA was hybridized with a ^{32}P-labeled mouse C_ε gene fragment—i.e., the 0.3-kb *Hin*dIII/*Hha* I fragment of M·Igε-7 (2) that contains the sequence encoding the C_H3 domain. Under a mild hybridization condition, the 5.3-kb *Xba* I and 3.0-kb *Bam*HI fragments of Ch4A·H·Igε-11 hybridized with the mouse C_ε gene probe (Fig. 3, lane d). Such weak hybridization is expected from the diversity of the amino acid sequences of the human and mouse ε chains (ref. 2; unpublished data).

Partial Nucleotide Sequence Determination. Cloning of the C_ε gene in H·Igε-11 was directly proved by partial nucleotide sequence determination. The 3.0-kb *Bam*HI fragment (fragment c in Fig. 2) was subcloned in plasmid pBR322; the clone obtained is referred to as pH·Igε-11. The nucleotide sequences at both ends of the 580-base pair (bp) *Ava* II fragment of pH·Igε-11 (fragment e) were determined and shown to encode amino acid sequences of parts of C_H2 (residues 214–253) and C_H3 (residues 328–359) domains of the ε chain secreted by the IgE myeloma 266B1 (36), as shown in Fig. 4.

The amino acid sequences predicted from the nucleotide sequences disagree with the known amino acid sequence at two positions: at residues 234 and 244. The difference could be due to errors in the amino acid sequence or mutations introduced during the long-term propagation of the 266B1 cell. Nonetheless, the results clearly demonstrate that H·Igε-11 contains the structural sequence of the C_ε gene and that the direction of transcription is from left to right. Because H·Igε-11 also contains the J_H segment, only a single copy of which is present in the myeloma DNA, H·Igε-11 must be the active ε chain gene. The putative locations of the V_H and C_ε exons are schematically represented in Fig. 2.

Cloning and Characterization of Germ-line C_ε Gene. To obtain the germ-line C_ε gene, the Charon 4A library of the *Hae* III/*Alu* I partial digests of human fetal liver DNA (27) was screened with ^{32}P-labeled fragment c of Fig. 2 as the C_ε gene probe. Out of 2×10^6 phages screened, seven positive clones were obtained. Restriction maps of three of them indicate that they are the overlapping clones (Fig. 2). These clones are named Ch4A·H·Igε-12, Ch4A·H·Igε-13, and Ch4A·H·Igε-14, and their inserts are called H·Igε-12, H·Igε-13, and H·Igε-14, respectively. The remaining four clones were indistinguishable from Ch4A·H·Igε-12. Restriction cleavage sites of the 3′ portion of the germ-line clones are similar to those of the C_ε coding region of H·Igε-11 (Fig. 2). When the two *Bam*HI fragments (3.0 kb) derived from H·Igε-11 and H·Igε-12 (fragments c and d, respectively) were digested with *Sac* I, *Ava* II, *Hha* I, *Hin*cII, *Hin*fI, or *Hap* II, no difference was observed in sizes of the restriction fragments (data not shown).

Heteroduplex analyses of the cloned C_μ and C_α genes of human and mouse revealed that S regions, which are responsible for the class switch recombination and consist of characteristic tandem repetitive sequences (14, 37–39), are more conserved than the coding sequences (10, 11). It is of interest to know the homology of the S_ε sequence between human and mouse, because the amino acid sequence of the ε chain is less conserved than are the sequences of the μ and α chains (ref. 2; unpub-

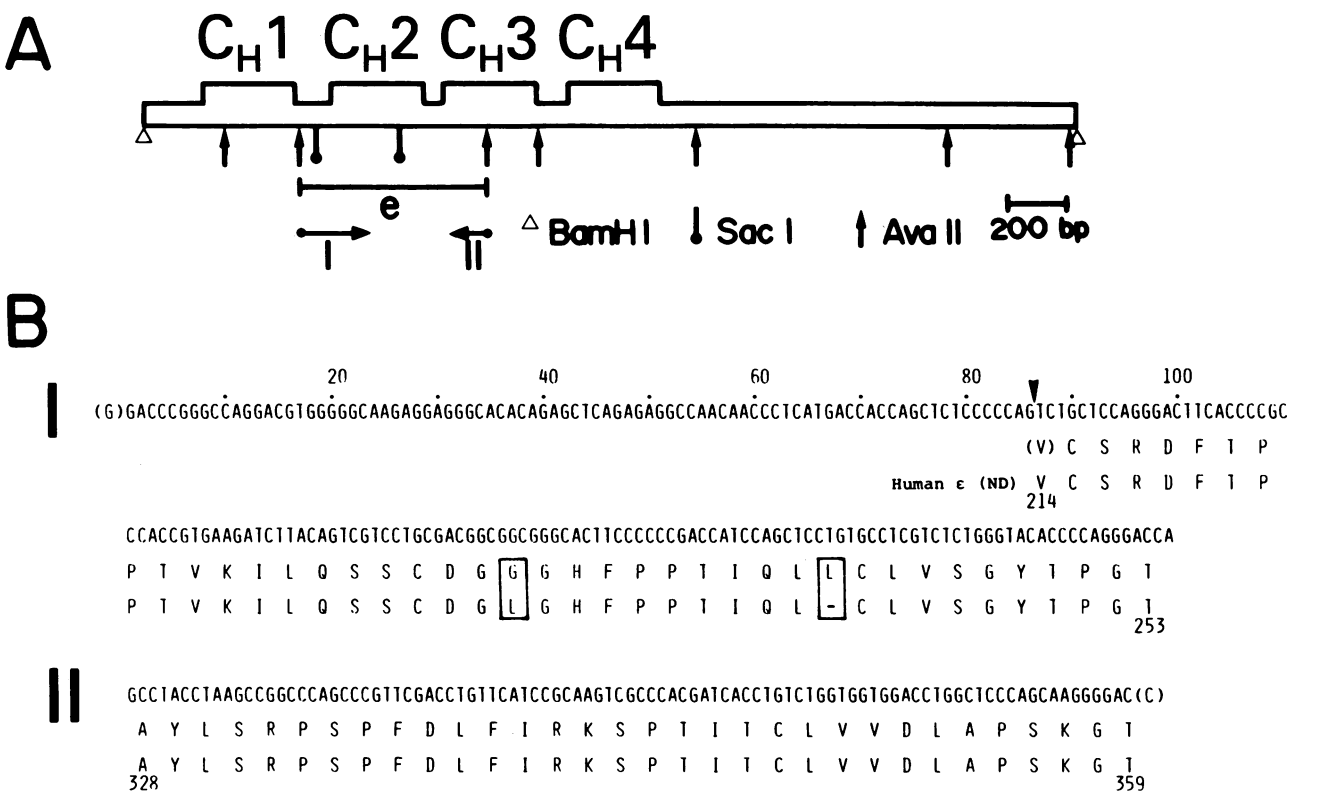

FIG. 4. Partial nucleotide sequences of the C_ε gene. (*A*) Strategy for nucleotide sequence determination. The 580-bp *Ava* II fragment (fragment e) was purified from the subcloned 3.0-kb *Bam*HI fragment (fragment c in Fig. 2) and its 5′ termini were labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP after bacterial alkaline phosphatase treatment. The 460- and 120-bp fragments produced by the *Hin*fI digestion of the *Ava*II fragment were subjected to sequence determination (29). Ranges and directions of sequences read are shown by horizontal arrows I and II. (*B*) Nucleotide sequences at both termini of fragment e. The nucleotide sequence at region II was translated into the complementary strand. The amino acid sequences predicted by the nucleotide sequences are shown under the coding sequences. The amino acid sequences at the C_H2 domain (residues 214–253) and the C_H3 domain (residues 328–359) of the human ε chain (ND) (36) are shown in the bottom row. Disagreeing amino acid residues are boxed. Amino acids are expressed by one-letter code (2). Numbers indicate positions of amino acid residues according to the published amino acid sequence (36). The arrowhead indicates the possible splicing site.

lished data). When a Southern blot filter of *Bam*HI-digested Ch4A·H·Igε-12 DNA was probed with the mouse S_ε fragment—i.e., a 3.0-kb *Bam*HI fragment of M·Igε-7 (2), a 4.0-kb fragment located immediately 5′ to the coding region hybridized even under the stringent washing conditions. On the other hand, hybridization with the mouse C_ε probe was detectable only under the mild washing conditions (Fig. 3*C*). These results demonstrate that the S_ε sequence is more conserved than the C_ε sequence even though the S_ε sequence is never translated into proteins. The mouse S region is characteristic of tandem repetition of the unit sequence that is unique to each class but shares the short common sequences A-G-C-T-G and T-G-G-G-G (37–39). Such structural features of the S region seem to be conserved in humans as well (10–12) and are of general importance for the immunoglobulin H chain gene system.

Three C_ε Genes in the Human Genome. The human C_γ gene family consists of, at least, four C_γ genes and a pseudo-C_γ gene (13), whereas the mouse genome contains the four C_γ genes but no conserved pseudogene. At least two copies of the C_α gene are represented in the human genome (ref. 11; unpublished data), whereas a single copy each of the C_α and C_ε genes has been found in mouse. To test the possibility that the human genome contains multiple C_ε genes, the human placenta DNA was digested with restriction enzymes, blotted onto a nitrocellulose filter, and hybridized with the C_ε probe (Fig. 5). The *Bam*HI digestion of the placenta DNA produced three bands of 3.0, 6.5, and 9.2 kb, of which the 3.0-kb band corresponds to the *Bam*HI fragment of H·Igε-11, the expressed C_ε gene in the 266B1 cell. *Eco*RI digestion of the placenta DNA also produced three distinct bands of 35, 30, and 10.5 kb. The results strongly suggest that three types of the C_ε gene are present in the human genome. The possibility was excluded that the extra bands might be due to cross-hybridization with the repetitive sequences flanking the C_ε probe (fragment c in Fig. 2) because the same three fragments hybridized with the C_ε-specific 580-bp *Ava* II fragment (fragment e in Fig. 4). We arbitrarily named the C_ε genes containing 3.0-, 6.5-, and 9.2-kb *Bam*HI fragments the $C_\varepsilon 1$, $C_\varepsilon 2$, and $C_\varepsilon 3$ genes, respectively.

On the other hand, the *Bam*HI digestion of the IgE myeloma DNA produced only two C_ε gene bands of 9.2 and 3.0 kb (Fig. 5, lane b). The $C_\varepsilon 2$ gene seems to be deleted from the IgE myeloma that expresses the $C_\varepsilon 1$ gene, suggesting that the order of the C_ε genes is 5′-$C_\varepsilon 2$-$C_\varepsilon 1$-$C_\varepsilon 3$-3′ unless the $C_\varepsilon 3$ gene is located on a different chromosome. The *Eco*RI digestion of the myeloma DNA yielded two C_ε gene bands of 37 and 10.5 kb, of which the 37-kb *Eco*RI fragment is likely to be the rearranged active C_ε gene—i.e., $C_\varepsilon 1$—because the expected *Eco*RI fragment of H·Igε-11 is larger than 17 kb (Fig. 2).

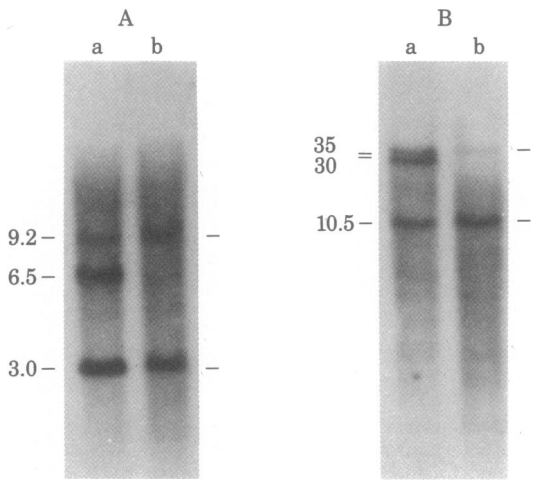

FIG. 5. Southern blot hybridization of human DNA with the C_ε probe. Human placenta and 266B1 DNAs were digested with *Bam*HI (A) or *Eco*RI (B). Southern blots were hybridized with the C_ε probe (fragment c in Fig. 2). Lanes a and b contain placenta and 266B1 DNAs, respectively. Numbers indicate sizes of hybridized bands in kb.

The 10.5-kb *Eco*RI fragment of the myeloma DNA is, therefore, derived from the $C_\varepsilon 3$ gene. The fragment was purified by agarose gel electrophoresis and ligated with λgt*WES* arms. By using the C_ε probe, one positive clone was obtained; it was named WES·H·Igε-31, and its insert was called H·Igε-31. The restriction map of H·Igε-31 is quite distinct from that of H·Igε-12 as shown in Fig. 2. The 4-kb *Sac* I fragment of H·Igε-31 hybridized with the $C_\varepsilon 1$-coding probe (fragment e in Fig. 4) and the mouse C_ε probe, albeit weakly. A preliminary study showed that H·Igε-31 does not contain sequences homologous to the S_ε region. The results lead us to suspect that the $C_\varepsilon 3$ gene (H·Igε-31) might be a pseudo-C_ε gene similar to a pseudo-C_γ gene found in the human genome (13).

To exclude the possibility that the multiple C_ε gene fragments are due to polymorphism of the human individual genome, we have analyzed DNAs of peripheral lymphocytes from seven unrelated individual Japanese donors, two cultured cell lines originated from Japanese patients, and two cultured myeloma cell lines originated from Caucasians. All of the DNAs examined contained the identical three restriction fragments (*Eco*RI or *Bam*HI) hybridizing to the C_ε probe, confirming that there are at least three C_ε genes, including the putative pseudogene in the human genome.

The significance of the multiple C_ε genes is not certain because duplication of this gene might be disadvantageous. One possibility is that the ancestral $C_\varepsilon 1$ gene was duplicated together with other C_H genes such as C_γ and C_α genes. Inactivation of the newly produced C_ε gene could be easily accomplished by deleting the S_ε sequence as observed in the $C_\varepsilon 3$ gene. It is also possible that only the $C_\varepsilon 3$ coding sequences might have been transposed to the region outside of the H chain gene complex. It should be noted that the intensity of the $C_\varepsilon 2$ band (*Bam*HI) is comparable to that of the $C_\varepsilon 1$ band, indicating that the $C_\varepsilon 2$ gene is highly homologous to the functional $C_\varepsilon 1$ sequence (Fig. 5*A*). It is of interest to know whether the $C_\varepsilon 2$ gene is functional or not.

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