Characterization and molecular cloning of the mRNA for the heavy (ε) chain of rat immunoglobulin E

(rat immunocytoma/primary structure/$C_ε 3$ domain)

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ABSTRACT We report a study of the mRNA for the heavy (ε) chain of rat IgE. Cytoplasmic RNA was prepared from the two rat immunocytomas IR2 and IR162 and fractionated by sucrose gradient centrifugation. An enriched fraction containing ≈5% mRNA for the ε chain was obtained in this way. When translated in vitro, it produced a 59,000-dalton polypeptide, which in the presence of a membrane fraction yielded a 90,000-dalton polypeptide, presumably through posttranslational modification. Both polypeptides were precipitated by rabbit antisera that were monospecific for rat ε chains. The ε chain mRNA was estimated to be ≈2200 nucleotides long and constitutes a minute fraction in the total mRNA both in the IR2 and the IR162 tumors, unlike the mRNA for light chains. Double-stranded cDNA copies prepared from the RNA fraction, which was enriched for ε chain mRNA, were inserted into the Pst I cleavage site of the pBR322 vector. Twenty clones with inserts exceeding 1000 base pairs were used for selection of mRNA from the IR2 tumor. By in vitro translation of the selected mRNA, one clone was identified that yielded a polypeptide with the same size as the unprocessed ε chain. The nucleotide sequence was determined for part of the inserted cDNA in this candidate clone and was found to be homologous to a sequence in the constant region (C) of the human ε chain. In this communication we report a sequence from the $C_ε 3$ domain of the rat IgE. When compared to the corresponding sequence of human IgE, 55% of the amino acids in the rat sequence were found to be conserved.

During the past decade, considerable attention has been focused on the genes which specify immunoglobulins. Thanks to the rapid development of recombinant DNA technology it has been possible to isolate immunoglobulin genes and to investigate their structure at the molecular level. It is now generally agreed that the genes which specify the variable (V) and constant (C) parts of immunoglobulins are rearranged during B-cell differentiation (1). Much is also known, particularly in the mouse system, about the locus that specifies the heavy chains for the different immunoglobulin classes (2–4). One class of immunoglobulins that has been studied in less detail so far is the IgE class, the reason being that IgE-secreting plasma cell tumors are rare and have been found in only two species, man and the LOU strain of rats (5, 6). IgE is of particular interest because it has been found to serve as the major mediator of immediate-type allergic reactions (7). Individuals with atopic allergy have an increased serum concentration of IgE, which serves as an important marker in clinical medicine. In order to improve our current understanding of allergic disorders, it is important to study the genetics of the IgE system in a suitable experimental animal. The rat has been successfully exploited as an experimental animal for studies of allergic reactions (8, 9). In this re-

port we describe the characterization and molecular cloning of the mRNA for the rat ε chain. The source of our material was two rat immunocytomas, which originally were isolated and characterized by Bazin and Beckers (5).

MATERIALS AND METHODS

Preparation of RNA. Two rat tumors, IR2 and IR162 (provided by H. Bazin), were propagated intraperitoneally in LOU/M rats. When the animals developed palpable tumors, they were killed and the tumors were excised and immediately processed. After two brief cycles of washing, the cells were disrupted in a Potter–Elvehjem homogenizer. The cytoplasmic fraction was freed of nuclei by low-speed centrifugation. Total, cytoplasmic RNA or the membrane-bound polysome fraction were prepared as described by Marcu et al. (10). RNA was finally extracted with phenol (11) and fractionated by oligo(dT)-cellulose chromatography prior to separation in a linear 15–30% sucrose gradient in 1 mM EDTA/0.2% NaDodSO₄/0.01 M Tris·HCl, pH 7.9, at 18°C.

In Vitro Translation and Immunoprecipitation. A rabbit reticulocyte lysate was used, and the procedure of Persson et al. (12) was followed. Protein A-Sepharose (Pharmacia) was used for collection of the immunoprecipitates.

Preparation of cDNA and Cloning. The procedure of Monahan et al. (13) was essentially followed. RNA, purified by sucrose gradient centrifugation, was used as a template for avian myeloblastosis virus reverse transcriptase (gift from J. Beard) using oligo(dT) as a primer. The synthesis was carried out at 42°C for 1 hr in the absence of actinomycin D. The RNA moiety of the resulting RNA·DNA hybrid was removed by treatment with 0.24 M NaOH at 42°C for 2 hr. The second strand of the cDNA copy was prepared with reverse transcriptase under the same conditions as those used for the first reaction, and the resulting hairpin was gently removed with S1 nuclease. Homopolymeric (dC) tails were added to the double-stranded cDNA with terminal transferase, and the cDNA preparation was fractionated in an exponential 5–30% sucrose gradient in the SW40 rotor at 35,000 rpm for 22 hr. Fractions containing cDNA with an estimated length of 1000 base pairs or more were pooled and concentrated by ethanol precipitation before insertion into the pBR322 vector. The vector was linearized by cleavage with endonuclease Pst I, and homopolymeric (dG) tails (10–15 nucleotides long) were added with terminal transferase. Uncleaved vector molecules were eliminated by sucrose gradient centrifugation. The cDNA was joined to the vector by hybridization, and the mixture was then used for transformation of the Escherichia coli K-12 strain 259. Transformants were isolated on tetracycline-containing plates.

Abbreviation: C, constant region.

Screening of Recombinant Clones. Plasmid DNA from individual recombinants was prepared by the method of Birnboim and Doly (14), and 20-μg samples were immobilized on nitrocellulose membrane discs with a diameter of 1 cm. A modification of the method of Harpold *et al.* (15) was used for RNA selection. The filters were washed 10 times at room temperature in 0.01 M Tris·HCl, pH 7.9/1 mM EDTA/0.5% NaDodSO$_4$ and once with hybridization buffer at 37°C for 30 min. The eluted RNA was translated in a rabbit reticulocyte lysate as described (12).

DNA Sequence Determination. The protocol of Maxam and Gilbert (16) was followed.

Gel Electrophoresis. Plasmid DNA or fragments were separated in 1% agarose or 5% polyacrylamide gels. RNA samples were treated with formaldehyde prior to separation in 1.0% agarose gels.

RESULTS

***In Vitro* Translation of mRNA from the Two Rat Immunocytomas IR2 and IR162.** Both the total cytoplasmic RNA and the membrane-bound polysome fraction were prepared and translated *in vitro*. For each RNA preparation, numerous polypeptide bands were observed when analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis. One band was very prominent and had the same electrophoretic mobility as the light chains of the respective IgE-producing tumor (Fig. 1). Analysis by immunoprecipitation confirmed that this band was precipitable with an antiserum monospecific for light chains (κ) of rat IgE (Fig. 1). No prominent bands within the expected size range of the rat ε chain were seen, indicating that either the mRNA is present in small amounts or that it is poorly translated *in vitro*. By immunoprecipitation with monospecific antisera against the rat ε chain, the presence of a 59,000-dalton band was revealed, however (Fig. 2, lane B).

The product, synthesized *in vitro*, that could be isolated by immunoprecipitation had an estimated molecular mass of 59,000 daltons in the case of both the IR2 and the IR162 immunocytomas in contrast to the *in vivo* synthesized counterpart, which migrated as an $\approx$90,000-dalton polypeptide in NaDodSO$_4$/polyacrylamide gel electrophoresis. This discrepancy was presumed to be caused by posttranslational modifications, which was confirmed by carrying out *in vitro* translations in the presence of membranes from dog pancreas (17). By immunoprecipitation it was shown that, under these conditions, a polypeptide was synthesized *in vitro* that comigrated with ε chains purified from ascites fluid.

Fractionation of mRNA. Total cytoplasmic RNA from the IR2 tumor and RNA from the membrane-bound polysome fraction of the IR162 tumor were separated by sucrose gradient centrifugation, and RNA samples from the individual fractions were analyzed by *in vitro* translation (Fig. 3). A polypeptide band corresponding to the light chain of IgE was translated from RNA in the 13S region of the gradient, both in the case of the IR2 tumor and of the IR162 tumor. This polypeptide had a molecular mass of 25,000 daltons when RNA from the IR2 tumor was analyzed, a molecular mass of 22,000 daltons in the case of the IR162 tumor, and was shown to consist of light chains by immunoprecipitation with a monospecific antiserum (data not shown). When fractionated RNA from the IR162 tumor was translated, an additional prominent polypeptide band appeared that was estimated to be 10,500 daltons (Fig. 3). This polypeptide apparently was translated from a separate mRNA that sedimented at 10 S and consisted of truncated light chains, as revealed by immunoprecipitation.

When the different RNA fractions were screened by *in vitro* translation, no prominent polypeptide band was observed in the
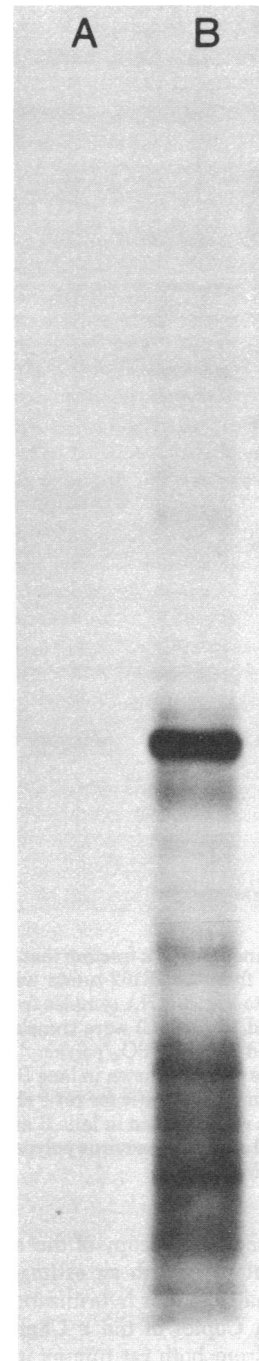

FIG. 1. Autoradiogram showing the *in vitro* translation of mRNA from the IR2 tumor (lane B). The total cytoplasmic RNA was extracted and translated *in vitro* in a rabbit reticulocyte cell-free system. The *in vitro* synthesized polypeptides were analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis. Part of the sample was immunoprecipitated by an antiserum monospecific for IgE light chains before analysis (lane A).

region of the unprocessed ε chain. However, after immunoprecipitation of the *in vitro* synthesized products from individual gradient fractions, it became apparent that a polypeptide band corresponding to the unprocessed ε chain was translated from a 20S mRNA (Fig. 2).

Cytoplasmic RNA from the IR2 myeloma also was fractionated on a 1.0% agarose gel under denaturing conditions in order to determine the size of the ε chain mRNA more accurately. The RNA was transferred to a nitrocellulose membrane (18) and
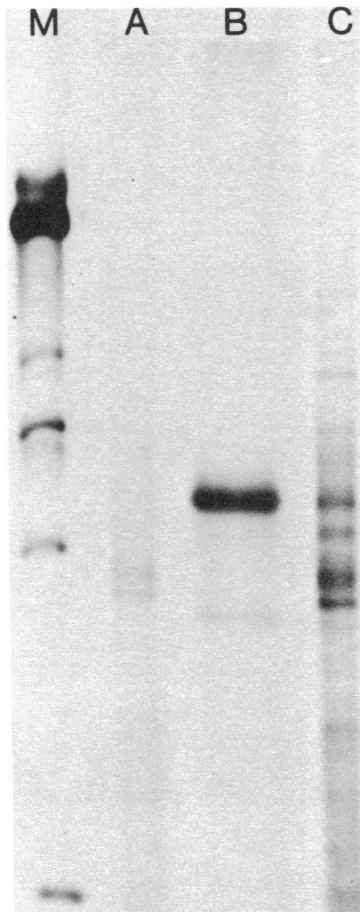
FIG. 2. *In vitro* translation of a fraction that had been enriched for ε chain mRNA. RNA from the IR162 tumor was fractionated as described in the legend to Fig. 3. RNA samples from fractions 12, (lane A), 12–15 (lane B) and 15 (lane C) were translated *in vitro*, and the products were analyzed by NaDodSO₄/polyacrylamide gel electrophoresis. The polypeptides that are shown in lane B were immunoprecipitated by an antiserum monospecific for rat ε chains. Approximately 20 times more sample was analyzed in lane B as compared to sample in lanes A and C. ³⁵S-Labeled adenovirus polypeptides were included as size markers (lane M).

hybridized to a cloned cDNA copy of the ε chain mRNA (see below). A prominent band with an estimated length of 2200 nucleotides was visualized after hybridization (Fig. 4).

Cloning of cDNA Copies of the ε Chain mRNA. *In vitro* translation of RNA from both rat tumors indicated that the ε chain mRNA is present in low amounts. Therefore, the poly(A)-containing RNA from the IR2 tumor was first fractionated by sucrose gradient centrifugation, and the fractions containing ε chain mRNA were identified by *in vitro* translation and pooled. Double-stranded cDNA copies were prepared as described before; cDNAs with a length exceeding 1000 nucleotides were prepared by sucrose gradient centrifugation and inserted into the *Pst* I cleavage site of the pBR322 vector. The resulting hybrids were transformed into the 259 strain of *E. coli* and yielded ≈1500 recombinants. Quick alkaline lysates (14) were prepared from 200 recombinants, and the sizes of the inserts were estimated by gel electrophoresis by using recombinants with known inserts as size markers. In this way, 20 recombinants with the largest inserts were selected for further analysis. Plasmid DNA was prepared from each recombinant and immobilized on nitrocellulose filters, which were used for selection of mRNA from the IR2 tumor. Out of the 20 analyzed clones, 1 was found to
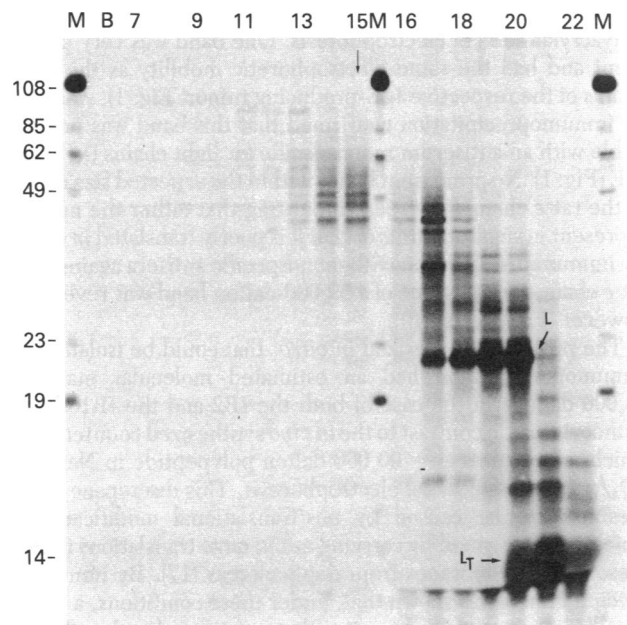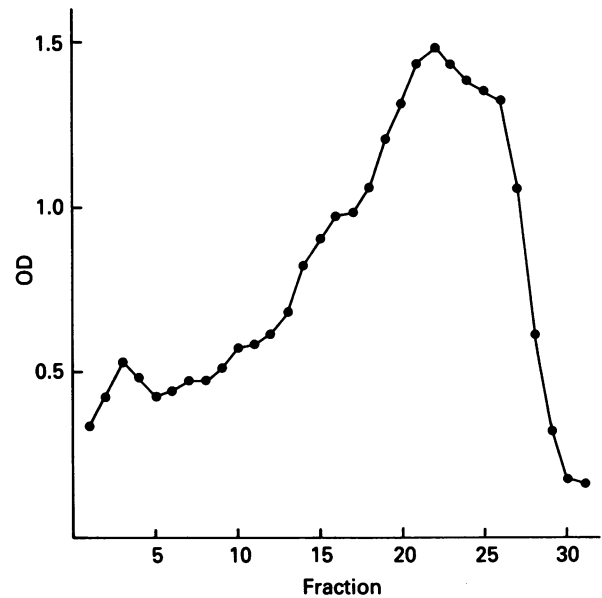

FIG. 3. Fractionation of RNA from the IR162 tumor in a 15–30% sucrose gradient. (*Upper*) Optical density profile. (*Lower*) Fractions in lanes 7–22 were analyzed by *in vitro* translation followed by NaDodSO₄/polyacrylamide gel electrophoresis. The polypeptide bands corresponding to light chains (L) and the truncated light chains (Lᴛ) are indicated. The fraction that contained the highest amount of ε chain mRNA is indicated by an arrow. ³⁵S-Labeled adenovirus polypeptides were included as size markers (lane M) and their molecular masses in kilodaltons are indicated. The lane numbers refer to different gradient fractions in *Upper*.

select mRNA that gave rise to a 59,000-dalton polypeptide upon *in vitro* translation (Fig. 5). This was regarded as a candidate clone, and the identity between the *in vitro* translated product and the ε chain was established by immunoprecipitation (data not shown).

Partial Sequence Analysis of the Insert Present in the Candidate Clone. The nucleotide sequence of the cDNA insert was studied by the strategy outlined in Fig. 6. A sequence covering 306 nucleotides was established and is shown in Fig. 7. The results revealed only one open translational reading frame

FIG. 4. Analysis of RNA from the IR2 tumor by electrophoresis. The RNA was denatured by formaldehyde before separation in a 1% agarose ge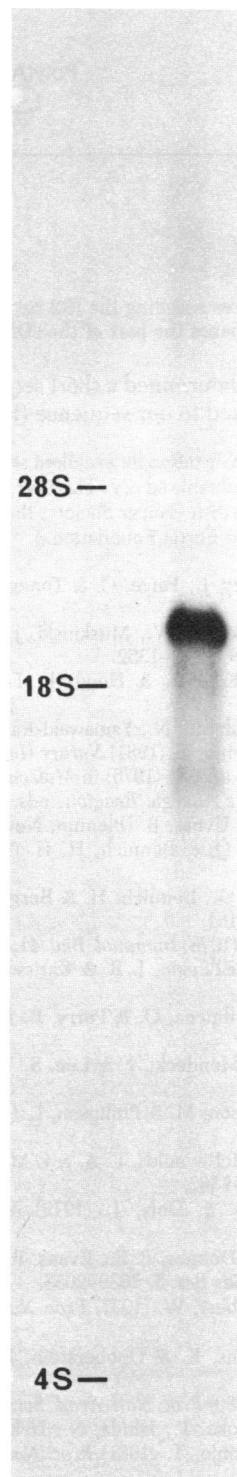l. The RNA was transferred to a nitrocellulose filter and hybridized to a ^{32}P-labeled cDNA clone that contained rat ε chain sequences. Unlabeled ribosomal and tRNA were used as size markers.

FIG. 5. Identification of a cDNA clone that contained sequences encoding the ε chain. Plasmid DNA was extracted from several recombinant clones and immobilized on nitrocellulose filters. RNA from the IR2 tumor was hybridized to the filters, and the hybridized RNA was eluted and used to program an *in vitro* protein-synthesizing system. Lanes: A, ^{35}S-labeled adenovirus polypeptides used as size markers; B, products that were synthesized with mRNA selected on a filter containing pBR322 DNA; C, clone-selected mRNA that gave rise to a polypeptide chain with the same electrophoretic mobility as unprocessed rat ε chains (arrow).

DISCUSSION

Although a wealth of information has accumulated during the past decade concerning the molecular mechanisms for generation of antibody diversity, our current understanding of immunopathological conditions in man is still very poor. In allergic reactions of the immediate type, antibodies of the IgE class are known to play an important role as mediators and are, hence, of considerable interest.

Our knowledge about the IgE system is very limited, primarily because IgE-secreting tumors are rare both in man and other mammals. Most of our current knowledge about the structure of IgE (20) stems from one specific human IgE-producing plasma cell tumor, IgE(ND), originally described by Johansson and Bennich (6). Later Bazin and coworkers (5) discovered the high incidence of immunocytomas in the LOU strain of rats and isolated the IgE-producing tumors IR2 and IR162, which were used in the present study. These provide interesting model systems for molecular analysis of the IgE system. In this report we describe the properties of the mRNA for the rat ε chain and its cloning in a bacterial vector. The clone, which was obtained with RNA from the IR2 myeloma, contains an approximately 1090-bp-long insert and covers most of the constant region of the ε chain, including the 3' noncoding part of the mRNA. In

which, when translated, would give rise to a sequence related to the known sequence for the $C_{\varepsilon}3$ domain of human IgE (20). A partial amino acid sequence also has been obtained for rat ε chains (unpublished data), which is in agreement with the sequence deduced from the clone. Fig. 7 shows a comparison between $C_{\varepsilon}3$ sequences of human, rat, and mouse (19) origin.
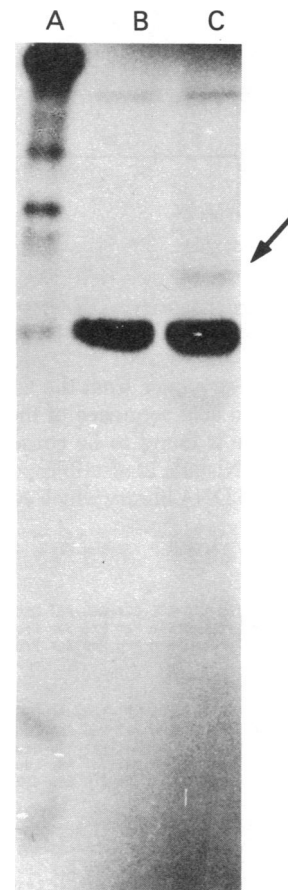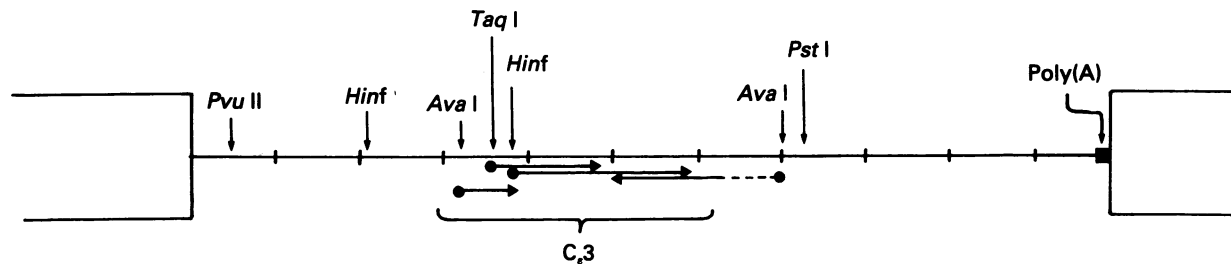
FIG. 6. A partial restriction enzyme cleavage map for the cDNA clone which contained sequences encoding the IR2 rat ε chain. The complete cDNA insert is 1090 base pairs. The horizontal arrows illustrate the strategy that was used to sequence the part of the cDNA shown in Fig. 7.

this study we report a sequence from the $C_\varepsilon 3$ domain. When compared to the amino acid sequence of the human ε chain, ≈55% of the sequence is found to be conserved. While this work was in progress, Nishida *et al.* (19) reported the isolation of a clone from a mouse DNA library which encodes the ε chain of the mouse. They determined a short sequence of their clone which is closely related to our sequence (Fig. 7).

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    1                                                          50
    :                                    Taq           :
                                          !
(A)CCC-CGG-GGT-GTG-ATT-ACC-TAC-CTG-ATC-CCA-CCC-AGT-CCC-CTC-GAC-CTG-TAT-GAA-
(B)Pro Arg Gly Val Ile Thr Tyr Leu Ile Pro Pro Ser Pro Leu Asp Leu Tyr Glu
(C)Pro Arg Gly Val Ser Ala Tyr Leu Ser Arg Pro Ser Pro Phe Asp Leu Phe Ile

                                                           100
        Hin f                                               !
(A)AAT-GGG-ACT-CCC-AAA-CTT-ACC-TGT-CTG-GTT-TTG-GAC-CTG-GAA-AGT-GAG-GAG-AAT-
(B)Asn Gly Thr Pro Lys Leu Thr Cys Leu Val Leu Asp Leu Glu Ser Glu Glu Asn
(C)Arg Lys Ser Pro Thr Ile Thr Cys Leu Val Val Asp Leu Ala Pro Ser Lys Gly
(D)            Lys Leu Thr Cys Leu Val Val Asp Leu Glu Ser Glu Lys Asn
(E)            AAG CTT ACC TGT CTG GTG GTG GAC CTG GAA AGC GAG AAG AAT

                                                           150
                                                            !
(A)ATC-ACC-GTG---------ACG-TGG-GTC-CGA-GAG-CGT-AAG-AAG-TCT-ATA-GGT-TCG-GCA-
(B)Ile Thr Val---------Thr Trp Val Arg Glu Arg Lys Lys Ser Ile Gly Ser Ala
(C)----Thr Val Asn Leu Thr Trp Ser Arg Ala Ser Gly Lys-----Pro Val Asn His
(D)Val Asn Val---------Thr Trp-----Asn Gln Glu Lys Lys Thr Ser Val Ser Ala
(E)GTC AAT GTG---------ACG TGG-----AAC CAA GAG AAG AAG ACT TCA GTC TCA GCA

                                                           200
                                                            !
(A)TCC-CAG-AGG-AGT-ACC-----AAG-CAC-CAT-AAT-GCC-ACA-ACC-AGT-ATC-----ACC-TCC-
(B)Ser Gln Arg Ser Thr-----Lys His Asn Ala Thr Thr Ser Ile-----Thr Ser
(C)Ser Thr Arg Lys Glu Glu Lys Gln Arg Asn-----Gly Thr Leu Thr Val Thr Ser
(D)Ser Glu Trp Tyr Thr Lys His His Asn Asn Ala Thr Thr Ser Ile-----Thr Ser
(E)TCC CAG TGG TAC ACT AAG CAC CAC AAT AAC GCC ACA ACT AGT ATC-----ACC TCC

                                                           250
                                                            !
(A)ATC-TTG-CCA-GTG-GAT-GCC-AAG-GAC-TGG-ATC-GAA-GGT-GAA-GGC-TAC-CAG-TGC-AGA-
(B)Ile Leu Pro Val Asp Ala Lys Asp Trp Ile Glu Gly Glu Gly Tyr Gln Cys Arg
(C)Thr Leu Pro Val Gly Thr Arg Asp Trp Ile Glu Gly Glu Thr Tyr Gln Cys Arg
(D)Ile Leu Pro Val Val Ala Lys Asp Trp Ile Glu Gly Tyr Gly Tyr Gln Cys Ile
(E)ATC CTG CCT GTA GTT GCC AAG GAC TGG ATT GAA GGC TAC GGC TAT CAG TGC ATA

                                                           300
                                                            !
(A)GTG-GAC-CAC-CCT-CAC-TTT-CCC-AAG-CCC-ATT-GTG-CGT-TCC-ATC-ACC-AAG
(B)Val Asp His Pro His Phe Pro Lys Pro Ile Val Arg Ser Ile Thr Lys
(C)Val Thr His Pro His Leu Pro Arg Ala Leu Met Arg Ser Thr Thr Lys
(D)Val Thr-----Pro Asp Phe Gln Pro Trp Arg
(E)GTG ACA-----CCT GAT TTC CAG CCA TGG CG
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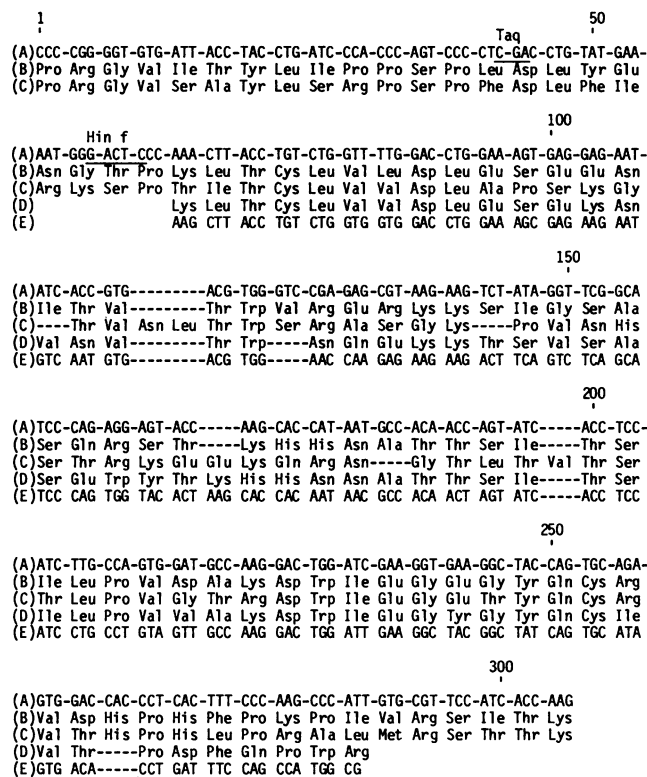
FIG. 7. An amino acid sequence from the $C_\varepsilon 3$ domain of rat IgE (row B) as determined from the DNA sequence (row A). A corresponding amino acid sequence from the human $C_\varepsilon 3$ domain (row C) is included as a reference, and partial amino acid (row D) and DNA (row E) sequence information from the mouse $C_\varepsilon 3$ domain is included for comparison (data from ref. 19). The first amino acid in the human sequence corresponds to amino acid 333 in the sequence of Bennich *et al.* (20). The gaps in the sequences were introduced to align them.

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