Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 7852–7856, December 1982
Immunology

Cloning and nucleotide sequence of mouse immunoglobulin ε chain cDNA

(immediate hypersensitivity/IgE/cell-free translation/cDNA cloning/protein primary structure)

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Communicated by Hans J. Müller-Eberhard, September 1, 1982

ABSTRACT **cDNA corresponding to mouse IgE heavy (ε) chain mRNA was cloned from mouse IgE-secreting hybridoma cells. A clone containing the ε cDNA insert was identified by hybridization to ε mRNA and subsequent translation *in vitro* to unprocessed ε chain reactive with anti-mouse IgE antibodies. This clone was used to select 20 other ε cDNA clones by colony hybridization. The clone containing the longest insert was selected and the ε cDNA insert was subjected to sequence analysis. The determined sequence is 1,279 nucleotides long and contains the coding regions for part of the constant region (C_ε)1 and all of the C_ε2, C_ε3, and C_ε4 domains and also the entire 3′ untranslated region of ε mRNA. When the amino acid sequence determined from the nucleotide sequence is compared to that of human ε chain, significant homologies between corresponding domains of the two ε chains are found, including conservations in cysteine and tryptophan residues and carbohydrate attachment sites.**

IgE has been established as the major class of antibody that mediates type I immediate hypersensitivity, in that allergic responses are associated with elevated levels of IgE antibodies specific for certain allergens (1). These IgE molecules bind to surface Fc receptors on mast cells and basophils and mediate the release of vasoactive amines and other pharmacologically active substances responsible for the clinical manifestation of hypersensitivity (2).

IgE also may play an important defense role against certain pathogens—e.g., parasites. Thus, both the total and specific IgE levels are dramatically elevated in certain parasitic infections and a striking correlation between the specific IgE antibody response and the development of immunity to reinfection has been demonstrated in the animal model (3).

Due to the intimate relatedness of IgE to the human allergic diseases and its possible role in the host defense, understanding the regulation of IgE production is a matter of great importance. Much effort has been directed toward this area of research and it has been found that IgE production is delicately controlled *in vivo*. The most enlightening observations have been that the level of IgE production is selectively controlled among other Ig classes. Thus, biologically active factors specifically regulating the IgE responses have been identified (reviewed in ref. 4). How the regulation is operating at the molecular level is awaiting exploration.

In recent years, significant advances have been made in understanding the genomic organization of Ig genes and their expression during lymphocyte differentiation. Most recently, the IgE heavy (ε) chain gene has been isolated and its position in the genome relative to other Ig genes has been established (5, 6). The differentiation of B cells into IgE-producing cells can be related to the rearrangement of the heavy chain variable (V) region gene initially proximal to the IgM heavy (μ) chain gene to a position next to the ε chain gene. One of the keys to understanding the regulation of IgE synthesis may then lie in the regulation at the DNA level in this class switching event.

Another important area of research for understanding the allergic responses is the establishment of the structure–function relationship of IgE molecules. Although the complete amino acid sequence of a human myeloma ε chain has been known for some time (7), the precise determinant involved in the binding of IgE to Fc receptors still has not been established. Once this determinant is defined, one can perhaps design effective therapeutic agents for allergy, acting at the level of inhibiting the binding of IgE. It appears that isolation of cDNA corresponding to mRNA of the ε chain would provide us with not only the protein sequence of ε chain but also a tool to explore the possibility of expressing in prokaryotic cells or chemically synthesizing biologically active fragments of ε chain of predetermined sequences.

Therefore, for the purpose of studying expression and structure of IgE, we have initiated the cloning of mouse ε chain cDNA. In this communication, we report the results of this cloning study, including the complete nucleotide sequence of an isolated cDNA that encodes most of the constant region (C_ε) of the ε chain.

MATERIALS AND METHODS

Cell Line. Murine anti-dinitrophenyl IgE hybridoma (H1-DNP-ε-26.82) was constructed previously (8) and maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum.

Preparation of RNA. RNA was isolated by a procedure adapted from that of Marcu *et al.* (9). Polyadenylylated RNA was isolated by oligo(dT)-cellulose chromatography and then was fractionated on a linear 15–30% sucrose gradient. Fractions were tested individually in the *in vitro* translation system described below and those fractions enriched in ε mRNA were pooled.

***In Vitro* Translation and Immunoprecipitation.** The mRNA was translated in the rabbit reticulocyte lysate system (New England Nuclear). For immunoprecipitation, the translation products were mixed with rabbit anti-mouse IgE (RAME) antibodies conjugated to Sepharose 4B (8) suspended in 0.5% bovine serum albumin in phosphate-buffered saline at pH 7.2 for 1 hr at 4°C. The beads were washed three times with the above saline and two times with 0.0625 M Tris·HCl at pH 6.8, and the bound polypeptides were released by boiling in 0.0625 M Tris·HCl, pH 6.8/10% glycerol/2% $NaDodSO_4$/5% 2-mercap-

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Abbreviations: ε chain, heavy chain of IgE; C_ε, constant region of ε chain; RAME, rabbit anti-mouse IgE; μ chain, heavy chain of IgM; CHO, carbohydrate.

toethanol for 1.5 min. Translation products and immunoprecipitates were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis by using the Laemmli system (10).

Preparation of Duplex cDNA for Cloning. Preparation of cDNA from mRNA with avian myeloblastosis virus reverse transcriptase, second-strand synthesis with DNA polymerase, nuclease S1 digestion, selection of the longest cDNA by fractionation on a column of Bio-Gel A-150m, addition of oligo(dC) tails and hybridizing of the tailed duplex cDNA with *Pst* I-cut and oligo(dG)-tailed pBR322 were performed essentially as described (11).

Transformation and Screening of Recombinant Bacteria. The hybrid DNA was used to transform calcium-shocked *Escherichia coli* strain C600 (12). Transformants were isolated on tetracycline-containing plates. ε cDNA clones were selected by colony hybridization (13) with ^{32}P-labeled cDNA made from the sucrose gradient-enriched ε mRNA by reverse transcription and hybridization selection and translation (14).

Characterization of ε cDNA. Restriction endonuclease sites within the inserts were identified and used to generate fragments that were subjected to nucleotide sequence analysis following the procedure of Maxam and Gilbert (15).

RESULTS

Identification of a Polypeptide Synthesized by *in Vitro* Translation of ε mRNA. The cloning of ε cDNA described in this report relied heavily on the *in vitro* translation of ε mRNA. When total polyadenylylated RNA isolated from IgE hybridoma cells was translated, numerous polypeptides were produced. However, no prominent band within the expected size range of ε chain was apparent on $NaDodSO_4$/polyacrylamide gels. By immunoprecipitation with RAME antibodies, a M_r 62,000 polypeptide was identified as the *in vitro* synthesized ε chain. This *in vitro* translation assay was used to identify fractions enriched in ε mRNA from the sucrose density gradient fractionation of crude polyadenylylated RNA. Fig. 1 presents a gel electrophoresis display of the *in vitro* translation products of the sucrose density gradient-enriched ε mRNA (lane b). The immunoprecipitates by RAME of the total translation products are shown in lane c. From the quantity of RAME-precipitable translation products, the purity of the enriched ε mRNA was estimated to be 5%.

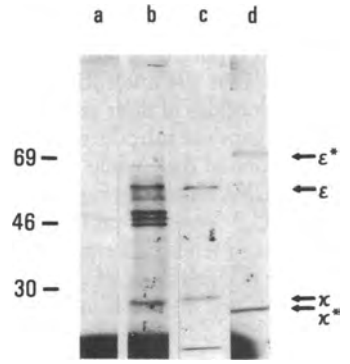

FIG. 1. Fluorograph of [^{35}S]methionine-labeled polypeptides synthesized by *in vitro* translation of mRNA (lanes a–c) or synthesized *in vivo* (lane d) and electrophoresed on 10% polyacrylamide gels. Lanes: a, no added mRNA; b, total translation products of sucrose density gradient-purified mRNA; c, polypeptides precipitated from translation products (lane b) by RAME antibodies conjugated to Sepharose 4B; d, RAME-Sepharose 4B-immunoprecipitated polypeptides from the supernatant of IgE-secreting hybridoma (H1-DNP-ε-26.82) cultured in the presence of [^{35}S]methionine. ε, ε*, κ, and κ* designate unprocessed and processed ε chain and unprocessed and processed κ chain, respectively. Molecular weights are shown as $M_r \times 10^{-3}$.

The ε chain polypeptide synthesized *in vitro* has an apparent molecular weight that is significantly lower than the *in vivo* synthesized product (M_r 72,000; Fig. 1, lane d). This difference most likely reflects the lack of glycosylation [the carbohydrate content of the mature mouse hybridoma IgE is 13% (8)] and other post-translational modifications of the polypeptides synthesized in the rabbit reticulocyte lysate system. Indeed, *in vitro* translation of ε mRNA in the presence of dog pancreatic microsomal membranes, which facilitates processing of the translation products (16), yielded a polypeptide that comigrated with ε chain synthesized *in vivo* (data not shown).

Another polypeptide synthesized *in vitro* from the partially purified ε mRNA was the light chain of IgE (Fig. 1, lane c). This light chain product migrated more slowly than the *in vivo*-produced polypeptide in $NaDodSO_4$/polyacrylamide gels (Fig. 1, lane d). Again, this is due to the lack of post-translational modification, because inclusion of microsomal membranes in *in vitro* translation resulted in a proper processing of the translation product (data not shown).

Molecular Cloning and Identification of ε cDNA Clones. From 20 ng of duplex cDNA, approximately 1,800 tetracycline-resistant and ampicillin-sensitive bacterial transformants were obtained. The number of potential ε cDNA clone candidates was decreased to about 300 by a screening procedure employing [^{32}P]cDNA derived from the partially purified ε mRNA as a hybridization probe. Hybridization selection and translation was used to identify an ε cDNA clone. In this procedure, plasmids from bacterial clones were bound to nitrocellulose filters and hybridized to total polyadenylylated RNA from IgE hybridoma cells; the hybridized mRNA then was eluted and translated *in vitro* and the products were analyzed by gel electrophoresis. The presence of a polypeptide comigrating with previously identified *in vitro*-synthesized ε chain indicated the presence of an ε cDNA clone. Immunoprecipitation of the polypeptide so obtained with RAME antibodies definitively established the identity of the ε cDNA clones.

To screen many clones in one assay, six pools of five clones each were screened first and one pool was found to be positive. Clones from this pool then were subjected individually to the same analysis and one *bona fide* ε cDNA clone was finally identified. As demonstrated in Fig. 2, DNA from this clone hybridized to a mRNA that could be translated to a polypeptide (lane d) with a mobility on the gel equal to unprocessed ε chain (lane g). Furthermore, the translated polypeptide was precipitated by RAME antibodies (lane h) and not by antibodies to mouse IgG (not shown). Polypeptides translated from mRNA hybridized with two other irrelevant clones (lanes e and f) were not precipitated by RAME (lanes i and j, respectively), demonstrating the specificity of the immunoprecipitation reactions.

The ε cDNA insert was excised from the plasmid by *Pst* I digestion and was used as a probe to hybridize with colonies of all 300 clones. Twenty clones hybridized strongly and were analyzed further. *Pst* I-digested plasmids obtained from these clones were analyzed on 1% agarose gels. Most of these clones yielded a single insert fragment with a size of 800–900 base pairs. One clone gave two fragments with sizes of *ca.* 900 and 400 base pairs. This clone ($C^2$30) was selected for further analysis.

Nucleotide Sequence of the Cloned ε cDNA. The nucleotide sequence of the ε cDNA $C^2$30 was determined and a sequence of 1,279 nucleotides was established as shown in Fig. 3. A single, long open reading frame was found and the amino acid sequence predicted from the nucleotide sequence is very homologous to the known human ε chain sequence (7) with which it is compared in Fig. 3. The mouse ε chain that was subjected to sequence analysis apparently includes about half of the $C_\varepsilon 1$

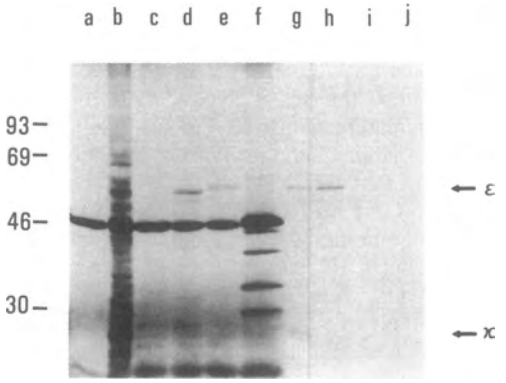

FIG. 2. Identification of an ε cDNA clone. Fluorograph of [^{35}S]-methionine-labeled polypeptides synthesized by *in vitro* translation of mRNA and electrophoresed on 10% polyacrylamide gels. In the hybridization-selection-translation procedure, plasmid DNA was bound to nitrocellulose filters and hybridized to total polyadenylylated RNA from the IgE hybridoma cells essentially as described (14). After extensive washing of the filters, the bound RNA was eluted as described (14) and then translated. Lanes: a, no added mRNA; b, total translation products of crude polyadenylylated RNA from IgE hybridoma cells; c–f, translation products from mRNAs hybrid-selected by recombinant plasmids (lane c, background control; lanes d–f, each with recombinant plasmid from one clone); g, h, i, and j, RAME-Sepharose 4B immunoprecipitated translation products from lanes b, d, e, and f, respectively. The positions of the ε chain (ε) and the light chain (κ) are indicated. Molecular weights are shown as $M_r \times 10^{-3}$.

domain (residues 164–224) and the complete $C_\varepsilon 2$ (residues 225–330), $C_\varepsilon 3$ (residues 331–437), and $C_\varepsilon 4$ (residues 438–547) domains. The boundary demarcation of each domain was guided by comparison with the known junctional sequences of mouse μ chain (18), which has a significant homology with ε chain. The cloned ε cDNA C^{2}30 also contains 93 nucleotides of 3′ untranslated region, which is comparable in length to that of mouse γ_1 and γ_{2b} mRNA (19, 20) and a poly(A) tail (19 nucleotides). The typical A-A-T-A-A-A sequence is found 14–19 bases before the poly(A) addition site.

DISCUSSION

The present study describes the isolation and structural characterization of a bacterial clone containing a DNA sequence corresponding to ε mRNA. The identification of this clone was based on its hybridization to a mRNA species that, when translated *in vitro*, directs the synthesis of a polypeptide with a mobility on $NaDodSO_4$/polyacrylamide gels of unprocessed ε chain and that is reactive with anti-mouse IgE antibodies.

A significant homology exists between the amino acid sequence of mouse ε chain (determined from the nucleotide sequence) and human ε chain. Homologies of 36%, 47%, and 51% between $C_\varepsilon 2$, $C_\varepsilon 3$, and $C_\varepsilon 4$ domains, respectively, were found. However, these values are significantly lower than those between constant region domains of mouse and human μ (21), γ (22), or α (23) chains. This is especially true of the COOH-terminal domains where homology of 78% was found between mouse and human μ (21) and α (23) chains. A number of gaps placed in the mouse and human ε sequences to maintain the homology alignment also may indicate extensive divergence. It is interesting to note that the gradient of homology from the NH_2-terminal to the COOH-terminal region as found for C_μ also is apparent in C_ε—that is, the COOH-terminal portion of the constant region appears more conserved than the NH_2-terminal portion, perhaps relating to the stronger selective constraints on the more COOH-terminal domains. This might be expected because the $C_\varepsilon 3$ and $C_\varepsilon 4$ domains have been implicated in the binding of IgE to the Fc receptors on mast cells and basophils (7).

Both the amino acids tryptophan and cysteine are believed to be important for maintaining the domain structure of immunoglobulins. Similar to other classes of immunoglobulins (21, 24), both of these residues are highly conserved in the ε chain. Of the nine tryptophan residues identified in the mouse ε chain, seven are conserved in the human counterpart. Similarly, all cysteine residues known to be involved in the $C_\varepsilon 2$, $C_\varepsilon 3$, and $C_\varepsilon 4$ intradomain disulfide bonds as well as the two involved in interheavy chain disulfide bridges are conserved (7). One extra cysteine residue is present in the $C_\varepsilon 2$ domain of the mouse chain. Whether there is an additional interheavy chain disulfide bridge in mouse IgE has yet to be determined.

Potential carbohydrate (CHO) attachment sites were revealed from the amino acid sequence. Six CHO attachment sites have been identified in the human ε chain and five of these are in the region where the mouse ε chain sequence has been determined in this study (7). The sequence Asn-X-Ser/Thr is found in identical positions in both the human and mouse ε chains at amino acid residues 173, 371, and 394. The sequence Asn-Glu-Ser is found in the mouse ε chain at amino acid residue 217 (see Fig. 3), which is near a CHO attachment site in the human ε chain (amino acid residue 219). Although the precise position varies between species, it appears that a glycosylation site in this region is biologically significant.

The amino acid sequence determined from the nucleotide sequence of the mouse ε chain has six additional amino acids at its COOH terminus as compared to the human ε chain. Interestingly, this COOH-terminal sequence is homologous to part of the COOH-terminal segment of the mouse μ chain (21) (Fig. 4). In particular, a potential CHO attachment site is present corresponding to the CHO attachment site already identified on the μ chain. Mouse C_γ genes code for a COOH-terminal lysine that is apparently removed proteolytically after translation (19, 20). Whether the mouse ε COOH-terminal sequence also is removed post-translationally and the significance of this segment remain to be determined. Furthermore, a potential RNA splicing site, T-G/G-T-A-A-C, is present at the junction of the $C_\varepsilon 4$ domain and the COOH-terminal segment. This site may permit the $C_\varepsilon 4$ coding region to be associated with a sequence coding for a membrane-bound form of the ε chain, as already demonstrated for μ and δ chains (25, 26).

The nucleotide sequence determined in this study is completely identical to that reported by Nishida *et al.* (6) for cloned genomic ε DNA at the first 194 nucleotides of their sequence. The remaining 23 nucleotides of their sequence can be aligned with ours (762–794) if gaps are introduced into their sequence. It also should be mentioned that, while this manuscript was in preparation, a rat ε cDNA clone was reported and the nucleotide sequence for the $C_\varepsilon 3$ domain of rat IgE was described (27). In the region where a sequence difference was found between our studies and those of Nishida *et al.* (6), there are only two base differences (G→C and T→C) at nucleotide numbers 772 and 774, respectively (Fig. 3), between mouse and rat ε cDNA, resulting in an amino acid change of Asp→His.

The cloned ε cDNA will surely be a useful probe for studying regulation of ε gene expression. A natural extension of this study would be the expression in bacteria of restriction fragments of the ε cDNA corresponding to various regions of the ε chain or the chemical synthesis of relevant regions. Identification of expression products or synthetic peptides with binding activity to mast cells would allow us to establish the peptide sequence in the ε chain that is involved in the binding to Fc receptors and, perhaps in the future, to artificially modulate immediate hypersensitivity reactions.

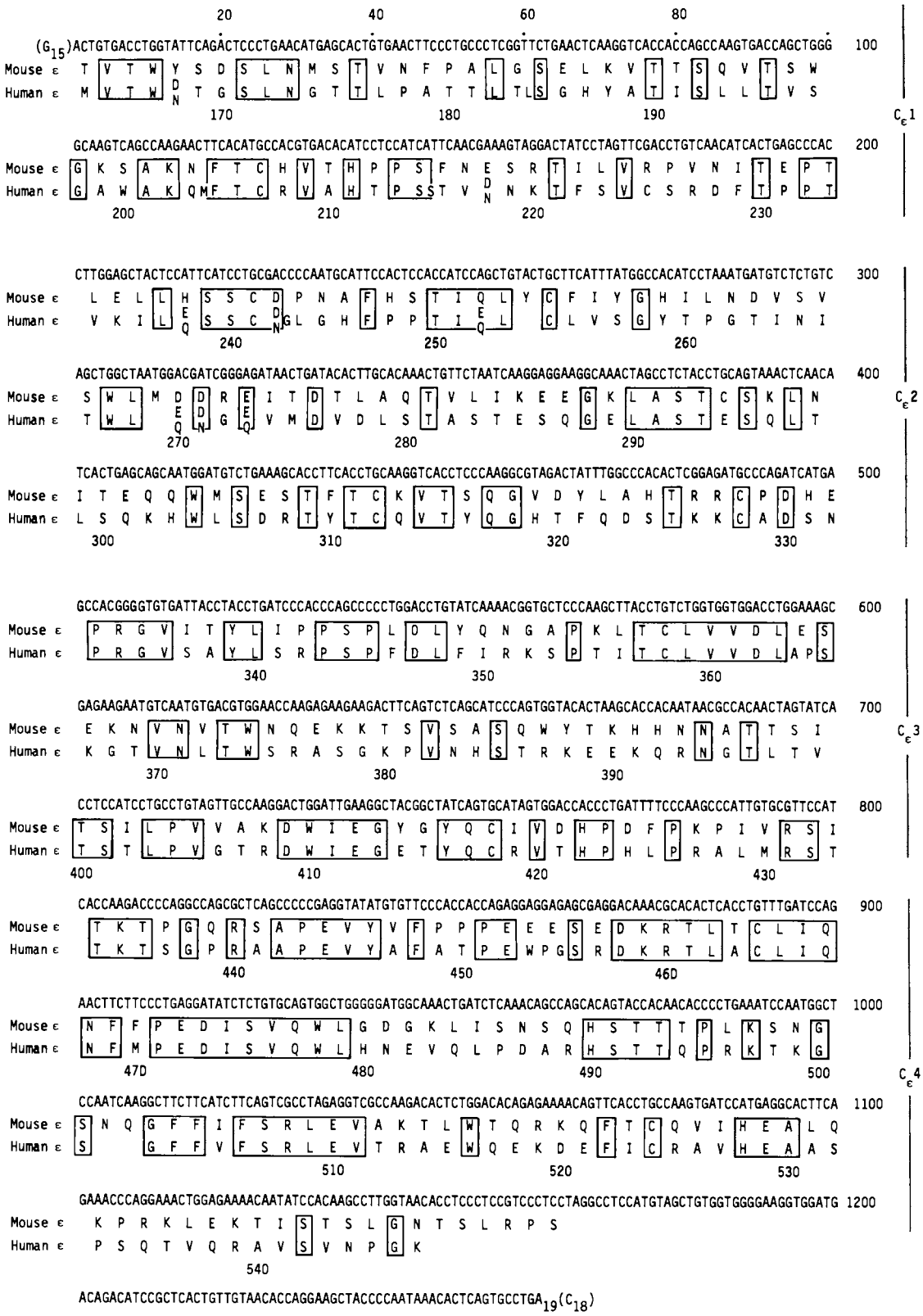

FIG. 3. Complete nucleotide sequence of cloned ε cDNA ($C^2$30). The entire cDNA insert was subjected to sequence analysis on both strands with the exception of a segment (nucleotides 1,229–1,260) that was subjected to sequence analysis on only one strand; 99.8% was subjected to sequence analysis more than once on one or both strands. The coding strand is inserted in the opposite orientation as the ampicillin resistance gene of pBR322 (17). The nucleotide sequence of the strand corresponding to the mRNA is displayed 5′ to 3′. The amino acids predicated from the nucleotide sequence are presented below the coding sequence. The amino acid sequence of human ε chain is shown below the mouse ε chain sequence. Boxes around the amino acid residues indicate identity between two sequences. Numbers below the amino acid residues refer to human ε chain (7). Protein domains are approximately demarcated in the right margin. Amino acids are expressed by a one-letter code as follows: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

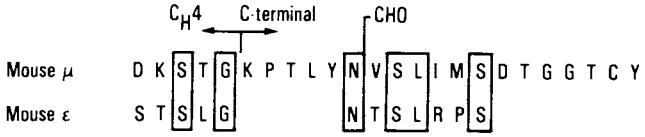

FIG. 4. The COOH-terminal (C-terminal) sequences of mouse μ and ε chains. A gap is placed in the ε chain sequence for a maximal homology with the μ chain. Homologous sequences are boxed.

We thank Dr. Robert Milner for help on the cloning procedures, Dr. Michael Wilson, Dr. Hans Spiegelberg, and Mary Ann Brow for helpful discussions, Dr. Ian Kennedy for critically reading the manuscript, Phil Van Hook for technical assistance, and Beverly Burgess for preparing the manuscript. This work was supported by National Institutes of Health Grant AI 19476. F.-T.L. is a Scholar of the Leukemia Society of America. This is publication no. 3 from the Medical Biology Institute, La Jolla, CA, and publication no. 2724 from the Research Institute of Scripps Clinic, La Jolla, CA.

1. Ishizaka, K., Ishizaka, T. & Hornbrook, M. M. (1966) *J. Immunol.* **97**, 840–853.
2. Bach, M., ed. (1978) *Immediate Hypersensitivity* (Dekker, New York).
3. Capron, A., Dessaint, J. P. & Capron, M. (1980) *J. Allergy Clin. Immunol.* **66**, 91–96.
4. Katz, D. H. (1982) in *Progress in Allergy*, ed. Ishizaka, K. (Karger, Basel, Switzerland), Vol. 32, p. 105.
5. Shimizu, A., Takahashi, N., Yamawaki-Kataoka, Y., Nishida, Y., Kataoka, T. & Honjo, T. (1981) *Nature (London)* **289**, 149–153.
6. Nishida, Y., Kataoka, T., Ishida, N., Nakai, S., Kishimoto, T., Bottcher, I. & Honjo, T. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1581–1585.
7. Dorrington, K. J. & Bennich, H. H. (1978) *Immunol. Rev.* **41**, 3–25.
8. Liu, F.-T., Bohn, J. W., Ferry, E. L., Yamamoto, H., Molinaro, C. A., Sherman, L., Klinman, N. R. & Katz, D. H. (1980) *J. Immunol.* **124**, 2728–2737.
9. Marcu, K. B., Valbuena, O. & Perry, R. P. (1978) *Biochemistry* **17**, 1723–1733.
10. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–689.
11. Gough, N. M., Webb, E. A., Cory, S. & Adams, J. M. (1980) *Biochemistry* **19**, 2702–2710.
12. Tanaka, T. & Weisblum, B. (1975) *J. Bacteriol.* **121**, 354–362.
13. Grunstein, M. & Hogness, D. S. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3961–3965.
14. Parnes, J. R., Velan, B., Felsenfeld, A., Ramanathan, L., Ferrini, U., Appella, E. & Seidman, J. G. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2253–2257.
15. Maxam, A. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560.
16. Scheele, G., Dobberstein, B. & Blobel, G. (1978) *Eur. J. Biochem.* **82**, 593–599.
17. Sutcliffe, J. G. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 77–90.
18. Kawakami, T., Takahashi, N. & Honjo, T. (1980) *Nucleic Acids Res.* **8**, 3933–3945.
19. Honjo, T., Obata, M., Yamawaki-Kataoka, Y., Kataoka, T., Kawakami, T., Takahashi, N. & Mano, Y. (1979) *Cell* **18**, 559–568.
20. Tucker, P. W., Marcu, K. B., Newell, N., Richards, J. & Blattner, F. R. (1979) *Science* **206**, 1303–1306.
21. Kehry, M., Sibley, C., Fuhrman, J., Schilling, J. & Hood, L. E. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2932–2936.
22. Adetugbo, K. (1978) *J. Biol. Chem.* **253**, 6068–6075.
23. Robinson, E. A. & Appella, E. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4909–4913.
24. Lin, L.-C. & Putnam, F. W. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 504–508.
25. Rogers, J., Early, P., Carter, C., Calame, K., Bond, M., Hood, L. & Wall, R. (1980) *Cell* **20**, 303–312.
26. Tucker, P. W., Liu, C.-P., Mushinski, J. F. & Blattner, F. R. (1980) *Science* **209**, 1353–1360.
27. Hellman, L., Pettersson, U. & Bennich, H. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1264–1268.