Characterization and Expression of the Gene for the Human Fc Receptor γ Subunit

DEFINITION OF A NEW GENE FAMILY*

Helmut Küster‡, Helen Thompson‡, and Jean-Pierre Kinet

From the Arthritis and Rheumatism Branch, National Institute of Arthritis, Musculoskeletal, and Skin Diseases and the §Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892

The high affinity IgE receptor, Fc,RI, is one of the key molecules involved in allergic reactions. It is a tetrameric complex ($\alpha$βγδ). The γ chains from Fc,RI are also subunits of other Fc receptors.

We have isolated, characterized, and sequenced the gene for the human γ chain of Fc,RI. It consists of five exons and spans 4 kilobases. The leader sequence is encoded by two exons, the second of which also contains the short extracellular domain, the hydrophobic transmembrane region, and the beginning of the cytoplasmic tail. Three short exons encode the remaining of the polypeptide and the 3' untranslated flanking sequence. The transcription initiation sites have been mapped.

Comparison between the gene structures of the γ chain of the Fc receptor and of the $\gamma$ chain of the T-cell receptor indicates that these genes have evolved from a common ancestor by duplication and that they define a new gene family. In addition to being localized on the same chromosome, both genes show an analogous organization of their exons. A high level of homology is found in three of their respective exons, and the splice sites between them are identical. Furthermore, γ and $\gamma$ chains are essential for surface expression of their respective receptors. Therefore, γ chains of Fc receptors and $\gamma$ chains of T-cell receptors may also define a new family of functionally related polypeptides.

Expression studies in COS cells show that the human γ chain alone is sufficient to achieve expression of the human α chain on the cell surface, whereas both β and γ chains are required for the surface expression of the rodent α chain.

The binding of immunoglobulins to Fc receptors mediates important functions of the immune defense system such as phagocytosis and antibody-dependent cytotoxicity. One of these Fc receptors, Fc,RI, triggers cellular degranulation and the release of potent mediators of the allergic reaction (1, 2). The same receptor also induces the secretion of important lymphokines (3-5). With the exception of Fc,RI, Fc receptors were, until recently, thought to consist of a single polypeptide.

However, Fc,RI is a tetrameric complex of one α, one β, and two identical disulfide-linked γ chains. Recently, we found (6) that one of the Fc receptors on mouse macrophages, Fc,RIIa, is not a single-chain receptor but consists of a multisubunit complex with γ subunits identical to those from Fc,RI. Furthermore, the Fc receptor on human natural killer cells (Fc,RIII-2) appears to be also associated with γ subunits (7).

Here we characterize the genomic structure of the human gene for γ chains including its exon-intron borders and transcription initiation sites. We compare the genomic structures of the γ chain and of the $\gamma$ chain of the T-cell receptor and demonstrate that gene duplication occurred during evolution to generate a new gene family. Therefore, the homologies previously detected between γ and $\gamma$ chains are significant and not simply due to exon shuffling as it is observed, for example, between the low density lipoprotein receptor and complement factor C9 (8).

It had been generally assumed that the requirements for cell-surface expression are identical between species. We have recently shown (9) that surface expression of rodent Fc,RI requires cotransfection of the three genes for α, β, and γ chains, whereas expression of the human α chain on the cell surface may be achieved by cotransfecting the human α chain with the rodent γ chain alone without apparent need for the β chain. Therefore, we suggested that the chimeric nature of the receptor in those experiments could be responsible for the differences observed between rodent and human α chains. Here we demonstrate that this is not the case. Rodent and human Fc,RI receptors are different in their requirements for surface expression. Whereas both species need γ chains, only the rodent receptor also requires β chains.

EXPERIMENTAL PROCEDURES

Extraction of Human Basophil RNA—Basophils were isolated from venous blood following leukopheresis of 2 liters of blood (National Institutes of Health Blood Bank) by single-step isopycnic banding on Percoll (10). Human (4 ml) polymorphonuclear cells were layered onto discontinuous Percoll gradients made from solutions with densities of 1.068, 1.079, and 1.079 g/ml. Final adjustment of density was facilitated by measurement of the refractive index at 22 °C with a Bausch & Lomb refractometer. After centrifugation at 700 × g for 25 min at 22 °C, most of the basophils were found in the band at a density of 1.070-1.079 g/ml. Purification ranged from 5 to 15% with a yield of 1.5 ± 0.4 × 10⁸ basophils/leukopheresis pack. The cDNA library was constructed as described before (9).

Screening and Isolation of Genomic and cDNA Clones—The 5' and 3' ends of the open reading frame of the rat Fc,RI γ subunit cDNA (bp 1-156 and 157-253 of a HincII digest, respectively) (11) were

1 The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) J05285.

‡ To whom correspondence should be addressed: Dept. of Health and Human Services, Bldg. 10, Rm. 9N-256, NIH, Bethesda, MD 20892. Tel.: 301-496-1565.
Gene for γ Subunit of Fc Receptors

RESULTS AND DISCUSSION

Genomic Cloning—A 33-kb genomic clone was isolated from a human leukocyte genomic library by screening with rat γ subunit cDNA probes. Two restriction fragments were generated from this clone. One of these hybridized with a probe corresponding to the 5' end of the rat γ chain, and the other hybridized with a 3' end-containing probe. Both fragments were entirely sequenced (Fig. 1). The organization of the human γ subunit gene was determined from consensus sequences for exon-intron boundaries (14). The transcription initiation sites were mapped using primer extension assays (Fig. 2) and confirmed by S1 nuclease protection assay (data not shown). These experiments showed the major start site to be 25 bp upstream of the start codon. Indeed, this site corresponds to the beginning of one of the cDNA clones that we isolated (see below). Other potential initiation sites are represented by negative numbers. The start and stop codons, the polyadenylation signal, and intron/exon boundaries are presented by upper-case letters and introns by small letters, respectively (Fig. 1). The start codon as position +1; nucleotides preceding it are represented by negative numbers. The start and stop codons, the polyadenylation signal, and the CAAT and the GC boxes are in boldface. Exons are indicated by underlined upper-case letters and introns by lower-case letters. The major transcription initiation site is shown as an arrow. The first exon of the human γ subunit gene, as shown has now been sequenced. The complete sequence of the gene has been submitted to GenBank. Bases which could not be assigned with certainty are denoted by n.

---

Gene for γ Subunit of Fc Receptors

RESULTS AND DISCUSSION

Genomic Cloning—A 33-kb genomic clone was isolated from a human leukocyte genomic library by screening with rat γ subunit cDNA probes. Two restriction fragments were generated from this clone. One of these hybridized with a probe corresponding to the 5' end of the rat γ chain, and the other hybridized with a 3' end-containing probe. Both fragments were entirely sequenced (Fig. 1). The organization of the human γ subunit gene was determined from consensus sequences for exon-intron boundaries (14). The transcription initiation sites were mapped using primer extension assays (Fig. 2) and confirmed by S1 nuclease protection assay (data not shown). These experiments showed the major start site to be 25 bp upstream of the start codon. Indeed, this site corresponds to the beginning of one of the cDNA clones that we isolated (see below). Other potential initiation sites are represented by negative numbers. The start and stop codons, the polyadenylation signal, and the CAAT and the GC boxes are in boldface. Exons are indicated by underlined upper-case letters and introns by lower-case letters. The major transcription initiation site is shown as an arrow. The first exon of the human γ subunit gene, as shown has now been sequenced. The complete sequence of the gene has been submitted to GenBank. Bases which could not be assigned with certainty are denoted by n.
The complete gene spans 4 kb, contains five exons, and has been entirely sequenced (Fig. 1). The first exon contains the entire 5' untranslated region and most of the leader peptide. It is followed by a large intron of 2.6 kb, which represents 67% of the total gene and contains Alu sequences. The second exon encodes the last 5 base pairs of the leader peptide, the short extracellular domain, the transmembrane domain, and the first 9 base pairs of the intracellular portion. It is followed by two short exons of 21 and 21 base pairs. Introns 2 to 4 span 163, 424, and 157 bp, respectively. The remainder of the intracellular tail is found in the fifth exon, which is 368 bp long and includes the entire 3' untranslated sequence.

A unique polyadenylation signal (AATAAA) is found 25 bp upstream of the end of the last exon. The latter is immediately followed by a T-rich sequence probably involved in the termination of the mature mRNA (17). The calculated total length of the mRNA (591 bp) is in agreement with results obtained by Northern blots (750 bp) (18) if one accounts for the poly(A) tail.

The five exons should generate transcripts encoding a polypeptide of 86 residues, identical in length to the previously sequenced mouse (9) and rat (11) γ chains (Fig. 3). A leader peptide of 18 amino acids is followed by a short extracellular segment of 5 residues, a transmembrane domain of 21 residues, and an intracellular tail of 42 amino acids. Among mouse, rat, and human γ chains, 86% of the bases are identical in the open reading frame compared to only 60% in the overall sequence. The polypeptide contains 86% identical amino acid residues among the three species. The high level of conservation in the γ chain polypeptide contrasts with a low level in the α chain polypeptide, where only 36% identical amino acids are found in the corresponding species (9). It is reasonable to postulate that the structure of the γ chain has been maintained because of its functional importance.

Comparison of Fc Receptor γ Chain and TCR δ Chain Genes: A New Family of Genes—The genes for the γ chain of FcRI and of other Fc receptors (see below) and for the δ chain of the TCR (16) show analogous organizations of their respective exons (Fig. 4). Exon 1 of both genes encodes most of the leader peptide. Exon 2 corresponds to the last 2 amino acids of the leader peptide, the short extracellular region, the transmembrane domain, and the first 3 residues of the intracellular segment. The remainder of the cytoplasmic tail is encoded by short exons of 21–81 bp. When compared to the γ subunit gene, there is an insertion of three additional exons in the δ subunit gene, resulting in a cytoplasmic tail that is 70 amino acids longer. The last exon of the γ chain (exon 5) encodes the final 20 amino acids and that of the δ chain (exon 8) encodes the final 21 amino acids; and in both cases, the last exon also contains the entire 3' untranslated sequence.

The second exon of the γ chain is homologous to the second exon of the δ chain: 58% identical amino acids are found between human γ and δ chains and 55% between mouse γ and δ chains (9). Similarly, the last two exons of γ and δ chains show a significant level of homology which was previously undetected: 30 and 44% identities between mouse γ and δ chains and between human γ and δ chains, respectively. It should be noted that the δ chain, unlike the γ chain, has been proposed to contain a consensus sequence for an ATP-binding site (19) within the C-terminal part homologous to the γ chain, although binding of ATP has not yet been shown directly. The localization of the γ and δ subunit genes on mouse chromosome 1 (20, 21), their similar genomic and structural organization, and the sequence homologies imply that gene duplication has generated these two genes. A later insertion or deletion of three exons could explain the difference of length between γ and δ chains.

In addition to sharing a common genomic structure, both γ and δ chains form disulfide-linked homodimers. Since the δ chain contains only 1 cysteine residue located at the external border of the transmembrane domain, this residue must be involved in the dimerization of δ chains. Therefore, the corresponding cysteine in the γ chain is likely to participate in a disulfide bridge between γ chains. Whether the second cysteine, present in the γ chain at the internal border of the transmembrane helix, is also involved in an additional disulfide bridge remains to be determined. Furthermore, both polypeptides associate noncovalently with other subunits to yield multimeric complexes. The type of interaction of the γ chain with α and β chains in FcRI is reminiscent of the association of the δ chain with αβ chains and the CD3 complex in the case of the TCR. Both γ and δ chains dissociate easily during solubilization, but their association with the other
Gene for γ Subunit of Fc Receptors

subunits can be maintained when the appropriate detergent and concentration are used (22-24).

Differential Requirements in Expression of Human and Rodent Fc,RI Receptors—To facilitate expression studies, we isolated cDNA clones corresponding to the human γ chain. A basophil-enriched leukocyte cDNA library was screened with rodent γ chain probes, and four clones of 1.6 kb were isolated. Their sequence in the open reading frame was identical and corresponded exactly to the predicted sequence from the gene. Interestingly, the flanking regions contained Alu sequences with sequence variations between the clones (data not shown). These variations are not necessarily surprising since the library was prepared from the blood of several individuals. Whether they correspond to allelic differences, to polymorphisms, or possibly to the existence of more than one gene is currently under investigation.

Four independent transfections of COS-7 cells with the human α chain resulted in only 0.007% of the cells expressing IgE receptors as measured by an IgE rosetting assay (Table I). In contrast, cotransfection of human α and γ subunit cDNAs yielded 6.5 ± 1.4% rosetting cells. Cotransfection of rodent β subunit cDNA together with human α and γ subunit cDNAs did not increase the efficiency of expression: 5.6 ± 3.1% rosetting cells were observed. In contrast, rodent Fc,RI can only be expressed efficiently on the cell surface by cotransfection of all three cDNAs. Therefore, the requirements for efficient expression of the α chain on the cell surface are clearly different between rodent and human.

Moreover, we found that the surface expression of other Fc receptors, mouse Fc,RIIA (25) and human Fc,RIII-2 (26-28), also required γ chains identical to those from Fc,RI (6, 7). Taken together, these observations underline the critical role played by γ chains in allowing Fc,RI and these other Fc receptors to reach the cell surface.

The ability of the γ chain to allow surface expression of α chains of Fc,RI is analogous to the role of the ζ chain in the expression of the TCR-CD3 complex. In the intracellular sorting of the murine T-cell receptor, the ζ chain is necessary for the complete complex to reach the cell surface (29, 30). In its absence, the TCR pentameric complex (αβγδζ) assembles in the endoplasmic reticulum and is then targeted to lysosomes. The ζ chain specifically prevents this degradation pathway and permits only the complete heptameric complex (αβγδζ) to reach the plasma membrane. It will be interesting to assess whether differences between the murine and human systems will be observed as in the case of Fc,RI. Future studies will have to address whether the role of the γ chain for the Fc receptors is similar to that of the ζ chain for the TCR. Since signal transduction studies have already shed light on the possible role of the ζ chain (31), it will now be of great

![Gene organization](image)

**FIG. 3.** Genomic organization of γ subunit of human Fc receptor (top) and comparison of γ chain polypeptide sequences between species (bottom). The relative positions of the exons and introns are depicted. The alignment of the three species was accomplished with the program MacVector. In the consensus sequence, an upper-case letter indicates a perfect match between all three species, and a lower-case letter indicates a match between two. Interruptions by vertical lines refer to the exon boundaries of the human gene. The black diamond shows the presumed post-translational cleavage site (11).
The γ chain family may contain more members and be important in signal transduction. It is likely that the γ chain of the T-cell receptor will be another member. In addition to sharing structural similarities with the ζ chain, the γ chain forms a heterodimer with the ζ chain and seems to be involved in signaling (32). Other potential candidates are the recently discovered γ chain that is disulfide-linked to the p subunit of the T-cell receptor will be another member. In addition to sharing structural similarities with the ζ chain, the γ chain forms a heterodimer with the ζ chain and seems to be involved in signaling (32). Other potential candidates are the recently discovered γ chain that is disulfide-linked to the β subunit of the interleukin-2 receptor and the 12-kDa molecule associated with the ζ chain in CD3 negative natural killer cells (33).

Acknowledgments—We wish to thank M. Baniyash and R. D. Klausner for allowing us to see their data on the genomic organization of the ζ chain before publication and Eric Sarejaki and George Poy for their excellent technical assistance.

REFERENCES

Characterization and expression of the gene for the human Fc receptor gamma subunit. Definition of a new gene family.

H Küster, H Thompson and J P Kinet


Access the most updated version of this article at http://www.jbc.org/content/265/11/6448

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/265/11/6448.full.html#ref-list-1