We have isolated and characterized three genes coding for hFcγRIIA, IIB, and IIC. Each gene spans 15–19 kilobases of DNA and consists of eight exons. Two exons encode the 5'-untranslated region and signal peptides, two exons code for homologous Ig-like extracellular domains, a single exon encodes the transmembrane spanning region, and three exons encode the cytoplasmic domains and 3'-untranslated regions. Analysis of gene structures support the concept that the hFcγRIIA and hFcγRIIB genes originated via gene duplication and divergence processes. The hFcγRIIC gene, however, showed a remarkable homology at its 5' end with the hFcγRIIB gene, whereas its 3' region was highly homologous with the hFcγRIIA gene, suggesting that the hFcγRIIC gene results from an unequal crossover event between the hFcγRIIA and IIB genes. This hypothesis was supported by nucleotide sequence analyses of the putative break-point region. The proposed site of recombination was located approximately 300 nucleotides downstream from the sixth (Cl) exon. These data provide a unique model for the evolutionary generation of a receptor family with multiple biological functions.

Human IgG Fc receptors comprise a family of glycoproteins that can bind the Fc moiety of immunoglobulin type G. Interaction of antigen-antibody complexes with FcγR triggers multiple cell type-specific functions (reviewed in Refs. 1, 2). On human leukocytes three distinct hFcγR classes are currently recognized, hFcγRI (CD64), hFcγRII (CD32), and hFcγRIII (CD16). The second class consists of different, albeit structurally related, 40-kDa molecules with low affinity for IgG (3–6). These molecules were found to be encoded by albeit structurally related, 40-kDa molecules with low affinity for IgG (3–6). These molecules were found to be encoded by

### Materials and Methods

**Screening and Isolation of Genomic DNA Encoding Human FcγRII**—A genomic DNA library, constructed in EMBL3 (14) using Sau3AI partial digests of human leukocyte DNA derived from a CML patient, was a generous gift of Dr. J. Hooijmakers (Erasmus University, Rotterdam, The Netherlands). A second genomic DNA library, constructed in λ FIX, using Sau3AI partial digests of human placental DNA, was obtained from Stratagene (La Jolla, CA). Approximately 2 × 10^6 plaques of each library were screened with: 1) an EcoRI probe of cDNA clone pPW3 (nt: 1–1460, Ref. 6), recognizing all coding regions for hFcγRIIA, and in addition the extracellular domain encoding regions of hFcγRIIB, and IIC, and the intracellular region encoding part of hFcγRIIC; 2) a 160-bp XbaI-PstI fragment (SIG B) of cDNA clone pIP4 (nt: 820–1050, Ref. 10), recognizing the signal peptide encoding region of hFcγRIIB, and IIC; 3) a 230-bp PstI fragment (CYT B) of cDNA clone pIP4 (nt: 820–1050, Ref. 10), recognizing the intracellular domain encoding part of hFcγRIIB.

The probes were labeled with [α-32P]dCTP using a random primer labeling kit (Gibco, Paisley, Scotland). Hybridizing phage clones were isolated by repeated plaque purification and rescanning. Genomic DNA inserts from phage clones were isolated according to standard procedures (15).

**Characterization of Genomic DNA Clones**—Genomic DNA inserts were isolated from a total of 39 hybridizing phage clones, digested with Sall (in poly linker of EMBL3 and λ FIX) and BamHI, separated on 0.8% agarose gels, and analyzed with different probes (see below). Several independent phage clones were selected for further analysis. The DNA inserts were analyzed by restriction endonuclease mapping using BamHI, EcoRI, HindIII, and Sall, followed by Southern blotting.

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‡ The abbreviations used are: h, human; bp, base pair(s); FcγRII, low-affinity Fc receptor for IgG; kb, kilobase pair(s); nt, nucleotide(s); RFLP, restriction fragment length polymorphism.

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**The Human Low Affinity Immunoglobulin G Fc Receptor IIC Gene Is a Result of an Unequal Crossover Event***

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Human IgG Fc receptors comprise a family of glycoproteins that can bind the Fc moiety of immunoglobulin type G. Interaction of antigen-antibody complexes with FcγR triggers multiple cell type-specific functions (reviewed in Refs. 1, 2). On human leukocytes three distinct hFcγR classes are currently recognized, hFcγRI (CD64), hFcγRII (CD32), and hFcγRIII (CD16). The second class consists of different, albeit structurally related, 40-kDa molecules with low affinity for IgG (3–6). These molecules were found to be encoded by
analyses (16). Eight phage clones were selected, based on their restriction maps and hybridization patterns. Nucleotide sequences of the DNA inserts or subcloned DNA fragments were determined by the dideoxy chain termination method (17) using a T7 polymerase-sequencing kit (Pharmacia) and synthetic oligonucleotides (18-19). The data obtained from Southern blotting and sequence analyses showed that the inserts of three phage clones contained parts of the hFcyRIIA gene, three phage clones parts of hFcyRIIC, and two phage clones hFcyRIIB gene fragments.

**Southern Blot Analyses**—High molecular weight genomic DNA was isolated from human leukocytes of 19 healthy donors by standard procedures (15). The genomic DNA was digested with BamH1, EcoRI, or HindIII, separated on 0.8% agarose gels, and blotted to Zeta-probe nylon membranes (Bio-Rad). Southern blots were hybridized in 0.25 M NaCl, 7% SDS, 1 mM EDTA, 50% formamide, supplemented with 50 μg/ml denatured salmon sperm DNA at 42°C with either of the following cDNA probes: SIG A, EcoRI-PstI fragment of pPW3 (nt: 1-140, Ref. 6), recognizing the signal peptide encoding region of hFcyRIIa; SIG B, Xbal-PstI fragment of pIP9 (nt: 50-210, Ref. 10), recognizing the signal peptide encoding regions of hFcyRIIB, and IIC; EC2-TM, PstI-XbaI fragment of pPW3 (nt: 480-770, Ref. 6), recognizing the intracellular region encoding domains of hFcyRIIa, Iib, and Iic; CYT A, PstI-HindIII fragment of pPW3 (nt: 770-985, Ref.6), recognizing the intracellular region encoding domains of hFcyRIIa, and Iic; CYT B, PstI fragment of pIP4 (nt: 820-1050, Ref. 10), recognizing the intracellular encoding region of hFcyRIIB.

After overnight hybridization, blots were washed at stringency in 0.3 x SSC, 1% SDS at 65°C, and exposed for 20-72 h to Kodak XAR5 x-ray film.

**RESULTS AND DISCUSSION**

**Organization of Three Distinct hFcyRII Genes**—Several overlapping phage clones were isolated upon screening of two human genomic libraries with three hFcyRII-specific cDNA probes (see "Materials and Methods"). Restriction endonuclease mapping, Southern blotting analysis, and nucleotide sequencing of eight independent clones resulted in the construction of a complex genomic pattern consistent with three distinct genes encoding hFcyRII: A, B, and C (Fig. 1). All three genes were composed of eight exons located on =15-19 kb of DNA. The first two exons (S1 and S2) encoded the signal peptides, with the predicted signal peptide cleavage sites located in the second, 21-nucleotide, mini-exon (S2). A corresponding mini-exon has been found in all members of the FcγR gene families both in man and mouse, as well as in the gene encoding the α-chain of FcγRI (7, 18-21). In all three hFcyRII genes, two exons (EC1 and EC2) were found to encode a single C2 set Ig-like domain (22), and a single exon (TM) for a hydrophobic transmembrane segment. The sixth exon (C1), present in all three genes, was previously found to be alternatively spliced in hFcyRII transcripts (3, 5, 10). Interestingly, this exon as well as its splice borders displayed extensive homology in all three genes, but until now no hFcɣRIIa or IIC cDNA clones have been identified which include information encoded by this C1 exon. Moreover, insertion of the C1 exon in hFcγRIIa or IIC transcripts does not change their reading frames. Furthermore, two exons (C2 and C3) were found to encode the main parts of the cytoplasmic regions. The deduced amino acid sequence of both of these exons of hFcγRIIa and Iic is remarkably similar (98% homology), in contrast to that of Iib (8% homology) (4-7).

The 3′-untranslated region of gene IIA can be ≥1 kb longer than that of IIC, due to the presence of one versus two polyadenylation signals in IIC, and IIA, respectively.

**Conclusions**—The physical maps of several overlapping independent genomic clones clearly show the presence of the three distinct hFcyRII genes. The organization of the hFcγRII genes show a remarkable resemblance between genes IIB and IIC upstream from C1, whereas downstream from C1 the organization of gene IIC was virtually identical to that of gene IIA (Fig. 1).

**Southern Blotting Analyses**—Leukocyte DNA from 19 Caucasian individuals was digested with BamH1, EcoRI, and HindIII and subjected to Southern blotting (Fig. 2 shows data from six individuals). Upon digestion with HindIII, three hybridizing bands sized 5.9, 9.5, and ≥19 kb were observed using an EC2-TM probe. The three hybridizing bands were found present in all 19 individuals and were consistent with the maps for genes IIA, IIC, and IIB, respectively. Southern blots probed with the SIG A probe revealed a single hybridizing fragment for each digest (≥20-kb BamH1, 3.8-kb EcoRI, and 1-kb HindIII fragments), as predicted from the restriction map of hFcyRII genomic clones. Southern analyses with the SIG B probe revealed single hybridizing fragments of 6.4 kb (BamH1) and 4.2 kb (HindIII) in 19 individual. This pattern was again consistent with the maps from the 5′ region of genes IIB and IIC. One hybridizing band of 5.8 kb was noted with EcoRI-digested DNA in most individuals tested. However, in two out of 19 unrelated donors (one of which is shown in Fig. 2, lane 4, SIG B probe), we found two hybridizing bands, 5.8 and ≥8 kb. This extra band may reflect an EcoRI restriction fragment length polymorphism (RFLP) in the 5′ region of either the hFcγRIIB or IIC gene. Moreover, the relative intensities of the two bands (EcoRI-digested DNA, probed with SIG B) in donor 4 versus the single ones in the other individuals, supported the hypothesis that the single hybridizing band represented identical fragments derived from two genes. Southern analyses of the 3′ parts of the three hFcγRII genes further supported a strong homology between the hFcγRIIIA and IIC genes. The 4.1-kb BamH1, 4.3-kb EcoRI, and 7.5-kb HindIII fragments observed to hybridize with the CYT A probe were compatible with sizes predicted from genomic hFcγRIIa and IIC clones. Southern blots with the CYT B probe revealed 3.5-kb BamH1, 4.6-kb EcoRI, and ≥20-kb HindIII fragments, all corresponding with the hFcγRIIB structural map (Fig. 1). Notably, a weakly hybridizing 4.2-kb BamH1 band was visible upon longer exposure in genomic DNA probed with CYT B. This fragment corresponds to a BamH1 fragment containing the C2 exon of hFcγRIIB, and the inefficient hybridization is likely attributable to the exon size (38 bp).

Taken together, the Southern analyses of genomic DNA from 19 unrelated Caucasian donors proved to be consistent...
Fig. 2. Southern analyses of human genomic DNA. Genomic DNA from six individuals (designated 1–6) was digested with BamHI, EcoRI, or HindIII, separated on agarose gels, and transferred to nylon membranes. The Southern blots were analyzed using five different probes, SIG A, SIG B, EC2-TM, CYT A, and CYT B, as indicated. Hybridizing fragments from the three genes are indicated by A, B, or C. HindIII fragments of phage λ-DNA served as size markers, indicated by horizontal bars, and are given in kb.

with the genomic organization of the three hFcγRII genes shown in Fig. 1.

The identification of three distinct genes encoding hFcγRII is consistent with the number of genes described by Qiu et al. (7). However, we found several differences in the genomic organization of these genes, which lead to a significantly different concept of the evolution of this gene family. We identified two HindIII sites between the C2 and C3 exons in hFcγRIIA, whereas a single HindIII site was found by Qiu et al. (7). The presence or absence of this restriction site could reflect a HindIII RFLP. However, such a polymorphism may then be rare in Caucasians because we did not observe a HindIII RFLP in genomic DNA of 19 individuals. Next, we localized the EC1 exon within the hFcγRIIA gene at a different position than Qiu et al. (7). To confirm our data, we determined the location of EC1 in four independent genomic clones (two of which are shown in Fig. 1). In all four clones, EC1 was found located in a 0.9-kb HindIII-EcoRI fragment and not in the adjacent 1.8-kb EcoRI fragment (7).

The structural organization of hFcγRIIC was not entirely consistent with the IIC gene (referred to as hFcγRIIa') characterized in Ref. 7. Briefly, this study points out differences in the 5' regions between gene IIC and gene IIB and in the 3' region between IIC and IIA. Furthermore, the structural map of gene IIC concerning the region in which C1 and C2 exons were located in Ref. 7 was observed to be different from the corresponding region of the hFcγRIIC gene presented in this paper. Unfortunately, however, no Southern data were shown by Qiu et al. (7) precluding the verification of both the 5' and 3' regions of hFcγRIIC. Our data, however, argue against restriction length differences in the 5' regions of genes IIB and IIC (Fig. 2, probe SIGB). The differences in the 3' regions of genes IIA and IIC (7) could not be confirmed, neither in our genomic clones, nor by Southern analyses (Fig. 2, probe CYTA). The most outstanding differences, located in the region containing the C1 and C2 exons, were further analyzed in two genomic hFcγRIIC clones isolated from different libraries. Both clones contained a 1.4-kb EcoRI fragment (including the C1 exon), identical to such a fragment in gene IIA.

Apart from the differences described above, the Southern blotting data presented by Qiu et al. (7) showed two BamHI and three HindIII hybridizing bands with a probe recognizing EC1. The hybridizing fragments and the corresponding genes (HindIII: ≈20 kb (B), ≈10 kb (a'/C), and 3.3 kb (A); and for BamHI: ≈18 kb (A) and 6 kb (B and a'/C) Ref. 7) are in perfect agreement with the hFcγRII genomic organization shown in Fig. 1.

Evidence for an Unequal Crossover Event—The striking homology between genes IIB and IIC upstream from the C1 exon, and downstream between genes IIA and IIC, prompted us to evaluate whether an unequal crossover event between
IIA and IIB genes resulted in the generation of IIC. Therefore, we sequenced the region surrounding the C1 exon from single phage clones containing each of the three genes. The EC2, TM, and C2 exons were also sequenced to identify the genetic origin of the genomic clones. Fig. 3 contains a schematic representation of the sequencing data of clones encoding genes IIA (upper row), IIC (center row), and IIB (bottom row). Each vertical line represents a single nucleotide difference between the genes IIA/IIB and gene IIC. The 5' region was found to be remarkably similar (99.8% homology) between IIA and IIB. In contrast, homology between genes IIA and IIC in this region is approximately 80%, including several insertions and deletions relative to the corresponding regions of genes IIB and IIC. The 3' portion, the sequences of genes IIA and IIC displayed 98.7% sequence homology, with less homology between genes IIB and IIC (≈78%). Remarkably, the region indicated between the arrows (Fig. 3) exhibited the highest homology between all three hFcγRII genes (99.8% homology between IIA and IIC, 98.9% between IIB and IIC, and 97.9% between IIA and IIB). This strong homology, as well as the absence of insertions or deletions render this region a likely candidate for a homologous recombination event. We, therefore, postulate that the hFcγRIIC gene resulted from an unequal crossover event between the IIA and IIB genes with a break-point located ≈300 bp downstream from the sixth (C1) exon. It is important to note that the pattern of gene evolution supported by our data is different from that derived from work of Qiu et al. (7). These authors suggested that the hFcγRIIA gene was generated via recombination of the hFcγRIIC and hFcγRIIA genes.

It is noteworthy that we found an at instead of a regular gt (23) splice border of the C2 exon in gene IIC. This unusual splice border was also identified by Qiu et al. (7) in ≈75% of the donors and was found to be accompanied by a stop codon in exon EC1. Upon sequencing the genomic hFcγRIIC clone, however, we did not observe such a stop codon in exon EC1 and can, therefore, exclude that the genomic IIC clone (shown in Fig. 3) contains a pseudogene.

In conclusion, we have characterized three genes encoding the class II Fc receptor for IgG. Our data support the concept that hFcγRIIA and IIB genes are derived from a common ancestral gene. An unequal crossover event between the hFcγRIIA and IIB genes most likely lead to the generation of a third gene, hFcγRIIC (as depicted in Fig. 4). This recombination event may have occurred relatively recent in evolution since intron sequences displayed a high degree of homology. This notion is further supported by the observation that in the murine system only a single gene has been identified for FcγRI.

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