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# Human lymphocyte Fc receptor for IgE: Sequence homology of its cloned cDNA with animal lectins

(IgE binding factor/isotype regulation/lymphocyte receptor)

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ABSTRACT **We have purified the human lymphocyte Fc receptor specific for IgE (Fcε receptor) and its soluble form by using the anti-Fcε receptor monoclonal antibody H107. Using an oligonucleotide probe corresponding to the partial amino acid sequence of the soluble Fcε receptor related to IgE binding factor, we cloned, sequenced, and expressed a cDNA for the receptor. The Fcε receptor has 321 amino acid residues with no $NH_2$-terminal signal sequence. The receptor was separated into two domains by a putative 24-amino acid residue transmembrane region located near the $NH_2$-terminal end. The Fcε receptor showed a marked homology with animal lectins including human and rat asialoglycoprotein receptors, chicken hepatic lectin, and rat mannose binding proteins.**

The lymphocytes are known to express Fc receptors for the various classes of immunoglobulins. Beside high-affinity IgE receptors on mast cells and basophiles, low-affinity Fc receptors for IgE (Fcε receptors) are expressed on a variety of hematopoietic cells including T and B lymphocytes (1, 2). In atopic and nonatopic patients as well as animals with elevated IgE levels, there is an increased expression of Fcε receptors on lymphocytes (3, 4). *In vitro* studies have shown that the expression of the receptor is enhanced by IgE and also by lymphokines (5, 6), suggesting that the receptor itself plays an important role in the regulation of IgE metabolism.

The Fcε receptor on human B-cell lines is a glycoprotein of 43–45 kDa (7). We have obtained H107, a monoclonal antibody (mAb) specific for Fcε receptors on human lymphocytes (4, 8) that also binds solubilized 43-kDa Fcε receptors from Fcε receptor-positive B-cell lines such as RPMI 8866 cells. From H107-conditioned culture medium, we obtained the soluble form of Fcε receptor recognized by H107 mAb. Fcε receptors and the soluble form of the Fcε receptor were affinity purified with H107 mAb. Using the oligonucleotide probe corresponding to the partial amino acid sequence of the $NH_2$ terminus of the soluble Fcε receptor, we have cloned and characterized the cDNA encoding the receptor.

## MATERIALS AND METHODS

**Cell Lines and Antibodies.** Fcε receptor-positive (RPMI 8866 and IM9) and Fcε receptor-negative (Daudi) human B-lymphoblastoid cell lines, an Fcε receptor-negative MLA 144 monkey and an Fcε receptor-negative type I human T-cell leukemia virus-positive human T-cell line (HuT 102) were maintained in RPMI 1640 medium with 10% (vol/vol) heat-inactivated fetal calf serum. The cells were also cultured in RPMI 1640 medium with 1% fetal calf serum at the density of $5 \times 10^5$ cells per ml for 72 hr, to obtain conditioned medium for the purification of soluble Fcε receptor. H107 anti-Fcε receptor mAb (8) was purified from the conditioned medium of the hybridoma cells using protein A-Sepharose affinity chromatography. Anti-interleukin 2 receptor mAb (anti-Tac) was a kind gift of T. Uchiyama.

**Inhibition of IgE-Specific Rosette Formation.** IgE-coated erythrocytes were prepared by sensitizing 1% (vol/vol) fixed ox erythrocytes with human IgE at 1 mg/ml in 0.1 M acetic acid, pH 5.0. IgE binding activity of soluble Fcε receptor was assessed by the inhibition of rosette formation of RPMI 8866 cells with IgE-coated erythrocytes, as described (3, 8, 9).

**Immunofluorescence Study of Fcε Receptors.** The cells were incubated with H107 mAb, anti-Tac mAb (0.1 μg/10 μl), or human IgE from U266 hybridomas (2 μg/20 μl), provided by Takeda Pharmaceutical (Osaka, Japan) for 30 min at 4°C, and then with fluoresceinated goat anti-mouse IgG or anti-human IgE immunoglobulin (Tago, Burlingame, CA). Binding of IgE to Fcε receptors was blocked by the treatment of the cells with H107 mAb before the incubation with IgE.

**Affinity Purification of Fcε Receptors.** H107 mAb, bovine serum albumin, human gamma globulin, and DNase I were conjugated to Tresyl-Sepharose (Pharmacia) at 5 mg/1 ml of beads (10). Soluble Fcε receptor was purified from the conditioned medium of RPMI 8866 cells using H107-Sepharose after absorption with the human gamma globulin-Sepharose. The Fcε receptor was purified as described (8). The cell lysate of RPMI 8866 cells in 0.5% Nonidet P-40 was absorbed with bovine serum albumin-, human gamma globulin-, and DNase I-conjugated Sepharose, and then incubated with the H107-Sepharose. After extensive washing with a high-salt buffer (10 mM sodium phosphate, pH 7.5/0.5 M NaCl/0.1% Nonidet P-40), soluble Fcε receptor and Fcε receptors were eluted from H107-Sepharose with an elution buffer (0.1 M acetic acid, pH 4.0/0.5 M NaCl/0.1% Nonidet P-40). Affinity-purified, soluble Fcε receptor was further fractionated on Superose column with Fast Protein Liquid Chromatography (FPLC; Pharmacia), to elucidate its IgE binding activity.

**$NaDodSO_4$/PAGE and Protein Amino Acid Sequence.** The method of $NaDodSO_4$/PAGE is described elsewhere (8). For immunoblots, nonreducing 13% polyacrylamide gels were used. After electrotransfer to a nitrocellulose filter, the membrane, blocked with 3% (wt/vol) bovine serum albumin, was incubated with or without H107 mAb (1 μg/ml) and then with goat anti-mouse IgG [$F(ab')_2$] conjugated to horseradish peroxidase (Tago). The antibody-bound bands were visualized with 3,3′-diaminobenzidine tetrahydrochloride and $H_2O_2$. For amino acid sequencing, both affinity-purified, soluble Fcε receptor and Fcε receptor were further purified by reverse-phase HPLC (using a Synchropak RP-P $C_{18}$ column) (Beckman) with a linear gradient of 2-propanol with

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Abbreviations: Fcε receptor, Fc receptor for IgE; IgE-BF, IgE binding factor; mAb, monoclonal antibody.

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0.1% trifluoroacetic acid. The N-terminal amino acid sequence of the soluble Fcε receptor and Fcε receptor was analyzed using a gas-phase protein sequencer (Applied Biosystems, Foster City, CA).

**RNA Gel Blot Hybridization.** Poly(A)$^+$ RNAs (5 μg each) were electrophoresed on a 1% agarose gel and transferred to a nitrocellulose filter (11). The filter was hybridized (12) with a $^{32}$P-labeled cDNA fragment B (see Fig. 3*A*) probe labeled by nick-translation (13).

**Cloning of Fcε Receptor cDNA and Expression on COS-7 Cells.** cDNA library was constructed from RPMI 8866 cells according to Okayama and Berg (14). *Escherichia coli* HB101 transformants ($1.1 \times 10^5$ colonies) were screened by the method of Hanahan and Meselson (15). Nucleotide sequences were determined by the dideoxy method using the pUC plasmid as a vector (16). Amino acid sequence homology was analyzed by integrated data base and extended analysis system for nucleic acid and proteins according to M. Kanehisa at Kyoto University. The COS-7 cells were transfected and cultured as described (17).

## RESULTS

**Purification and Analysis of Soluble and Cell-Bound Fcε Receptors.** Ten liters of RPMI 8866 cell-conditioned medium (RPMI 1640 medium with 1% fetal calf serum) was concentrated 10-fold and applied to an H107-Sepharose column. After extensive washing with high-salt buffer, the column was eluted with 0.1 M acetate buffer, pH 4.0. Analysis of the eluate of the column by 13% NaDodSO$_4$/PAGE revealed two adjacent protein bands of ≈25 kDa (Fig. 1*A*). FPLC gel filtration through Superose column showed that the activity to inhibit the rosette formation of RPMI 8866 cells with IgE-coated erythrocytes was associated with a molecule of 20–25 kDa (data not shown). The result suggested that the 20- to 25-kDa proteins are the fragments of Fcε receptor.

In contrast to the soluble Fcε receptor, Fcε receptor on the cell surface has been shown to be 43 kDa (7, 8). To compare

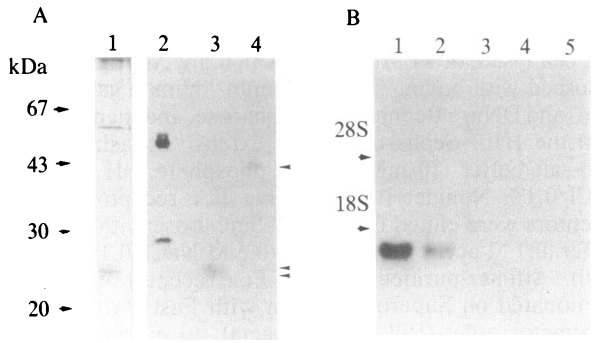


FIG. 1. Analysis of soluble H107 antigen by NaDodSO$_4$/PAGE, immunoblot analysis of the affinity-purified cell-bound and soluble H107 antigens, and RNA gel blot analysis of Fcε receptor mRNA. (*A*) Lane 1: NaDodSO$_4$/PAGE analysis of soluble H107 antigen from RPMI 8866 cells. Soluble H107 antigen was obtained from the culture supernatant and applied to 13% reducing gels. The protein bands were stained with silver. Lanes 2–4: immunoblot analysis. Samples were analyzed with nonreducing 13% NaDodSO$_4$/PAGE. After electroblotting to a nitrocellulose membrane, the membrane was incubated with H107 mAb followed by horseradish peroxidase-conjugated anti-mouse IgG. Lane 2, mouse H107 antibody (IgG2b) applied to the reducing gel as a molecular marker; 3, soluble Fcε receptor obtained from the RPMI 8866 cell-conditioned medium affinity-purified with a H107-Sepharose column; 4, H107 antigen purified from the lysate of RPMI 8866 cells. The molecular size markers are indicated on the left. (*B*) RNA gel blot analysis of Fcε receptor mRNA. Filters were hybridized with the Fcε receptor probe (fragment B in Fig. 3*A*). Origins of RNAs. Lane 1, IM9 cells; 2, RPMI 8866 cells; 3, Daudi cells; 4, MLA 144 cells; 5, HuT 102 cells.

soluble Fcε receptor with the 43-kDa cell-bound Fcε receptor, we analyzed affinity-purified soluble and cell-bound Fcε receptors using the immunoblot method. As shown in Fig. 1*A*, H107 mAb reacted not only with the 43-kDa Fcε receptor but also with two 25-kDa proteins with slightly different mobilities on the blot. When affinity-purified 43-kDa Fcε receptor was kept at room temperature overnight, a portion of the Fcε receptor spontaneously degraded to an 18-kDa fragment as determined by NaDodSO$_4$/PAGE and silver stain (data not shown).

**Partial Amino Acid Sequences of Fcε Receptors.** Affinity-purified soluble Fcε receptor was further purified by reverse-phase HPLC (Synchropak RP-P, $C_{18}$ column) to obtain a single protein peak. However, NaDodSO$_4$/PAGE analysis showed that the peak still consisted of two components with slightly different molecular sizes. Analysis of the $NH_2$-terminal amino acid sequence of the 25-kDa protein revealed two overlapping sequences (Fig. 2*A*, sequences a and b). In contrast, no sequence was obtained from the 43-kDa Fcε receptor that was purified by affinity chromatography on H107-Sepharose followed by reverse-phase chromatography, probably because of the blocking of the $NH_2$ terminus. However, we could obtain an $NH_2$-terminal sequence of 25 amino acid residues from the 18-kDa fragment purified from spontaneously degraded 43-kDa Fcε receptor (Fig. 2*A*, sequence c).

The sequences from the 25-kDa soluble Fcε receptor and the 18-kDa fragment had an overlapping sequence (Ser-Gly-Phe-Val) (Fig. 2*A*). We constructed a mixture of 14-base synthetic oligonucleotides probe corresponding to the 5 amino acid residue (Met-Glu-Leu-Gln-Val) of the sequence of 25-kDa Fcε receptor (Fig. 2*B*).

**Cloning of cDNA for Fcε Receptor.** A cDNA library complementary to poly(A)$^+$ RNA of RPMI 8866 cells was screened with the mixture of $^{32}$P-labeled 14-base oligonucleotides as a probe. The probe hybridized with two out of $1.1 \times 10^5$ colonies of the cDNA library. Two clones, designated as Fcε R-1 and Fcε R-2, contained 1.1- and 1.7-kb insert, respectively. Restriction site mapping and nucleotide sequence determination showed that the two clones are identical, except for the extra 0.6-kb sequence in the 5′ end of Fcε R-2 (Fig. 3*A*). The complete nucleotide sequence (1531 base pairs) of the Fcε R-2 clone was determined according to the strategy shown in Fig. 3*A*. The derived amino acid sequence contained a stretch matching with the 12 amino acid residues in the $NH_2$-terminal portion of the 25-kDa soluble Fcε receptor (Fig. 2*C*).

The restriction map and the putative structure of the receptor are shown in Fig. 3*A*. In the complete 321-amino acid sequence of Fcε receptor cDNA, there is no hydrophobic signal sequence, which is present in many membrane-bound glycoprotein genes. The Fcε receptor molecule has a putative hydrophobic transmembrane domain of 24 amino acid residues in the $NH_2$-terminal region after a 23-amino acid hydrophilic sequence (Fig. 3*B*). The average hydrophilicity values of $NH_2$-terminal 23 residues, hydrophobic 24 residues, and COOH-terminal 274 residues are 0.9, −1.5, and 0.3 kCal, respectively. The amino acid sequence of the Fcε receptor has an unexpected homology with several animal lectins including human and rat asialoglycoprotein receptors (18, 19), chicken hepatic lectin (20), and rat mannose binding proteins (21, 22). Homology among these proteins is particularly significant in the COOH-terminal portions (Fig. 4). Within C-terminal 120 amino acid residues including gaps that were introduced to obtain maximum homology, 42 (35%), 42 (35%), 39 (33%), and 27 (23%) residues of Fcε receptor were identical with those of human and rat asialoglycoprotein receptors, chicken hepatic lectin, and rat mannose binding protein-C, respectively. Fcε receptor has no substantial

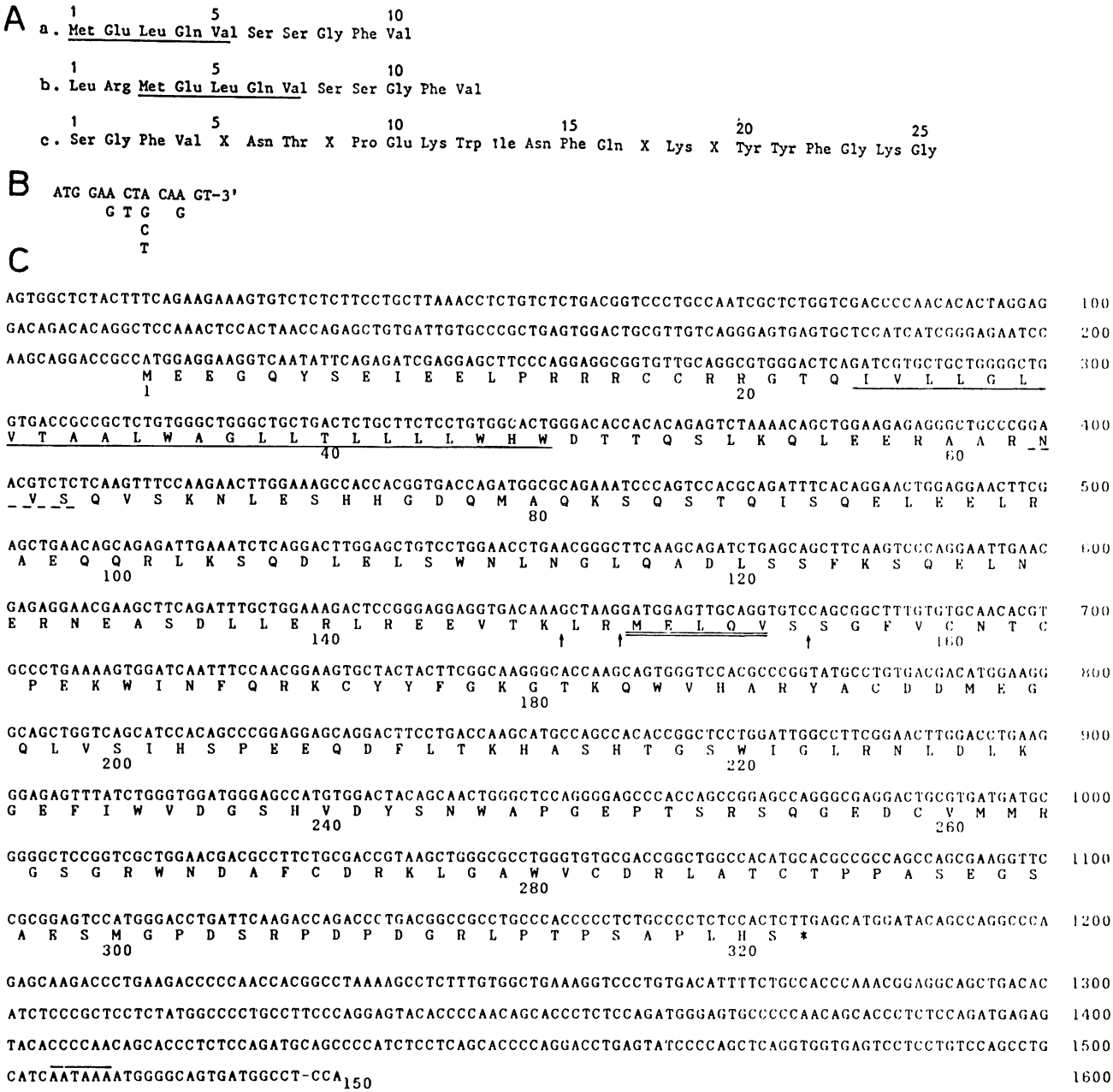


FIG. 2. Nucleotide and amino acid sequences of Fcε receptor and IgE binding factor (IgE-BF). (*A*) Amino acid sequence of soluble and cell-bound Fcε receptors. Sequences a and b, N-terminal amino acid sequences of the 25-kDa soluble Fcε receptors. Sequence c, N-terminal amino acid sequence of the 18-kDa protein that was separated by reverse-phase chromatography from degraded Fcε receptor. (*B*) The mixture of synthetic oligonucleotides used as a probe. This nucleotide sequence was for the underlined five amino acid residues in *A*, sequences a and b. (*C*) Nucleotide and predicted amino acid sequences of Fcε receptor. Nucleotides are numbered at right and amino acids are numbered throughout. Double underline, broken line, underline, and overline indicate the probe sequence, sites of potential asparagine-linked glycosylation, putative transmembrane region, and poly(A) addition signal, respectively. Asterisk indicates termination codon. Arrows indicate the cleavage site of Fcε receptor.

homology with proteins in the immunoglobulin gene superfamily.

**Detection of Fcε Receptor mRNA.** Poly(A)$^+$ RNAs prepared from various lymphoid cell lines were examined for Fcε receptor mRNA by RNA gel blots, using $^{32}$P-labeled fragment B chains (Fig. 3*A*) of the Fcε R-2 clone cDNA as probe. There was only one species of the Fcε receptor gene transcript of 1.7–1.8 kb long (Fig. 1*B*) in the Fcε receptor-positive cell lines (IM9 and RPMI 8866 cells), whereas the Fcε receptor-negative cell lines (Daudi, MLA 144, and HuT 102 cells) did not give any detectable signals. Lack of an alternatively spliced mRNA for Fcε receptor indicates the possibility that the soluble Fcε receptor in culture supernatant is a proteolytic cleavage product of Fcε receptor.

**Expression of Fcε Receptor on COS Cells Transfected with Fcε R-2 cDNA.** Fcε R-2 cDNA inserted in pKCRH2 (pKCR-ε R2A) (Fig. 3*C*) was transfected to COS-7 cells. After 48 hr of culture, the expression of H107 antigen on transfected cells was detected by treatment with H107 mAb and fluoresceinated anti-mouse IgG (Table 1). In contrast, H107 antigen was not detected on control COS-7 cells transfected with the same cDNA in the inverted orientation (pKCR-ε R2B). The presence of Fcε receptor on the COS-7 cells transfected with pKCR-ε R2A was also confirmed by the treatment of the cells with human IgE and fluoresceinated anti-human IgE. Furthermore, the binding of IgE to the transfected cells was

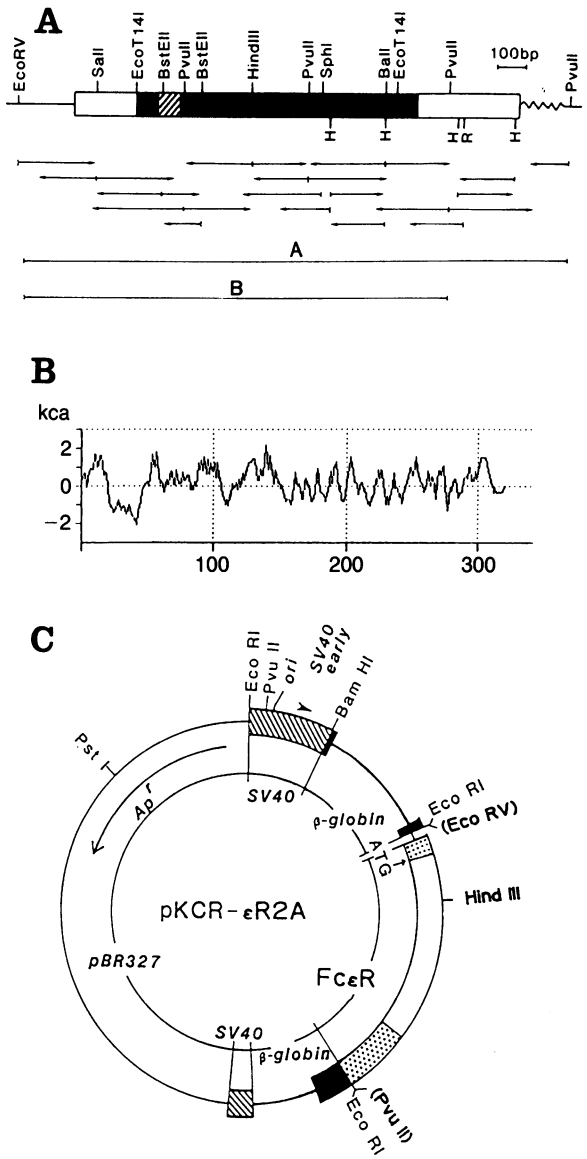


FIG. 3. Restriction map of Fcε R-2 clone, sequence strategy, hydrophilicity, and structure of the expression vector. (*A*) Restriction map of Fcε R-2 clone and sequence strategy. Solid and zigzag lines indicate vector and poly(A) tail, respectively. Open, hatched, and filled rectangles indicate untranslated, putative transmembrane, and coding regions, respectively. The directions and ranges of nucleotide sequence determination are shown by horizontal arrows. Restriction site of *Hae* III (H) and *Rsa* I (R) enzymes are only partially shown. Fragment A indicates the insert of pKCR-ε R2A (in *C*). (*B*) Hydrophilicity plot. Hydrophilicity of the transcript peptide was analyzed by using a software DNASIS (Hitachi Software Engineering, Yokohama, Japan). (*C*) Structure of expression vector for Fcε receptor cDNA (pKCR-ε R2A). Open rectangles, coding sequences of Fcε receptor cDNA; closed rectangles, noncoding sequences; closed boxes, β-globin sequence; hatched rectangles, simian virus 40 sequences; solid line, β-globin gene introns, pBR327 and pBR322; Ori, origin of replication. The cDNA insert had around 200 base pairs of pBR322 sequence at its 5′ terminus.

completely blocked by pretreatment of the cells with H107 mAb but not with irrelevant anti-interleukin 2 receptor mAb (anti-Tac) (Table 1).

## DISCUSSION

The coexpression of the H107 antigen and the binding sites for IgE on the COS-7 cells transfected with the Fcε R-2 cDNA suggested that the cDNA encodes for Fcε receptor having the H107 epitope. The presence of H107 epitope on both the 43-kDa Fcε receptor and the 25-kDa soluble Fcε receptor is consistent with the deduced amino acid sequence of the Fcε receptor protein. The $NH_2$-terminal 12 amino acid sequence of the 25-kDa soluble Fcε receptor resides in amino acid residues (148–159) of the Fcε receptor protein, indicating that the soluble Fcε receptor is cleaved by proteolysis. IgE binding properties of the 25-kDa soluble Fcε receptor as determined by rosette inhibition suggests that IgE binding sites reside in the COOH-terminal domain of Fcε receptor. The molecular size of Fcε receptor protein calculated from the deduced 321-amino acid sequence was 36.1 kDa, while that of soluble Fcε receptor corresponding to amino acid sequence 148–321 was 19.4 kDa. The discrepancy between the estimated molecular size and the calculated molecular size of both proteins may be explained by the post-transcriptional modification such as glycosylation (9).

The hydrophilicity plot of human lymphocyte Fcε receptor showed the lack of an $NH_2$-terminal signal sequence, and the presence of a putative transmembrane portion near the $NH_2$-terminal end (residues 24–45) (Fig. 3*B*). Several integral membrane proteins such as human and rat asialoglycoprotein receptors (18, 19), influenza virus neuraminidase (23), human invariant γ chain of class II histocompatibility antigens (24), and transferrin receptor (25) have a structure similar to the Fcε receptor at $NH_2$-terminal portion. In the case of influenza virus neuraminidase (23) and transferrin receptor (26), the transmembrane portion was shown to provide a signaling function using chimeric cDNA. The COOH-terminal portion of these proteins is outside of the cells.

The putative hydrophobic transmembrane domain near the $NH_2$ terminus, together with the marked homology with animal lectins (Fig. 4) suggests that Fcε receptor has its $NH_2$ terminus in the cytoplasm and that the COOH terminus is outside of the cells. Although we cannot exclude the possibility that Fcε receptor is an extrinsic membrane protein, the transmembrane orientation suggested above is supported by the reactivity of H107 mAb, which reacts with the 25-kDa soluble Fcε receptor as well as with the Fcε receptor on the cell surface. Indeed, the polypeptide sequence of the 25-kDa protein is encoded in the COOH-terminal portion of Fcε receptor cDNA.

An important question is the relationship between soluble Fcε receptor and IgE-BFs regulating *in vitro* IgE synthesis (9, 27). Indeed, Fcε receptors and IgE-BFs have a common

Table 1. Expression of Fcε receptor cDNA in COS-7 cells

| Source of DNA | Antibody | | | % positive |
|---|---|---|---|---|
| | First | Second | Third | |
| pKCR-ε R2A | H107 | αmIgG$^F$ | — | 4.7 |
| | Tac | αmIgG$^F$ | — | 0 |
| | IgE | αhIgE$^F$ | — | 3.7 |
| | αmIgG$^F$ | — | — | 0 |
| | αhIgE$^F$ | — | — | 0 |
| | H107 | IgE | αhIgE$^F$ | 0 |
| | Tac | IgE | αhIgE$^F$ | 3.9 |
| pKCR-ε R2B | H107 | αmIgG$^F$ | — | 0 |
| | IgE | αhIgE$^F$ | — | 0 |
| pKCR-Tac2A | Tac$^F$ | — | — | 7.7 |

pKCR-ε R2A with the same orientation to β-globin and pKCR-ε R2B with the reverse orientation to β-globin were the expression vectors for Fcε receptor cDNA. pKCR-Tac2A, the interleukin 2 receptor cDNA expression vector (17), was employed as positive control. For the inhibition of IgE binding to the Fcε receptor on the transfected COS-7 cells, the cells were preincubated with H107 mAb or Tac mAb as a control before incubation with IgE. The positive cells were counted by fluorescence and phase-contrast microscopy. αmIgG$^F$, αhIgE$^F$, and Tac$^F$ are fluorescein-conjugated goat anti-mouse IgG, goat anti-human IgE, and Tac, respectively.

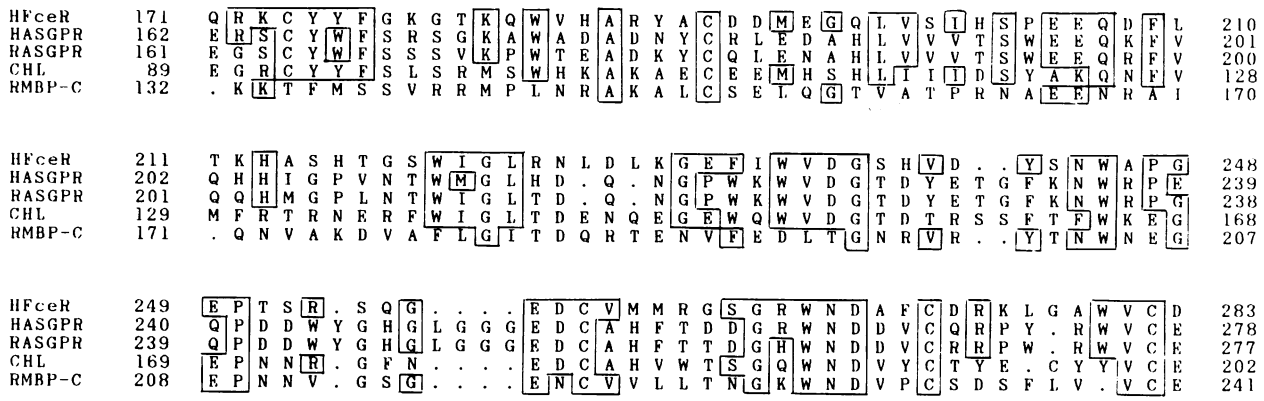

FIG. 4. Comparison of human Fcε receptor (HFce R), human and rat asialoglycoprotein receptors (HASGPR and RASGPR, respectively) (18, 19), chicken hepatic lectin (CHL) (20), and rat mannose binding protein-C (RMBP-C) (21, 22). Only residues that are conserved between human Fcε receptor and any of the other proteins are boxed. The numbers of amino acid residues are shown on both sides.

antigenic determinant (2). However, our human Fcε receptor cDNA has no homology to that of the rat IgE-BFs cloned (28). The molecular relationship between human Fcε receptor and the IgE-BFs is to be clarified. Furthermore, human Fcε receptor has no homology to the IgE binding protein from rat basophil leukemia cells (29). There may be several distinct sets of the receptors and/or soluble factors having affinity for IgE.

The unexpected homology between Fcε receptor and animal lectins is of interest. These lectins have specific sugar binding activities. For example, asialoglycoprotein receptors recognize terminal galactose, and chicken hepatic lectin recognizes terminal *N*-acetylglucosamine. The homology among these proteins including Fcε receptor is confined to the COOH-terminal portion (Fig. 4), which may contain the ligand-binding domain. From the evolutionary standpoint, the marked homology of Fcε receptor with animal lectins suggests that Fcε receptor and animal lectins have a common ancestral gene, which evolved before immunoglobulins. Fcε receptor and these animal lectins may constitute a gene superfamily. Fcε receptor cDNA may facilitate the study of the various diseases with the dysfunctions of the Fc receptor/immunoglobulin binding factor system (30, 31).

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