

The Nucleotide Sequence of Dinitrophenyl-Specific IgE and Fc ϵ RI α -Subunit Obtained from FE-3 Hybridoma Cells

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ABSTRACT

FE-3 cells were established by Hanashiro et al. by hybridizing mouse myeloma cells (Sp2/0-Ag14/SF) with rat spleen cells that were freshly isolated from Brown–Norway rats sensitized with DNP-As. FE-3 cells can constitutively secrete IgE without stimulation by cytokines. Our preliminary experiments demonstrated that the IgE secretion was decreased at 3 days after start of culture and the addition of exogenous IgE into culture media depressed the secretion of IgE. Thus, we hypothesized that the IgE production in FE-3 cells may be regulated by a signal transduction through the binding of IgE to its high affinity receptor (Fc ϵ RI) or to an IgE binding protein on the cell surface. In this study, we aimed to identify the nucleotide sequence of IgE FE-3 and compared with those of mouse IgE and IgE IR162 to find a structural heterogeneity in the Fc region of IgE FE-3. We also tested if the mRNA of Fc ϵ RI was expressed in FE-3 cells using the reverse transcriptase-polymerase chain reaction (RT-PCR) method with the combination of sequencing analysis. Consequently, the cDNA sequence of IgE FE-3 was identical to that of the CH3 and CH4 domains in the ϵ -chain of rat IgE IR162, whereas the cDNA of Fc ϵ RI was identical to that of mouse, suggesting that the genes of IgE FE-3 and Fc ϵ RI was derived from that of rat spleen cells and mouse myeloma cells, respectively.

INTRODUCTION

MANY STUDIES demonstrated that the type I allergy is mediated by the interaction between the IgE and its high affinity receptor (Fc ϵ RI), followed by cross-linking with a specific allergen. To study the type I allergy, we have used rats because the rat is a more useful animal than the mouse in terms of experimental procedures such as drug administration, blood sampling, and recording the resistance of airway in response to a specific allergen. It was also necessary to measure the precise IgE level in circulating blood, body fluid, and culture media to clarify the role of IgE in the type I allergy. However, only rat IgE derived from IR162 myeloma cells (IgE IR162) was commercially available for the research.^(1–10) Furthermore, IgE IR162 is not an antigen-specific IgE that is produced in the process of allergic reaction.

Antigen-specific IgE antibodies were desperately needed to study the mechanisms that underlie allergic reactions in response to a specific antigen. Therefore, IgE-secreting hybridoma (FE-3) was established by the cell fusion of mouse myeloma cells with the spleen cells from the Brown–Norway

rats, which were sensitized by dinitrophenyl (DNP)-conjugated proteins of *Ascaris suum* (DNP-As).⁽¹¹⁾ It was reported that there was a structural heterogeneity in the Fc region of rat IgE. Hanashiro et al.⁽¹²⁾ demonstrated that the purified rat IgE from sensitized rats by DNP-As was structurally different from IgE IR162 using Western blot with anti-rat myeloma IgE (Fc) antibody. Therefore, we first identified the nucleotide sequence of IgE FE-3 and compared with those of mouse IgE and IgE IR162 to find a structural heterogeneity in the Fc region.

In physiological condition, IgE-producing cells can produce and secrete IgE by the stimulation of various cytokines such as interleukin-4 (IL-4), IL-13, and CD40.^(13–15) However, FE-3 cells can constitutively secrete IgE without the stimulation by cytokines. In addition, in our preliminary experiments, we found that IgE secretion from FE-3 was decreased at 3 days after start of cell culture and that the addition of exogenous IgE into culture media depressed the secretion of IgE. Therefore, we hypothesized that IgE production in FE-3 cells may be regulated by a signal transduction through the binding of IgE to an IgE receptor (e.g., Fc ϵ RI) or an IgE binding protein on the cell surface.

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TABLE 1. PRIMERS FOR β -ACTIN, IgE, AND IgE RECEPTOR (Fc ϵ RI)

Name	Sense/antisense	Sequence (5' to 3')	Position	DDBJ* accession number
BA-S	sense	GAGCTATGAGCTGCCTGACGG	794–814	X03672 (mouse β -actin)
BA-AS	antisense	TTGCGGTGCACGATGGAGGG	1179–1198	X03672 (mouse β -actin)
A-1-1	sense	CTTACCTGTCTGGTTTTGGACCTGG	1179–1203	J00744 (rat IgE)
RT-1S	antisense	AAGCAGTGGTAACAACGCAGAGG		adopter region of RT-1
D-1-A	antisense	CGGAGGGAAGTGTTACCAAGGC	1744–1765	J00744 (IgE)
RIA-S1	sense	CCTGTGCAGTGGTAGCTGCC	56–75	J03606 (rat Fc ϵ RI α)
RIA-S2	sense	CTGTAGTGTCTTGGACCCACC	136–157	J03606 (rat Fc ϵ RI α)
RIA-AS	antisense	CCTGTGCAGTGGTAGCTGCC	594–614	J03606 (rat Fc ϵ RI α)
MIR-S	sense	GGTCACTGGAAGGTCTGCCC	42–61	J05018 (mouse Fc ϵ RI α)
MIR-AS	antisense	CTAGGGATCACATGAGTGGCC	881–901	J05018 (mouse Fc ϵ RI α)

*DDBJ: DNA Data Bank of Japan.

In this study, we aimed to identify the nucleotide sequence of IgE FE-3 and to test if Fc ϵ RI is expressed on the cell membrane of FE-3 cells.

MATERIALS AND METHODS

Reagents

Guanidinium thiocyanate, phenol, chloroform, isoamyl alcohol (3-methyl-1-butanol), isopropyl alcohol (2-propanol, isopropanol), ethanol, sodium acetate, sodium citrate, sarcosyl, and ethidium bromide were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Moloney murine leukemia virus reverse transcriptase(M-MLV RTase) was purchased from GIBCO BRL, Life Technologies, Inc. (Gaithersburg, MD). *Taq* DNA

polymerase was purchased from Promega Co. (Madison, WI). NucleoSpin Extraction Kits and Nucleospin RNA II Kits were purchased from CLONTECH Laboratories, Inc. (Palo Alto, CA). BigDye terminal cycle sequencing FS ready reaction kits were purchased from Perkin-Elmer Co. (Foster City, CA). NuSieve™ GTG agarose was purchased from FMC Bioproducts Co. (Rockland, ME). The primers used in this study were purchased from Hokkaido System Science Co. Ltd. (Hokkaido, Japan) and listed in Table 1. Unless otherwise stated, only reagent grade chemicals were used as other reagents.

Cell culture

FE-3 cells were established by Hanashiro et al.⁽¹¹⁾ by hybridizing mouse myeloma cells, Sp2/0-Ag14/SF (American Type Culture Collection, Rockville, MD) with rat spleen cells

β -actin FE-3	---GAGCTATGAGCTGCCTGACGG Y CAGGTCATCACTAT Y GGCA Y GAGCGGTTCGGATG	57
β -actin Mouse	GAAGAGCTATGAGCTGCCTGACGGCCAGGTCATCACTATTGGCAACGAGCGGTTCGGATG	850
β -actin Rat	GAAGAGCTATGAGCTGCCTGACGGTCAGGTCATCACTATCGGCAATGAGCGGTTCGGATG	850
β -actin FE-3	CCC Y GAGGCTCT Y TTCCAGCCTTCCTTC Y TGGGTATGGAATCCTGTGGCATCCATGAAC	117
β -actin Mouse	CCCTGAGGCTCTTTTCCAGCCTTCCTTCCTTGGGTATGGAATCCTGTGGCATCCATGAAC	910
β -actin Rat	CCCCGAGGCTCTCTTCCAGCCTTCCTTCCTGGGTATGGAATCCTGTGGCATCCATGAAC	910
β -actin FE-3	TACATTC AATTCCATCATGAAGTGTGACGTTGACATCCGTAAGACCTCTATGCCAACAC	177
β -actin Mouse	TACATTC AATTCCATCATGAAGTGTGACGTTGACATCCGTAAGACCTCTATGCCAACAC	970
β -actin Rat	TACATTC AATTCCATCATGAAGTGTGACGTTGACATCCGTAAGACCTCTATGCCAACAC	970
β -actin FE-3	AGTGCTGTCTGGTGG Y ACCACCATGTACCCAGGCAT Y GCTGACAGGATGCAGAAGGAGAT	237
β -actin Mouse	AGTGCTGTCTGGTGGTACCACCATGTACCCAGGCATTGCTGACAGGATGCAGAAGGAGAT	1030
β -actin Rat	AGTGCTGTCTGGTGGCACCACCATGTACCCAGGCATCGCTGACAGGATGCAGAAGGAGAT	1030
β -actin FE-3	TACTGC Y CTGGCTCCTAGCACCATGAAGATCAAGATCATTGCTCCTCCTGAGCGCAAGTA	297
β -actin Mouse	TACTGCTCTGGCTCCTAGCACCATGAAGATCAAGATCATTGCTCCTCCTGAGCGCAAGTA	1090
β -actin Rat	TACTGCCCTGGCTCCTAGCACCATGAAGATCAAGATCATTGCTCCTCCTGAGCGCAAGTA	1090
β -actin FE-3	CTCTGTGTGGAT Y GGTGGCTC Y ATCCTGGCCTCACTGTCCACCTTCCAGCAGATGTGGAT	357
β -actin Mouse	CTCTGTGTGGATCGGTGGCTCCATCCTGGCCTCACTGTCCACCTTCCAGCAGATGTGGAT	1150
β -actin Rat	CTCTGTGTGGATTGGTGGCTCTATCCTGGCCTCACTGTCCACCTTCCAGCAGATGTGGAT	1150
β -actin FE-3	CAGCAAGCAGGAGTACGATGAGTCCGGCCCCCTCCATCGTGACCCGCAA	405
β -actin Mouse	CAGCAAGCAGGAGTACGATGAGTCCGGCCCCCTCCATCGTGACCCGCAA	1198
β -actin Rat	CAGCAAGCAGGAGTACGATGAGTCCGGCCCCCTCCATCGTGACCCGCAA	1198

FIG. 1. The nucleotide sequence of β -actin obtained from FE-3 hybridoma cells. For comparison, the corresponding nucleotide sequences of β -actin obtained from the mouse and rat are shown. It was found that 11 bp of 405 bp in the β -actin gene from the FE-3 cells showed overlapping differences from both the mouse and rat nucleotide sequences. The Ys in bold of β -actin FE-3 represent heterozygous nucleotides (C and T), indicating that the nucleotide sequence of the β -actin gene in the FE-3 cells was heterozygous between the mouse and rat genes. The numbers in right-hand side indicate the positions of nucleotide at the end of each row from the start of cDNA (β -actin Mouse, X03672; β -actin Rat, V01217).

that were freshly isolated from Brown–Norway rats sensitized with DNP-As. The FE-3 cells were grown in RPMI 1640 medium (Nissui Pharmaceutical Co., Tokyo, Japan) containing 0.3 mg/mL of L-glutamine (Wako Pure Chemical Industries), 0.1 mg/mL of pyruvic acid (Sigma Chemical Co., St. Louis, MO), 0.2 μg/mL of insulin (Takeda Chemical Industries, Ltd., Osaka, Japan), 100 μg/mL of penicillin (Takeda Chemical Industries), 100 μg/mL of streptomycin (Meiji Seika Kaisha, Ltd., Tokyo, Japan) and 2% NaHCO₃ and supplemented with 10% fetal bovine serum (FBS) (Sanko Junyaku, Tokyo, Japan), as described previously.⁽¹¹⁾ The number of FE-3 cells in the collected culture medium was counted using a hemocytometer and were adjusted to 1 × 10⁵ cells/mL and cultured in 25-cm² flasks (Corning Inc., Corning, NY) at 37°C in a CO₂ incubator (Astec Inc., Fukuoka, Japan) under 5% CO₂-95% moisture. The cell viability was evaluated by the Trypan blue dye exclusion test. The collected culture medium was then centrifuged at 3000 rpm for 10 min at 4°C. After the cell pellet was washed, it was resuspended in 100 μL of cold phosphate-buffered saline (PBS).

Extraction of total RNA from FE-3 cells

The total cellular RNA was extracted from the FE-3 cells (1 × 10⁶ cells) using the acid guanidinium-phenolchloroform (AGPC) method as described by Chomczynski and Sacchi⁽¹⁶⁾ or NucleoSpin RNA II kit (CLONTECH Laboratories, Inc.). Briefly, 1 × 10⁶ cells of FE-3 cells were homogenized with 500 μL of the denaturing solution: 4 M guanidinium thiocyanate,

25 mM sodium citrate, pH 7.0; 0.5% sarcosyl, 0.1 M 2-mercaptoethanol. Subsequently, 50 μL of 2 M sodium acetate (pH 4.0), 500 μL of water-saturated phenol, and 100 μL of chloroform-isoamyl alcohol mixture (49:1) were added to the homogenate. The suspension was shaken vigorously for 10 sec and cooled on ice for 15 min and centrifuged at 15,000 rpm for 20 min at 4°C. After centrifugation, RNA in the aqueous phase was transferred to a new tube and precipitated by isopropanol, and then the RNA pellet was rinsed by 75% ethanol. Finally, the RNA pellet was dissolved in RNase free water. When using the NucleoSpin RNA II kit, total RNA was extracted according to the manufacturer's instructions. In this case, FE-3 cells were homogenized by passing through 21-gauge needle 10 times. Concentration of total RNA was determined by reading the absorbance at 260 nm using spectrophotometer.

Reverse transcription of mRNA

Reverse transcriptions (RTs) were carried out according to the standard procedures.^(17–19) In brief, 500 ng of total RNA in 22.5 μL of reaction mixture (5 μL of 5 × M-MLV buffer, 5 μL of 10 mM dNTP, 2.5 μL of 100 mM DTT, and mess up to 22.5 μL with RNase free water) and 0.5 μL of 50 μM synthetic oligo(dT)-adaptor primer (RT-1, 15'-AAGCAGTG-GTAAACAACGCAGAGGCC (dT)₃₁VN-3') were mixed. After denaturing at 75°C for 3 min, the reaction was cooled down on ice and initiated by adding 2 μL of M-MLV RTase and incubated for 2 h at 37°C. The synthesized first strand cDNA was stored at -20°C until use.

		CH3 domain ----->	
IgE FE-3	-----CTTACCTGCTCTGGTTTTGGACCTGGAAAGTGAGGAGAATATCACCGTGACGT		52
IgE IR162	TGTGATTACTTACCTGCTCTGGTTTTGGACCTGGAAAGTGAGGAGAATATCACCGTGACGT		1230
IgE FE-3	GGGTCGAGAGCGTAAGAAGTCTATAGGTTTCGGCATCCCAGAGGAGTACCAAGCACCATA		112
IgE IR162	GGGTCGAGAGCGTAAGAAGTCTATAGGTTTCGGCATCCCAGAGGAGTACCAAGCACCATA		1290
IgE FE-3	ATGCCACAACCAAGTATCACCTCCATCTTGCCAGTGGATGCCAAGGACTGGATCGAAGGTG		172
IgE IR162	ATGCCACAACCAAGTATCACCTCCATCTTGCCAGTGGATGCCAAGGACTGGATCGAAGGTG		1350
IgE FE-3	AAGGCTACCAAGTGCAGAGTGGACCAACCTCACTTTCCCAAGCCCATTTGCGTTCCATCA		232
IgE IR162	AAGGCTACCAAGTGCAGAGTGGACCAACCTCACTTTCCCAAGCCCATTTGCGTTCCATCA		1410
IgE FE-3	CCAAGGCCCCAGGCAAGCGCTCAGCCCCAGAGGTATATGTGTTCTGCCACCGGAGGAGG	CH4 domain ----->	292
IgE IR162	CCAAGGCCCCAGGCAAGCGCTCAGCCCCAGAGGTATATGTGTTCTGCCACCGGAGGAGG		1470
IgE FE-3	AGGAGAAGGACAAACGAACACTCACCTGCTTGATCCAGAATTTCTTCCCCGAGGATATCT		352
IgE IR162	AGGAGAAGGACAAACGAACACTCACCTGCTTGATCCAGAATTTCTTCCCCGAGGATATCT		1530
IgE FE-3	CTGTGCAGTGGCTGCAGGATAGCAAGCTGATCCCCAAAAGCCAACATAGTACCACGACAC		412
IgE IR162	CTGTGCAGTGGCTGCAGGATAGCAAGCTGATCCCCAAAAGCCAACATAGTACCACGACAC		1590
IgE FE-3	CTCTGAAATACAATGGCTCCAACCAACGCTTCTTCATCTTTAGCCGCCTGGAGGTCACCA		472
IgE IR162	CTCTGAAATACAATGGCTCCAACCAACGCTTCTTCATCTTTAGCCGCCTGGAGGTCACCA		1650
IgE FE-3	AGGCACCTCTGGACACAGACAAAACAGTTCACCTGCCGAGTGATCCATGAGGCACTTCGGG		532
IgE IR162	AGGCACCTCTGGACACAGACAAAACAGTTCACCTGCCGAGTGATCCATGAGGCACTTCGGG		1710
IgE FE-3	AACCCAGGAAGCTGGAGAGAACATATCCAAGAGCCTTGGTAACACTTCCCTCCG		587
IgE IR162	AACCCAGGAAGCTGGAGAGAACATATCCAAGAGCCTTGGTAACACTTCCCTCCG		1764

FIG. 2. The nucleotide sequences of the CH3 and CH4 domains of IgE obtained from FE-3 hybridoma cells (IgE FE-3). For comparison, the corresponding nucleotide sequence of IgE obtained from IR162 rat myeloma cells IgE IR162) is shown. It can be seen that 587 bp in the CH3 and CH4 domains revealed an overall homology of 100% at the DNA level. The numbers in right-hand side indicate the positions of nucleotide at the end of each row from the start of cDNA (IgE IR162, J00744). In the case of IgE IR162, CH3 domain starts from 1101 and ends at 1422, and CH4 domain starts from 1423 and ends at 1788, as indicated with two arrows on the alignment of the two nucleotide sequences.

Amplification of cDNA by polymerase chain reaction (PCR) and nested PCR

The cDNAs of the IgE FE-3 CH3, CH4 domain, FcεRI α subunit, and β-actin genes were amplified by the PCR method⁽²⁰⁾ with Program Temp Control System PC-800 (Astec Inc., Fukuoka, Japan). The Hot Start PCR was performed according to the method of D'Aquila et al.⁽²¹⁾ Briefly, 1st strand cDNA, *Taq* DNA polymerase enzyme and a pair of synthetic oligonucleotide primers were employed for amplification. Synthetic oligonucleotide primers were designed on the basis of the published sequences of β-actin (DDBJ accession number, X03672), ε-chain of IgE (J00744), and FcεRI α subunit (J03606). The primers used in this study were listed in Table 1. A pair of synthetic oligonucleotide primers, BA-S and BA-AS, was used for amplification of β-actin gene. A pair of synthetic oligonucleotide primers, A-1-1 and RTAS-1, was used for amplification of the cDNA fragment of IgE FE-3 CH3, CH4 domain. The nested PCR was then performed using the PCR product and a pair of synthetic oligonucleotide primers, A-1-1 and D-1-A. The cDNA fragment of FcεRI α subunit extracellular domain was amplified by the primers, RIA-S1 and RIA-AS with a subsequent nested PCR using RIA-S2 and RIA-AS. Condition for both PCR and nested PCR were: a single 2 min at 94°C, 30 cycles of 30 s at 94°C, 30 s at 58°C, 1 min at 74°C, followed by a single 5-min extension. PCR and nested PCR products were analyzed on NuSieve™ GTG agarose/Tris borate EDTA gel, visualized with ethidium bromide.

DNA sequencing

After the amplified cDNA fragments of β-actin, IgE and FcεRI α subunit by PCR was purified by NucleoSpin™ Extraction Kits, the nucleotide sequences were analyzed by an ABI PRISMTM 310 genetic analyzer (Perkin-Elmer Co.) with the BigDye terminal cycle sequencing FS ready reaction kits (Perkin-Elmer Co.). This sequencing analysis is based on the dideoxy nucleotide chain termination methods by Sanger et al.⁽²²⁾ The synthetic oligonucleotides, BA-S, BA-AS, A-1-1, D-1-A, RIA-S2, and RIA-AS, were used as primers. The cycle sequencing was carried out according to the manufactures' instructions.

RESULTS

Determination of the nucleotide sequences of β-actin and IgE FE-3 in FE-3 cells

The primers (BA-S and BA-AS) specific for β-actin gene detected a single band at the size of 405 bp by PCR. The sequencing results of the PCR product demonstrated that 11 bp of 405 bp in the β-actin gene from the FE-3 cells showed overlapping differences from both the rat⁽²³⁾ and mouse^(24,25) nucleotide sequences (Fig. 1). The primers (A-1-1 and D-1-A) specific for ε-chain of IgE detected a single band at the size of 587 bp by nested PCR, following the first PCR with the primers

FcεRIα Rat	AGCTC ACT GGTGCAGTTAGCACCTGAAGG CAC AGGGGCAATGG ATA CTGGAG AT CTGCC	74
FcεRIα Mouse	AGCTCTCTGGTGCAGTTAGCACCTGAAGGTGCAGGGGCGATGGTCACTGGAAAGGTCTGCC	60
RIA-S1 →		
FcεRIα Rat	CGGCTGTGCCTAGCATT AGT GCATATCTCTGGGTGTCATGCT AA CAGCCACT CAG AAA	134
FcεRIα Mouse	CAGCTGTGCCTAGCAGTCTGTTTCATGTCTCTTGATGTCATTCTGCAGCCACTGAGAAA	120
RIA-S2 →		
FcεRIα Rat	TCTGT AGT GTCTTGGACCCACCGTGGATTAGAATACTTACAGGAGAGTAAAGTGACTCTT	194
FcεRIα Mouse	TCTGTACTGACCTTGGACCCACCATGGATTAGAATATTTACAGGAGAGAAAGTGACCCTT	180
FcεRIα Rat	ATAT GC AA TGGGAACAAT TCCT CTCAAATGAACT---CTACTAAATGGATCCACAATGAT	251
FcεRIα Mouse	TCCTGCTATGGGAACAATCACCTTCAAATGAACTCTACTACTAAATGGATCCACAATGGT	240
FcεRIα Rat	AGC AT CTCT AA TGTGAAATCGTCACATTGGGTCAATTGTGAGTGCCACCA ATT CAAGACAGT	311
FcεRIα Mouse	ACCGTCTCTGAGGTGAACCTTTCACATTGGTTCATTGTGAGTGCCACCGTTCAAGACAGT	300
FcεRIα Rat	GGAAAATACATATGTGAGAAGCAAGGATTT ATA AGAGCAAACCTGTGTACTTGAA CGTG	371
FcεRIα Mouse	GGAAAATACATATGTGAGAAGCAAGGATTTGTTTAAGAGTAAACCTGTGTACTTGAAATGTA	360
FcεRIα Rat	ATGCAAGAGTGGCTGCTGCT C CA AT CTCTGCTGACG TGGT CTTAG ACA ATGGATCCTTT	431
FcεRIα Mouse	ACGCAAGATTGGCTGCTCCTTCAGACATCTGCTGACATGATCTTAGTCCATGGATCCTTT	420
FcεRIα Rat	GACATCAGATGCC GT AGCTGGAAGAA AT GGAA AGT CC ACA AGGTGATCTACTACA AGGAC	491
FcεRIα Mouse	GACATCAGATGCCATGGCTGGAAGAACTGGAATGTCCGCAAGGTGATCTACTACAGGAAT	480
FcεRIα Rat	GAC ATT GTCTTT CAG TACTCTTATGACAGCA ACA CACTCTCCATTAGAA AGGCCACATTT	551
FcεRIα Mouse	GACCATGCTTTCAACTACAGTTATGAGAGCC---CCGTCTCCATTAGAGAGGCCACACTG	538
RIA-AS ←		
FcεRIα Rat	AATGACAGTGGCAGCTACCACTGC ACAGGC	581
FcεRIα Mouse	AATGACAGTGGCACCTACCACTGCAAGGGC	568

FIG. 3. Comparison of the established nucleotide sequences of IgE high affinity receptor (FcεRI) α subunit from rat RBL-2H3 and mouse mast cell line PT18. Rat-specific nucleotides are represented in bold. The designed primers (RIA-S1, RIA-S2, and RIA-AS) are indicated with arrows on the alignment of the two nucleotide sequences. The numbers in right-hand side indicate the positions of nucleotide at the end of each row from the start of cDNA (FcεRI α Rat, J03606; FcεRI α Mouse, J05018).

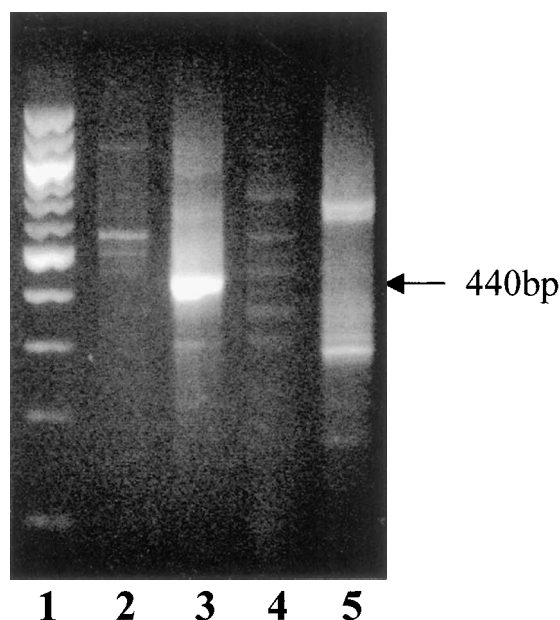


FIG. 4. Detection of mRNA of FcεRI α subunit by nested PCR following RT-PCR. Lane 1: 100bp DNA stepladder marker. Lane 2: RT-PCR product with RIA-S1/RIA-AS primers. Lane 3: nested PCR product with RIA-S2/RIA-AS primers using the 1:100 diluted RT-PCR product (as in Lane 2) as a template. Lane 4: PCR product with RIA-S1/RIA-AS primers using total RNA as a template. Lane 5: Nested PCR product with RIA-S2/RIA-AS primers using the 1:100 diluted PCR product (as in Lane 4) as a template.

(A-1-1 and RTAS-1). The sequencing results of the PCR product demonstrated a complete identity with the previously published sequence of IgE IR162, which corresponds the constant region (CH3 and CH4 domains) in the ϵ -chain (Fig. 2). The nucleotide sequence data reported in this paper will be appeared in the DDBJ nucleotide sequence databases with the accession number AB028845, AB028846, and AB028847.

Detection of mRNA of FcεRI α subunit by nested PCR following RT-PCR

To test if the mRNA of FcεRI was expressed in FE-3 cells, RT-PCR was carried out. Primers were designed on the basis of the previously published sequences of FcεRI subunits (α , β , and γ).⁽²⁶⁾ Consequently, the homology of FcεRI α subunit between rat and mouse (87%) is higher than those of other subunits (β and γ), and the extracellular domain of α subunit was evidenced to serve as the binding site for IgE.^(27,28) Thus, the specific primers (RIA-S1, RIA-S2, and RIA-AS) targeting the extracellular domain of FcεRI α subunit was designed by comparing the two established nucleotide sequences: FcεRI α subunit from rat RBL-2H3,⁽²⁹⁾ mouse mast cell PT18⁽²⁶⁾ (Fig. 3).

PCR was carried out with the RIA-S1/RIA-AS primers, followed by nested PCR with the RIA-S2/RIA-AS primers. PCR and nested PCR products were analyzed by agarose electrophoresis, and then visualized with ethidium bromide by UV transilluminator (Fig. 4). In Lanes 2 and 4, there were many weak nonspecific bands over the lane. In Lane 3, there was an

intense band at 440 bp, the size of which was the same as the target. Because there was no specific band at 440 bp when total RNA was used as a template for nested PCR following PCR, the 440 bp band in Lane 3 was amplified from mRNA, not from contaminated DNA. The nested PCR product in Lane 3 was extracted from agarose gel and its sequence was analyzed. Consequently, the sequence of nested PCR product was identical to mouse FcεRI α subunit extracellular domain cDNA corresponding 158–534. Therefore, it was evidenced that the mRNA of FcεRI α subunit extracellular domain was expressed in FE-3 cells.

Next, we tried to identify the cDNA coding all amino acids of FcεRI α subunit protein except for signal peptide. The specific sense primer, MIR-S, was designed by referring to the nucleotide sequence of mouse FcεRI α subunit cDNA (corresponding 42–61 of mouse FcεRI α subunit cDNA). RT-1S, which has the adopter sequence of RT-1 primer, was used as an anti-sense primer. Nested PCR was carried out using RIA-S1/MIR-AS primers following RT-PCR with MIR-S/RT-1S primers. The PCR and nested PCR products were analyzed by agarose electrophoresis with 2.5% agarose gel (Fig. 5). Lane 2 is smeared with nonspecific unidentified bands and there are also several clear nonspecific bands. In Lane 3, there was an intense band at 850 bp, the size of which was the same as the target.

The nested PCR product (Lane 3 in Fig. 5) was extracted from the agarose gel and the sequence was analyzed. Consequently, 794 nucleotides out of 850 nucleotides could be sequenced, with the resultant sequence being 100% identical to mouse FcεRI α subunit cDNA. Therefore, it was evidenced that the cDNA of FcεRI α subunit was expressed in FE-3 cells (Fig. 6).

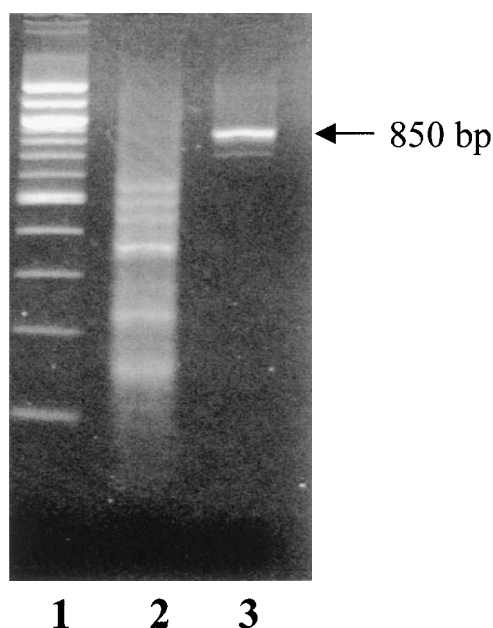


FIG. 5. Amplification of mRNA of FcεRI α subunit including all amino acids except for signal peptide by nested PCR following RT-PCR. Lane 1: 100 bp DNA step ladder marker. Lane 2: RT-PCR product with MIR-S/RT-1S primers. Lane 3: Nested PCR product with RIA-S2/RIA-AS primers using the 1:100 diluted RT-PCR product (as in Lane 2) as a template.

FcεRIα	FE-3	-----	→ signal peptide	1
FcεRIα	Mouse	AGCTCTCTGGTGCAGTTAGCACCTGAAGTGCAGGGCCATGGTCACTGGAAGGTCTGCC		60
FcεRIα	FE-3	-----GTCTCTTGATGTCAATTCTGACAGCCACTGAGAAA	→ extracellular domain	34
FcεRIα	Mouse	CAGCTGTGCCTAGCACTGCTGTTTCATGTCTCTTGATGTCAATTCTGACAGCCACTGAGAAA		120
FcεRIα	FE-3	TCTGTACTGACCTTGGACCCACCATTGGATTAGAATATTTACAGGAGAGAAAAGTGACCCTT		94
FcεRIα	Mouse	TCTGTACTGACCTTGGACCCACCATTGGATTAGAATATTTACAGGAGAGAAAAGTGACCCTT		180
FcεRIα	FE-3	TCCTGCTATGGGAACAATCACCTTCAAATGAACTCTACTACTAAATGGATCCACAATGGT		154
FcεRIα	Mouse	TCCTGCTATGGGAACAATCACCTTCAAATGAACTCTACTACTAAATGGATCCACAATGGT		240
FcεRIα	FE-3	ACCGTCTCTGAGGTGAACTCTTCACATTGGTCATTGTGAGTGCCACCGTTCAAGACAGT		214
FcεRIα	Mouse	ACCGTCTCTGAGGTGAACTCTTCACATTGGTCATTGTGAGTGCCACCGTTCAAGACAGT		300
FcεRIα	FE-3	GGAAAAATACATATGTGAGAAGCAAGGATTGTTTAAAGAGTAAACCTGTGTAATGTA		274
FcεRIα	Mouse	GGAAAAATACATATGTGAGAAGCAAGGATTGTTTAAAGAGTAAACCTGTGTAATGTA		360
FcεRIα	FE-3	ACGCAAGATTGGCTGCTCCTTCAGACATCTGCTGACATGATCTTAGTCCATGGATCCTTT		334
FcεRIα	Mouse	ACGCAAGATTGGCTGCTCCTTCAGACATCTGCTGACATGATCTTAGTCCATGGATCCTTT		420
FcεRIα	FE-3	GACATCAGATGCCATGGCTGGAAGAACTGGAATGTCCGCAAGGTGATCTACTACAGGAAT		394
FcεRIα	Mouse	GACATCAGATGCCATGGCTGGAAGAACTGGAATGTCCGCAAGGTGATCTACTACAGGAAT		480
FcεRIα	FE-3	GACCATGCTTTCAACTACAGTTATGAGAGCCCCGTCTCCATTAGAGAGGCCACACTGAAT		454
FcεRIα	Mouse	GACCATGCTTTCAACTACAGTTATGAGAGCCCCGTCTCCATTAGAGAGGCCACACTGAAT		540
FcεRIα	FE-3	GACAGTGGCACCTACCACTGCAAGGGCTATCTTAGGCAGGTGGAATATGAATCTGACAAA		514
FcεRIα	Mouse	GACAGTGGCACCTACCACTGCAAGGGCTATCTTAGGCAGGTGGAATATGAATCTGACAAA		600
FcεRIα	FE-3	TTCAGAATTGCTGTAGTAAAAGCTTACAAATGCAAGTATTATTGGCTACAACATAATTTTC	→ transmembrane domain	574
FcεRIα	Mouse	TTCAGAATTGCTGTAGTAAAAGCTTACAAATGCAAGTATTATTGGCTACAACATAATTTTC		660
FcεRIα	FE-3	CCATTGTTGGTGGCGATTCTGTTTGTCTGTGGACACGGGGTTATTGCTCTCAACCGAAGAA	→ cytoplasmic domain	634
FcεRIα	Mouse	CCATTGTTGGTGGCGATTCTGTTTGTCTGTGGACACGGGGTTATTGCTCTCAACCGAAGAA		720
FcεRIα	FE-3	CAGTTCAAATCAGTCTTGAGATTGAGAAGCTGGAAAATACAAGAAAGTTGAAACCGAA		694
FcεRIα	Mouse	CAGTTCAAATCAGTCTTGAGATTGAGAAGCTGGAAAATACAAGAAAGTTGAAACCGAA		780
FcεRIα	FE-3	CTCCTAACCTAGATACATATAGGAACTAATGTCATTGCTGAAGAAGCAATTCTTAACAGT	→ 3'-UTR	754
FcεRIα	Mouse	CTCCTAACCTAGATACATATAGGAACTAATGTCATTGCTGAAGAAGCAATTCTTAACAGT		840
FcεRIα	FE-3	AATTTTCTGCAGAACTCCTCAATAGCTTCTCCACTGTCAAA-----		794
FcεRIα	Mouse	AATTTTCTGCAGAACTCCTCAATAGCTTCTCCACTGTCAAAGGCCACTCATGTGATCCCTA		900

FIG. 6. Comparison of the nucleotide sequences between FcεRI α subunit in FE-3 cells and the established sequence of mouse mast cell PT18. In the case of FcεRI α (Mouse), open reading frame starts at the initiation codon (ATG) and ends at the termination codon (TAG) corresponding 40 and 792 of mouse FcεRI, respectively. Sixty-nine nucleotides from 40 to 108 are coding the signal peptide of 23 amino acids. The FcεRI α subunit protein (Mouse) is coded by the nucleotides from 109 to 789 and consists of three domains: extracellular domain, transmembrane domain, and cytoplasmic domain. The numbers in right-hand side indicate the positions of nucleotide at the end of each row from the start of cDNA (FcεRI α Mouse, J05018).

DISCUSSION

Our working hypothesis is that the IgE production in FE-3 cells may be regulated by a signal transduction through the binding of IgE to its receptor or binding protein on the cell surface *in vivo*. In the present study, RT-PCR with the combination of the sequence analysis revealed that mRNAs of IgE FE-3 and FcεRI α subunit were expressed in FE-3 cells.

It was also very interesting from which species the expressed mRNAs of IgE and FcεRI in FE-3 cells were derived because FE-3 cells were established by the cell fusion of mouse myeloma cells and rat spleen cells. It was revealed that the cDNA sequence of IgE FE-3 was identical to that of the CH3 and CH4 domains in the ε-chain of rat IgE IR162,^(6,30,31) whereas the cDNA of FcεRI was identical to that of mouse, suggesting that the genes of IgE FE-3 and FcεRI was derived from that of rat spleen cells and mouse myeloma cells, respec-

tively. In addition, one of housekeeping genes, β-actin, was expressed in FE-3 cells and sequencing analysis revealed that 11 bp of 405 bp in the β-actin cDNA were derived from rat and mouse, demonstrating the simultaneous expressions of rat and mouse β-actin cDNA.⁽²³⁻²⁵⁾ It was thus clarified that the nucleotide sequence of the β-actin gene in the FE-3 hybridoma cells was heterozygous between the rat and mouse genes.

In our preliminary study, we found that the secretion of IgE from FE-3 cells was decreased at 3 days after start of culture. We also found that secretion of IgE from FE-3 cells was decreased by the addition of purified IgE FE-3 into culture media. In the present study, it was evidenced that FcεRI α subunit mRNA was expressed in FE-3. These results suggested that the binding of IgE FE-3 to FcεRI α subunit cells might negatively regulate the IgE production in FE-3 cells. However, it remains unclear whether the FcεRI α subunit protein is expressed on the cell surface of FE-3 cells. Simth et al.⁽³²⁾ reported that blood eosinophils from atopic

donors expressed mRNA of FcεRI α subunit in the cell but did not express α subunit protein on the cell surface.

The type I hypersensitivity reactions are mediated by the interaction between the IgE produced by B cell and its high affinity receptor (FcεRI), followed by cross-linking with a specific allergen. It has been reported that IgE were bound to Fcε RI, which are expressed on target cells including mast cell,^(26,29,33,34) basophil,^(34–37) and eosinophil.^(32,39,40) When the Fcε RI-bound IgE antibodies are cross-linked by an antigen, the target cells are activated for degranulation and release bioactive mediators. In addition, it was also evidenced that Fcε RI was expressed in many types of cells other than target cells, including macrophages,^(39,41) Langerhans cells,^(39,42) megakaryocytes,^(43,44) platelets,^(43,44) monocytes,^(41,45,46) dendritic cells,^(36,47) and bronchial epithelial cells.⁽⁴⁸⁾ These cells do not produce and secrete IgE. Conversely, IgE secreting cells such as B cells do not express FcεRI receptor. Thus, it was unique that FE-3 cells actually expressed those two mRNAs. It has not yet been clarified how those two mRNAs are simultaneously expressed in FE-3 cells. The possible answers to this question are as follows: (1) The FcεRI gene may translocate to 5'- or 3'-flanking region of IgE ε-chain gene, resulting in constructing a consecutive single gene. Thus, the two different genes might be separately expressed by alternative splicing. (2) The two different genes may become to have a common promoter sequence at their upstream regions and to share a common transcription factor in the process of hybridization.

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