

TRANSFECTOMAS EXPRESSING BOTH SECRETED AND MEMBRANE-BOUND FORMS OF CHIMERIC IgE WITH ANTI-VIRAL SPECIFICITY¹

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Because of the lack of a cell line expressing on surface and secreting human IgE of known Ag specificity, the construction of a transfectoma line possessing such properties would be useful for studying the roles of surface IgE and the effects of anti-IgE antibodies on IgE-producing B cells. Toward this goal, the human genomic DNA segment encompassing the two exons encoding the membrane anchor peptide of ϵ -chain and their flanking regions was sequenced. Hybrid ϵ and κ genomic DNA comprising the C regions of human ϵ - and κ -chains and the H and L chain V regions of the murine mAb BAT123, which reacts with the gp120 envelope protein of HIV-1, were constructed. Mammalian expression vectors containing these fusion genes were used to transfect murine myeloma Sp2/0 cells, and transfectants stably expressing on surface and secreting into culture medium chimeric IgE were obtained. The chimeric IgE showed identical Ag-binding properties as the murine mAb BAT123. Acting in concert with the specific peptide Ag polyvalently coupled to a protein carrier, the chimeric antibody could induce histamine release from human blood basophils. These results demonstrate the potential utility of the transfectoma cells and the chimeric IgE in studying the roles of membrane-bound IgE and effects of anti-IgE antibodies on IgE-producing B cells.

IgE plays a central role in mediating various immediate-type hypersensitivity reactions, by virtue of its isotype-specific, high affinity binding to the IgE·FcR on mast cells and basophils (1). During an allergic reaction in a sensitized individual, the multivalent allergens cross-link IgE on the surface of mast cells and basophils, aggregate the underlying receptors, and hence trigger the release of histamine and other pharmacologic mediators (2), leading to various manifestations of allergic diseases (3–5). This effector function of IgE and its cytotropism and roles in mediator release have dominated IgE studies since it was discovered as a reagin about 25 years ago (3–5). In recent years, the regulation of IgE synthesis has gradually drawn substantial interest, and much work is being done

to study the development and maturation of surface IgE-expressing and IgE-producing cells (6, 7) and the roles of various lymphokines and cytokines on these processes (8–10).

The Ig on the surface of B cells are Ag receptors and believed to play regulatory roles in the activation, differentiation, and isotype switching of B cells (11, 12). Antibodies against various Ig isotypes have been shown to exert a range of effects on the activity and development of B cells in vitro and in vivo (13–15). In clinical trials, antibodies against the Id of Ig on the surface of B cells have been shown to suppress the growth of or to eliminate those malignant cells in some patients with B cell lymphomas (16, 17). Recently, we have identified a number of anti-IgE mAb, which exhibit binding specificity for IgE on B cells, but not for IgE bound to high and low affinity IgE·FcR (18). We are attempting to investigate the effects of such mAb on IgE-expressing B cells. The practical implication is that if these mAb could mediate the suppression or removal of the IgE-expressing B cells, they might be used in patients with severe allergic diseases to alleviate symptoms.

A variety of human lymphoblastoid cell lines have been used as experimental tools in the studies of the roles of surface Ig and of the effects of anti-Ig antibodies on B cells. Reflective of the minute levels of IgE in the circulation of man (4), IgE-expressing and IgE-producing B cells account for very small fractions of human B cells (6, 19). The occurrence of IgE myelomas is also very rare and only one human IgE-producing cell line, U266, has ever been established (20). Despite the ample applications of the human myeloma U266 cell line and its subclone, the SKO-007 cell line, for various experiments, the low density of IgE on the cell surface and the unknown Ag specificity limit its further application. Neuberger et al. (21) have constructed a transfectoma secreting a hapten-specific chimeric IgE with human immune effector functions. In the present studies, we have constructed a cell line that both expresses on surface and secretes into medium a mouse/human chimeric IgE with specificity for the envelope protein of HIV-1.

MATERIALS AND METHODS

DNA sequencing. DNA sequencing of recombinant plasmids was performed by using a Sequenase sequencing kit (U. S. Biochemicals, Cleveland, OH), following the protocol for sequencing double-stranded templates as specified by the manufacturer. Oligonucleotide primers needed for the sequencing were synthesized on a Bio-search Cyclone DNA synthesizer (San Rafael, CA).

RNA isolation and analysis. Cytoplasmic RNA was extracted from actively dividing cell cultures (22). RNA samples were dena-

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tured at 60°C with 50% formamide and 2.2 M formaldehyde and electrophoresed on 1% agarose gels containing 2.2 M formaldehyde. RNA was transferred to nitrocellulose and hybridized with ³²P-labeled probes. ³²P-labeled probes were prepared by nick translation or random primer extension with the use of hexadeoxynucleotide mixtures (Pharmacia, Piscataway, NJ).

Gene construct. DNA fragments derived from phage clone 38A (23) (a gift from Dr. P. Leder) were used to reconstruct the human ϵ gene-containing exons encoding the four constant region domains and the transmembrane anchor peptide. Because no convenient restriction enzyme sites were present to allow for the direct isolation of the complete ϵ gene from phage clone 38A, the 5.5-kb *HindIII*/*XbaI* fragment containing domains CH1 to 4 and the 900-kb *XbaI*/*SacI* fragment containing the membrane exons were subcloned into pUC 18 separately. The complete ϵ gene was then reconstructed by adding the 900-bp *XbaI*/*SacI* fragment at the 3' end of the 5.5-kb *HindIII*/*XbaI* fragment. The resulting 6.4-kb DNA segment containing domains CH1 to 4 and membrane exons was used to construct the expression vector containing the chimeric ϵ gene.

Cell culture. Mouse myeloma Sp2/0 and transfectoma cells were cultured in 5% CO₂ at 37°C in Iscove's modified Dulbecco's medium (GIBCO, Grand Island, NY) supplemented with epidermal growth factor (GIBCO) at 10 µg/liter, insulin (Sigma, St. Louis, MO) at 5 mg/liter, human transferrin (Sigma) at 5 mg/liter, and 2.5% FCS (GIBCO). The human IgE myeloma cell line SKO-007 (American Type Chemical Co., Rockville, MD) was maintained in RPMI 1640 medium (GIBCO) containing 5% FCS.

Gene transfer by electroporation. DNA of the chimeric Ig plasmid was introduced into mouse myeloma Sp2/0 cells by electroporation with the use of a CellZap.II apparatus (Andersen Systems, Brookline, MA) set at an electric field of 180 V and a capacitance of 1250 µF. A total of 8×10^6 Sp2/0 cells was washed with PBS and resuspended in 0.8 ml of PBS containing 25 µg of DNA. After being left on ice for 5 min, they were electroporated (24), left on ice for another 0.5 h, diluted 10-fold in growth medium, incubated for 0.5 h at room temperature, and plated at 5×10^4 cells/well in 96-well microtiter plates. After 48-h incubation, the transfectants were selected in medium containing G418 (GIBCO) at 0.2 mg/ml for the κ -chain plasmid uptake and mycophenolic acid (Sigma) at 1 µg/ml, xanthine (Sigma) at 250 µg/ml, and hypoxanthine (Sigma) at 15 µg/ml for the ϵ -chain plasmid uptake. The frequency of transfectants resistant to both drugs was approximately 2×10^{-5} .

Quantitation of chimeric IgE production. Stable drug-resistant transfectants were screened for the production of human IgE by ELISA. Immulon 2 microassay plates (Dynatech Laboratories, Chantilly, VA) were coated with 100 µl of murine anti-human IgE mAb HP6029 at 1 µg/ml in PBS overnight at room temperature. Wells were then treated with PBSB for 1 h and washed with PBST. Fifty microliters of culture medium from the wells with cell growth were added to the anti-IgE mAb-coated well and incubated for 1 h. After the plates were rinsed with PBST,³ 100 µl of biotin-conjugated anti-human IgE mAb TES 19 (18), diluted at 1/1000 in PBSTB, were added to each well. The plates were incubated for another hour and washed with PBST. A total of 100 µl of peroxidase-conjugated streptavidin (Kirkegaard & Perry Laboratory, Gaithersburg, MD), diluted at 1/50,000 in PBSTB, was added to each well, and plates were incubated for 1 h. Peroxidase substrate solution containing 0.1% 3',3',5',5'-tetramethyl benzidine (Sigma) and 0.003% hydrogen peroxide (Sigma) was added at 200 µl/well and incubated at room temperature for 0.5 h. The reaction was stopped by the addition of 50 µl of 2 M sulfuric acid and the OD of the reaction mixture in each well was read with a BioTek ELISA auto-reader (Winooski, VT) at 450 nm. To determine the concentration of antibody produced by the transfectomas, purified human IgE (Pharmacia) was used to generate a standard curve.

Immunofluorescence flow cytometric analyses. Membrane-bound chimeric or myeloma IgE on the cell surface was detected by fluorescence flow cytometry. A total of 2×10^6 cells was washed and incubated at 4°C for 0.5 h in 100 µl of anti-human IgE mAb TES 19 at 10 µg/ml in PBSB. After washing with PBSB, the cells were incubated in 20 µl fluorescein-conjugated goat (Fab')₂ anti-mouse IgG (H + L) (Boehringer Mannheim, Indianapolis, IN) at 1/20 dilution in PBSB at 4°C for 0.5 h. The cells were washed in PBSB, fixed in 1% paraformaldehyde (Polysciences, Warrington, PA) in PBS, and analyzed on a Coulter EPICS flow cytometer.

Antibody purification and analysis. For purifying IgE from culture supernatants of transfectomas or myelomas, an affinity column packed with anti-IgE mAb-conjugated resin was prepared. A murine

anti-human IgE mAb TES 19 was coupled to Affigel-10 (BioRad) according to the manufacturer's suggested procedure. After culture supernatant had been passed through the column, the column was extensively washed with PBS, and bound chimeric IgE or myeloma IgE was eluted with 50 mM diethylamine at pH 11.5. Purified IgE was dialyzed against PBS and concentrated. Antibody samples were analyzed by SDS-PAGE on 10% gels under reducing and nonreducing conditions. Protein was also transferred to nitrocellulose by using a Bio-Rad mini Trans-Blot electrophoretic transfer cell apparatus at 100 V for 1 h. The nitrocellulose filters were treated with Blotto (5% nonfat dry milk in PBS) for 0.5 h and then with horseradish peroxidase-labeled goat anti-human IgE (Kirkegaard & Perry) at 25 µg/ml, or horseradish peroxidase-labeled goat anti-human kappa (Fisher Scientific, Orangeburg, NY) at 20 µg/ml in Blotto overnight at room temperature. After three washes in PBST, the filters were developed by incubation in 0.3 mg/ml 4-chloronaphthol and 0.009% H₂O₂ at room temperature.

Binding inhibition assay. Ag-binding specificity of the chimeric IgE was assayed by ELISA for its ability to compete with peroxidase-conjugated mAb BAT123. Wells of 96-well plates were coated with 50 µl of affinity-purified gp120 at 0.5 µg/ml in PBS overnight at room temperature, and then blocked with PBSB for 1 h. After the wells were washed with PBST, 100 µl purified antibody solution of chimeric IgE or IgG were added to each well containing peroxidase-conjugated mAb BAT123 at 1/50,000 dilution (stock was about 1 mg/ml) in PBSB. The plate was incubated for 1 h, washed, and peroxidase substrate solution at 200 µl/well, and incubated for another 0.5 h. The reaction was stopped by the addition of 50 µl of 2 M sulfuric acid and the OD was read with a Bio-Tek ELISA auto-reader at 450 nm.

Histamine release assay. Basophils from healthy volunteers were partially purified from the peripheral blood leukocytes of donors by centrifugation on a single step Percoll gradient (1.080 g/ml) as previously described (25). More than 50% of the total cell histamine content could be released from these basophil preparations in response to polyclonal anti-human IgE at 0.1 µg/ml. After the basophils were incubated for 0.5 h at 37°C with chimeric IgE or chimeric IgG at 10 µg/ml, they were washed free of the sensitization buffer, and then challenged with various amounts of the Ag. After another 45 min at 37°C, the cell supernatants were harvested and their histamine contents measured by using a fluorometric assay (26). Histamine release is expressed as percentage of total histamine release induced by cell lysis. Polyvalent Ag, R15K-OVA, was prepared by conjugating to OVA a synthetic peptide, R15K, which consists of amino acid residues 308 to 322 of gp120 of HTLV-III_B strain of HIV-1. The control Ag, HGE20K-OVA, was a conjugate of OVA with a synthetic peptide of 20 amino acid residues, the sequence of which is derived from a segment of human Cγ1 region. PS myeloma IgE was a gift from Dr. K. Ishizaka.

RESULTS

Identification of membrane-specific exons in human ϵ locus. Recombinant λ phage clone 38A containing a human germ line ϵ constant gene (23) was analyzed to determine the 3' boundary of the insert DNA. Based on restriction enzyme analyses, it was estimated that there was an additional DNA segment of approximately 3 kb downstream from the 3-kb *Bam*HI fragment containing the four constant domains of the ϵ gene. By assuming an analogy to the reported mouse (27) and rat (28) ϵ loci, the membrane exons were estimated to be located within the 1-kb *SacI* fragment 2.3 kb from the 3' end of the ϵ gene (Fig. 1). This 1-kb *SacI* fragment was subcloned into pUC19 and its nucleotide sequence determined (Fig. 2). The sequence from position 369 to 717 was compared with the corresponding published murine sequence. The positions of the exon/intron boundaries were deduced from a comparison with the proposed genomic structures of the rat and mouse ϵ genes. The nucleotide sequences indicate that M1 is 122 bp long, encoding 41 amino acids, and M2, located 82 bp 3' to M1, is 81 bp long, encoding 27 amino acids. The homology of these two exons between human and murine sequences are 68% and 51% at the nucleotide and amino acid levels, respectively.

The cloning of the V genes of BAT123 and the construc-

³ Abbreviations used in this paper: PBST, PBS + 0.05% Tween 20; PBSB, PBS + 1% BSA; PBSTB, PBST + 1% BSA; *gpt*, *Escherichia coli* xanthine-guanine phosphoribosyl transferase gene; *neo*, Tn5 neomycin resistance gene.

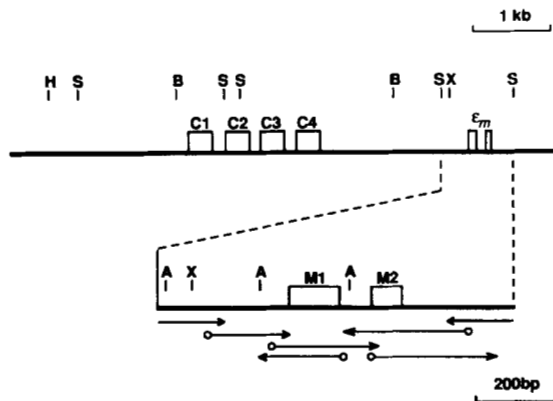


Figure 1. Partial restriction map and sequencing strategy. The upper part of the figure shows the restriction map of a human ϵ -chain genomic DNA clone. C1, C2, C3, and C4 are exons encoding the CH1-CH4 domains of ϵ -chain. ϵ_m denotes the location of presumed exons encoding the transmembrane anchor peptide of ϵ -chain. Restriction endonuclease abbreviations: B, BamHI; H, HindIII; S, SacI; X, XbaI; and A, ApaI. The lower part of the figure summarizes the sequencing strategy. Nucleotide sequence was determined by Sanger's procedure with the use of synthetic oligonucleotide primers represented by open circles. The arrows indicate the direction and extent of sequencing. M1 and M2 are exons encoding the membrane anchor peptide.

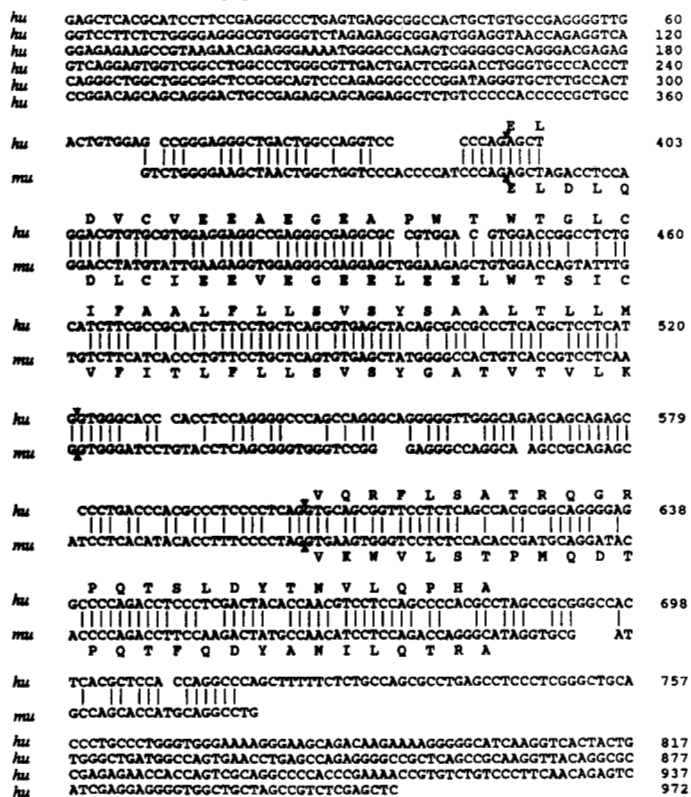


Figure 2. Sequence comparison between the human and mouse ϵ -chain membrane exons. The human sequence was obtained from the 970-bp SacI fragment containing the two membrane exons and the introns, together with 250 bp of the 3'-flanking sequences. The corresponding murine sequence (23) from nucleotides 369 to 717 is shown in the bottom line. The human and murine sequences are aligned to assume maximum homology. Solid triangles indicate RNA splicing positions.

tion of expression vectors containing chimeric κ and γ 1 genes have been reported (29). The human $C_{\gamma 1}$ region in the expression vector was replaced by the 6.4-kb human C_{ϵ} gene segment containing domains CH1 to CH4 and the membrane exons. The resulting chimeric ϵ -chain expression plasmid, pSV Δ HgptBAT123V $_H$ -hC $_{\epsilon}$, together with the chimeric κ -chain plasmid, pSV184 Δ HneoBAT123V $_{\kappa}$ -hC $_{\kappa}$ (Fig. 3), were coinjected into murine myeloma Sp2/



Figure 3. Schematic representation of the chimeric κ - and ϵ -chain vectors, pSV184 Δ HneoBAT123V $_{\kappa}$ -hC $_{\kappa}$ and pSV2 Δ HgptBAT123V $_H$ -hC $_{\epsilon}$. Ig DNA is represented by thick lines and exons by solid boxes. Vector sequences are shown as thin lines. The transcriptional directions of the *neo* and *gpt* genes are as indicated. The ranges of the DNA sequences are indicated with two-way horizontal arrows. L, leader exon; V, VJ or VDJ exon; C, C region exon; C1, C2, C3, and C4, exons encoding the CH1-CH4 domains of ϵ -chain. Broken line between BAT123 V $_H$ and human ϵ gene represents residual S107 intervening sequences carried over during the derivation of this plasmid.

0 cells by electroporation to generate transfectoma lines producing chimeric IgE as described in *Materials and Methods*.

Analysis of transfectomas by ELISA and immunofluorescent cell staining. From 2 to 3 wk after the transfection Sp2/0 cells were selected in medium containing G418, mycophenolic acid, xanthine, and hypoxanthine, culture supernatant from each well of the 96-well plates was assayed for the presence of secreted chimeric IgE by ELISA with the use of purified human IgE as a standard. The amount of chimeric IgE was estimated to be in the range of 1 to 40 μ g/ml/ 10^6 cells for different transfectoma lines. Six stable transfectants were further analyzed for the expression of membrane-bound IgE. Cells were incubated with a murine anti-human IgE mAb, then fluorescein-conjugated goat anti-mouse IgG, and analyzed for cell surface fluorescence intensity by flow cytometry. Figure 4 shows that 43% of the positive control, SKO-007 cells, and 0% of the parental Sp2/0 cells shifted to higher channels of fluorescence. Among the chimeric IgE-producing cell lines, 10 to 95% of total cells were positive. For one transfectoma, SE44, 95% of cells shifted to higher fluorescence channels. Among the six transfectoma lines analyzed, the level of secreted Ig correlated with the extent of surface IgE staining. The transfectoma cell line, SE44, showed highest levels of both secreted and membrane-bound chimeric IgE, and was used in subsequent experiments.

Biochemical and immunologic characterization of the chimeric IgE. Secreted chimeric IgE was purified from SE44 cell culture supernatant and subjected to SDS-PAGE under reducing conditions (lanes 4 to 6 in Fig. 5A). The H chains of the chimeric IgE secreted by SE44 cells and of the IgE from SKO-007 cells both showed multiple bands near 80 kDa, presumably caused by heterogeneous glycosylation. It is known that the ϵ -chain is much more glycosylated than the γ H chains, and unglycosylated ϵ -chain gives a less diffuse band of approximately 60 kDa (30). The mobility of an unreduced sample of the chimeric IgE on SDS-PAGE gels (lane 2 in Fig. 5A) was consistent with the tetrameric H $_2$ L $_2$ structure.

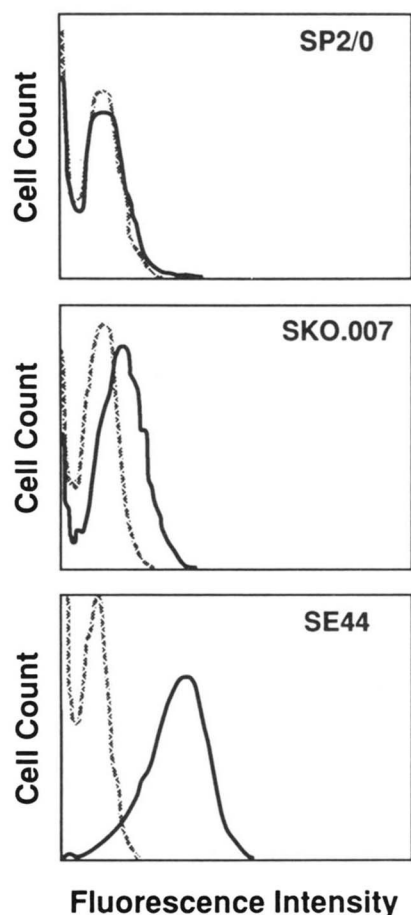


Figure 4. Detection of human ϵ -chain expression on SKO-007 and SE44 cells by fluorescence flow cytometry. (A), Sp2/0. (B), SKO-007. (C), SE44. The cells were incubated with an anti-human IgE mAb TES 19, followed by fluorescein-conjugated goat anti-mouse IgG. Broken lines represent background fluorescence profiles obtained by incubation with fluorescein-conjugated goat anti-mouse IgG only.

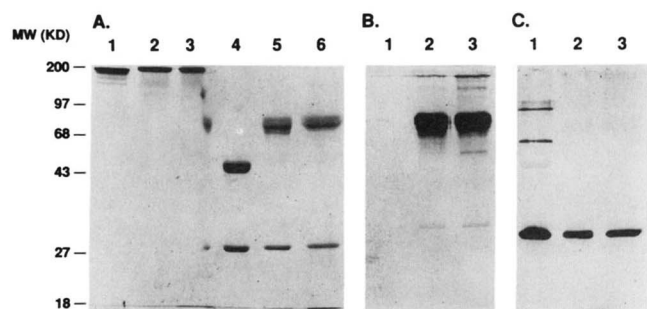


Figure 5. SDS-PAGE and Western immunoblot analysis of chimeric IgE. (A), Coomassie blue staining; 3 μ g of purified antibody, either non-reduced (lanes 1 to 3) or reduced (lanes 4 to 6) were electrophoresed through a 10% SDS-PAGE gel and stained with Coomassie blue. (B) and (C), immunostaining; 3 μ g of purified antibody was electrophoresed and electroblotted onto nitrocellulose. The filter was reacted with horseradish peroxidase-labeled goat anti-human IgE (B), or horseradish peroxidase-labeled goat anti-human κ (C), and then developed with 4-chloro-1-naphthol. Lanes 1 and 4, chimeric IgG ($\gamma 1, \kappa$) with V regions derived from BAT123; lanes 2 and 5, chimeric IgE produced by SE44 cells; lanes 3 and 6, human myeloma IgE produced by SKO-007 cells. The chimeric IgG ($\gamma 1, \kappa$) and chimeric IgE share the same chimeric κ -chain with a BAT123-derived V_{κ} domain and human C_{κ} domain.

In Western immunoblot analyses purified chimeric and myeloma IgE were subjected to reducing SDS-PAGE on a 10% gel, transferred to nitrocellulose filter, reacted with peroxidase-labeled goat anti-human ϵ -chain or goat anti-human κ -chain antibody, and developed with the peroxidase substrates. Figure 5B shows that goat anti-human

ϵ -chains react strongly with the ϵ -chains of both SE44 chimeric (lane 2) and SKO-007 myeloma (lane 3) IgE, and not with the chimeric BAT123 IgG (lane 1). Goat anti-human κ -chain antibody reacts strongly with the L chains derived from all these three antibodies (Fig. 5C).

Identification of membrane-bound ϵ -chain mRNA. The sequence unique to the membrane-bound forms of the ϵ -chain is presumably encoded by the membrane exons, designated M1 and M2 (Fig. 1). To confirm that M1 and M2 sequences are expressed in the mRNA of ϵ -chain, probes corresponding to M1 and M2, as well as to domains CH1 to 4, were used in the RNA hybridization analysis. The 970-bp *SacI* segment containing the membrane exons was cut into three segments by *Apal*. The two 250-bp fragments containing M1 and its 5' flanking region were used as the probe specific for exon 1 (probe c in Fig. 6). The 450-bp fragment containing M2 and 3'-untranslated region was used as the exon 2 probe (probe a in Fig. 6). These probes were used separately to hybridize with cytoplasmic RNA from SE44 and SKO-007 cells. Both probes yielded similar results and the RNA filter hybridized with probe a is shown in Figure 6A, which revealed mRNA species of approximately 3.0 kb and 3.6 kb in SE44 and SKO-007 cells, respectively. When an identical RNA filter was probed with the 5.5-kb *HindIII/XbaI* DNA fragment containing domains CH1 to CH4 of ϵ -chain (probe b in Fig. 6), transcripts of approximately 2.3 kb in length were detected in SE44 and SKO-007 cells (Fig. 6B). Bands characteristic for membrane IgE-specific messages (Fig. 6C) were only visible after prolonged exposure of the same filter (Fig. 6B). In both SE44 and SKO-007 cells, the 2.3-kb mRNA that encodes the secreted form of ϵ -chain was estimated from the hybridization intensities to be about 50 to 100 times more abundant than the transcripts for the membrane-bound forms.

Because there was no polyadenylation recognition sequence present in the 288 bp 3'-flanking region of the ϵ membrane exon 2 (Fig. 2), the termination/polyadenylation of the membrane-specific chimeric ϵ transcripts probably utilized the bidirectional SV40-derived poly(A)

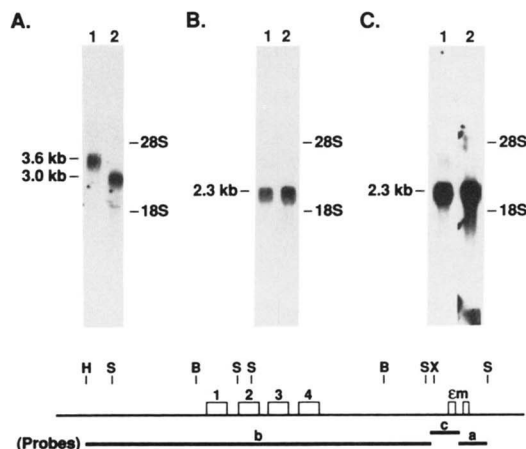


Figure 6. Northern blot analysis of RNA from SE44 cells. Twenty micrograms of cytoplasmic RNA were fractionated on 1% formaldehyde agarose gels, transferred to nitrocellulose, and hybridized with probe a representing a DNA segment containing membrane exon M2 of human ϵ gene (A) or probe b corresponding to a DNA segment containing CH1 to CH4 of human ϵ gene (B, C). Autoradiographs were exposed for 4 h (B) and 20 h (C). Lane 1, SKO-007; lane 2, SE44. A restriction map of the human ϵ locus is shown with the probe-specific regions indicated by heavy lines underneath.

recognition sequence within the *gpt* gene construct (Fig. 3). This may account for the observation that the ϵ membrane-specific mRNA of SE44 cells was shorter than that of SKO-007 cells.

Ag-binding specificity. Binding inhibition assays were performed to determine whether chimeric SE44 IgE could compete effectively with the murine mAb BAT123 to bind to HTLV-III_B envelope glycoprotein gp120. Figure 7 shows the binding of peroxidase-labeled BAT123 to solid phase gp120 in the presence of BAT123, chimeric IgE, or chimeric IgG, all of which have the same V regions. The data indicate that the replacement of mouse C γ 1 regions in BAT123 with human ϵ or γ 1 regions does not affect its Ag-binding affinity.

Induction of histamine release from basophils. Histamine release assay was carried out to determine whether chimeric IgE purified from the culture supernatant of SE44 cells exhibits the physiologic function of human IgE. Partially purified human basophils were incubated with chimeric IgE before the sensitization with the Ag, R15K-OVA, which was prepared by the polyvalent conjugation of R15K peptide to OVA. R15K is a synthetic peptide having the amino acid sequence of residues 308 to 322 of gp120 of HTLV-III_B strain of HIV-1. The neutralizing mAb BAT123 reacts with both gp120 and R15K strongly (29). Three independent experiments were carried out by using basophils purified from different donors. The results of one representative experiment are shown in Figure 8. The R15K-OVA Ag is able to trigger release of histamine by basophils preincubated with chimeric IgE at 10 μ g/ml. The dose response curve indicates that optimal release occurred between 10 and 200 ng/ml of the R15K-OVA Ag, yielding 80 to 84% release as compared with total release induced by cell lysis (Fig. 8). When chimeric BAT123 IgG was used, no specific release was noted. In addition, no histamine release was observed when an irrelevant polyvalent peptide Ag, HGE20K-OVA, was added to the basophils that had been preincubated with either the chimeric SE44 IgE or BAT123 IgG. Finally, preincubation of basophils with PS myeloma IgE at 10 μ g/ml, followed by sensitization with chimeric IgE, eliminated their response to the R15K-OVA Ag whereas their response to the polyclonal anti-human IgE antibody remained the same (date not shown).

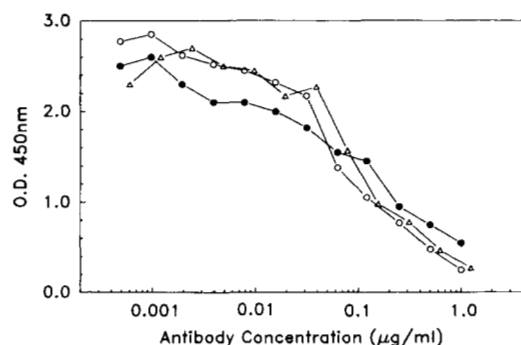


Figure 7. Binding inhibition assay. Various concentrations of chimeric IgE (Δ), chimeric IgG ($\bullet$), and murine mAb BAT123 ($\circ$) were added to compete with the binding of peroxidase-labeled BAT123 to solid phase gp120. The OD readings at 450 nm are plotted against the concentration of the added antibody. An irrelevant antibody, anti-human IgE mAb TES 1, did not show any effect at concentrations up to 2.0 and 40 μ g/ml.

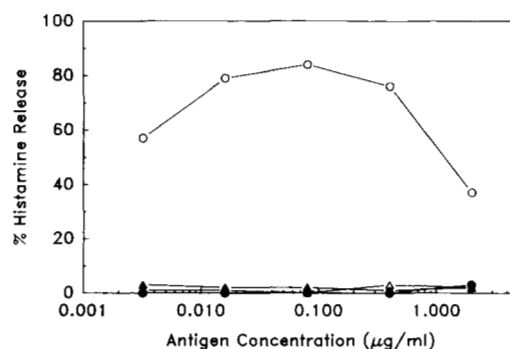


Figure 8. The induction of histamine release from human basophils sensitized with chimeric IgE by R15K-OVA. The data shown are from one of three separate experiments with the use of leukocytes from different donors. Histamine release ($\circ$) is plotted against the concentration of the Ag R15K-OVA. Each point is an average of two measurements with variations smaller than 2%. Under similar conditions, an irrelevant peptide Ag, HGE20K-OVA, did not induce noticeable amounts of histamine release ($\bullet$). Chimeric BAT123 IgG with the same Ag-binding specificity as the chimeric IgE gave background histamine release when either R15K-OVA (Δ) or HGE20K-OVA ($\blacktriangle$) was used.

DISCUSSION

By employing a combination of molecular and cell biologic methods generally referred to as the transfectoma technology, many chimeric IgG mAb comprising murine V regions and human γ -chain C regions have been prepared. In contrast, only one chimeric IgE, which has a specificity for the hapten 4-hydroxy-3-nitrophenacetyl, has been reported (21). The human C ϵ region gene, which was used for constructing the hybrid H chain gene of this hapten-specific IgE, contained the segments encoding CH1 to CH4 domains but not the membrane exons encoding the membrane anchor peptide. The resulting transfectant cells should, therefore, not express membrane-bound IgE on the cell surface. With a goal to establish cell lines that express IgE of both secreted and membrane-bound forms, we used a human ϵ gene segment containing both the exons encoding CH1 to CH4 domains and the membrane exons to construct the chimeric ϵ gene. In addition, we derived the V regions of the chimeric IgE from the murine mAb BAT123 specific for a viral Ag, with a purpose that the chimeric IgE may be used to explore pertinent questions relating to the possible deployment of IgE-mast cell mechanisms in the defense against a natural infection.

Because the genomic segment encoding the membrane anchor peptide of human ϵ -chain had not been characterized and reported, we first determined the nucleotide sequence of this genomic DNA region and identified the membrane exons. In the murine Ig H chain locus, one or two exons encoding the presumed isotype-specific membrane peptides are located at approximately 2 kb downstream from the C region genes of each H chain isotype and subclass (27, 31–33). The mRNA splicing sites and the coding sequence for mouse γ 1 chain has been further confirmed by cDNA cloning (34). Although the putative membrane exons for human Ig were identified only for μ - (35) and δ - (36) chains, it had generally been assumed that the membrane exons are organized similarly for other human H chains. By comparing with the proposed genomic structure of murine ϵ -chain (27), we located two putative membrane exons, M1 and M2, in the human ϵ gene about 2 kb downstream from the CH4 exon. According to this proposed structure, the splicing between CH4

and M1 occurs within the codon for a glycine residue, the second to the last amino acid of the human secretory ϵ -chain. The splicing sites in CH4, M1, and M2, and the resulting coding sequence have been confirmed by sequence analyses of DNA segments amplified by PCR using mRNA isolated from both SE44 and SKO-007 cells (C. Peng, L. K. Sun, R. S. Liou, T. W. Chang, manuscript in preparation).

The V regions of the chimeric IgE constructed in the present studies were derived from murine mAb BAT123, which reacts with a dominant neutralizing epitope of the HIV-1 envelope glycoprotein, gp120 (29). Our previous studies demonstrated that a chimeric BAT123 IgG ($\gamma 1$, κ) retained Ag-binding properties of the parental BAT123 (murine IgG1) with enhanced biologic activities (29). The present paper reported that the chimeric IgE exhibited similar Ag-binding properties as the chimeric BAT123 IgG ($\gamma 1$, κ) and murine mAb BAT123. The interaction of the murine V regions of BAT123 with HIV-1 virions, purified gp120, various recombinant gp120 Ag, and synthetic peptides is well studied. The binding of BAT123 to HIV-1 infected cells, which express gp120 on the cell surface, has also been investigated (37). Moreover, mAb against the Id of mAb BAT123 have been prepared (38), and provided a useful tool to study the cross-linking of the chimeric IgE. The in vitro experiments described in this paper indicate that polyvalent HIV-1 envelope Ag can induce the discharge of pharmacologic mediators from basophils that have been preincubated with IgE specific for HIV-1. It would be interesting to study the effects of virions and native viral Ag on the sensitization of mast cells and basophils. Increased serum IgE has been found in patients with respiratory infections to various viral agents (39). Recently, it was also reported that HIV-1 infected individuals had elevated levels of serum IgE (40), even though the physiologic significance of these IgE in immune defense are unclear.

Recently, Haba and Nisonoff (41) reported that when mice were treated with polyclonal syngeneic anti-mouse IgE, the IgE-secreting B cells were greatly diminished and the levels of circulating IgE remained very low for extended periods after treatment. It is not yet clear how IgE-producing cells are decreased by the anti-IgE treatment. One possible mechanism is that anti-IgE causes the lytic removal of IgE-expressing cells. Another possibility is that anti-IgE suppresses the IgE-secreting cells through a regulatory process by binding to CD23 receptor via bound IgE (42). In an earlier paper, we proposed that mAb binding to surface IgE-expressing B cells, but not mast cells and basophils, might be used for down-regulating IgE-producing B cells in allergic patients (18). Thus, a practical purpose for constructing a mouse myeloma cell line expressing human ϵ -chains is for studying the effects of murine anti-human IgE mAb on human IgE-expressing cells in mice. The IgE-expressing myeloma, SKO-007, is not ideal for these studies in the mouse because, unlike SE-44 cells, SKO-007 cells bear xenogeneic Ag that can induce other immune responses in BALB/c mice, and complicate the examination of the anti-IgE effects. Mouse model systems like this may help us understand the effects of anti-IgE treatment and the regulation of IgE synthesis in vivo.

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