The Two Membrane Isoforms of Human IgE Assemble into Functionally Distinct B Cell Antigen Receptors

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Summary

The human Cε gene expresses two membrane IgE heavy chain mRNAs which differ in the sequence that encodes their extracellular membrane–proximal domain. In the long IgE isoform (m_LIgE), this domain contains a stretch of 52 amino acids which are absent in the short variant (m_SIgE). We have now generated B cell transfectoma cell lines that express these two isoforms and show that both types of mIgE form functional B cell antigen receptors (BCR). Both receptors associate with the Ig-α/Ig-β heterodimer, as well as with protein kinases that are capable of phosphorylating this complex. Upon their cross-linking, both receptors can activate protein tyrosine kinases that phosphorylate the same substrate proteins. Both IgE receptors also associate with two novel proteins that do not bind to mIgM. Apart from these similarities, the two IgE-BCRs show several differences of which some are analogous to the differences between the IgM- and IgD-BCRs. First, the m_SIgE is transported to the cell surface at a higher rate than the m_LIgE. Second, the two IgE-BCRs associate with differently glycosylated Ig-α proteins, the m_LIgE associates with the completely glycosylated form, whereas the m_SIgE associates with an Ig-α glycoform that is partially sensitive to endoglycosidase H. Third, the kinetics of protein tyrosine phosphorylation induced by receptor cross-linking is significantly different for the two IgE-BCRs. Finally, cross-linking of the m_SIgE-BCR leads to growth inhibition of the B cell transfectoma, whereas signaling through the m_LIgE-BCR does not affect the cellular proliferation. These data show that the two human membrane IgE isoforms assemble into functionally distinct antigen receptors which can induce different cellular responses.

Antigen receptors on B lymphocytes are expressed on the plasma membrane as a complex of disulfide-bonded Ig heavy and light chains that are noncovalently associated with at least two other glycoproteins, Ig-α (CD79a) and Ig-β (CD79b) (1–5). Ig-α and Ig-β are two glycosylated transmembrane proteins of the Ig superfamily that are encoded by the B cell–specific genes mb-1 and B29, respectively (6, 7). These proteins form a disulfide-linked heterodimer which appears to be a prerequisite for the transport and cell-surface expression of the membrane-bound Igs (mIg)[1] (2, 3, 8). While the mIg molecule serves as the antigen-binding component of the receptor, the noncovalently associated Ig-α/Ig-β heterodimer has been shown to be the signal transduction unit of the B cell antigen receptor (BCR) (9–12). The Ig-α/Ig-β heterodimer is directly involved in the coupling of the BCR to several protein tyrosine kinases (PTKs) expressed in B cells, such as the src-related PTKs Lyn, Fyn, Lck, and Blk, and the cytoplasmic PTK Syk (13–17). Signal transduction from the cross-linked BCR involves the rapid activation of these enzymes which phosphorylate several substrate proteins in B cells, including the Ig-α and Ig-β components themselves (18).

Depending on their developmental stage, B cells express different classes of mIg. Immature B cells carry only the IgM antigen receptor, whereas IgM and IgD are coexpressed at a later stage of differentiation (19, 20). After class switching, B cells which express either IgG, IgA, or IgE antigen receptors are generated. Engagement of the Ig receptors by antigen can lead to cell proliferation, differentiation into antibody-secreting plasma cells, anergy, or apoptosis (21).

The human Ig constant ε gene (Cε) appears to be peculiar in its capacity to produce a number of alternatively spliced ε mRNAs that encode two membrane-type and several secretory-type IgE H chains (22–29). We have recently characterized the protein products of the secretory ε transcripts and found that only two of them encode prop-

[1]*Abbreviations used in this paper:* BCR, B cell antigen receptor; Cε, constant ε gene; Endo H, endoglycosidase H; mIg, membrane-bound Ig; m_LIgE, long IgE isoform; m_sIgE, short IgE isoform; NIP, 4-hydroxy-5-iodo-3-nitro phenacetyl; PTK, protein tyrosine kinase; PNGase, recombinant N-glycosidase.

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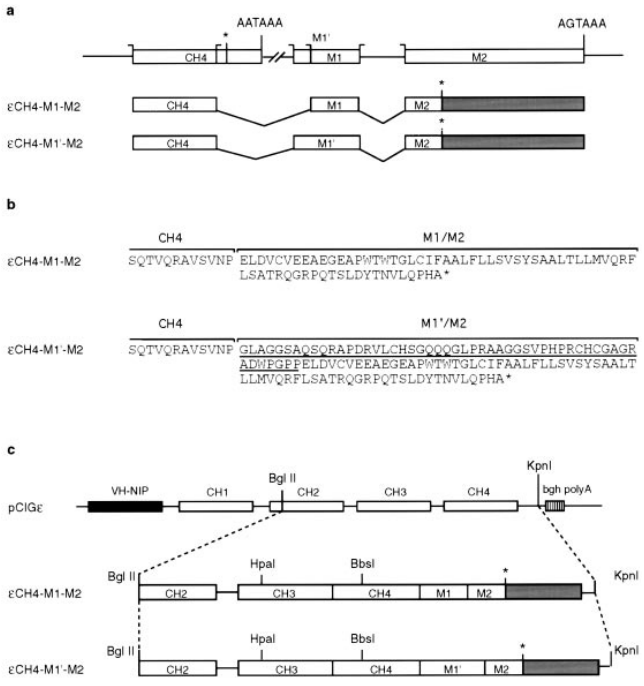

Figure 1. Schematic representation of the two membrane IgE H chain isoforms. (*a*) Diagram of the 3′ part of the Cε and the pattern of alternative splicing that generates the εCH4-M1-M2 and εCH4-M1′-M2 membrane transcripts. Open boxes represent coding sequences and shadowed boxes represent 3′ untranslated regions; stop codons are indicated by asterisks. (*b*) Amino acid sequence of the COOH terminus of the short (*εCH4-M1-M2*) and long (*εCH4-M1′*-M2) ε chains. The extra 52 amino acids present in the extracellular membrane–proximal domain of the long ε chain are underlined. (*c*) Diagram of the chimeric mouse V_H-NIP/Cε membrane gene constructs. The 3′ ends of the two membrane ε isoforms were cloned in their cDNA form (starting from the CH3 exon) into pCIG-Cε, using the indicated restriction enzyme sites.

erly assembled and secreted IgE molecules (30). All other isoforms were apparently aberrantly spliced byproducts which were retained and degraded by cellular posttranslational quality control mechanisms (22).

We have now investigated the expression and function of the IgE molecules encoded by the two types of membrane ε transcripts. These two ε mRNA species differ only in the 5′ part of the first membrane exon that encodes the extracellular membrane proximal domain (Fig. 1 *a*). The longer variant (εCH4-M1′-M2) contains 156 extra nucleotides as a consequence of alternative splicing between the donor splice site at the 3′ end of the CH4 exon and the upstream acceptor splice site in the M1 exon. Thus, the two putative membrane proteins have the same constant ε region and the same transmembrane and intracellular domains, but differ in a 52–amino acids segment which is present only in the long membrane IgE variant (Fig. 1 *b*).

The long membrane transcript was initially described as the predominant ε mRNA species in humans because it was found at significantly higher levels than the short species in IL-4 plus anti-CD40–stimulated PBL and in IgE-producing myeloma cell lines (27, 29). However, we have recently shown that the short membrane transcript (εCH4-M1-M2), which is homologous to the murine membrane transcript, is predominantly expressed by unstimulated PBL, indicating that the expression of the two membrane ε mRNAs may depend on the stage of B cell differentiation (23).

Materials and Methods

Cell Lines and Transfections. The WEHI 231 B cell lymphoma (provided by Dr. Roberto Sitia, Dipartimento di Ricerca Biologica e Tecnologica-San Raffaele Scientific Institute, Milan, Italy) was maintained in DMEM supplemented with 10% FCS, penicillin (100 U/ml), streptomycin (100 μg/ml), and 50 μM 2-mercaptoethanol. Transfections were performed by electroporation as described elsewhere (30), except that 800 μg/ml of G418 (Geneticin; Life Technologies, Inc., Gaithersburg, MD) were used for selection of the WEHI 231 clones. Cells were cloned by limiting dilution, and positive clones were identified by immunofluorescence.

Construction of Vectors for the Expression of the εCH4-M1-M2 and εCH4-M1′-M2 H Chains. The construction of the pCIG-CεCH4-M1′-M2 has been described in detail previously (22). The pCIG-CεCH4-M1-M2 vector was similarly constructed by replacing the BglII/KpnI fragment of pCIG-CεCH4-M1′-M2 with a corresponding fragment containing the M1 instead of the M1′ exon (Fig. 1 *c*).

Flow Cytometric Analysis. The expression of surface IgE was examined by flow cytometry on a FACScan®, (Becton Dickinson Immunocytometry Sys., Mountain View, CA) using rabbit anti–human IgE (ε chain) (Dako Corp., Carpinteria, CA), and FITC-conjugated swine anti–rabbit IgG (Dako Corp.).

Immunoprecipitations. Metabolic labeling and immunoprecipitations were performed as previously described (30). Briefly, 5×10^6 cells/ml were labeled with [^{35}S]methionine (Amersham Intl., Buckinghamshire, England) at 100–250 μCi/ml (1 Ci = 37 GBq), and chased with cold methionine as indicated in the figures. Cell lysates were immunoprecipitated with rabbit Ig's to human IgE (ε chains) or rabbit Igs to mouse IgM (μ-chains) (Dako Corp.) and purified by protein A–Sepharose. The samples were analyzed by SDS-PAGE in the presence or absence of mercaptoethanol, as indicated in the figure legends. Treatments of labeled supernatants with recombinant N-glycosidase F (PNGase F) and endoglycosidase H (Endo H) were performed according to the protocols provided by the manufacturer (New England Biolabs Inc., Beverly, MA).

Surface Biotinylation and Immunoprecipitation of IgE and IgM BCRs. Twenty million cells were washed twice with PBS and incubated in 1 ml of PBS containing 0.5 mg/ml of sulfo-NHS-biotin (Pierce Chem. Co., Rockford, IL) at room temperature for 15 min. Free succinimide groups were blocked by the addition of 10 ml of nonsupplemented medium at room temperature for 10 min. Cells were washed twice with PBS and resuspended in 500 μl lysis buffer containing 1% digitonin (Sigma Chemical Co., Steinheim, Germany), 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA, and the protease inhibitors PMSF (1 mM), aprotinin (10 mg/ml), and leupeptin (10 mg/ml) (all from Sigma Chemical Co.). Lysates were incubated on ice for 30 min and centrifuged at 10,000 *g* for 30 min at 4°C. The supernatants were precleared three times with 30 μl of protein A agarose beads (GIBCO BRL) at 4°C for 30 min. Immunoprecipitations were performed by incubating the lysates with 6 μl of rabbit Igs to human IgE (ε chains) (Dako Corp.), rabbit Igs to mouse IgM (μ chains) (Dako Corp.), or goat Igs to human IgE (ε chains) (Kirkegaard & Perry Labs., Inc., Gaithersburg, MD), at 4°C for

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60 min. 20 μl of protein A agarose were then added and incubated for a further 30 min at 4°C. The Ig-α/Ig-β heterodimer was immunoprecipitated using a polyclonal antibody against Ig-α (provided by Dr. J.C. Cambier, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO). Beads were preincubated with 10 mg/ml bovine serum albumin for 20 min and washed three times with lysis buffer before use. The beads were pelleted by centrifugation (the supernatants were saved for reprecipitation), washed twice with high salt lysis buffer (50 mM Tris, pH 7.5, 500 mM NaCl, 5 mM EDTA, 0.2% digitonin, plus inhibitors), and twice with low salt buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 5 mM EDTA, 0.2% digitonin, plus inhibitors). For reprecipitation, supernatants were precleared twice with 30 μl beads before adding a different primary antibody.

In Vitro Kinase Assay. Immunoprecipitates from digitonin lysates were resuspended in kinase buffer (50 mM Tris, pH 7.5, 10 mM $MnCl_2$, 1 mM EDTA, 1 mM sodium orthovanadate [Sigma Chemical Co.], 1% digitonin, plus inhibitors) containing 10 μCi [γ^{32}P] (Amersham Intl.), and incubated at room temperature for 5 min. The reaction was terminated by addition of kinase buffer containing 50 mM EDTA, and the pellets were collected by centrifugation before resuspending in SDS-PAGE reducing sample buffer. The samples were boiled and electrophoresed on a 10% SDS-PAGE. The gel was dried without fixing and autoradiographed at −70°C.

Detection of Tyrosine Phosphorylated Proteins by Western Blotting. Tyrosine phosphorylated proteins were detected as previously described (31). Briefly, 4×10^6 cells were resuspended in 0.5 ml of DMEM and stimulated with either goat anti–human IgE (ε chain) (Kirkegaard & Perry Labs., Inc.) (20 μg/ml) or anti–mouse IgM (5 μg/ml) at 37°C for the indicated periods of time. After washing twice with ice-cold PBS containing 1 mM sodium orthovanadate, cells were lysed with 100 μl of Triton X-100 lysis buffer containing 20 mM Tris-HCl (pH 8.0), 137 mM NaCl, 10% glycerol, 1% Triton X-100, 2 mM EDTA, plus inhibitors, and 1 mM sodium orthovanadate. After incubation for 10 min on ice, the supernatants were cleared by centrifugation at 10,000 *g* for 15 min at 4°C. Subsequently, 30 μl of supernatant was subjected to 10% SDS-PAGE and Western blotting. The PVDF membrane (Amersham Intl.) was incubated with the antiphosphotyrosine monoclonal antibody PY20 (Transduction Laboratories, Lexington, KY), followed by horseradish peroxidase–coupled anti–mouse Ig antibody (Dako Corp.). The bound antibodies were visualized by the enhanced chemiluminescence detection system (Amersham Intl.).

Cellular Proliferation Assay. Two B cell transfectomas that expressed comparable levels of the long IgE isoform (m_LIgE) and the short IgE isoform (m_SIgE) were cultured in triplicate in 96-well plates at a cell density of 2×10^4/well. The cells were incubated for 24 h with various amounts of goat anti–human IgE (ε chain) (Kirkegaard & Perry Labs., Inc.) in 0.2 ml medium. Proliferation was determined by incorporation of [methyl-^{3}H]thymidine (1 μCi/well, 94.0 Ci/mmol; Amersham Intl.). After 14 h of incubation, cells were harvested and levels of incorporated [^{3}H]thymidine were measured by scintillation counting.

Results

Expression, Assembly, and Transport of m_SIgE and m_LIgE. To investigate the properties of the two membrane IgE isoforms, we generated two chimeric mouse/human ε-chain gene constructs which contained the mouse heavy chain variable region segment from an Ab with anti–4-hydroxy-5-iodo-3-nitro phenacetyl (NIP) specificity and the human Cε region with a COOH terminus corresponding to the m_LIgE or the m_SIgE isoform. A schematic diagram of the two constructs is depicted in Fig. 1 *c*. The two constructs were independently transfected into WEHI-231 cells, and G418 resistant transfectants were selected for the expression of mIgE by immunofluorescence.

To investigate the assembly and transport of the m_LIgE and m_SIgE isoforms, we perfomed pulse-chase studies in the WEHI cell lines. Cells were given a 15 min pulse with

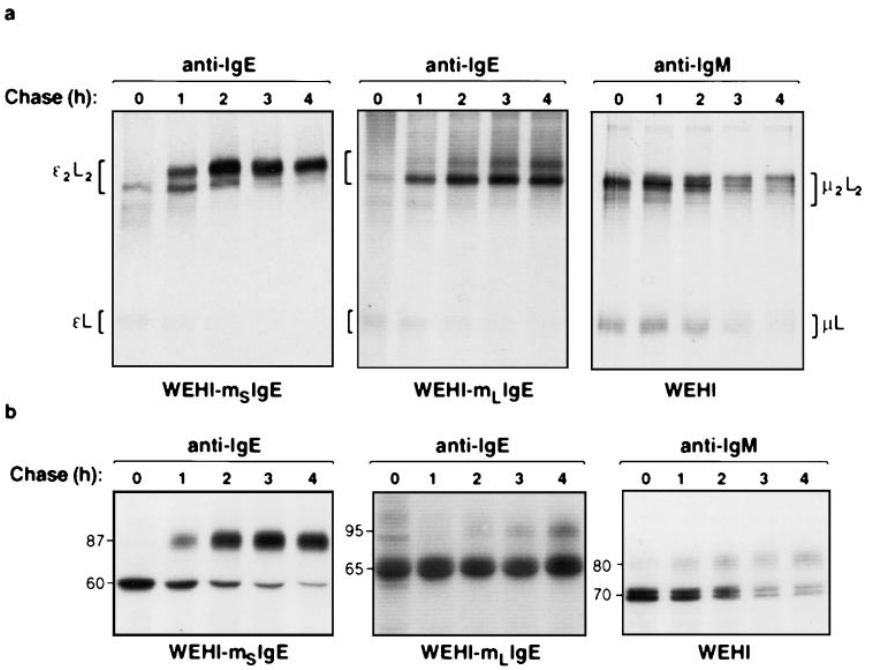

Figure 2. Assembly and transport of m_LIgE and m_SIgE. WEHI-m_SIgE, WEHI-m_LIgE, and wild-type WEHI cells were pulse labeled with [^{35}S]methionine for 15 min and chased for the indicated times in the presence of excess cold methionine. Cellular extracts were immunoprecipitated with the indicated antibodies and (*a*) analyzed on a nonreducing 6% SDS-PAGE or (*b*) treated with Endo H and separated on reducing 10% SDS-PAGE.

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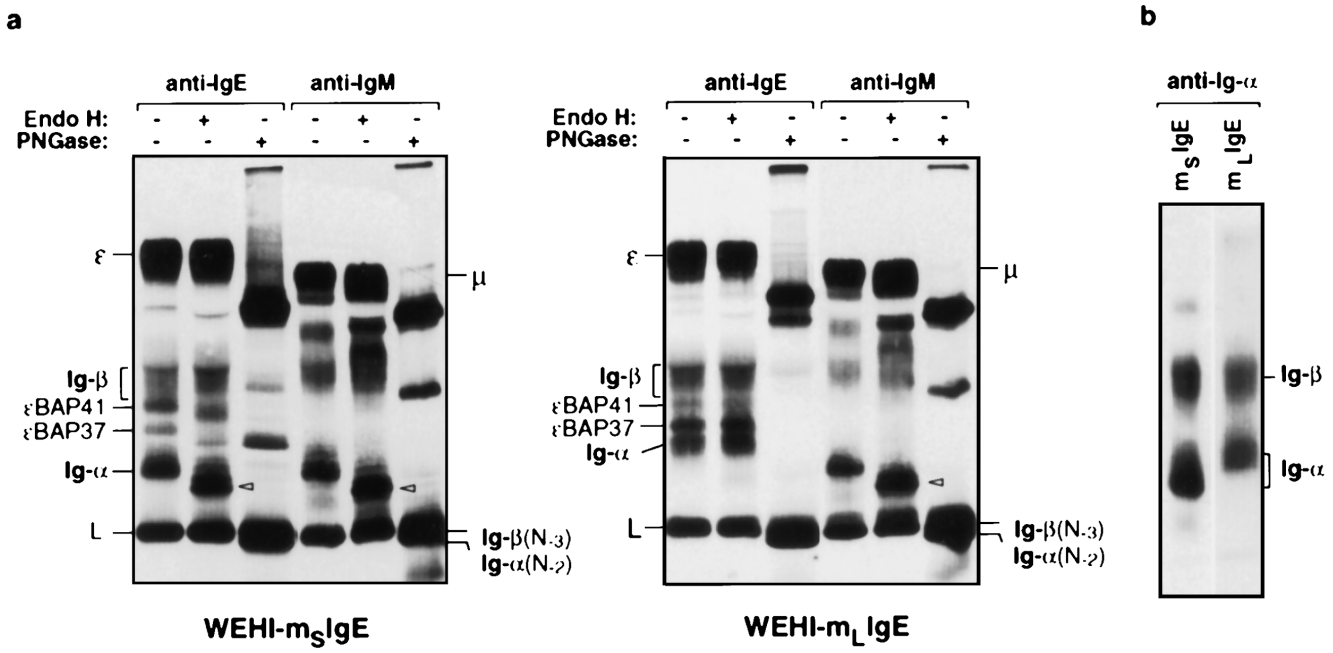

Figure 3. Immunoprecipitation of BCRs from biotin-labeled cell-surface proteins. WEHI-m_SIgE and WEHI-m_LIgE were surface biotinylated and treated with digitonin lysis buffer to preserve the BCR complexes. (*a*) Lysates were treated first with anti-IgE, and then with anti-IgM sera to immunoprecipitate the IgE-BCR and endogenous IgM-BCR complexes, respectively. Immunoprecipitated material was treated with Endo H or PNGase as indicated, and analyzed by reducing 10% SDS-PAGE. Arrowheads indicate the position of the Ig-α polypeptide after removal of one of the two N-linked carbohydrate moieties by Endo H. Ig-α(N_{-2}) and Ig-β(N_{-3}) correspond to the Ig-α and Ig-β polypeptides after removal by PNGase of the two or three N-linked carbohydrate moieties, respectively. (*b*) Reprecipitation of the Ig-α/Ig-β heterodimer dissociated by NP-40 from the m_SIgE-BCR and m_LIgE-BCR. The immunoprecipitation was done with an anti Ig-α serum and analyzed on a 10% SDS-PAGE under reducing conditions.

[^{35}S]methionine and then chased for 1, 2, 3, and 4 h. mIgE was immunoprecipitated from the WEHI-m_SIgE or WEHI-m_LIgE cell extracts and analyzed on a nonreducing 6% SDS-PAGE (Fig. 2 *a*). Two major species of more than 240 kD were observed in both cell lines, demonstrating that both membrane isoforms are assembled into H_2L_2 molecules. During the course of the chase, the quantity of immunoprecipitated proteins remained the same, indicating that there is no intracellular degradation of either membrane isoform. The accumulation of higher molecular weight species in the course of the chase suggested that both proteins are terminally glycosylated during their transport to the cell surface. This was confirmed by treatment of the immunoprecipitated material with Endo H which cleaves N-linked high mannose oligosaccharides of glycoproteins before they arrive in the medial Golgi, but fails to cleave terminally processed carbohydrate moieties. As shown in Fig. 2 *b*, after Endo H treatment two ϵ species were observed in each case (*a*) a 60-(m_SIgE) or 65-(m_LIgE) kD species corresponding to Endo H–sensitive ϵ chains that carried unprocessed high mannose carbohydrates and (*b*) an 87-(m_SIgE) or 90-(m_LIgE) kD species corresponding to mature ϵ chains that bore processed N-linked carbohydrates which were resistant to the enzyme. In the WEHI-m_LIgE transfectoma, only 20–30% of the newly synthesized membrane ϵ chains were of the mature processed type after 4 h chase (Fig. 2). This was similar to the rate of transport observed for the endogenous mouse mIgM. Interestingly, three to four times more m_SIgE H chains were present in the mature form after the same period of time. This clearly demonstrated that the m_SIgE is more efficiently transported to the cell surface than the m_LIgE, or even the endogenous mIgM.

Characterization of IgE-BCR Components. To characterize the IgE-BCR, and to determine the m_SIgE and m_LIgE associated proteins, we immunoprecipitated the IgE antigen receptor complex from digitonin lysates of surface biotinylated WEHI-m_SIgE and WEHI-m_LIgE cells. The immunoprecipitations were perfomed in duplicate using two different anti–human IgE antibodies. As a control, the endogenous IgM-BCR was subsequently immunoprecipitated from the same cell extracts. The immunoprecipitated material was resolved on a 10% SDS-PAGE under reducing conditions (Fig. 3 *a*). Analysis of the mIgM complex yielded the expected characteristic pattern of an 80-kD band corresponding to the μ heavy chain, a 25-kD band corresponding to the light chain, and the 32- and 40–47-kD bands corresponding to Ig-α and Ig-β polypeptides, respectively (18). A similar pattern was observed after immunoprecipitation of the m_SIgE- and m_LIgE-BCRs, indicating that Ig-α and Ig-β chains are also present in these receptors. This was confirmed by analysis of the material that was dissociated from the immunoprecipitated IgE-BCRs by treatment with NP-40. Reprecipitation of this material with an anti–Ig-α specific antiserum produced only the Ig-α and Ig-β subunits (Fig. 3 *b*).The Ig-β subunit was of the same molecular mass in the three BCRs, whereas the Ig-α of the m_LIgE (m_LIgE-α) showed a slower

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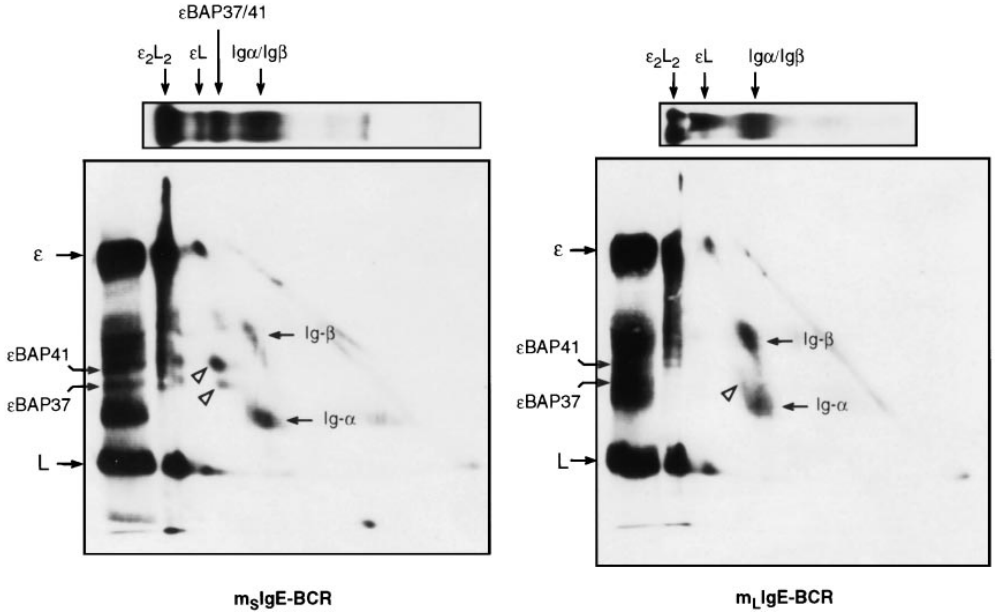

Figure 4. Bidimensional analysis of surface-biotinylated IgE-BCRs. Anti-IgE immunoprecipitates from the two transfectomas were analyzed by bidimensional (nonreducing/reducing) PAGE. The migration of the same material run only under reducing conditions or nonreducing conditions is shown in the left lane of each gel, or as a separate lane on top of each gel, respectively. Arrowheads indicate the position of the dissociated ϵBAP37 and ϵBAP41 proteins. The other components of the IgE-BCRs are indicated in the figure.

mobility than the IgM-α and m_SIgE-α (Fig. 3). Slower mobility of the Ig-α chain has also been observed in the case of the IgD-BCR. This difference has been shown to be the consequence of different terminal glycosylation of the two N-glycosylation sites in the polypeptide (8, 32, 33). To establish if this is the case with the m_LIgE-α, immunopreciptated materials were treated with endoglycosidases. Fig. 3 *a* shows that Endo H treatment decreased the molecular weight of the IgM- and m_SIgE-associated Ig-α polypeptides. On the other hand, the m_LIgE-α remained at the same position, indicating that both glycosylation sites are terminally glycosylated. Treatment with PNGase, which cleaves all sugar residues, eliminated the difference in size between the different Ig-α polypeptides.

Two additional proteins of 37 and 41 kD were detected in the m_SIgE- and m_LIgE-BCR immunoprecipitations (Fig. 3 *a*). These proteins were not present in the IgM-BCR and were immunoprecipitated with either of the two anti-IgE Abs, indicating that they are specifically associated with mIgE. The relative amount of these IgE-BCR associated proteins (further referred to as ϵBAP37 and ϵBAP41) was different in the two IgE-BCRs, with significantly lower quantities of ϵBAP41 in the m_LIgE-BCR. These proteins were not immunoprecipitated with the anti–Ig-α antiserum, indicating that they are not covalently associated to Ig-α or antigenically related to this polypeptide (Fig. 3 *b*).

To further characterize the components of the IgE-BCRs, two dimensional analysis of anti-IgE immunoprecipitated material was performed on digitonin lysates from biotinylated WEHI-m_SIgE and WEHI-m_LIgE cells. The analysis was done on a 10% SDS-PAGE under nonreducing conditions in the first dimension and reducing conditions in the second (Fig. 4). In addition to the bands corresponding to ϵ_2L_2 and ϵL, a complex of ~75 kD was present in the nonreducing PAGE analysis of the m_SIgE-BCR. A slightly higher mol wt complex (80 kD) was detected in the m_LIgE-BCR. In the second dimension, these complexes dissociated into free Ig-α and Ig-β chains. This experiment also indicated that the ϵBAP37 and ϵBAP41 proteins form a disulfide-bound complex (ϵBAP37/41) which, in the nonreducing PAGE analysis of the m_SIgE-BCR, migrated distinctly from the Ig-α/Ig-β heterodimer. In the m_LIgE-BCR the ϵBAP37 apparently formed a homodimer which comigrated with the Ig-α/Ig-β complex.

In Vitro Phosphorylation of IgE-BCR Associated Proteins. To investigate if the IgE-BCRs contain protein kinases capable of phosphorylating the Ig-α/Ig-β heterodimers as shown for the IgM-, IgD- and IgG-BCRs (34), we performed in vitro [γ^{32}P]ATP labeling of immunoprecipitated BCRs. The endogenous IgM-BCR from both transfectomas was analyzed as an internal control. As shown in

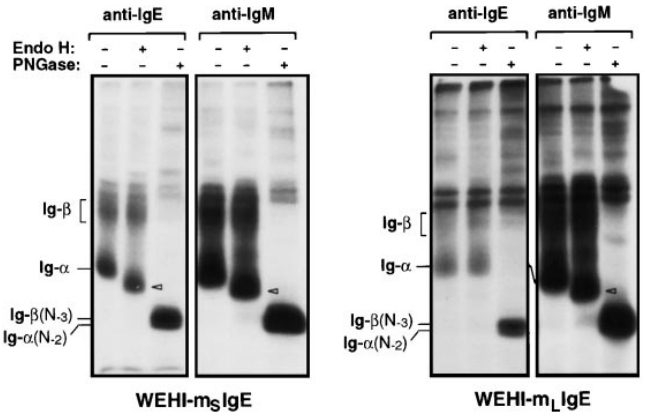

Figure 5. In vitro phosphorylation of BCR associated proteins. IgE- and IgM-BCR from the WEHI-m_SIgE and WEHI-m_LIgE transfectomas were immunoprecipitated, incubated with [γ^{32}P]ATP, and analyzed on reducing 10% SDS-PAGE. Treatment with Endo H or PNGase is indicated on top of each gel. Arrowheads, Ig-α(N_{-2}), and Ig-β(N_{-3}) as in legend to Fig. 3.

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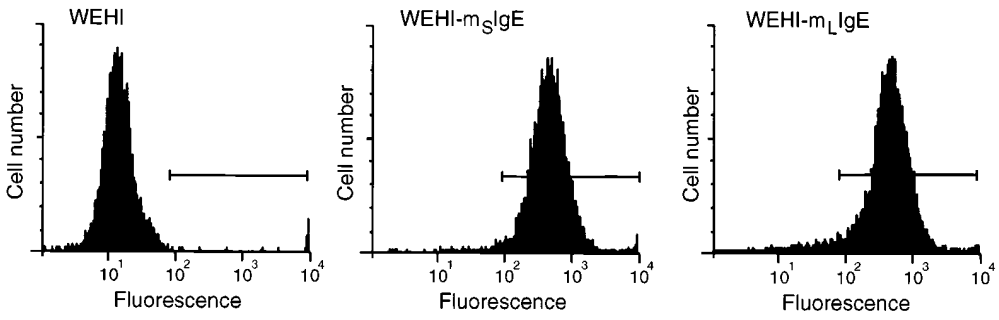

Figure 6. Quantitation of m_SIgE- and m_LIgE-BCR levels on the surface of transfected WEHI cells. The level of surface IgE was determined in each cell line by fluorescent flow cytometry analysis with a rabbit anti–human IgE antibody and FITC-conjugated swine anti–rabbit IgG.

Fig. 5, bands corresponding to the Ig-α and Ig-β proteins were found to be phosphorylated when incubated with [γ^{32}P]ATP in all three BCRs. The identity of these bands was again confirmed by treatment with Endo H and PN-Gase F. The two newly identified ϵBAPs were not detected in this in vitro assay, suggesting that they are not substrates for the BCR-associated protein kinases.

Protein Tyrosine Phosphorylation upon Cross-linking of the IgE-BCRs. To analyze the activation of PTKs upon engagement of the IgE-BCRs, we used transfectant cell lines (WEHI-m_LIgE and WEHI-m_SIgE) that expressed comparable levels of m_LIgE and m_SIgE (Fig. 6). Wild-type WEHI cells were analyzed in parallel as control. The three cell lines were incubated for different periods of time (1–40 min) in the presence of either anti-IgE or anti-IgM. The PTK activation in these cells was monitored by the increase in tyrosine phosphorylation of PTK substrate proteins in total Triton X-100 cell lysates. Cross-linking the IgM- or the IgE-BCRs resulted in a similar pattern of phosphorylation, indicating that all three BCRs induce the phosphorylation of the same substrate proteins. However, the kinetics of phosphorylation of the two IgE-BCRs was quite different. After cross-linking the m_LIgE-BCR, the substrate phosphorylation reached its maximum in 1 min and drastically declined within 5 min (Fig. 7). In contrast, in the m_SIgE-producing transfectant, the substrate phosphorylation still increased after 1 min of stimulation and reached its maximum after 5 min. Increased tyrosine phosphorylation was still evident after 40 min, although for several polypeptides it had declined. These data showed that the kinetics of protein tyrosine phosphorylation induced by receptor cross-linking is significantly different for the two IgE-BCRs.

In subsequent experiments we observed that the kinetics of tyrosine phosphorylation were independent of the amount of IgE expressed on the cell surface. Analysis of WEHI-m_SIgE and WEHI-m_LIgE clones expressing different amounts of IgE-BCR showed a difference only in the intensity of the signal, whereas the kinetics of both receptors remained the same (data not shown). Moreover, cross-linking of the endogenous IgM-BCR in the two transfectants and in wild-type WEHI cells showed the same kinetics of tyrosine phosphorylation, and was consistent with published data (35). These experiments excluded the possibility that the differences in signal transduction between the two IgE-BCRs were due to peculiarities of the transfected cell lines rather than the type of mIgE.

Cellular Responses Induced by Cross-linking of m_SIgE- and m_LIgE-BCR. Cross-linking of the endogenous IgM-BCR in WEHI cells leads to their growth inhibition (36). On the other hand, cross-linking of the IgD-BCR in WEHI cells transfected with a δ chain does not affect their proliferation (37). To investigate whether signaling through the IgE-BCRs has an effect on cellular proliferation, WEHI-m_SIgE and WEHI-m_LIgE cells were grown for 24 h in the presence of various concentrations of anti-IgE Ab and were subsequently incubated for 14 h with [^{3}H]thymidine. A marked inhibition in proliferation was observed only in the WEHI-m_SIgE transfectoma; cross-linking of the m_LIgE had no ef-

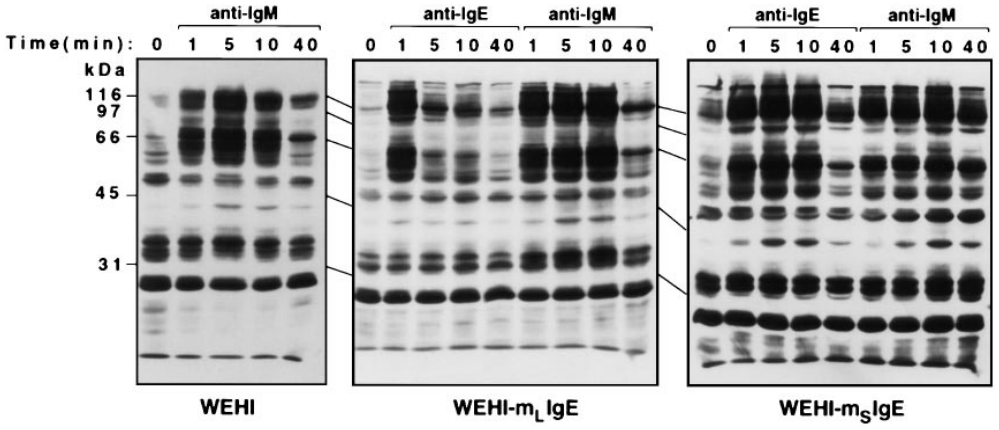

Figure 7. Kinetics of PTK substrate phosphorylation upon cross-linking of the m_SIgE-, m_LIgE-, and IgM-BCR. Wild-type WEHI, WEHI-m_LIgE, or WEHI-m_SIgE cells were incubated for various times with anti-IgE or anti-IgM antibodies, as indicated on top of each gel. Cellular extracts were then prepared and tyrosine phosphorylated proteins detected by immunoblotting with an anti-phosphotyrosine mAb.

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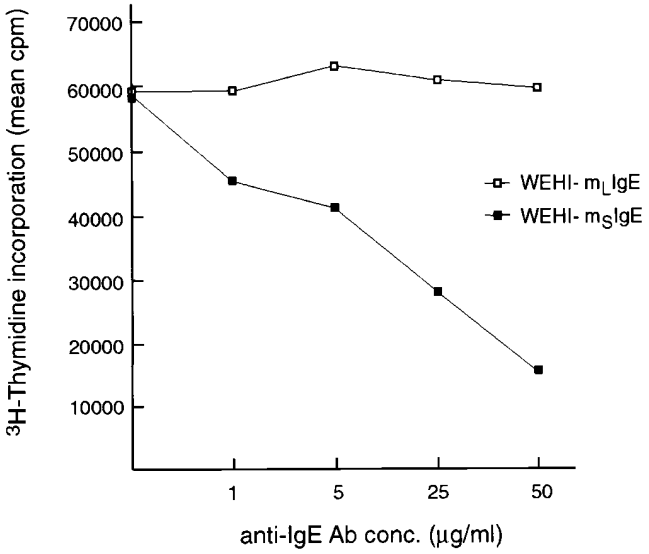

Figure 8. Proliferation of WEHI-m_LIgE and WEHI-m_SIgE cells after cross-linking of mIgE. 2×10^4 cells were incubated with 0, 1, 5, 25, or 50 μg/ml of polyclonal anti–human IgE anti-serum. Results show one representative of three separate experiments with similar results. Each point represents an average of triplicate cultures (SEM <5%).

fect (Fig. 8). The above results indicate that different cellular responses can occur after IgE-BCR signaling, depending on the type of mIgE expressed on the cell surface.

Discussion

The data presented in this paper are the first demonstration of the existence of two functional human IgE B cell receptors that are generated by alternative splicing. Although both receptors were found to be correctly assembled, transported to the cell surface, and capable of signal transduction upon receptor engagement, a number of differences were noted between them that could only be attributed to the different extracellular membrane proximal domains. The first difference was in the rate of their transport to the plasma membrane. The transit time of the m_LIgE was much longer, since only 20% of the molecules were found to be terminally glycosylated within 3 h of their synthesis, whereas almost all of the m_SIgE molecules were converted into the mature form during this period. Interestingly, a different rate of transport has also been shown for IgM and IgD with a shorter transit time for the former (38). In our experiments the endogenous IgM was transported at a similar rate as the m_LIgE, indicating that the transport of the m_SIgE is extremely efficient.

Both mIgE isoforms were found to associate into complete BCR complexes that contain the Ig-α/Ig-β heterodimer as well as the PTKs involved in their phosphorylation. However, a difference was also noted in the pattern of glycosylation of the associated Ig-α; when associated with m_SIgE, the Ig-α polypeptide had only one of the sites terminally glycosylated and resistant to Endo H cleavage, whereas, when associated with m_LIgE, both sites were resistant to the enzyme. This is identical to what has been reported for IgM and IgD, which associate with partially or completely processed Ig-α, respectively (8, 32). Several possibilities have been considered to explain the difference in glycosylation of the Ig-α polypeptide associated with IgD, including the length of the extracellular membrane proximal domain, the presence of interchain disulfide bonds in this region, and the transit time of the complex through the Golgi (33). These possibilities could also account for the different processing of the IgE-associated Ig-α polypeptides, since, analogous to IgD, the m_LIgE contains a much longer extracellular domain than the m_SIgE, has three extra cysteines in this region, and also has a lower rate of transport to the cell surface.

An unexpected observation in this study was the finding that both IgE receptors associate with two proteins (εBAP37 and εBAP41) that were not present in the endogenous IgM-BCR of the WEHI lymphoma. Their association with the IgE receptors appeared to be very specific, since they were coprecipitated with two different anti-IgE antisera. These proteins obviously differ from other BCR-associated proteins, such as BAP32 and BAP37 which are exclusively associated with IgM (39), and BAP29 and BAP31 which are preferentially associated with IgD molecules (40), for the following reasons: (*a*) they have an extracellular domain which was labeled in the cell-surface biotinylation experiment, (*b*) both proteins were glycosylated, and (*c*) they form disulfide-bonded complexes. The εBAP41 appeared to be underrepresented in the m_LIgE-BCR, suggesting that the longer extracellular membrane–proximal domain affects the association of εBAP41 to the BCR or its accessibility to biotinylation.

The most striking difference between the two IgE-BCRs was related to the response elicited by their cross-linking. Both receptors appeared to activate the same PTKs, as judged by the pattern of tyrosine phosphorylation. However, a clear difference was noted in the maximal phosphorylation time point as well as in the duration of the response. More specifically, the phosphorylation induced via the m_LIgE-BCR reached its maximum after 1 min of receptor engagement and declined within the following 5 min. In contrast, the signal induced by cross-linking of the m_SIgE-BCR reached its maximum after 5 min and remained for a prolonged period. Differences in the kinetics of the response have also been observed for the IgM- and IgD-BCRs (31). Experiments with these receptors or receptor chimeras have indicated that the extracellular membrane proximal and/or transmembrane domains influence the duration of the response (31). Since in the IgE-BCRs the difference resides only in the extracellular membrane–proximal domain, it can be concluded that this region determines the kinetics of protein tyrosine phosphorylation upon receptor engagement. The mechanisms through which this could occur are unknown, but could be mediated through association and/or interaction with the different Ig-α glycoforms or other proteins of the BCR complex.

The different kinetics of signal transduction through the IgE-BCRs were associated with different cellular responses

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of the WEHI transfectomas. Cross-linking of the m_SIgE-BCR led to growth inhibition, whereas signaling through the m_LIgE-BCR had no effect on cellular proliferation. These cellular responses are analogous to those observed after signaling through the IgM- and IgD-BCRs in WEHI cells (37). In this case the IgM-BCR, which, like m_SIgE, has a short extracellular spacer, is capable of transmitting a growth inhibitory signal.

In conclusion, we have shown that both human mIgE isoforms assemble into functional B cell receptors. When expressed on immature B cells such as the WEHI lymphoma, the two IgE-BCRs transmit qualitatively distinct signals after cross-linking. Work is in progress to determine the properties of these receptors in more mature B cells which have undergone Ig H chain class switching. However, it should be noted here that B cells coexpressing IgM and IgE have been detected in peripheral blood (41, 42). Clearly, it would be of interest to determine the type of mIgE expressed by these cells and whether the two mIgE-BCRs can be coexpressed by the same cell. Such studies should further delineate the relevance of having two receptors with identical specificity for the ligand and may allow characterization of additional components of the BCR signal-transduction pathways.

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