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

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Summary

The human Ce 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 (mIgE), this domain contains a stretch of 52 amino acids which are absent in the short variant (mIgE). 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 mIgE is transported to the cell surface at a higher rate than the mIgE. Second, the two IgE-BCRs associate with differently glycosylated Ig-α proteins, the mIgE associates with the completely glycosylated form, whereas the mIgE 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 mIgE-BCR leads to growth inhibition of the B cell transfectoma, whereas signaling through the mIgE-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.

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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 (Ce) 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-

1Abbreviations used in this paper: BCR, B cell antigen receptor; Ce, constant ε gene; Endo H, endoglycosidase H; mIg, membrane-bound Ig; mIgE, long IgE isoform; mIgE, short IgE isoform; NIP, 4-hydroxy-5-iodo-3-nitro phenacetyl; PTK, protein tyrosine kinase; PNGase, recombinant N-glycosidase.
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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 Sita, 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-εCeCH4-M1-M2 has been described in detail previously (22). The pCIG-CεCeCH4-M1-M2 vector was similarly constructed by replacing the BglII/KpnI fragment of pCIG-εCeCH4-M1′-M2 with a corresponding fragment containing the M1 instead of the M1′ exon (Fig. 1).

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 x 10⁸ cells/ml were labeled with [35S]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 Ig's 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 BCeRs. Two 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 10 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 1 ml of PBS 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 5 μl of rabbit Ig's to human IgE (ε chains) (Dako Corp.), rabbit Ig's to mouse IgM (μ-chains) (Dako Corp.), or goat Ig's to human IgE (ε chains) (Kirkegaard & Perry Labs., Inc., Gaithersburg, MD), at 4°C for 30 min.
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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 MnCl₂, 1 mM EDTA, 1 mM sodium orthovanadate [Sigma Chemical Co.], 1% digitonin, plus inhibitors) containing 10 μCi [γ³²P] (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 × 10⁶ cells were resuspended in 0.5 ml of DMEM and stimulated with either goat anti-human IgE (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-Cl (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 transfecteds that expressed comparable levels of the long IgE isoform (mLIgE) and the short IgE isoform (mSIgE) were cultured in triplicate in 96-well plates at a cell density of 2 × 10⁵/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-³H]thymidine (1 μCi/well, 94.0 Ci/mmol; Amersham Intl.). After 14 h of incubation, cells were harvested and levels of incorporated [³H]thymidine were measured by scintillation counting.

Results

Expression, Assembly, and Transport of mSIgE and mLIgE. 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 Ce region with a COOH terminus corresponding to the mLIgE or the mSIgE isoform. A schematic diagram of the two constructs is depicted in Fig. 1c. The two constructs were independently transfected into WEHI-231 cells, and G418 resistant transfectants were selected for the expression of mlIgE by immunofluorescence.

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

![Figure 2. Assembly and transport of mSIgE and mLIgE. WEHI-mSIgE, WEHI-mLIgE, and wild-type WEHI cells were pulse labeled with [³⁵S]methionine for 15 min and chased for the indicated periods of time in the presence of excess cold methionine. Cellular extracts were immuno-precipitated 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.](image-url)
[35S]methionine and then chased for 1, 2, 3, and 4 h. mIgE was immunoprecipitated from the WEHI-mSIgE or WEHI-mLIgE cell extracts and analyzed on a nonreducing 6% SDS-PAGE (Fig. 2a). Two major species of more than 240 kD were observed in both cell lines, demonstrating that both membrane isoforms are assembled into H2L2 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. 2b, after Endo H treatment two species were observed in each case (a) 60–(mSIgE or 65–(mLIgE kD species corresponding to Endo H–sensitive ε chains that carried unprocessed high mannose carbohydrates and (b) an 87–(mSIgE or 90–(mLIgE kD species corresponding to mature ε chains that bore processed N-linked carbohydrates which were resistant to the enzyme. In the WEHI-mLgE 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 mlgM. Interestingly, three to four times more mSIgE H chains were present in the mature form after the same period of time. This clearly demonstrated that the mSIgE is more efficiently transported to the cell surface than the mLIgE, or even the endogenous mlgM.

**Characterization of IgE-BCR Components.** To characterize the IgE-BCR, and to determine the mSIgE and mLIgE associated proteins, we immunoprecipitated the IgE antigen receptor complex from digitonin lysates of surface biotinylated WEHI-mSIgE and WEHI-mLIgE cells. The immunoprecipitations were performed 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. 3a). Analysis of the mlgM complex yielded the expected characteristic pattern of an 80-kD band corresponding to the m heavy chain, a 25-kD band corresponding to the light chain, and the 32- and 40–47-kD bands corresponding to Igα and Igβ polypeptides, respectively (18). A similar pattern was observed after immunoprecipitation of the mSIgE- and mLIgE-BCRs, indicating that Igα and Igβ 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–Igα specific antiserum produced only the Igα and Igβ subunits (Fig. 3b). The Igβ subunit was of the same molecular mass in the three BCRs, whereas the Igα of the mSIgE (mSIgE–α) showed a slower
mobility than the IgM-α and mLIgE-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 mLIgE-α, immunoprecipitated materials were treated with endoglycosidases. Fig. 3a shows that Endo H treatment decreased the molecular weight of the IgM- and mSIgE-associated Ig-α polypeptides. On the other hand, the mLIgE-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 mSIgE- and mLIgE-BCR immunoprecipitations (Fig. 3a). 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 eBAP37 and eBAP41) was different in the two IgE-BCRs, with significantly lower quantities of eBAP41 in the mLIgE-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. 3b).

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-mSIgE and WEHI-mLIgE 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 εL2 and εL, a complex of ~75 kD was present in the nonreducing PAGE analysis of the mSIgE-BCR. A slightly higher mol wt complex (80 kD) was detected in the mLIgE-BCR. In the second dimension, these complexes dissociated into free Ig-α and Ig-β chains. This experiment also indicated that the eBAP37 and eBAP41 proteins form a disulfide-bound complex (eBAP37/41) which, in the nonreducing PAGE analysis of the mLIgE-BCR, migrated distinctly from the Ig-α/Ig-β heterodimer. In the mLIgE-BCR the eBAP37 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 [γ32P]ATP labeling of immunoprecipitated BCRs. The endogenous IgM-BCR from both transfectomas was analyzed as an internal control. As shown in
Fig. 5, bands corresponding to the Ig-α and Ig-β proteins were found to be phosphorylated when incubated with [γ32P]ATP in all three BCRs. The identity of these bands was again confirmed by treatment with Endo H and PNGase F. The two newly identified eBAPs 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-mIgE and WEHI-mIgE) that expressed comparable levels of mIgE and mIgE (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 mIgE-BCR, the substrate phosphorylation reached its maximum in 1 min and drastically declined within 5 min (Fig. 7). In contrast, in the mIgE-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-mIgE and WEHI-mIgE 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 mIgE- and mIgE-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-mIgE and WEHI-mIgE cells were grown for 24 h in the presence of various concentrations of anti-IgE Ab and were subsequently incubated for 14 h with [3H]thymidine. A marked inhibition in proliferation was observed only in the WEHI-mIgE transfectoma; cross-linking of the mIgE had no ef-
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 mIgE 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 mSIgE 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 mSIgE, indicating that the transport of the mSIgE 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 mIgE, the Ig-α polypeptide had only one of the sites terminally glycosylated and resistant to Endo H cleavage, whereas, when associated with mSIgE, 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 mIgE contains a much longer extracellular domain than the mSIgE, 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 mIgE-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 mIgE-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 mSIgE-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.
of the WEHI transfectedomas. Cross-linking of the mIgE-BCR led to growth inhibition, whereas signaling through the mIgE-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 mIgE, 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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References


