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Biochemistry

Identification of an IgE-binding protein by molecular cloning

(immediate hypersensitivity/IgE receptor/cDNA cloning/nucleotide sequence)

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ABSTRACT **The synthesis and function of IgE are dependent on IgE-binding proteins, which include cell surface IgE receptors and IgE-binding lymphokines. To further our understanding of the IgE system, we have engaged in the molecular cloning of genes for some of these proteins. In studying the *in vitro* translation products of mRNA from rat basophilic leukemia (RBL) cells, we have identified a M_r 31,000 polypeptide that binds IgE and is also reactive with antibodies to proteins affinity-purified from RBL cells with IgE immunoadsorbent. For the molecular cloning, double-stranded cDNA was synthesized from sucrose gradient-fractionated RBL mRNA, inserted into plasmid pBR322, and used to transform *Escherichia coli*. By screening transformants with a hybridization-selection/*in vitro* translation procedure, we identified one clone containing cDNA that hybridized to mRNA coding for a M_r 31,000 IgE-binding protein. The DNA sequence of this cloned cDNA (571 base pairs) was determined and the amino acid sequence corresponding to part of the protein was deduced. In RNA blot analysis, the cDNA hybridized with a mRNA of 1100 nucleotides found in RBL cells but absent in cells not expressing IgE receptors. This cloned cDNA most likely codes for the M_r 31,000 IgE-binding protein identified in RBL cells, which appears to be related to the IgE-binding phenotype of the cells and which may have a significant role in the IgE-mediated activation of basophils and mast cells.**

Both the production and function of immunoglobulin E (IgE) molecules are intimately dependent on IgE-binding proteins. One type of IgE-binding proteins includes the cell surface receptors of mast cells, basophils, lymphocytes, and other cell types (1, 2). The receptors on mast cells and basophils are responsible for IgE-mediated immediate hypersensitivity reactions (3, 4), whereas those on lymphocytes play important roles in the regulation of IgE antibody responses (5, 6). Another type of IgE-binding proteins includes the lymphokines that function in either potentiating or suppressing IgE antibody production (5). To further our understanding of the IgE system, it is important to establish the structural relatedness of these proteins, the structure–function relationship for each of these proteins, and the regulation of their gene expression. Toward these goals, we have initiated the molecular cloning of DNA coding for these proteins.

We chose to work first with rat basophilic leukemia (RBL) cells, which have been used extensively to study a high-affinity IgE receptor present on basophils and mast cells, and from which IgE-binding proteins have already been isolated and characterized (reviewed in refs. 1, 4, 7). One well-studied IgE-binding protein is a glycoprotein of $M_r \approx 55{,}000$ which has been studied in a number of laboratories (8–10). It has been definitively established that this protein is expressed on the surface of RBL cells, mast cells, and basophils and is responsible for the high-affinity IgE-binding property of these cells.

The biochemistry of the high-affinity IgE receptor became somewhat complex when the multisubunit nature of the receptor was revealed by the identification of other protein components in the efforts to purify the receptor from RBL cells. One group of scientists has isolated two additional proteins of M_r 33,000 (11, 12) and 10,000 (13), respectively, in the affinity purification of IgE-receptor complexes. They also demonstrated that these two proteins could be chemically crosslinked to the M_r 55,000 glycoprotein by treating RBL cells or cell lysates with bifunctional crosslinking reagents and therefore designated them as the β and γ subunits, respectively, of the high-affinity IgE receptor, with the M_r 55,000 glycoprotein being designated as the α subunit.

Another group of scientists has isolated a M_r 30,000–33,000 protein, in addition to the M_r 55,000 glycoprotein, in the purification of RBL IgE receptor by repetitive affinity chromatography with IgE immunoadsorbent (14–16). Although this protein shares certain characteristics with the protein designated as the β subunit, their exact relationship remains to be firmly established. Furthermore, it remains to be determined whether the isolation of either protein can be attributed to its ability to bind IgE independently. More recently, proteins of M_r 30,000–33,000 that are distinct from the β subunit but bind IgE have been identified in RBL cells (17).

In studying the *in vitro* translation products of RBL mRNA, we have identified a M_r 31,000 protein that clearly possesses an intrinsic IgE-binding property. A cDNA clone hybridizable to a mRNA species that can be translated to this M_r 31,000 IgE-binding protein then was isolated and characterized, thereby definitively establishing the existence of this protein in RBL cells. These results are the subject of this communication.

MATERIALS AND METHODS

Cell Lines and Reagents. Cell lines used include RBL (18) (kindly provided by H. Metzger of the National Institutes of Health), mouse mastocytoma P815 (19), rat lymphoma IR983F (20) (originally from H. Bazin and provided by M. Zanetti of the Medical Biology Institute), and mouse hybridomas secreting either IgE or IgG1 antibody to dinitrophenyl (DNP) (21). Mouse monoclonal IgE (HI-DNP-ϵ-26.82) and IgG1 (HI-DNP-γ_1-109.3) specific for DNP (21), goat anti-rabbit IgG antibodies (21), and rabbit antiserum prepared against proteins affinity-purified from RBL cells by repetitive chromatography on IgE immunoadsorbent (anti-ϵBP; ref. 15) have been described previously. Immunoadsorbents were prepared by conjugating 10 mg of protein to 1 g (dry weight) of CNBr-activated Sepharose 4B (Pharmacia).

Preparation of RNA and *in Vitro* Translation of RNA. Extraction of total cytoplasmic RNA from various cell sources by the standard phenol/chloroform method, isolation of

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Abbreviations: NRS, normal rabbit serum; RBL, rat basophilic leukemia; kb, kilobase(s).

poly(A)$^+$ RNA by oligo(dT)-cellulose affinity chromatography, and sucrose density gradient fractionation of RBL cell poly(A)$^+$ RNA have been described (22). *In vitro* translation of mRNA was achieved with the rabbit reticulocyte lysate system (New England Nuclear) in the presence of [^{35}S]methionine.

cDNA Cloning and Screening. Double-stranded cDNA was prepared from sucrose gradient-fractionated RBL cell mRNA, inserted into the *Pst* I site of pBR322, and used to transform *Escherichia coli* C600 (23). A hybridization-selection/*in vitro* translation procedure was used to screen for positive clones (24). Briefly, pools of 12 chimeric plasmids were bound to nitrocellulose filters, the filters were incubated with total RBL cell poly(A)$^+$ RNA, and the hybridized RNA was eluted and translated *in vitro*. The translation products were immunoprecipitated (see below) to detect an IgE-binding protein. A single positive clone was identified by subjecting the individual clones of a positive pool to the same procedure.

Immunoprecipitation. *Procedure A. Isolation of an IgE-binding protein with IgE-Sepharose 4B; one-cycle procedure used for* in vitro *translation products.* The translation mixture (20 μl) was diluted with an equal volume of 2× solution A (1× = 20 mM Tris Cl, pH 7.5/10 mM EDTA/150 mM NaCl/1% Trasylol) containing 2% Triton X-100 and then was mixed with 50 μl (packed volume) of rabbit γ-globulin-Sepharose 4B for 1 hr at 4°C. The supernatant was mixed with 50 μl of IgE-Sepharose 4B for 3 hr at 4°C. The beads were washed four times with 4 ml of solution A/1% Triton X-100 and once with 4 ml of solution A/0.05% NaDodSO$_4$. The bound protein was eluted with 75 μl of 62.5 mM Tris Cl, pH 6.8/2% NaDodSO$_4$/10% glycerol/5% 2-mercaptoethanol by boiling for 3 min.

Procedure B. Isolation of an IgE-binding protein by repetitive affinity purification with IgE-Sepharose 4B; used for cell lysates. This procedure is essentially the same as previously reported (14, 15) with slight modifications as described (22). Briefly, the cell lysate (typically 5 × 10^7 cells) was subjected to the first cycle of affinity purification as described in procedure A, and the bound proteins were eluted with three 200-μl portions of 0.5 M acetic acid/1% Triton X-100, neutralized with 75 μl of 2 M Tris Cl, pH 8.8/1% Triton X-100, and subjected to a second cycle of affinity purification. At the end of the first cycle of affinity purification, proteins were radiolabeled, while still bound to IgE-Sepharose 4B, by the chloramine-T method (25) with 1 mCi (1 Ci = 37 GBq) of Na^{125}I and 10 μg of chloramine-T in 30 μl of 0.1 M sodium phosphate (pH 7.5) for 60 sec at 4°C. The beads were further washed three times with 4 ml of solution A/1% Triton X-100, and the bound proteins were eluted as described above.

Procedure C. Immunoprecipitation with rabbit anti-εBP serum. To *in vitro* translation products (20 μl) or proteins isolated from RBL cells by using IgE-Sepharose 4B (200 μl) were added 3 μl of normal rabbit serum (NRS). The mixture was incubated for 1 hr and then reacted with goat anti-rabbit IgG-Sepharose 4B (50 μl) for 1 hr. The supernatants were incubated with 3 μl of rabbit anti-εBP serum for 1 hr and then mixed with goat anti-rabbit IgG-Sepharose 4B (50 μl) for 1 hr. The beads were washed and the bound proteins were eluted as described above. All the above procedures were done at 4°C.

Procedure D. Adsorption with lentil lectin-Sepharose 4B. Proteins bound to IgE-Sepharose 4B, iodinated and eluted as described in procedure B, were mixed with lentil lectin-Sepharose 4B (Pharmacia; 100 μl of a 1:1 suspension in 10 mM Tris Cl, pH 7.4/0.15 M NaCl/1% Triton X-100) for 1 hr at 4°C.

Electrophoresis, RNA Blot Hybridization, and DNA Sequence Analysis. NaDodSO$_4$/PAGE of proteins was conducted using the Laemmli system (26). Gels containing ^{35}S-labeled proteins were treated with EN^{3}HANCE (New England Nuclear), dried, and fluorographed; gels containing ^{125}I-labeled proteins were dried and autoradiographed. For blot analysis, RNAs were glyoxylated, electrophoresed in 2% agarose gels, transferred to nitrocellulose filters, and hybridized with cDNA probe labeled with ^{32}P by nick-translation (27). The DNA sequence was determined by the dideoxy primer-extension method (28).

RESULTS

Identification of M_r 31,000 IgE-Binding Protein in the *in Vitro* Translation Products of RBL mRNA. In previous work, the expression of IgE-binding activity on the surface of *Xenopus* oocytes injected with RBL cell mRNA was detected and a M_r 31,000 translation product from those oocytes was isolated by use of an IgE immunoadsorbent (22). The extraordinary intensity of the M_r 31,000 protein band on NaDodSO$_4$/polyacrylamide gels in comparison to the presumed α subunit of the IgE receptor suggested that this protein might have an intrinsic IgE-binding activity. We subjected sucrose gradient-fractionated RBL cell mRNA to *in vitro* translation in the rabbit reticulocyte lysate system and subjected the translation products (composed of numerous translated proteins; data not shown) to affinity purification with IgE immunoadsorbent. From the translation products of 13–14S RNA, a M_r 31,000 protein was isolated virtually free of other proteins (Fig. 1*A*).

As mentioned in the Introduction, a M_r 30,000–33,000 protein, in addition to another higher molecular weight protein, was isolated from RBL cell lysates by using IgE immunoadsorbent (14–16). It has been reported that polyclonal rabbit antisera raised against these proteins appeared to react with both protein components (15). We postulated that the M_r 31,000 protein detected by us corresponded to the M_r 30,000–33,000 protein and that the antisera would be useful to facilitate the study of the M_r 31,000 protein. Therefore, the reactivity of this translation product with the serum was examined. (For simplicity, this antisera is designated as anti-IgE binding proteins; anti-εBP.) A M_r 31,000 protein was immunoprecipitated from the translation products of RBL mRNA by anti-εBP serum (Fig. 1*B*, lane a) but not by normal rabbit serum (lane b). The M_r 31,000 protein was clearly the major protein in the immunoprecipitates and was the only

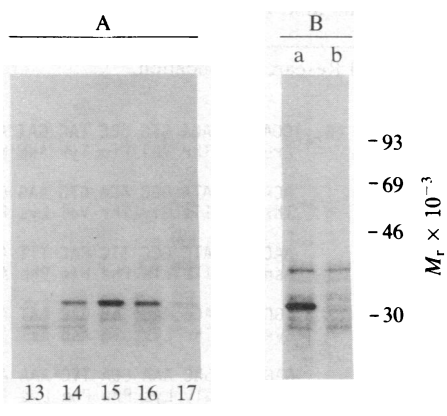

FIG. 1. NaDodSO$_4$/10% PAGE analysis of IgE-binding proteins isolated from the *in vitro* translation products of RBL mRNA. (*A*) RBL poly(A)$^+$ RNA was fractionated on a 10–30% sucrose gradient (see ref. 22 for the RNA sedimentation coefficient in each fraction) and individual fractions were translated *in vitro*. IgE-binding proteins in the translation products then were isolated with IgE-Sepharose 4B. (*B*) *In vitro* translation products from sucrose gradient-fractionated RNA (pool of fractions 13–16) were subjected to immunoprecipitation with rabbit anti-εBP serum (lane a) or NRS (lane b).

protein that was immunoprecipitated specifically.

Cloning of cDNA Coding for the M_r 31,000 IgE-Binding Protein. RBL mRNA from sucrose density gradient fractions 14–16 was used to generate a cDNA library, which was screened by a hybridization-selection/*in vitro* translation procedure for clones coding for the IgE-binding protein. From about 1000 clones, one positive clone (136C9.13) was identified. cDNA from this clone hybridized to RBL mRNA that could be translated to a M_r 31,000 protein that binds IgE (Fig. 2, lane c) but not bovine serum albumin (lane a) or IgG1 (lane b). Furthermore, the translated protein was shown to react specifically with the anti-εBP antiserum (lane d vs. lane e). Insert analysis indicated that the cloned cDNA was about 570 base pairs long.

The nucleotide sequence of the 136C9.13 cDNA was determined and a single open reading frame was identified. As shown in Fig. 3, this cDNA contains a coding sequence for 138 amino acids representing approximately the carboxyl-terminal half of the protein. The cDNA sequence also contains 121 nucleotides of 3′ untranslated region and a typical AATAAA sequence 15–21 bases before the poly(A) addition site. Searches for homologous sequences showed that this cDNA sequence was not in the nucleic acid data base‡ and that there is no significant homology to other known protein or DNA sequences. The predicted protein appears rather hydrophilic as indicated by a hydropathy plot (29); inspection of the sequence does not identify any potential transmembrane sequences or any N-linked carbohydrate attachment sites. A full-length cDNA is obviously necessary for more complete information on the protein structure.

The M_r 31,000 IgE-Binding Protein Is Expressed by RBL Cells and Not by Cells Lacking IgE Receptors. To relate the 136C9.13 cDNA to the IgE-binding property of RBL cells with confidence, we had first to show that this cDNA detects a mRNA species present in RBL cells but not in other cells that lack the IgE receptor. Thus, RNA blot hybridization experiments were performed (Fig. 4). The 136C9.13 cDNA hybridized specifically with 1.1-kilobase (kb) and 1.6-kb mRNAs from RBL cells (lane c). Under more stringent conditions, only the 1.1-kb mRNA band was detected. The 1.1-kb band but not the 1.6-kb band was also detected with much lower intensity in mRNA from mouse mastocytoma line P815 (lane d). Neither of these two mRNAs were found in a rat lymphoma (lane e) or in either of two mouse hybridomas secreting IgE (lane a) and IgG1 (lane b), respectively. A 1.1-kb mRNA has also been detected in mouse mastocytoma cell line CXBG ABMCT-1 but not in mouse macrophage line P388D1 (data not shown).

‡National Biomedical Research Foundation.

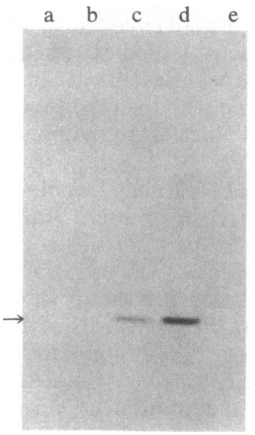

FIG. 2. $NaDodSO_4$/10% PAGE analysis of the *in vitro* translation product from mRNA hybridized to a cloned cDNA. mRNA hybridizing with 136C9.13 cDNA immobilized on nitrocellulose filters was translated *in vitro* and the translation products were immunoprecipitated with Sepharose 4B conjugated with bovine serum albumin (lane a), mouse monoclonal IgG1 (lane b), or mouse monoclonal IgE (lane c) or were subjected to two-step immunoprecipitation with rabbit anti-εBP serum (lane d) or NRS (lane e) followed by goat anti-rabbit IgG-Sepharose. Arrow indicates position of M_r 31,000 protein.

The discovery of an RBL mRNA coding for a M_r 31,000 IgE-binding protein prompted us to document that this protein was actually translated *in vivo* in certain cell types that bind IgE. When an RBL cell lysate was adsorbed with IgE-Sepharose and the bound proteins were iodinated, eluted, and readsorbed with IgE-Sepharose,§ a M_r 31,000 protein was indeed isolated in addition to a protein of $M_r \approx 55{,}000$, which has been designated as the α subunit of the IgE receptor (Fig. 5*A*, lane a). Neither protein was isolated from mouse mastocytoma P815 cells (lane b) or from rat B-lymphoma cells (lane c).

Next, we wanted to establish that the RBL cell-derived M_r 31,000 protein detected above is an IgE-binding protein.

§The two-cycle affinity-purification procedure was found to be necessary to eliminate proteins binding to IgE nonspecifically (14, 15). We found it convenient, in conjunction with this procedure, to radiolabel the protein at the end of the first cycle of affinity purification; proteins of high specific radioactivity thus were obtained.

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(G14)CCA CTG ACA GTG CCC TAC GAT ATG CCC TTG CCT GGA GGA GTC ATG CCT CGC ATG CTG ATC    60
     Pro Leu Thr Val Pro Tyr Asp Met Pro Leu Pro Gly Gly Val Met Pro Arg Met Leu Ile

     ACA ATC ATA GGC ACA GTG AAG CCC AAC GCA AAC AGT ATC ACT CTG AAT TTC AAG AAA GGG   120
     Thr Ile Ile Gly Thr Val Lys Pro Asn Ala Asn Ser Ile Thr Leu Asn Phe Lys Lys Gly

     AAC GAC ATC GCC TTC CAC TTT AAC CCC CGC TTC AAT GAG AAC AAC AGA AGA GTC ATC GTG   180
     Asn Asp Ile Ala Phe His Phe Asn Pro Arg Phe Asn Glu Asn Asn Arg Arg Val Ile Val

     TGC AAC ACG AAG CAG GAC AAT AAC TGG GGA AGG GAA GAA AGA CAG TCA GCT TTC CCC TTT   240
     Cys Asn Thr Lys Gln Asp Asn Asn Trp Gly Arg Glu Glu Arg Gln Ser Ala Phe Pro Phe

     GAG AGC GGC AAA CCA TTC AAA ATA CAG GTC CTG GTT GAA GCC GAC CAC TTC AAG GTT GCG   300
     Glu Ser Gly Lys Pro Phe Lys Ile Gln Val Leu Val Glu Ala Asp His Phe Lys Val Ala

     GTC AAT GAT GTT CAT CTG TTG CAG TAT AAC CAT CGG ATG AAG AAC CTC AGG GAA ATC AGC   360
     Val Asn Asp Val His Leu Leu Gln Tyr Asn His Arg Met Lys Asn Leu Arg Glu Ile Ser

     CAA CTG GGG ATC ATT GGT GAC ATA ACC CTC ACC AGC GCT TCC CAC GCC ATG ATC TAAGCCA   421
     Gln Leu Gly Ile Ile Gly Asp Ile Thr Leu Thr Ser Ala Ser His Ala Met Ile

     GAAGGGGTGGGCCGGCACCAGAACTGCCCTGTGTGTTATGAGCGGGAAACTTTGCATTTCTCTCTCCTTATACTTCTTG   500
     TAAGACATCCATTTAATAAAGTCTCGTGCTGAGAGA9(C13)
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FIG. 3. Nucleotide sequence of cloned cDNA 136C9.13. The amino acids predicted from the nucleotide sequence are presented below the coding sequence.

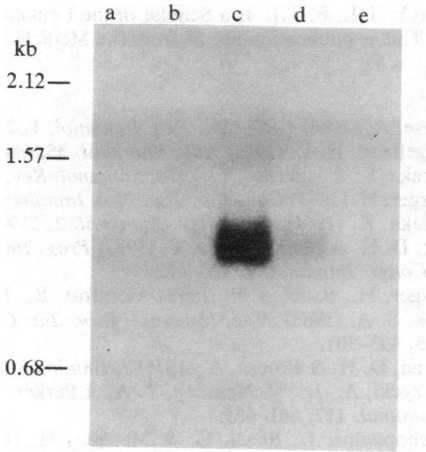

FIG. 4. Blot hybridization analysis of RNA from various cell lines with cloned 136C9.13 cDNA probe. Total poly(A)$^+$ RNA samples (5 μg each) from mouse IgE-secreting hybridoma 26.82 (lane a), mouse IgG1-secreting hybridoma 109.3 (lane b), RBL cells (lane c), mouse mastocytoma P815 (lane d), and rat lymphoma IR983F (lane e) were glyoxylated, electrophoresed in 2% agarose gels, transferred to nitrocellulose filters, and hybridized with ^{32}P-labeled 136C9.13 probe. Hybridization conditions: 0.04% Ficoll/0.04% bovine serum albumin/0.04% polyvinylpyrrolidone/25 mM Pipes, pH 6.8/25 mM EDTA/0.75 M NaCl/50% formamide/0.2% NaDodSO$_4$/heat-denatured tRNA (50 μg/ml)/heat-denatured salmon sperm DNA (50 μg/ml); 42°C, 16 hr. Washing conditions: 15 mM NaCl/1.5 mM Na citrate/0.1% NaDodSO$_4$ at 51°C.

Since it may be argued that in the above experiments, the M_r 31,000 protein was isolated from RBL cells by virtue of its association with the M_r 55,000 protein, it was necessary to determine whether the M_r 31,000 protein could be isolated independently from the higher molecular weight component. This was approached by employing a lectin adsorbent, since the α-subunit protein, which is highly glycosylated, is known to bind to various lectins (30), whereas the M_r 31,000 protein, which is not (or lightly) glycosylated should bind to lectins only weakly or not at all. Thus, the protein bound to and subsequently eluted from IgE-Sepharose 4B was incubated with lentil lectin-Sepharose 4B, and material that did not bind to the lectin adsorbent then was treated with IgE-Sepharose 4B. As shown in Fig. 5*B* (lane a), the M_r 31,000 protein was indeed the major protein bound. This result indicates that the M_r 31,000 protein found in RBL cells is an IgE-binding protein.

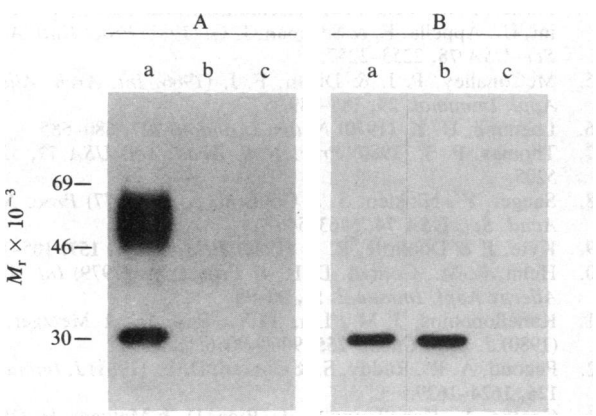

FIG. 5. NaDodSO$_4$/10% PAGE analysis of ^{125}I-labeled IgE-binding protein from RBL cells. (*A*) Cell lysates from RBL cells (lane a), mouse mastocytoma line P815 (lane b) or rat lymphoma line IR983F (lane c) were subjected to repetitive affinity purification with IgE-Sepharose 4B as described in *Materials and Methods*. The proteins were labeled with ^{125}I at the end of the first cycle of affinity purification. (*B*) RBL cell lysates were subjected to affinity purification with IgE-Sepharose 4B and the bound proteins were iodinated, eluted, and treated with lentil lectin-Sepharose 4B. The material not bound to the lectin adsorbent then was immunoprecipitated with IgE-Sepharose 4B (lane a) or subjected to two-step immunoprecipitation with rabbit anti-εBP (lane b) or NRS (lane c) followed by goat anti-rabbit IgG-Sepharose 4B.

The success of the above experiment allowed us to also establish that the isolated M_r 31,000 protein reacts with the anti-εBP antiserum, as is the case for the M_r 31,000 protein translated *in vitro*. Thus, supernatants obtained following the adsorption with lentil lectin-Sepharose 4B described above were immunoprecipitated with anti-εBP serum, and the M_r 31,000 protein was found to be specifically immunoprecipitated (Fig. 5*B*, lane b vs. lane c). This result strongly supports the relatedness of this protein to the one identified in the *in vitro* translation products.

DISCUSSION

This study has established the following: (*i*) A M_r 31,000 IgE-binding protein, translated *in vitro* from RBL mRNA, and its *in vivo* counterpart in RBL cells have been identified. The *in vitro*- and *in vivo*-derived proteins are related as they both are of a similar size, exhibit an intrinsic and specific IgE-binding activity, and are reactive with a specific antiserum raised against highly purified proteins isolated from RBL cells by using IgE immunoadsorbent. (*ii*) cDNA coding for this protein has been cloned, and the amino acid sequence of part of the protein (as predicted from the nucleotide sequence) has been found to be novel. (*iii*) This protein has been shown to be expressed specifically in certain cell types known to possess IgE-specific receptors.

The significance of this newly defined IgE-binding protein and its relationship to IgE receptors on basophils and mast cells remains to be determined. Several possibilities must be considered. First, this may be the protein core or a precursor of the M_r 55,000 IgE-binding glycoprotein (the α subunit) of the high-affinity RBL cell IgE receptor. Although the size of this protein is in close agreement with that estimated for the protein core of the α subunit (7, 15, 31) and the unglycosylated (or partially glycosylated) form(s) of the α subunit is known to possess IgE-binding activity (15, 32), the following considerations argue against this possibility. (*a*) We have been unable to detect the processing of the primary translation product of the 136C9.13 cDNA-selected mRNA to one of a significantly higher molecular weight either in *Xenopus* oocytes or in the rabbit reticulocyte lysate system in the presence of microsomal membranes (data not shown). (*b*) There are five methionine residues in the partial deduced amino acid sequence for the M_r 31,000 IgE-binding protein, whereas the amino acid composition determined for the purified α subunit indicates the presence of only three methionine residues (31). (*c*) It has been reported that the α subunit can be divided into two heavily glycosylated fragments (33), yet no potential N-glycosylation sites are found within the 138 amino acid partial sequence we obtained from the cDNA.

The second possibility is that the M_r 31,000 IgE-binding protein is identical to the protein designated as the β subunit of the high-affinity receptor. The β subunit has a $M_r \approx$ 33,000, it can be chemically crosslinked to the α subunit, and its isolation has been attributed to a physical association with the α subunit (11, 12, 34). Although evidence supporting the association of this protein with the α subunit is convincing, the possibility that this protein has an affinity for IgE has not been excluded. The M_r 31,000 IgE-binding protein and the β subunit in fact share certain similarities; beside

their similar sizes, neither protein can be labeled when RBL cells are subjected to surface radioiodination (ref. 12; unpublished observation). If the M_r 31,000 IgE-binding protein were the β subunit of the IgE receptor, we would have an interesting situation wherein both the receptor and a receptor-associated protein have a ligand (IgE)-binding capacity. The following model may then be proposed: The binding of an IgE molecule to the high-affinity IgE-binding protein (the α subunit) of basophils or mast cells could be followed by the recognition of a second site on the IgE molecule by the M_r 31,000 IgE-binding protein (the β subunit). This secondary event, facilitated by the initial event, could then conceivably be linked to some cellular activation events.

The third possibility is that this newly defined protein is unrelated to the well-studied high-affinity IgE receptor. It may then be identical to one of the IgE-binding proteins recently identified in RBL cells (17). In this case, one may speculate on the biological function of this protein: It might represent a second type of IgE receptor, since two types of IgE receptors, one of high affinity and one of low affinity, have been reported for RBL cells (35). Alternatively, it may be an intracytoplasmic IgE-binding protein. The identification of IgE in the cytoplasm of one type of mast cell (36) suggests a function for intracellular IgE and possibly the need for an intracellular IgE receptor. In addition, the possibility that this protein represents a secretory protein analogous to IgE-binding factors found for lymphocytes (5) cannot be excluded.

A protein of M_r 30,000 has been isolated from lymphoid cells and characterized as an IgG Fc (Fcγ) receptor (37). In addition, one type of lymphocyte-derived IgE-binding factor has an apparent M_r of 30,000 (38). It is reasonable then to consider the existence of a family of proteins in this size range that possess immunoglobulin-binding properties. With regard to the IgE-binding protein identified in this work, it is important to point out that a protein having similar properties has also been isolated from normal mast cells (16, 39).

The detection of an RNA species from P815 cells that hybridizes with the cDNA probe for the M_r 31,000 IgE-binding protein and that is similar in size to that detected in RBL cells (Fig. 4) merits discussion. P815 cells, although of mast-cell lineage, do not bind IgE (18). The M_r 31,000 protein was not detected in P815 cell lysates (Fig. 5) or in the translation products of P815 mRNA (data not shown). Therefore, if the RNA detected by blot hybridization codes for a protein analogous to the one detected in RBL cells, the RNA may very well be nonfunctional. This may underlie a defect in this mastocytoma line that results in the lack of IgE receptor expression.

Although the significance of the M_r 31,000 IgE-binding protein has not been established, it is important to note that the mRNA coding for this protein cosedimented in sucrose density gradients with mRNA(s) that induce IgE-binding activity in oocytes (compare Fig. 1 with figure 2 of ref. 22). This may simply be coincidental but is consistent with the involvement of the M_r 31,000 protein in the IgE-binding activity of RBL cells. These observations, together with both the specific IgE-binding property and the expression of this protein demonstrated in this work, suggest that this protein may be uniquely related to the IgE-binding phenotype of basophils and mast cells.

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