The EMBO Journal vol.6 no.1 pp.109-114, 1987

Cloning and expression of the cDNA coding for a human lymphocyte IgE receptor

Christoph Lüdin, Hans Hofstetter, Marie Sarfati[1], Claude A.Levy, Ueli Suter, Danielle Alaimo, Erich Kilchherr[2], Heiner Frost[2] and Guy Delespesse[1]

Departments of Biotechnology and [2]Biology, Ciba-Geigy Ltd., 4002 Basel, Switzerland, and [1]MRC Group for Allergy Research, Department of Immunology, University of Manitoba, Winnipeg R3E OW3, Canada

Communicated by M.Birnstiel

Low-affinity receptors ($Fc_\epsilon R$) and secreted factors (IgE-BF) which bind to immunoglobulins of the IgE isotype play a key role in the regulation of human IgE synthesis. We report here the cloning of a cDNA coding for the $Fc_\epsilon R$ of the human B-lymphoblast cell line RPMI 8866. The nucleotide sequence of this cDNA predicts a polypeptide with 321 amino acids and a mol. wt of 36 281 daltons. A functional $Fc_\epsilon R$ capable of binding IgE was expressed in Chinese hamster ovary cells after stable transformation with the cDNA which had been cloned into a mammalian expression vector. Amino acid sequence analysis of IgE-BF purified from RPMI 8866 cells revealed an amino-terminal sequence of 19 residues which coincides with the predicted amino acid sequence of the $Fc_\epsilon R$, starting at residues 148 and 150. A computer search with the translated amino acid sequence of the $Fc_\epsilon R$ revealed a domain of 120 amino acids having striking homology to the human asialoglycoprotein receptors.

Key words: lymphocyte IgE receptor/IgE-binding factor/cDNA cloning/asialoglycoprotein receptors

Introduction

Immunoglobulins of the IgE isotype are responsible for the immediate hypersensitivity reactions that occur in diseases such as hay fever, allergic asthma and anaphylaxis (Ishizaka and Ishizaka, 1967). As shown in animal models, the production of IgE antibodies is regulated not only by antigen-specific mechanisms but also by isotype-specific and non-antigen-specific mechanisms (Ishizaka, 1985). The latter are mediated by lymphocytes capable of expressing low-affinity receptors for the Fc region of IgE ($Fc_\epsilon R$) and secreting IgE-binding factors (IgE-BFs), which may selectively potentiate or suppress IgE synthesis. Similar mechanisms have been identified in human IgE regulation. Particular B-cell lines bearing $Fc_\epsilon R$ were shown to produce IgE-BFs capable of modulating the *in vitro* synthesis of human IgE (Sarfati *et al.*, 1984a,b). In particular, $Fc_\epsilon R$ and IgE-BF expressed by the human B-lymphoblast cell line RPMI 8866 are structurally related proteins which share several antigenic determinants and which have identical peptide fragments after proteolytic digestion (Delespesse *et al.*, 1986b; Suemura and Kishimoto, 1986). These results strongly suggest that human IgE-BFs are processed products of $Fc_\epsilon R$.

Several different monoclonal antibodies have been prepared which react with $Fc_\epsilon R$ on the B-cell line RPMI 8866 (Rector *et al.*, 1985). They have been used to identify, in the Nonidet-P40 lysates of RPMI 8866 cells, two main protein components with mol. wts of 45 and 65-96 kd. Both components bind to IgE-Sepharose independently and react with all monoclonal antibodies directed against $Fc_\epsilon R$, suggesting that they have at least some amino acid sequences in common (Nakajima and Delespesse, 1986). The present experiments were undertaken to isolate cDNA clones which encode a human lymphocyte $Fc_\epsilon R$. We have identified one type of cDNA coding for a protein which reacts with $Fc_\epsilon R$-specific monoclonal antibodies and binds to human IgE.

Results

Cloning of $Fc_\epsilon R$ cDNA

The human B-lymphoblastoid cell line RPMI 8866 which expresses $Fc_\epsilon R$ (Sarfati *et al.*, 1984a; Peterson and Conrad, 1985) and secretes IgE-BF (Sarfati *et al.*, 1984a) was used as the source of poly(A) mRNA. This was fractionated on sucrose density gradients and then injected into *Xenopus laevis* oocytes. After overnight incubation, the synthesis of $Fc_\epsilon R$ was monitored by allowing oocyte homogenate to react with $Fc_\epsilon R$-specific monoclonal antibodies in a radioimmunoassay. The 17S mRNA fraction produced a strong signal (Figure 1). It was used to prepare a cDNA library in a pUC9-based plasmid vector. Since no protein sequence was available initially, the cDNA library was screened by hybrid-selection and translation in *X. laevis* oocytes. One clone (pCL1) was identified which hybridized to mRNA coding for $Fc_\epsilon R$. DNA sequencing revealed that the cDNA insert of pCL1 contained only 420 bp. It was used to prepare a radioactive DNA probe in order to select by hybridization several additional clones with longer cDNA inserts containing up to ~1500 bp.

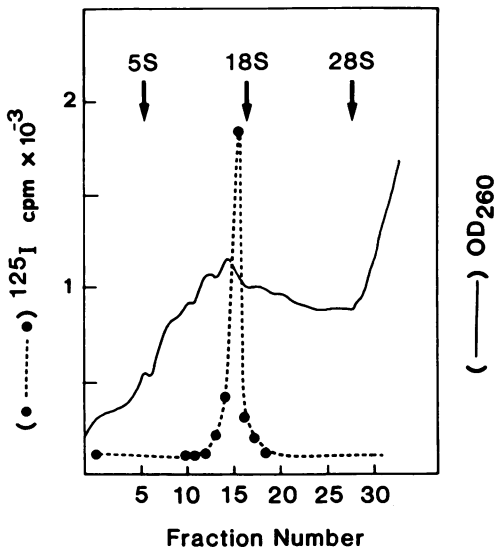

Fig. 1. Sedimentation analysis of human $Fc_\epsilon R$ mRNA. Poly(A) mRNA isolated from RPMI 8866 cells was centrifuged through a 5-23% sucrose gradient. RNA from individual fractions was injected into *X. laevis* oocytes and the synthesis of $Fc_\epsilon R$ protein was measured in a radioimmunoassay. rRNA from RPMI 8866 cells was centrifuged in a parallel tube as a size marker.

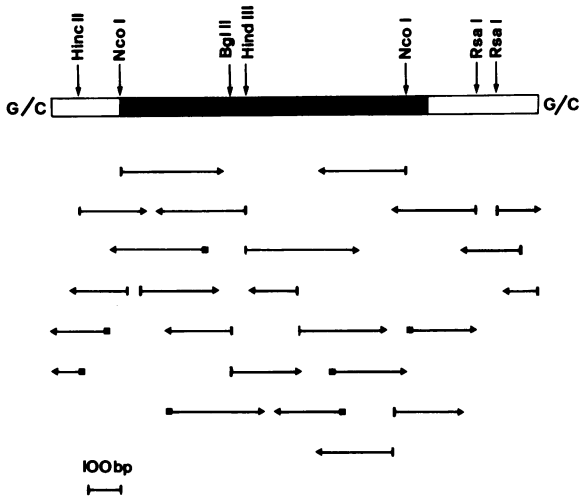

Fig. 2. Restriction map of the $Fc_\epsilon R$ cDNA and the strategy for determining the nucleotide sequence. The dark bar indicates the protein coding region; the open bar indicates the untranslated region. Arrows show the extent of sequencing of each segment.

Nucleotide sequence analysis

The nucleotide sequence of several cDNA inserts was determined by the dideoxy chain termination method of Sanger *et al.* (1977). Figure 2 shows the restriction map and sequencing strategy for the longest cDNA insert (clone pCL4). The nucleotide sequence and the translated amino acid sequence of the human lymphocyte $Fc_\epsilon R$ protein are shown in Figure 3. The apparent large open reading frame predicts a protein with 321 amino acids and a mol. wt of 36 281 daltons. This result was confirmed by *in vitro* translation of RNA produced from the cDNA using the SP6 RNA polymerase system (Melton *et al.*, 1984; data not shown). These findings compare favorably with the mol. wt of the natural human $Fc_\epsilon R$ (Nakajima and Delespesse, 1986), estimated to be ~45 kd. The difference in mol. wt can be attributed to N- and O-linked glycosylations of the natural $Fc_\epsilon R$ protein. Note that one N-linked glycosylation is predicted from the cDNA sequence at amino acid position 63.

The predicted amino acid sequence of the $Fc_\epsilon R$ indicates no characteristic signal sequence at the amino terminus commonly found on membrane-bound or secreted proteins. Instead, the hydropathy plot (Kyte and Doolittle, 1982) identified a very hydrophobic region characteristic of membrane anchoring domains between amino acid residues 24 and 44, preceded by clusters of charged amino acids. Several other membrane proteins which span the membrane only once are devoid of a signal sequence, and consequently have an opposite membrane orientation (N terminus inside/C terminus outside; Spiess and Lodish, 1986). By analogy, a similar arrangement is suggested for the $Fc_\epsilon R$ protein.

Expression of functional $Fc_\epsilon R$

To assess the biological activity of the protein encoded by the cDNA, the latter was expressed in cultured mammalian cells. To this end, a 1257-bp cDNA fragment (nucleotide residues 82–1338 of clone pCL4) was transferred into the plasmid pSVd, an eukaryotic expression vector which contains the SV40 early enhancer/promoter, as well as SV40-derived splicing and poly(A) signals (Figure 4a). This plasmid was mixed with the plasmid pSV2neo carrying the neomycin selection marker (Southern and Berg, 1982), and the mixture co-transfected into Chinese hamster ovary (CHO) cells using the calcium phosphate precipitation method. Several G418-resistant cell lines were selected and shown to be recognized by $Fc_\epsilon R$-specific monoclonal antibodies (Figure 4b).

The rosetting technique, a widely used method for the demonstration of low-affinity F_c receptors, was used to show expression of functional $Fc_\epsilon R$. The transformed CHO cells were incubated with IgE-coated bovine erythrocytes. After gentle washing, ~ 20% of the still adherent cells formed rosettes, including many cells densely covered with erythrocytes (Figure 4c). No rosettes could be observed with untransformed CHO cells, or with the transformed cells pre-incubated with human IgE or $Fc_\epsilon R$-specific monoclonal antibodies. Pre-incubation with human IgA and IgG had no inhibitory effect on IgE-rosette formation. Interestingly, the culture supernatants of the transformed CHO cells contained factors reacting in a radioimmunoassay with monoclonal antibodies directed against the $Fc_\epsilon R$ and IgE-BF. It is most likely that these factors are IgE-BFs.

$Fc_\epsilon R$ and IgE-BF share amino acid sequences

IgE-BF was purified from culture supernatants of RPMI 8866 cells in three steps. The resulting material migrated in the mol. wt range of 24–26 kd on SDS–polyacrylamide gels (Figure 5a). The protein was >90% pure as judged from the SDS–polyacrylamide gel. The amino-terminal sequence of this purified IgE-BF was determined by gas phase sequence analysis. A double sequence was obtained with one sequence starting two amino acids later than the other. Seventeen amino acids were determined and these matched the sequence deduced from the cDNA of the $Fc_\epsilon R$ (Figure 5b). The two amino termini of the IgE-BF correspond to the leucine residue at position 148 and the methionine residue at position 150 of the deduced $Fc_\epsilon R$ amino acid sequence. This result agrees with previous findings (Delespesse *et al.*, 1986b; Suemura and Kishimoto, 1986) which indicated that IgE-BF is a cleavage product of membrane-bound $Fc_\epsilon R$.

Amino acid sequence homology

The predicted amino acid sequence of clone pCL4 was compared initially with the protein sequence database of the National Biomedical Research Foundation (NBRF, Washington) using the programs ALIGN and SEARCH. This analysis revealed a long domain of striking homology between residues 76–193 of the chicken hepatic lectin (Drickamer, 1981) and residues 158–273 of the human $Fc_\epsilon R$. The homology search was extended to include published sequences of rat (Holland *et al.*, 1984) and human (Spiess and Lodish, 1985) asialoglycoprotein receptors. Figure 6 shows the homology between residues 163–282 of the human $Fc_\epsilon R$ and residues 153–276 and 177–300 of the human asialoglycoprotein receptors 1 and 2, respectively. 38% of the amino acid residues in these regions are identical. Most interesting is the finding that the cysteine residues are at identical positions, suggesting similar folding of the homology domains.

Discussion

In this paper we reported the cloning of a cDNA coding for a human lymphocyte $Fc_\epsilon R$. Expression of the cDNA in CHO cells showed that it codes for a functional membrane-bound receptor capable of binding specifically to human IgE. These results support earlier biochemical studies and demonstrate that the human lymphocyte $Fc_\epsilon R$ consists of a single polypeptide chain (Nakajima and Delespesse, 1986).

The translated amino acid sequence predicts a protein of 321 amino acid residues and a mol. wt of 36 281 daltons. Inspection of its hydropathy plot revealed no obvious signal sequence at the

```
AGTGGCTCTACTTTCAGAAGAAAGTGTCTCTCTTCCTGCTTAAACCTCTGTCTCTGACGGTCCCTGCCAATCGCTCTGGTCGACCCCAAC     90

ACACTAGGAGGACAGACACAGGCTCCAAACTCCACTAACCAGAGCTGTGATTGTGCCCGCTGAGTGGACTGCGTTGTCAGGGAGTGAGTGC   181

TCCATCATCGGGAGAATCCAAGCAGGACCGCCATG GAG GAA GGT CAA TAT TCA GAG ATC GAG GAG CTT CCC AGG AGG   258
                                  Met Glu Glu Gly Gln Tyr Ser Glu Ile Glu Glu Leu Pro Arg Arg    15

CGG TGT TGC AGG CGT GGG ACT CAG ATC GTG CTG CTG GGG CTG GTG ACC GCC GCT CTG TGG GCT GGG CTG   327
Arg Cys Cys Arg Arg Gly Thr Gln Ile Val Leu Leu Gly Leu Val Thr Ala Ala Leu Trp Ala Gly Leu    38

CTG ACT CTG CTT CTC CTG TGG CAC TGG GAC ACC ACA CAG AGT CTA AAA CAG CTG GAA GAG AGG GCT GCC   396
Leu Thr Leu Leu Leu Leu Trp His Trp Asp Thr Thr Gln Ser Leu Lys Gln Leu Glu Glu Arg Ala Ala    61

CGG AAC GTC TCT CAA GTT TCC AAG AAC TTG GAA AGC CAC CAC GGT GAC CAG ATG GCG CAG AAA TCC CAG   465
Arg Asn Val Ser Gln Val Ser Lys Asn Leu Glu Ser His His Gly Asp Gln Met Ala Gln Lys Ser Gln    84

TCC ACG CAG ATT TCA CAG GAA CTG GAG GAA CTT CGA GCT GAA CAG CAG AGA TTG AAA TCT CAG GAC TTG   534
Ser Thr Gln Ile Ser Gln Glu Leu Glu Glu Leu Arg Ala Glu Gln Gln Arg Leu Lys Ser Gln Asp Leu   107

GAG CTG TCC TGG AAC CTG AAC GGG CTT CAA GCA GAT CTG AGC AGC TTC AAG TCC CAG GAA TTG AAC GAG   603
Glu Leu Ser Trp Asn Leu Asn Gly Leu Gln Ala Asp Leu Ser Ser Phe Lys Ser Gln Glu Leu Asn Glu   130

AGG AAC GAA GCT TCA GAT TGG CTG GAA AGA CTC CGG GAG GAG GTG ACA AAG CTA AGG ATG GAG TTG CAG   672
Arg Asn Glu Ala Ser Asp Leu Leu Glu Arg Leu Arg Glu Glu Val Thr Lys Leu Arg Met Glu Leu Gln   153

GTG TCC AGC GGC TTT GTG TGC AAC ACG TGC CCT GAA AAG TGG ATC AAC TTC CAA CGG AAG TGC TAC TAC   741
Val Ser Ser Gly Phe Val Cys Asn Thr Cys Pro Glu Lys Trp Ile Asn Phe Gln Arg Lys Cys Tyr Tyr   176

TTC GGC AAG GGC ACC AAG CAG TGG GTC CAC GCC CGG TAT GCC TGT GAC GAC ATG GAA GGG CAG CTG GTC   810
Phe Gly Lys Gly Thr Lys Gln Trp Val His Ala Arg Tyr Ala Cys Asp Asp Met Glu Gly Gln Leu Val   199

AGC ATC CAC AGC CCG GAG GAG CAG GAC TTC CTG ACC AAG CAT GCC AGC CAC ACC GGC TCC TGG ATT GGC   879
Ser Ile His Ser Pro Glu Glu Gln Asp Phe Leu Thr Lys His Ala Ser His Thr Gly Ser Trp Ile Gly   222

CTT CGG AAC TTG GAC CTG AAG GGA GAG TTT ATC TGG GTG GAT GGG AGC CAT GTG GAC TAC AGC AAC TGG   948
Leu Arg Asn Leu Asp Leu Lys Gly Glu Phe Ile Trp Val Asp Gly Ser His Val Asp Tyr Ser Asn Trp   245

GCT CCA GGG GAG CCC ACC AGC CGG AGC CAG GGC GAG GAC TGC GTG ATG ATG CGG GGC TCC GGT CGC TGG  1017
Ala Pro Gly Glu Pro Thr Ser Arg Ser Gln Gly Glu Asp Cys Val Met Met Arg Gly Ser Gly Arg Trp   268

ACC GAC GCC TTC TGC GAC CGT AAG CTG GGC GCC TGG GTG TGC GAC CGG CTG GCC ACA TGC ACG CCG CCA  1086
Asn Asp Ala Phe Cys Asp Arg Lys Leu Gly Ala Trp Val Cys Asp Arg Leu Ala Thr Cys Thr Pro Pro   291

GCC AGC GAA GGT TCC GCG GAG TCC ATG GGA CCT GAT TCA AGA CCA GAC CCT GAC GGC CGC CTG CCC ACC  1155
Ala Ser Glu Gly Ser Ala Glu Ser Met Gly Pro Asp Ser Arg Pro Asp Pro Asp Gly Arg Leu Pro Thr   314

CCC TCT GCC CCT CTC CAC TCT TGAGCATGGATACAGCCAGGCCCAGAGCAAGACCCTGAAGACCCCCAACCACGGCCTAAAAGC  1239
Pro Ser Ala Pro Leu His Ser  *                                                               321

CTCTTTGTGGCTGAAAGGTCCCTGTGACATTTTCTGCCACCCAAACGGAGGCAGCTGACACATCTCCCGCTCCTCTATGGCCCCTGCCTTC  1330

CCAGGAGTACACCCCAACAGCACCCTCTCCAGATGGGAGTGCCCCCAACAGCACCCTCTCCAGATGAGAGTTACACCCCAACAGCACCCTC  1420

TCCAGATGCAGCCCCATCTCCTCAGCACCCCAGGACCTGAGTATCCCCAGCTCAGGGTGGTGAGTCCTCCTGTCCAGCCTGCATCAATAAA  1511

ATGGGGCAGTGATGGCC(A)n                                                                        1528
```

Fig. 3. Nucleotide sequence of the $Fc_\epsilon R$ cDNA (clone pCL4) and predicted amino acid sequence of the $Fc_\epsilon R$ protein. Clone pCL4 contains the longest cDNA insert amongst the $Fc_\epsilon R$-specific cDNA clones found. Nucleotides and amino acids are numbered at the right. The hydrophobic region encoding the putative membrane anchor is indicated with a line below the amino acid sequence; the potential site for N-glycosylation is boxed; the poly(A) signal is underlined.

amino terminus, although the $Fc_\epsilon R$ is anchored in the cytoplasmic membrane. The $Fc_\epsilon R$ shares this unusual feature with several other membrane-bound proteins which span the membrane only once (Spiess and Lodish, 1985). In particular, the human asialoglycoprotein receptor 1 has been shown to have an unusual membrane orientation, having the C terminus outside and the N terminus inside the cytoplasmic membrane (Spiess and Lodish, 1986). By comparison, this arrangement predicts that the $Fc_\epsilon R$ has a cytoplasmic amino-terminal domain of 23, mainly hydrophilic, amino acid residues. The neighbouring region of amino acid residues 24–44 shows a high degree of hydrophobicity which is indicative of membrane-spanning domains. This leaves the main body of the $Fc_\epsilon R$, from amino acid residue 45 to the carboxyl end, to form the extracellular domain, which binds to IgE and which is most probably cleaved off to become soluble IgE-BF.

In addition to the unusual orientation in the membrane, the translated amino acid sequence of the human $Fc_\epsilon R$ was found to share a long domain of homology with the human asialoglycoprotein receptors 1 and 2. Asialoglycoprotein receptors are cell surface proteins of hepatocytes and they bind to galactosyl-terminal glycoproteins. The homology domain spans a continuous sequence of 119 amino acids (residues 163–282) of which 42 are identical in the three proteins. Most remarkably, the positions of the cysteine residues are constant. In addition, two cysteine residues delimit the homology region. The similar spacings between three constant cysteine residues at each end of the homology domain are reminiscent of an inverted repeat. This suggests the formation of disulfide bridges between cysteine residues 163 and 282, 174 and 273 and 191 and 259. The extensive amino acid sequence homology together with the unusual membrane orientation are in keeping with the fact that IgE is

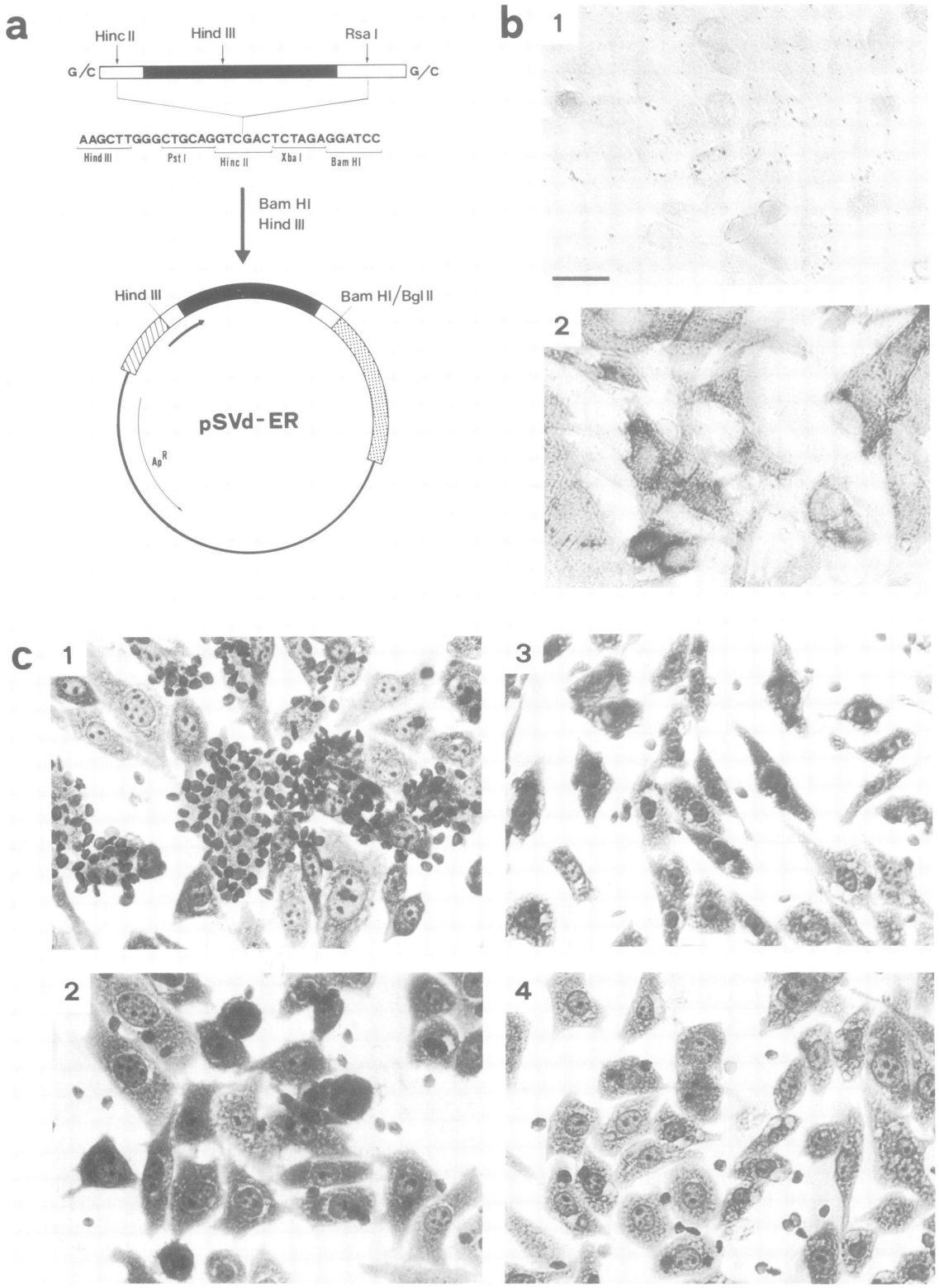

Fig. 4. Expression of functional $Fc_\epsilon R$ in CHO cells stably transformed with the expression plasmid pSVd-ER. **(a)** Construction of the expression plasmid pSVd-ER. The coding and non-coding regions of the $Fc_\epsilon R$ cDNA are shown as black and white boxes; the SV40-derived control elements are hatched (enhancer and promoter) or dotted [splicing and poly(A) signals]; sequences derived from plasmid pBR322 are indicated by a single line. **(b)** Detection of $Fc_\epsilon R$ proteins on transformed CHO cells by staining with peroxidase. Control (1) and transformed (2) CHO cells were fixed with glutaraldehyde and incubated successively with $Fc_\epsilon R$-specific monoclonal antibodies and rabbit anti-mouse antibodies coupled to peroxidase. Bar represents 10 μm. **(c)** Visualization of functional $Fc_\epsilon R$ by rosetting with IgE-coated bovine erythrocytes. IgE-rosette formation with control (2) and transformed (1,3,4) CHO cells after pre-incubation with BSA alone (1,2) or BSA supplemented with IgE (3) or $Fc_\epsilon R$-specific monoclonal antibodies (4).

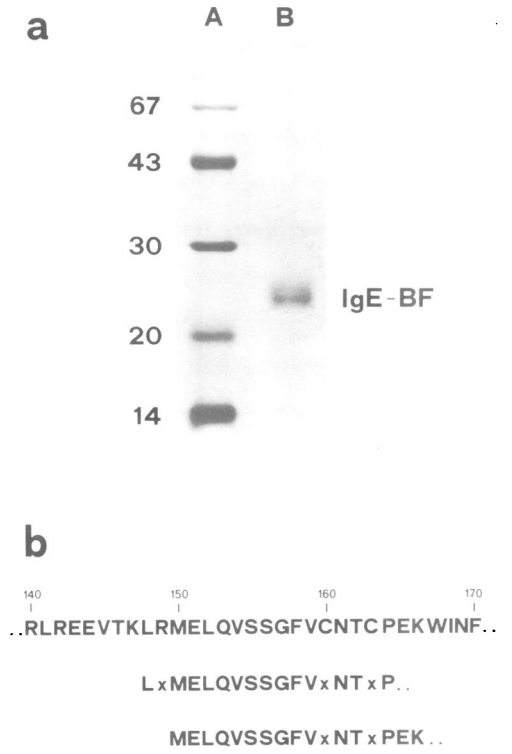

Fig. 5. Purification and N-terminal sequence analysis of IgE-BF. **(a)** SDS–polyacrylamide gel (12.5%) of IgE-BF isolated from RPMI 8866 supernatant. **Lane A**, mol. wt markers; **lane B**, purified IgE-BF. Numbers to the left give mol. wt (kd) of marker proteins. **(b)** Comparison of amino acid sequences of IgE-BF and $Fc_\epsilon R$. The amino acid sequence of the $Fc_\epsilon R$ (top line) was deduced from the nucleotide sequence shown in Figure 3. Amino acid sequence analysis of IgE-BF produced two amino ends (lower lines) displaced by two residues. x represents non-determined residues.

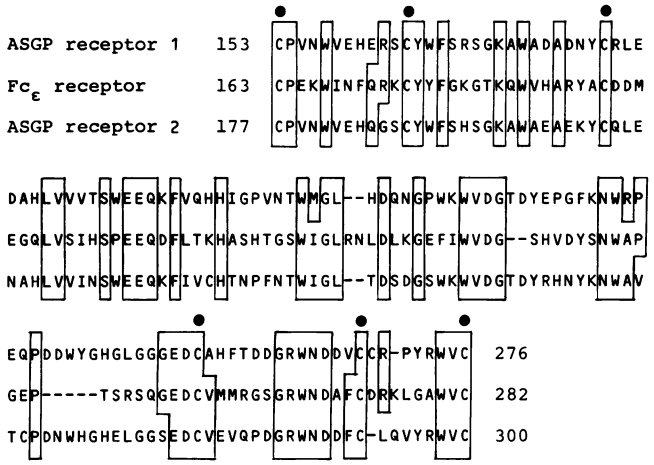

Fig. 6. Amino acid sequence homology between the human $Fc_\epsilon R$ and the human asialoglycoprotein receptors. Amino acids are boxed which are identical in the sequences of the $Fc_\epsilon R$ and the human asialoglycoprotein (ASGP) receptors 1 and 2. Cysteine residues present in all three sequences are marked with a filled circle above the boxes. Numbers on both sides of the amino acid sequences indicate the positions of the homology regions.

a heavily glycosylated immunoglobulin (Ishizaka, 1973), and suggest that homologous structural domains are used by functionally distinct receptors binding to glycoproteins.

The data presented here, together with our previous results, strongly suggest that soluble IgE-BF is derived from membrane-bound $Fc_\epsilon R$ by proteolytic cleavage. Alternative splicing of primary RNA transcripts would be another mechanism to account for the findings that $Fc_\epsilon R$ and IgE-BF react with the same monoclonal antibodies and that they have at least partially overlapping amino acid sequences. Four experimental results argue in favour of proteolytic cleavage. First, we have isolated a dozen independent plasmids carrying $Fc_\epsilon R$-specific cDNA inserts by hybridization with the cDNA insert shown in Figure 3. Restriction enzyme analysis and partial nucleotide sequencing revealed only one type of cDNA corresponding to the one shown in Figure 3. Second, translation of size-fractionated mRNA showed that only the 17S fraction led to the synthesis of proteins which reacted with the monoclonal antibodies directed against $Fc_\epsilon R$ and IgE-BF (Figure 1). Third, the leucine and methionine residues found at the amino terminus of the purified IgE-BF are on the carboxyl side of a lysine and an arginine residue at positions 147 and 149 of the $Fc_\epsilon R$ sequence, respectively (Figure 5b). This suggests the action of a protease with trypsin-like specificity. Fourth, the disappearance of $Fc_\epsilon R$ from surface-iodinated RPMI 8866 cells is associated with the appearance of IgE-BF in the culture supernatant (G.Delespesse, unpublished results).

Recently, Martens *et al.* (1985) reported the cloning of a cDNA coding for rodent IgE-BF produced by a rat–mouse T-cell hybridoma that was capable of regulating rodent IgE synthesis. The nucleotide sequence of the cDNA described by these authors has a strong homology to the *gag* and *pol* genes of the Syrian hamster intracisternal A particle, an endogenous retrovirus (Martens *et al.*, 1985; Toh *et al.*, 1985; Moore *et al.*, 1986). Furthermore, it codes for a secreted protein devoid of an obvious membrane anchoring domain. The protein sequence derived from the cDNA sequence reported here shows no sequence homology with the rodent IgE-BF. Hence, the two proteins seem unrelated and it is suggested that there might exist at least two types of IgE-BF, coded for by two different genes. One IgE-BF would be a processed product of the surface $Fc_\epsilon R$, whereas the other one would be a secreted protein. Since the IgE-BF cDNA described by Martens *et al.* (1985) is of T-cell origin, whereas the cDNA described in this study is of B-cell origin, it could be argued that T- and B-cells may employ different genes and effector molecules to achieve the same biological function. This possibility, however, seems unlikely since it was shown that the monoclonal antibodies which react with $Fc_\epsilon R$ and IgE-BFs of B-cell origin also bind to the same proteins expressed on or derived from T-lymphocytes (Sarfati *et al.*, 1986; Delespesse *et al.*, 1986a).

The availability of the cDNA clone coding for the human lymphocyte $Fc_\epsilon R$ will allow detailed analyses of the membrane-bound $Fc_\epsilon R$ as well as of the soluble IgE-BFs. It will also provide the means to study molecular mechanisms of human IgE synthesis for a better understanding and treatment of allergic diseases.

Materials and methods

Preparation of size-fractionated mRNA

RPMI 8866 cells were grown in tissue culture flasks in RPMI 1640 medium supplemented with 15% FCS, 100 units/ml penicillin and 100 μg/ml streptomycin to a cell density of $\sim 2 \times 10^6$ cells/ml. Total RNA was isolated from 5 g of cells by the guanidinium thiocyanate–CsCl (Glisin *et al.*, 1974) method and poly(A) mRNA was purified by oligo(dT)–cellulose chromatography (Aviv and Leder, 1972). The mRNA (130 μg) was dissolved in 1 mM EDTA, heated to 70°C for 3 min and fractionated on a 5–23% linear sucrose gradient in 0.1 M NaCl, 10 mM Tris– HCl pH 7.5, 1 mM EDTA and 0.5% SDS by centrifugation at 41 000 r.p.m. for 5 h in a Kontron TST41 rotor. RNA from individual fractions was precipitated with ethanol, dissolved in water at a concentration of

0.5 mg/ml and assayed for $Fc_\epsilon R$ mRNA by injection into *X. laevis* oocytes. After 40 h incubation at 20°C, the oocytes were homogenized and the cleared homogenate assayed for $Fc_\epsilon R$ receptor in a radioimmunoassay, essentially as described previously (Sarfati *et al.*, 1986). Briefly, a 96-well microtiter plate was coated with a first monoclonal antibody raised against the $Fc_\epsilon R$ receptor protein onto which the oocyte homogenate was adsorbed, followed finally by a second ^{125}I-radiolabelled monoclonal antibody directed against $Fc_\epsilon R$. Bound ^{125}I was determined in a γ-counter.

Molecular cloning and DNA sequencing

2.8 μg of single-stranded cDNA was synthesized from 6 μg mRNA of fraction 15 in Figure 1, elongated by dCMP residues and finally converted into double-stranded cDNA by using oligo$(dG)_{12-18}$ as primer for DNA polymerase I (Klenow fragment). The double-stranded cDNA was elongated with dCMP residues and molecules longer than 1300 bp were selected by electrophoresis through a 1% agarose gel. The size-selected cDNA was inserted into the *Pst*I site of the plasmid pUC-KO by the standard G/C-tailing method. pUC-KO is a derivative of pUC9 wherein the *lac* promoter and operator have been deleted between the *Hae*II restriction site just upstream of the *lac* promoter and the *Hin*dIII restriction site within the polylinker. After transformation of *Escherichia coli* HB101 by the hybrid plasmids, ~4500 colonies were screened by hybrid selection and frog oocyte injection (Nagata *et al.*, 1980). Individual colonies were grown to saturation in 2 ml LB broth. Groups of 96 cultures were pooled and the plasmid DNA was isolated. From each pool, 100 μg of alkali-denatured plasmid DNA was bound covalently to APT cellulose and hybridized overnight with total mRNA isolated from RPMI 8866 cells (Seed, 1982; Odink *et al.*, 1984). The hybridized mRNA was eluted, twice precipitated with ethanol and injected into *X. laevis* oocytes. The oocyte homogenates were tested for $Fc_\epsilon R$ in a radioimmunoassay as described above. After screening of ~50 pools, one pool gave a positive signal. Its 96 colonies were divided into 12 new pools of eight colonies and rescreened. Finally, a single colony containing a $Fc_\epsilon R$ cDNA was identified (clone pCL1). It was used to screen the remaining cDNA library by hybridization. Several additional clones with longer cDNA inserts were found. Their nucleotide sequence was determined by the dideoxy chain termination method (Sanger *et al.*, 1977) after subcloning of DNA restriction fragments into M13 bacteriophage derivatives mp8 and mp9.

Expression of $Fc_\epsilon R$ cDNA in CHO cells

A 1257-bp *Hin*cII/*Rsa*I DNA fragment obtained from the $Fc_\epsilon R$ cDNA (nucleotide positions 82–1338) was inserted into the *Hin*cII site within the polylinker of plasmid pSP64 (Melton *et al.*, 1984). This construction added a *Hin*dIII and a *Bam*HI site to the 5′ and 3′ end of the $Fc_\epsilon R$ cDNA, respectively. Partial cleavage with *Hin*dIII and complete cleavage with *Bam*HI restriction endonucleases produced a 1284-bp DNA fragment which was inserted between the *Hin*dIII and *Bgl*II sites of plasmid pSVd, thereby yielding the expression plasmid pSVd-ER. The plasmid pSVd was derived from plasmid pSVneo2911 (Asselbergs *et al.*, 1986) by cutting with *Sma*I and *Bgl*II restriction endonucleases and replacing the released DNA fragment with a *Bgl*II-oligonucleotide linker. 10 μg of pSVd-ER and 1 μg of pSV2neo plasmid DNA (Southern and Berg, 1982) were co-precipitated with calcium phosphate and used to transfect sub-confluent CHO cells ($dhfr^-$-mutant DXB-11; Urlaub and Chasin, 1980). The transformed cells were maintained in α-MEM medium supplemented with 5% fetal calf serum, gentamycin and 0.5 mg/ml G418. After 2 weeks, G418-resistant colonies grew out. Several colonies were picked and enlarged individually. $Fc_\epsilon R$ was visualized by successive incubation of glutaraldehyde-fixed cells with $Fc_\epsilon R$-specific monoclonal antibodies (10 μg/ml) at 37°C for 1 h, and rabbit anti-mouse antibodies coupled to peroxidase (Dakopatts P260; 1:50) at 37°C for 1 h. One positive colony was selected and established as a permanent $Fc_\epsilon R$-bearing cell line. IgE-specific rosettes were prepared essentially as described for $Fc_\epsilon R$-positive lymphocytes (Sarfati *et al.*, 1984a; Yodoi and Ishizaka, 1980). Briefly, ~10^4 CHO cells were seeded per well of a 96-well microtiter plate. After 2 days, the cells were washed and incubated for 2 h on ice with phosphate-buffered saline(PBS) supplemented with 3% bovine serum albumin alone, or in addition supplemented with human IgE (1 mg/ml) or $Fc_\epsilon R$-specific monoclonal antibodies (20 μg/ml). Bovine erythrocytes coated with human IgE were added and sedimented onto the CHO cells by centrifugation at 200 *g*. After incubation on ice for 2 h, unbound erythrocytes were removed by gentle washing.

Purification and N-terminal sequence analysis of IgE-BF

Ten litres of culture supernatant from RPMI 8866 cells were filtered through a 20 ml column of Affi-Gel 10 (Bio-Rad) coupled with $Fc_\epsilon R$-specific monoclonal antibodies. After extensive washing with PBS, the bound material was eluted with 50 ml 0.1 M glycine–HCl (pH 2.6). Fractions were collected in tubes containing an equal volume of 1 M Tris–HCl (pH 8.0) and 0.5% Tween 20. IgE-BF-containing fractions (as assayed by radioimmunoassay) were pooled, dialyzed against 10 mM Tris–HCl (pH 7.4) and loaded on a SynChropak AX 300 anion-exchange column (SynChrom Inc.). After washing the column with 10 mM Tris–HCl (pH 7.4), the protein was eluted with a gradient of 0–1 M NaCl. Fractions containing IgE-BF were collected into 0.1% octylpyranoglucoside (Sigma) and concentrated by lyophilization. Reversed-phase chromatography was performed on a SynChropak RP-4 (SynChrom Inc.) column in 0.1% trifluoroacetic acid (TFA)/5% acetonitrile. IgE-BF was eluted by applying a gradient of 5–54% acetonitrile in 0.1% TFA during 30 min at a flow-rate of 0.5 ml/min. Fractions were collected into 0.05% SDS and assayed for IgE-BF with the radioimmunoassay. The purity of the material was analysed by SDS–polyacrylamide gel electrophoresis (Laemmli, 1970). The N-terminal amino acid sequence was analysed with a positive-phase protein sequencer model 470 (Applied Biosystems) according to the method of Hunkapillar and Hood (1983).

Acknowledgements

We thank our colleagues Joseph Brüggen, Karel Odink, Fred Asselbergs, Norman Hardman and Jui Yoa Chang for their support and advice; Hans Rink for the initial finding of the protein sequence homology; Rene Knecht for the analysis of the amino terminus of the IgE-BF; and Doris Rüegg, Elisabeth Edelmann, Colette Kristofic and Silvie Antz for excellent technical assistance. M.S. is the holder of an MRC postdoctoral fellowship.

References

Asselbergs,F.A.M., Will,H., Wingfield,P. and Hirschi,M. (1986) *J. Mol. Biol.*, **189**, 401–411.
Aviv,H. and Leder,P. (1972) *Proc. Natl. Acad. Sci. USA*, **69**, 1408–1412.
Delespesse,G., Sarfati,M., Rubio-Trujillo,M. and Wolowiec,T. (1986a) *Eur. J. Immunol.*, **16**, 815–821.
Delespesse,G., Sarfati,M. and Nakajima,T. (1986b) In Reed,C.P. (ed.), *Proceedings of the Twelfth International Congress on Allergy and Clinical Immunology* Mosby, St. Louis. pp. 35–40.
Drickamer,K. (1981) *J. Biol. Chem.*, **256**, 5827–5839.
Glisin,V., Crkvenjakov,R. and Buys,C. (1974) *Biochemistry*, **13**, 2633–2637.
Holland,E.C., Leung,J.O. and Drickamer,K. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 7338–7342.
Hunkapillar,M.W. and Hood,L.E. (1983) *Methods Enzymol.*, **91**, 399–425.
Ishizaka,K. (1973) In Sela,M. (ed.), *The Antigens.* Academic Press, New York, Vol. 1, pp. 484–487.
Ishizaka,K. (1985) *J. Immunol.*, **135**, 1–10.
Ishizaka,K. and Ishizaka,T. (1967) *J. Immunol.*, **99**, 1187–1198.
Kyte,J. and Doolittle,R.F. (1982) *J. Mol. Biol.*, **157**, 105–132.
Laemmli,U.K. (1970) *Nature*, **227**, 680–685.
Martens,C.L., Huff,T.F., Jardieu,P., Trounstine,M.L., Coffmann,R.L., Ishizaka, K. and Moore,K.W. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 2460–2464.
Melton,D.A., Krieg,P.A., Rebagliati,M.R., Maniatis,T., Zinn,K. and Green, M.R. (1984) *Nucleic Acids Res.*, **12**, 7035–7056.
Moore,K.W., Jardieu,P., Mietz,J.A., Trounstine,M.L., Kuff,E.L., Ishizaka,K. and Martens,C.L. (1986) *J. Immunol.*, **136**, 4283–4290.
Nagata,S., Taira,H., Hall,A., Johnsrud,L., Streuli,M., Ecsödi,J., Boll,W., Cantell,K. and Weissmann,C. (1980) *Nature*, **284**, 316–320.
Nakajima,T. and Delespesse,G. (1986) *Eur. J. Immunol.*, **16**, 809–814.
Odink,K.G., Nicholls,S.C., Lockyer,M.J. and Hillman,Y. (1984) *Mol. Biochem. Parasitol.*, **10**, 55–66.
Peterson,L.H. and Conrad,D.H. (1985) *J. Immunol.*, **135**, 2653–2660.
Rector,E., Nakajima,T., Rocha,C., Duncan,D., Lestourgeon,D., Mitchell,R.S., Fischer,J., Sehon,A.H. and Delespesse,G. (1985) *Immunology*, **55**, 481–488.
Sanger,F., Nicklen,B. and Coulson,A.R. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463–5467.
Sarfati,M., Rector,E., Wong,K., Rubio-Trujillo,M., Sehon,A.H. and Delespesse, G. (1984a) *Immunology*, **53**, 197–205.
Sarfati,M., Rector,E., Rubio-Trujillo,M., Wong,K., Sehon,A.H. and Delespesse, G. (1984b) *Immunology*, **53**, 207–214.
Sarfati,M., Fonteyn,C., Nutman,T. and Delespesse,G. (1986) *Immunology*, in press.
Seed,B. (1982) *Nucleic Acids Res.*, **10**, 1799–1810.
Southern,P.J. and Berg,P. (1982) *J. Mol. Appl. Genet.*, **1**, 327–341.
Spiess,M. and Lodish,H.F. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 6465–6469.
Spiess,M. and Lodish,H.F. (1986) *Cell*, **44**, 177–185.
Suemura,M. and Kishimoto,T. (1986) *Int. Arch. Allergy, Appl. Immunol.*, **77**, 26–31.
Toh,H., Ono,M. and Miyata,T. (1985) *Nature*, **318**, 388–389.
Urlaub,G. and Chasin,L.A. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 4216–4220.
Yodoi,J. and Ishizaka,K. (1980) *J. Immunol.*, **124**, 934–946.

Received on 20 October 1986