

How antibodies work: focus on Fc receptors

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ABSTRACT

It is increasingly appreciated that the part of an antibody not involved in the binding of antigen—the Fc region—plays an important biological role. It activates a variety of receptors not only on so-called effector cells such as macrophages and granulocytes, but also on lymphocytes, and it can thereby modulate the immune response itself. Over the past 2 years much new information has been gained about the structure of such receptors, in large part through molecular genetics. In this review we describe the structure and some aspects of the function of the most complicated of the cellular Fc receptors so far identified: the receptor with high affinity for immunoglobulin E (IgE) on mast cells. The structure of its IgE-binding chain is strikingly similar to the corresponding polypeptide of an immunoglobulin G receptor. Like the latter and like a receptor that binds polymeric immunoglobulin, segments of the protein resemble immunoglobulin sequences. It is surprising that other IgE-binding proteins that putatively serve related functions have completely different structures. —METZGER, H.; KINET, J.-P. How antibodies work: focus on Fc receptors. *FASEB J.* 2: 3-11; 1988.

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MUCH OF THE USEFUL WORK PERFORMED BY antibodies is accomplished simply by the attachment of the immunoglobulin to the offending antigen. Such binding is sufficient to prevent penetration of tissues by a variety of organisms and to neutralize many toxic substances. In addition, however, after combining with antigens, antibodies can recruit a variety of defense systems such as phagocytosis, cell-mediated killing, and secretion of a variety of potent effector substances.

These two aspects of antibody function, the binding to antigen and the mobilization of defense systems, are carried out by discrete regions of the antibody molecule—the Fab and Fc regions, respectively. An intriguing question has been how these regions interact functionally. For example, does the attachment of antibody to an-

tigen lead to a subtle activation of the Fc region, thereby triggering effector functions? Although still espoused by some, the weight of evidence speaks against such an idea. It appears rather that aggregation of the Fc regions engendered by multiple antibodies binding to the same antigen is both sufficient and necessary (1, 2).

The antigen-combining site has been, until recently, the principal focus for those interested in the analysis of antibodies. The mechanisms by which highly similar proteins can express a rich diversity of combining sites has presented an exciting challenge to geneticists, protein chemists, and crystallographers alike. They have done their job well, and the broad outlines of the complex genetic mechanisms and the protean structures they generate are now defined (3). Because antibodies that bind small or modest-sized ligands can be selected for study, the ability to investigate the relationship between structure and function (binding) has been another attractive aspect of focusing on the antigen-combining sites.

Much less experimental attention had been accorded the Fc regions of the molecule. In part, this resulted from the paucity of information about the molecules that interact with this region, the complement component C1 being the outstanding exception (4). Furthermore, as these Fc receptors began to be identified, they were found to be large proteins and present only in tiny amounts. Both factors present formidable difficulties for obtaining satisfying high-resolution answers to questions about their interactions with Fc regions. Despite these problems, interest in the Fc region of antibodies and the proteins with which it interacts is now increasing. In part there is a growing recognition that such Fc receptors can greatly modulate control of antibody production (5)—a central problem in immunology. In addition, there is a developing appreciation that understanding the activation of cells by immunoglobulins provides an exciting scientific challenge as well as a potential approach to modifying harmful cellular reactions seen in allergy, autoimmunity, and inflammatory disorders.

In this review we focus first on the state of our knowledge about one of these receptors—the receptor with high affinity for immunoglobulin E (IgE). We then broaden the discussion to other molecules that interact with the Fc region of this immunoglobulin. Finally, we close with a wider glimpse at the world of Fc receptors to explain further why these proteins are stimulating renewed interest.

THE RECEPTOR WITH HIGH AFFINITY FOR IgE

Overview

Some 35 million Americans suffer from allergic disorders (6), disorders whose distressing symptoms in large part result from the actions of this receptor. Its role in allergy is as follows: The allergic individual produces an abnormal amount of IgE in response to seemingly innocuous antigens. This IgE then binds to the high-affinity receptor that is present on mast cells and basophils exclusively. The attachment of monomeric IgE via its Fc region to the receptor does not by itself stimulate the cells, but if even a small fraction of the cell-bound IgE reacts with antigen, the cells respond rapidly. They explosively discharge their granular contents (Fig. 1), convert the arachidonic acid side chains on the phospholipids of their plasma membrane into prostaglandins and leukotrienes, and then release these potent mediators. The latter and the granular mediators can account for many of the clinical symptoms of allergy. (In populations subject to parasitic infections, this same reaction likely has a more salutary role by assisting in the expulsion and killing of the invading organisms.)

New developments in three aspects of this system are particularly exciting: 1) the development of techniques that will permit identification of the sites on IgE that interact with the receptor with potential therapeutic implications, 2) increased knowledge about the structure of the receptor and isolation of the genes that code for this protein, and 3) increased knowledge about the complex reactions activated by the receptor.

Interaction of IgE with receptor

The availability of tumors that secrete large amounts of IgE (normally present in microgram or lower concentrations in serum) and, recently, of IgE monoclonal antibodies, as well as of mast cell-like tumor lines has permitted a rigorous analysis of the binding reaction (7). The reaction is consistent with a simple bimolecular forward reaction ($k_1 \cong 1-2 \times 10^5 \text{ M}^{-1} \cdot \text{s}^{-1}$) and a unimolecular back reaction ($k_{-1} \leq 1 \times 10^{-5}$). No cofactors are required and the stoichiometry indicates that one molecule of IgE binds to one molecule of receptor. Although both the receptor (below) and IgE are rich in carbohydrate, there is evidence that these moieties do not significantly influence the binding of IgE to the receptor (7, 8).

A fertile area to explore in developing therapies for allergic diseases is to prevent binding of the IgE to the receptor. Specifically, if one could identify those regions on the IgE that interact with the receptor, one could construct peptides or other molecules that could inhibit the binding. Several approaches are being employed. Inasmuch as crystallographic data on Fc regions exist only for immunoglobulin G (IgG), attempts have been made to model the Fc region of IgE to look for promising sites to investigate. Figure 2 is the model proposed by Padlan and Davies (9) using interactive computer graphics program, the data from a 2.9 Å analysis of human IgG Fc,

and the amino acid sequence deduced from the cloned gene for the human IgE heavy chain. Because IgE contains an extra set of domains compared with IgG, sequence homologies and other plausible assumptions were required to build the model.

Until direct crystallographic data become available, such models must suffice to assess whether potential sites implicated by direct experiment are plausible. One such experimental approach that has become practical is the *in vivo* synthesis of selected peptides of the Fc region by transfection of bacteria with modified cDNAs of the ϵ heavy chain. This has been the approach used by Gould and colleagues (10). The peptides were tested to see if they could block the binding of antigen-specific IgE to intact cells, thereby preventing degranulation when antigen was subsequently added. So far, the smallest active fragment they have discovered contains 76 residues from glutamine-301 in the carboxy-terminal portion of the $C_{\epsilon 2}$ domain to arginine-376 in the amino-terminal portion of the $C_{\epsilon 3}$ domain. These findings are consistent with the results from earlier studies in which we examined what portion of the Fc region was protected from cleavage when it was bound to the receptor. In these and other studies the interface between the $C_{\epsilon 2}$ and $C_{\epsilon 3}$ domains was implicated (7). Others have claimed to have isolated smaller active peptides. In one case this claim has repeatedly failed to be reproduced by others. In the second, more recent study (11) there are some disturbing features that remain to be explained. In particular, one can calculate that only a few percent of the total receptors were examined. Furthermore, although the concentrations of peptide required for inhibition suggested a relatively low affinity, once the peptides were bound the inhibition was not reversed by washing. These findings appear to be inconsistent with each other.

One aspect of this general approach deserves mention. Whereas there are good cell lines for studying the binding of IgE to rodent cells *in vitro*, no adequate human cell lines are available for similarly studying the binding to the human receptors—the system of primary interest. Thus, the studies by Geha et al. (10) had to be performed by skin testing—an assay that is much more cumbersome, potentially hazardous, and less quantitative. The recent success in cloning of the receptor (below) makes it likely that one will soon be able to prepare cells transfected with the human receptor and thereby be able to perform binding studies *in vitro*. Thus, through the use of molecular genetics the tools are now available, or soon will be, to explore rigorously the sites on IgE that interact with the receptor and to develop inhibitors of that reaction.

Transforming such inhibitors into therapeutically useful reagents will present additional problems, but these should not be insurmountable. On the other hand, one can envision certain limitations. The slow dissociation of IgE from its receptor makes it likely that inhibitors of the binding could not displace IgE that is already bound but could only prevent additional molecules from binding. Therefore, such inhibitors can be useful at best in preventing allergic attacks and are unlikely to be effective in aborting them.

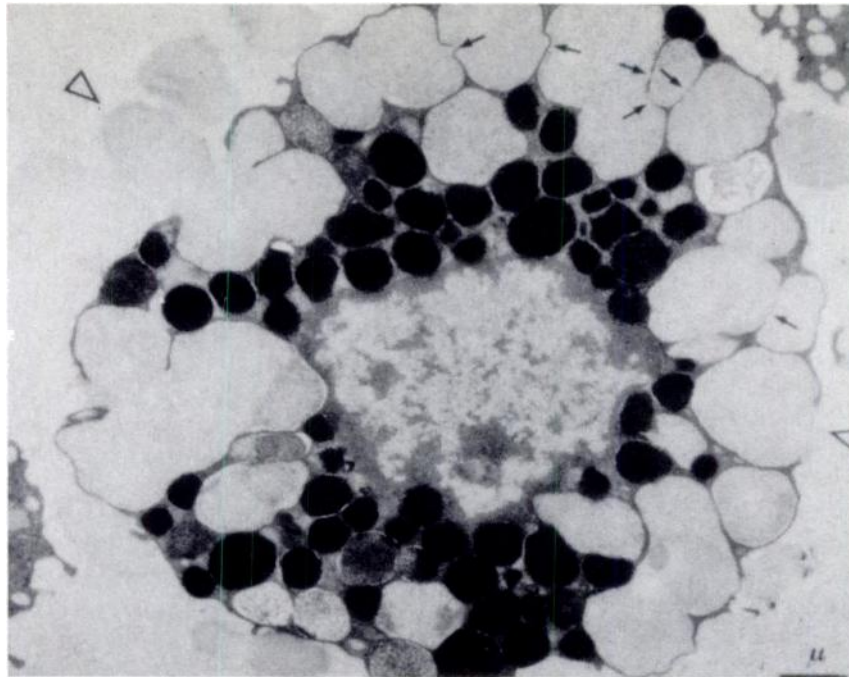


Figure 1. Electron micrograph of a rat peritoneal mast cell that was reacted with ovalbumin-specific IgE and then with ovalbumin. The cell was fixed at 60 s after addition of the antigen. Many of the perigranular membranes surrounding the granules show evidence of fusion or fission (small, filled arrows) and some of the granular contents have already been released (large, open arrows). From ref 6a.

Structure of the receptor

The IgE-binding polypeptide

Because of its high affinity, identification of that portion of the receptor that includes the combining site for IgE was accomplished rather easily. This single polypeptide — now called α — fully accounts for the binding activity. Using the rat basophilic leukemia cell line, one was able to isolate enough of this chain to study its structure in some detail. It was found to contain about 30% by weight of carbohydrate and to have a moderately hydrophobic amino acid composition (7, 12). By a variety of criteria this chain is exposed on the extracellular aspect of the plasma membrane whereas the evidence that it penetrates the lipid bilayer and has a cytoplasmic component was largely negative and at best ambiguous. We and our colleagues recently isolated and sequenced the cDNA that codes for this chain (13), and this has provided substantial new information about the topology of α and other aspects of its structure.

The gene was identified by synthesizing an oligonucleotide probe based on the sequence of a tryptic peptide isolated from a digest of purified α chain. (The amino terminus of the α chain is blocked so that direct sequencing of the intact chain was not possible.) The oligonucleotide was then used to screen a library of cDNA prepared from size-fractionated mRNA derived from the basophilic leukemia cells.

The characteristics of the cDNA are as follows: It predicts an amino acid sequence consistent with a leader sequence that would, after cleavage, leave a peptide of 227 residues. The amino-terminal 180 residues show

seven potential sites for N-glycosylation and therefore probably represent the extracellular portion of α . There are two moderately homologous presumptive cystine-bridged loops that bear some of the hallmarks of the disulfide loops in immunoglobulins. However, the loops in the receptor would be some 20 or more residues shorter than the corresponding ones in immunoglobulins.

This long hydrophilic sequence is followed by a very hydrophobic one of about 20 amino acids, which is suggestive of a transmembrane segment. Notably it contains a single charged residue—an aspartic acid. Finally, a highly basic sequence of 27 amino acids likely represents a cytoplasmic tail.

The sequence predicted by the cDNA gratifyingly conforms to all the known properties of the α chain determined by direct analysis, and conflicts with none of them (13). Further direct evidence that the isolated gene corresponds to the α chain is based on polyclonal antibodies elicited against peptides constructed on the basis of the cDNA sequence. Such antibodies will detect isolated α chains with immunoblot procedures (C. Ra, J.-P. Kinet, J. Kochan, and J. Hakimi, unpublished observations). Although at this writing successful expression has not yet been confirmed, there are several reasons why this may be difficult to demonstrate in the absence of coexpression of the other polypeptides that comprise the receptor (see below).

Preliminary data suggest that the cDNA for the rodent α chain can serve as a useful probe to identify its human analog (J. Kochan and J.-P. Kinet, unpublished data). Once this is accomplished and the human α is expressed, it can obviate the current difficulties in studying the binding of human IgE (see above).

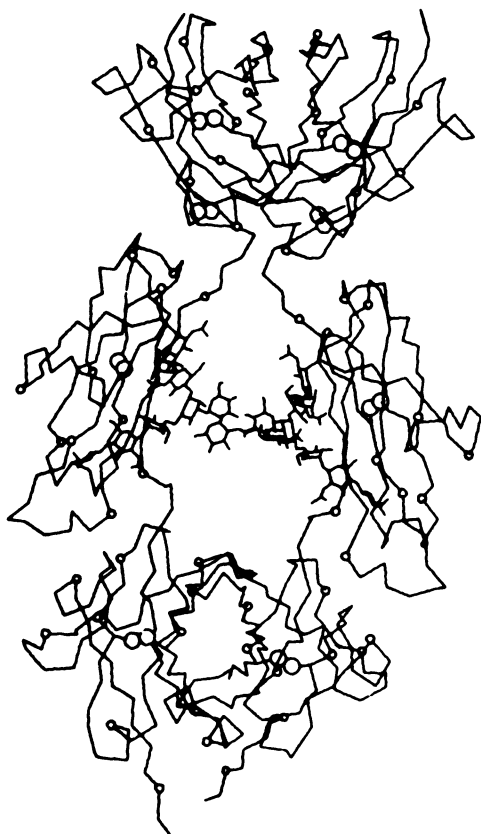


Figure 2. α -Carbon backbone of human IgE Fc. Every 10th residue is indicated by small circles and the disulfide bonds are indicated by the larger circles. The $C_{\epsilon 2}$ domains are at the top, the $C_{\epsilon 3}$ domains are in the middle, and the $C_{\epsilon 4}$ domains are at the bottom. The carbohydrate moieties shown between the two $C_{\epsilon 3}$ domains are as they are found in human IgG Fc. From ref 9.

Unusual instability of the receptor

The α chain was initially thought to comprise the receptor in its entirety. Measurements of the molecular weight of the solubilized receptors in nondenaturing solvents, labeling of the receptor with specific probes, and use of cross-linking reagents first alerted us to the possibility that components were being lost during the affinity purification of the receptor. This was surprising because we had already made a considerable effort to develop a double immunospecific purification procedure that eliminated use of conventional denaturants. We subsequently recognized—and similar observations are now being made in other systems—that the mild detergents commonly used to solubilize membrane proteins may not always be as harmless as they are commonly thought to be. We found that micellar detergent could dissociate the α chains from two other types of polypeptides (β and γ) (14, 15), but that by using appropriate mixtures of detergent and phospholipids throughout the purification, the three types of polypeptide could be copurified. Subsequently we determined that once solubilized, the receptor would remain in solution even in submicellar detergent, and at these low concentrations of the detergent, the receptor is also substantially stabilized.

The instability of the receptor can be put to good use. Thus, employing Triton X-114—a detergent that

separates into discrete phases at room temperature where it forms supramicellar aggregates—we can readily dissociate and separate the α from the β and γ chains for preparative purposes (16). Subsequent analyses have provided considerable structural information about the β and γ chains (Table 1), and good progress is being made on the isolation of the cDNAs that code for these chains. It is reasonable to anticipate that it will soon be possible to cotransfect cells with the cDNAs for all three polypeptides and in this way achieve adequate expression (see above).

What is a subunit of a membrane protein?

An interesting question raised by these findings is whether the β and γ chains should be considered part of the receptor or discrete entities. There are few useful criteria to resolve this dilemma. One expects that subunits of a single protein should have a simple, small-number stoichiometric relationship to each other. In addition, we think it appropriate to determine whether the putative subunits are coordinately synthesized and degraded. This has proved to be the case for the α , β , and γ chains (17). The recent failure to obtain expression (13) now suggests an additional criterion. If the expression of one chain is dependent on the coexpression of others, this is strong evidence that they should be considered as subunits of a single receptor.

Mechanism of action

Role of aggregation

As noted earlier, the simple binding of IgE to the receptor does not stimulate cells to degranulate and causes no known perturbation. Such stimulation occurs only when the receptor-bound IgE interacts with a multivalent antigen. Because the receptor to which the IgE is bound is mobile in the plane of the membrane, such an interaction automatically leads to aggregation of the receptors. This latter step is the essential one as shown by the finding that when receptors are aggregated even in the absence of IgE (e.g., by adding a multivalent antireceptor antibody), the cells are stimulated (18, 19). Therefore, the IgE determines which antigens can activate the cell, but mechanistically plays only a passive role.

TABLE 1. Subunits of the receptor with high affinity for IgE^a

Parameter	α	β	γ
M_r , kDa	37	33	7
No. per receptor	1	1	2
Carbohydrate	30%	0	0
Amino terminus	Blocked	Blocked	Blocked
Amino acid composition	Known ^b	Known ^c	Known ^c
Extracellular domain	180 residues ^b	?	?
Transmembrane segment	20 residues ^b	+	+
Cytoplasmic domain	27 residues ^b	+	+

^aData are for the receptor isolated from rat basophilic leukemia cells. ^bRef 13. ^cRef 12.

Several questions arise concerning the aggregation of the receptor: How many receptor molecules have to be aggregated to initiate a signal? How does the number of signals relate to the cellular response? What is the nature of the signal or signals and what is the role of the receptor in generating them?

Several years ago we attempted to define the minimal unit signal, i.e., the number of receptors that must be aggregated in any single complex to generate a response (20). For this purpose, we used preformed oligomers of IgE, i.e., dimers, trimers, and oligomers of higher multiplicity. We examined which of them would initiate degranulation or, in more recent studies (21), would activate one of the biochemical perturbations that has been implicated in the sequence of events leading to secretion. Under favorable circumstances such studies showed that dimers were sufficient, although higher oligomers stimulated the cells more vigorously. Recent observations by Menon et al. (22) show that the interpretation of such studies is not as straightforward as originally thought. Thus it appears that even small aggregates of receptors can spontaneously aggregate with each other. Therefore, although dimers of IgE, which generate dimers of receptors, are capable of initiating degranulation, the mechanistically important aggregate could be larger. On the other hand, there are considerable data to suggest that only a small fraction of the total available receptors need to be aggregated to obtain a substantial, even maximal, cellular response.

Nature of the signal

A variety of cellular alterations have been implicated as early events initiated by the receptor (Table 2). The evidence for their involvement stems in part from results of experiments employing inhibitors, e.g., the use of protease inhibitors or inhibitors of methyl transfer reactions. In other experiments, increases in incorporation of label into metabolites have been observed, e.g., into labeled inositol phosphates. The nature of the early signals initiated by the receptor is currently hotly debated and analyses of some of the contradictory claims have been published (7, 23). Here we discuss only some general aspects as well as a few selected items—in both cases focusing on questions that remain unresolved.

One of the principal barriers to exploring the functional questions is the lack of suitably simple preparations. Thus essentially all of the published work has been performed on intact cells, making it difficult or impossible to determine whether certain phenomena are direct or indirect consequences of the action of the receptor. Elaborate flow diagrams have been published illustrating a complicated array of biochemical reactions that are postulated to occur in the plasma membrane, but no membrane preparations that exhibit a quantitatively meaningful amount of activity have been reported. Obtaining such preparations to examine rigorously the receptor-mediated events deserves highest priority.

A further lack is the failure to identify other molecules that mediate some of the reported activities. The one possible exception stems from the work by Pecht and his col-

leagues on the so-called cromolyn-binding protein. Elsewhere we have summarized and critically evaluated these studies (7), and in the 2 years since that analysis was written, little further clarification has been published. These workers claim to have isolated a protein whose interaction with the aggregated receptor for IgE leads to the formation of a channel permeable to Ca^{2+} . The work has not yet been published in a manner that can be reproduced by others, and furthermore other workers have been unable to observe such channels with patch-clamp techniques (24, 25). Nevertheless, studies employing ^{45}Ca suggest that influx of Ca^{2+} is a prominent feature of receptor activation (26).

A substantial biochemical signal initiated by the receptor, one that is readily reproduced, is the hydrolysis of phosphoinositides into diacyl glycerol and inositol (poly)phosphate(s). This reaction is currently being explored in depth because there is evidence that it represents one of the earliest perturbations initiated by the receptor (23, 27). By analogy with other systems where inositol phosphates are generated, one anticipates that the receptor should interact with a GTP-binding protein, which in turn activates a phospholipase C (28). Isolation of these components and reconstituting them along with the receptor are the necessary next steps toward understanding this aspect of the receptor's function.

In general, such studies offer an attractive alternative approach to developing a therapy for allergic reactions. Because the receptor is found only on mast cells and basophils and does not appear to serve a critical homeostatic function, interfering with its action should lead to few side effects. Furthermore, by such inhibition one could hope to abort allergic attacks rather than only prevent them (cf. above).

OTHER PROTEINS THAT BIND IgE

Structure

Three other proteins that bind the Fc region of IgE have been identified and their putative cDNA has been cloned (Table 3). Each is distinctive and none resembles the IgE-binding chain of the high-affinity receptor. Liu and colleagues fractionated mRNA from rat basophilic leukemia cells and obtained a fraction that, when injected into frog oocytes, appeared to code for a 31,000-Da protein with IgE-binding capacity. A cDNA was subsequently isolated whose coding sequence predicts a 27,573-Da protein (29). The predicted amino acid sequence is completely different from that predicted by the cDNA we have isolated. Similarly, the composition predicted for the Liu protein is very different from that experimentally determined for any of the subunits of the high-affinity receptor. An amino-terminal domain shows distinctive repetitive sequences of nine amino acids rich in proline, glycine, and alanine. There is no apparent signal sequence and none for a transmembrane segment. The nature of this protein, its tissue distribution, and its functional role are still uncertain.

Three groups independently isolated a cDNA from the human B lymphoblastoid line RPMI-8866 that ap-

TABLE 2. *Early events stimulated by the receptor with high affinity for IgE^a*

Phenomenon	Comment
Morphological changes	Very rapid; molecular basis undefined
Immobilization of receptors	Possibly through interaction with actin
Internalization of receptors	Unlikely to be required for secretion
Activation of serine protease	Based exclusively on studies with inhibitors
Stimulation of lipid methylation	Controversial
Stimulation of adenylate cyclase	Not observed with some cells
Hydrolysis of phosphatidylinositides	Good correlation with aggregation of receptors
Rise in intracellular Ca ²⁺	Mechanism(s) controversial; insufficient alone to stimulate secretion

^aCitations for each of these phenomena are found in ref 7. See also ref 23.

pears to code for a receptor with low affinity for IgE (30-33). The cDNA predicts an amino acid sequence of 321 residues. This protein, like the transferrin receptor and a few other membrane proteins, appears to be

oriented such that the carboxy terminus is on the outside of the cell. This sequence shows considerable homology to chicken hepatic lectin.

A third protein has been identified by Ishizaka and colleagues on T lymphocytes, and a cDNA has been cloned that appears to code for it (33). Its sequence of 556 amino acids is distinct from that of the proteins already referred to and is unrelated to any previously described proteins other than polymerases of several retroviruses. Although the IgE-binding factor it codes for is immunologically related to a cell-bound T lymphocyte receptor, the cDNA so far isolated does not contain an obvious transmembrane segment.

Role in B cell differentiation

The aforementioned B and T lymphocyte proteins that bind IgE (and related ones that similarly bind other immunoglobulins) are of particular interest because of their apparent role in regulating production and secretion of immunoglobulins of the homologous isotype. In turn, the expression of these receptors or binding factors appears to be regulated by the concentration of the homologous isotype in the extracellular fluids. The molecular mechanisms of these fascinating regulatory circuits will be challenging to explore. This suggests a subtle role for the Fc regions of antibodies that has only recently been anticipated (5).

TABLE 3. *Cellular Fc receptors for which cDNA has been isolated*

Protein	Ref	Ligand	Cells	Location of amino terminus	Sequence length		Comment
					Total	Transmembrane domain	
High-affinity receptor, α chain	13	IgE	Mast cell, basophil	External	227	20	Two presumptive disulfide loops homologous to immunoglobulins
IgE-binding	19	IgE	RBL, ? other	No data	262	?	Repetitive sequences rich in proline, alanine, glycine
Low-affinity IgE receptor	30-32	IgE	B lymphocyte, ? other	Internal	321	23	Homology to hepatic lectin
IgE-binding factor	33	IgE	T-lymphocytes	No data	556	?	Homology to retroviral polymerases
Receptor for IgG α	34	IgG	Macrophage	External	231	20	Two presumptive disulfide loops homologous to immunoglobulins
β_1	34	IgG	Macrophage, T lymphocyte	External	300	26	Extracellular portion highly homologous to α
β_2	34	IgG	Macrophage, T lymphocyte	External	254	26	Identical to β_1 except for missing 138 residues in presumed intracellular domain

Fc RECEPTORS FOR IgG

Structure

There is evidence for a wide variety of membrane proteins that bind immunoglobulins other than IgE. The most detailed information is available for two of these: a receptor with moderate affinity for IgG on macrophages (and a related one on T lymphocytes) and a receptor on epithelial cells for polymeric immunoglobulins (see below). Ravetch et al. have isolated three discrete cDNAs from libraries prepared from mouse macrophages and T lymphocytes that code for highly homologous membrane proteins and that function as Fc receptors for IgG (34). One cDNA was derived from an mRNA that is expressed only in macrophages and was dubbed α . Its deduced amino acid sequence suggests a signal peptide, an amino-terminal extracellular domain of 185 residues, a transmembrane segment of 20 residues, and a carboxy-terminal cytoplasmic tail of 26 residues. The extracellular and transmembrane domains (but not the cytoplasmic tail) of this murine protein show substantial homology with the corresponding segments of the IgE-binding chain of the rat high-affinity receptor (see above). There is a 50% overall homology at the nucleotide level ($\sim 30\%$ at the amino acid level) and even greater homology in selected areas such as in the amino terminus of the extracellular domain and in the transmembrane portion. The latter contains an aspartic acid residue and is surrounded by eight amino acids that are identical to those in the corresponding domain of the IgE-binding sequence. Like the IgE-binding chain, there are two presumptive shortened disulfide loops in the extracellular domain and these are moderately homologous to immunoglobulin sequences. The two related sequences β_1 and β_2 (see also refs 35 and 36) are found in T lymphocytes and macrophages. They have identical extracellular domains that are 95% homologous over a 170-residue stretch with the α sequence. However, their identical transmembrane segments and related cytoplasmic tails are totally different from the analogous portions of α .

It is noteworthy that although it has been possible to express the β_2 gene, this has not been possible with the α gene. Perhaps the same factors that have so far precluded expression of the IgE-binding subunit of the high-affinity receptor may account for the failure to express the α gene of the IgG receptor. Conceivably the aspartic acid residue, in the transmembrane segment, must be neutralized by a positive charge in the transmembrane segment on one of the known subunits of the IgE receptor, or on an as-yet-unidentified subunit of the IgG receptor.

Fernandez-Botran and Suzuki (37) have attempted to characterize the Fc receptors on the same line of T lymphocytes used by Ravetch et al. (34), as well as on the P388D₁ murine macrophage-like line. Their preparations seem heavily contaminated (figure 2 in ref 37). The amino acid composition of the protein they characterized (38), whose peptide molecular weight most closely equals that predicted for the protein coded by β_1 , bears no relationship to the latter. Furthermore, Ravetch et al. failed to find a β_1 mRNA in the P388D₁ line (34).

Function of IgG receptors

The functional role of the proteins encoded by β_1 and β_2 on lymphocytes is uncertain. Possibly they represent the Fc receptors (and generate the IgG-binding factors) that regulate IgG production as has been described for the IgE-binding proteins on B and T cells (above). However, it is curious that there is no corresponding structural resemblance to the lymphocyte IgE-binding proteins.

The functional role of the β and α proteins in macrophages is also not clear although here they likely stimulate phagocytosis, lysosomal enzyme release, and arachidonic acid metabolism. Macrophages have previously been shown to express three receptors for IgG that have distinctive isotypic specificity. How these relate to α , β_1 , and β_2 has not yet been clarified. Although the receptor that is specific for IgG₁ and IgG_{2b} reacts with a widely used monoclonal antibody called 2.4G2 and this same antibody reacts with transfected β_2 (and presumably with β_1 , because the latter has an identical extracellular portion), further studies are needed. Young et al. (39) incorporated isolated receptors from macrophages into liposomes. When immune complexes were added, they observed the opening of Na⁺ channels (39). No further studies of this phenomenon have appeared and its functional significance is uncertain. Fernandez-Botran and Suzuki originally proposed that their preparations had intrinsic phospholipase A₂ activity although more recent publications refer to this activity as merely associated (38). The availability of the genes that presumptively code for these proteins may now allow a more rigorous assessment of these phenomena.

Fc RECEPTORS THAT TRANSPORT ANTIBODIES

So far, all of the Fc receptors we have considered appear to participate in either the regulation of antibody synthesis or in the recruitment of effector systems during an immune response. An additional family of Fc receptors appears to function as part of the normal biosynthetic pathway by which antibodies are delivered to where they can fulfill their intended activity. The best characterized is a receptor found at the basolateral membrane of epithelial cells that transports polymeric immunoglobulins (immunoglobulins A and M) through the cell and across the apical membrane (40). There a protease cleaves the receptor so that the noncovalently or covalently bound immunoglobulin is released with an attached fragment of the receptor, the secretory component. Like the IgE-binding chain of the high-affinity receptor and the IgG-binding chain of the IgG receptors, the receptor for poly Ig has repeating immunoglobulin-like domains. However, all five are of standard length and are not truncated like those in the aforementioned receptors for IgG and IgE (above). There is no evidence that these receptors are activated when interacting with the antibody. There are functionally related receptors for transporting IgG across various epithelial cells but molecular analysis of these is still in its infancy. The molecular mechanisms employed by the cells to direct the receptor and subsequently the receptor-Ig adduct to the appropriate membranes are of great interest.

CONCLUDING REMARKS

Molecular genetic techniques now permit rigorous comparative studies of the family of proteins that share the property of binding to the Fc region of immunoglobulins. It is already clear that this is a polyglot family whose diverse functional language is mediated by a correspondingly diverse set of structures. All except those whose principal function is transcellular transport require activation. Where available, the evidence suggests that this stimulation is engendered by aggregation of the receptor. It might be supposed that this common requirement would reflect some common structural motif in either the transmembrane or cytoplasmic domains, but such a pattern has not emerged. It is possible that the diversity of these domains reflects the diversity of other cellular proteins that are activated by the different receptors. An interesting alternative explanation is that the transduction elements of these receptors have not yet been identified. The only Fc receptor that has been demonstrated to consist of multiple subunits is the receptor with high affinity for IgE. The non-Ig-binding subunits of this receptor initially eluded detection because they dissociated during purification. It is possible that some of the other proteins described here are likewise only the ligand-binding part of the receptor and that the mechanistically important components remain to be discovered. An analogy may be drawn with the only protein whose activation by binding to antibody aggregates is relatively well understood: the C1 protein of the classical complement pathway referred to in the introduction (4). This oligomer of 22 polypeptide chains may presage the complexity with which we may ultimately have to cope in order to understand the world of Fc receptors.

One of the hallmarks of quantum progress in a field is when it becomes possible to analyze the important molecules and not just phenomena. The field of Fc receptors is now at this stage. Using the molecular knowledge to explain the phenomena will be the next step. FJ

REFERENCES

1. METZGER, H. The effect of antigen on antibodies: recent studies. *Contemp. Top. Mol. Immunol.* 7: 119-152; 1978.
2. METZGER, H. Antibodies as effector molecules. *Harvey Lect.* 80: 49-66; 1986.
3. YANCOPOULOS, G. D.; ALT, F. W. Regulation of the assembly and expression of variable region genes. *Annu. Rev. Immunol.* 4: 339-368; 1986.
4. SCHUMAKER, V. N.; ZAVODSKY, P.; POON, P. H. Activation of the first complement. *Annu. Rev. Immunol.* 5: 21-42; 1987.
5. KÖLSCH, E.; OBERBARNSCHEIDT, J.; BRÜNER, K.; HEUER, J. The Fc receptor: its role in the transmission of differentiation signals. *Immunol. Rev.* 49: 61-78; 1980.
6. KAPLAN, A. P. *Allergy*, New York: Churchill Livingstone; 1985.
- 6a. UVNÄS, B. Correlation between morphological and biochemical events on antigen-induced histamine release from mast cells. Goodfriend, L.; Schon, A. H.; Orange, R. P., eds. *Mechanisms in allergy*. New York: Dekker; 1973: 369-383.
7. METZGER, H.; ALCARAZ, G.; HOHMAN, R.; KINET, J.-P.; PRIBLUDA, V.; QUARTO, R. The receptor with high affinity for immunoglobulin E. *Annu. Rev. Immunol.* 4: 419-470; 1986.
8. GRANATO, D. A.; NEESER, J.-R. Effect of trimming inhibitors on the secretion and biological activity of a murine IgE monoclonal antibody. *Mol. Immunol.* 24: 849-855; 1987.
9. PADLAN, E. A.; DAVIES, D. R. A model of the Fc immunoglobulin E. *Mol. Immunol.* 23: 1063-1075; 1986.
10. GEHA, R. S.; HELM, B.; WOOD, N.; MARSH, P.; GOULD, H. IgE sites relevant for binding to type 1 Fc epsilon (FCER) receptors on mast cells. *J. Allergy Clin. Immunol.* 79: 129; 1987 (abstr.).
11. BURT, D. A.; STANWORTH, D. R. Inhibition of binding of rat IgE to rat mast cells by synthetic IgE peptides. *Eur. J. Immunol.* 17: 437-440; 1987.
12. ALCARAZ, G.; KINET, J.-P.; LIU, T.-Y.; METZGER, H. Further characterization of the receptor with high affinity for immunoglobulin E. *Biochemistry* 26: 2569-2575; 1987.
13. KINET, J.-P.; METZGER, H.; HAKIMI, J.; KOCHAN, J. A cDNA presumptively coding for the α subunit of the receptor with high affinity for immunoglobulin E. *Biochemistry* 26: 4605-4610; 1987.
14. RIVNAY, B.; WANK, S. A.; METZGER, H. Phospholipids stabilize the interaction between the alpha and beta subunits of the solubilized receptor for immunoglobulin E. *Biochemistry* 21: 6922-6927; 1982.
15. KINET, J.-P.; ALCARAZ, G.; LEONARD, A.; WANK, S.; METZGER, H. Dissociation of the receptor for immunoglobulin E in mild detergents. *Biochemistry* 24: 4117-4124; 1985.
16. ALCARAZ, G.; KINET, J.-P.; KUMAR, N.; WANK, S. A.; METZGER, H. Phase separation of the receptor for immunoglobulin E and its subunits in Triton X-114. *J. Biol. Chem.* 259: 14922-14927; 1984.
17. QUARTO, R.; KINET, J.-P.; METZGER, H. Coordinate synthesis and degradation of the α -, β - and γ -subunits of the receptor for immunoglobulin E. *Mol. Immunol.* 22: 1045-1051; 1985.
18. ISHIZAKA, T.; ISHIZAKA, K. Triggering of histamine release from rat mast cells by divalent antibodies against IgE-receptors. *J. Immunol.* 120: 800-805; 1978.
19. ISERSKY, C.; TAUROG, J. D.; POY, G.; METZGER, H. Triggering of cultured mastocytoma cells by antibodies to the receptor for IgE. *J. Immunol.* 121: 549-558; 1978.
20. SEGAL, D. M.; TAUROG, J. D.; METZGER, H. Dimeric immunoglobulin E serves as a unit signal for mast cell degranulation. *Proc. Natl. Acad. Sci. USA* 74: 2993-2997; 1977.
21. MAEYAMA, K.; HOHMAN, R. J.; METZGER, H.; BEAVEN, M. Quantitative relationships between aggregation of IgE receptors, generation of intracellular signals, and histamine secretion in rat basophilic leukemia (2H3) cells. Enhanced responses with heavy water. *J. Biol. Chem.* 261: 2583-2592; 1986.
22. MENON, A. K.; HOLOWKA, D.; BAIRD, B. Small oligomers of immunoglobulin E (IgE) cause large-scale clustering of IgE receptors on the surfaces of rat basophilic leukemia cells. *J. Cell Biol.* 98: 577-583; 1984.
23. BEAVEN, M. A.; CUNHA-MELO, J. R. Membrane phosphoinositide-activated signals in mast cells and basophils. *Prog. Allergy* In press.
24. LINDAU, J.; FERNANDEZ, J. M. IgE mediated degranulation of mast cells does not require the opening of ion channels. *Nature (London)* 319: 150-153; 1986.
25. NEHER, E.; ALMERS, W. Fast calcium transients in rat peritoneal mast cells are not sufficient to trigger exocytosis. *EMBO J.* 5: 51-53; 1986.
26. FEWTRELL, C.; SHERMAN, E. The IgE receptor-activated

- calcium permeability pathway in rat basophilic leukemia (2H3) cells. *Biochemistry* 26: 6995-7003; 1987.
27. PRIBLUDA, V.; METZGER, H. Ca-independent phosphoinositide breakdown in rat basophilic leukemia cells. *J. Biol. Chem.* 262: 11449-11454; 1987.
 28. GILMAN, A. R. Transducers of receptor-generated signals. *Annu. Rev. Biochem.* 56: 615-649; 1987.
 29. ALBRANDT, K.; ORIDA, N. K.; LIU, F.-T. An IgE-binding protein with a distinctive repetitive sequence and homology with an IgG receptor. *Proc. Natl. Acad. Sci. USA* 85: 6859-6863; 1987.
 30. KIKUTANI, H.; INUJ, S.; SATO, R.; BARSUMIAN, E. L.; OWAKI, H.; KYAMASAKI, K.; KAISHO, T.; UCHIBAYASHI, N.; HARDY, R. R.; HIRANO, T.; TSUNASAWA, S.; SAKIYAMA, F.; SUEMURA, M.; KISHIMOTO, T. Molecular structure of human lymphocyte receptor for immunoglobulin E. *Cell* 47: 657-665; 1986.
 31. IKUTA, K.; TAKAMI, M.; KIM, C. W.; HONJO, T.; MIYOSHI, T.; TAGAYA, Y.; KAWABE, T.; YODOI, J. Human lymphocyte Fc receptor for IgE: sequence homology of its cloned cDNA with animal lectins. *Proc. Natl. Acad. Sci. USA* 84: 819-823; 1987.
 32. LUDIN, C.; HOFSTETTER, H.; SARFATI, M.; LEVY, C. A.; SUTER, U.; ALAIMO, E.; KICHHER, E.; FROST, H.; DELASPESSE, G. Cloning and expression of the cDNA coding for a human lymphocyte IgE receptor. *EMBO J.* 6: 109-114; 1987.
 33. MARTENS, C. L.; HUFF, T. F.; JARDIEU, P.; TROUNSTINE, M. L.; COFFMAN, R. L.; ISHIZAKA, K.; MOORE, K. W. cDNA clones encoding IgE-binding factors from a rat-mouse T-cell hybridoma. *Proc. Natl. Acad. Sci. USA* 82: 2460-2464; 1985.
 34. RAVETCH, J. V.; LUSTER, A. D.; WEINSHANK, R.; KOCHAN, J.; PAVLOVEC, A.; PORTNOY, D. A.; HULMES, J.; PAN, Y.-C. E.; UNKELESS, J. C. Structural heterogeneity and functional domains of murine immunoglobulin G Fc receptors. *Science* 234: 718-725; 1986.
 35. LEWIS, V. A.; KOCH, T.; PLUTNER, H.; MELLMAN, I. A complementary DNA clone for a macrophage-lymphocyte Fc receptor. *Nature (London)* 324: 372-375; 1986.
 36. HIBBS, M. L.; WALKER, I. D.; KIRSZBAUM, L.; PIETERSZ, G. A.; DEACON, N. J.; CHAMBERS, G. W.; MCKENZIE, I. F. C.; HOGARTH, P. M. The murine Fc receptor for immunoglobulin: purification, partial amino acid sequence, and isolation of cDNA clones. *Proc. Natl. Acad. Sci. USA* 83: 6980-6984; 1986.
 37. FERNANDEZ-BOTRAN, R.; SUZUKI, T. Biochemical signal transmitted by Fc receptor for immunoglobulin G_{2a} of a mouse macrophage-like cell line, P388D1: mode of activation of adenylate cyclase mediated by immunoglobulin G_{2a} binding proteins. *Biochemistry* 25: 4388-4397; 1986.
 38. FERNANDEZ-BOTRAN, R.; SUZUKI, T. Biochemical properties of phosphatidylcholine-binding proteins that share common antigenic determinants with the Fc γ_{2b} receptor. *Biochemistry* 24: 1896-1903; 1985.
 39. YOUNG, J. D. E.; UNKELESS, J. C.; KABACK, H. R.; COHN, Z. A. Mouse macrophage-Fc receptor for IgG γ 2b/ γ 1 in artificial and plasma membrane vesicles functions as a ligand-dependent ionophore. *Proc. Natl. Acad. Sci. USA* 80: 1636-1640; 1983.
 40. UNDERDOWN, B. J.; SCHIFF, J. M. Immunoglobulin A: strategic defense initiative at the mucosal surface. *Annu. Rev. Immunol.* 4: 389-417; 1986.