

Antibody–Cell Interactions: Fc Receptors

Minireview

Jean-Pierre Kinet

National Institute of Arthritis and Musculoskeletal
and Skin Diseases
National Institutes of Health
Bethesda, Maryland 20892

Antibodies are involved in two main functions: the binding to antigen via the antigen-combining site and the mobilization of defense mechanisms via another part of the molecule, the Fc region. The attachment of the Fc region to receptors on many cells of the immune system triggers various functions such as phagocytosis, antibody-dependent cytotoxicity, and the secretion of potent mediators. Because the Fc receptors (FcRs) mediate these essential functions of the immune defense system, understanding their structure and function is a central problem in immunology. Such understanding may also help in designing new therapeutic approaches to allergy and autoimmunity, disorders in which these receptors are involved.

In this minireview I discuss the new information about the structures of FcRs that has been obtained by molecular cloning. It is becoming apparent that with one exception (FcεRI) the FcRs belong to the immunoglobulin (Ig) superfamily, so that both the antibodies and their receptors are members of the same general family. However, the structural diversity of these receptors is greater than initially expected. The analysis of homologous receptors from different species is now possible and may help to identify those portions of the molecule that are functionally important. Special emphasis is given here to the most complex of FcRs: the receptor with high affinity for IgE. One class of Fc receptors, the Ig transporters, is not discussed because of space limitations and because their mechanism of action is presumably quite different.

Fc Receptors for IgG

Distinct forms of FcγRs have been identified both in mice and in humans: a receptor (FcγRI) with high affinity for monomeric IgG, and other receptors (FcγRII and FcγRIII) that have only low affinity for monomeric IgG but that can effectively bind immune complexes by multiple receptor–ligand interactions (reviewed in Unkeless et al., 1988).

Several mouse and human FcγRs have been cloned. All belong to the Ig superfamily and with one exception (FcγRIII-PIG; see below) are integral membrane proteins with a leader peptide, an extracellular portion that is glycosylated, a single transmembrane segment, and a carboxy-terminal cytoplasmic “tail” (see figure). The amino-terminal extracellular portion contains two or three Ig-related domains that show significant homology with the C₂ set of Ig domains as defined by A. Williams. These domains are formed by disulfide loops of variable length (42–44 residues) that are too short to allow confident prediction of their folding relative to a typical Ig domain.

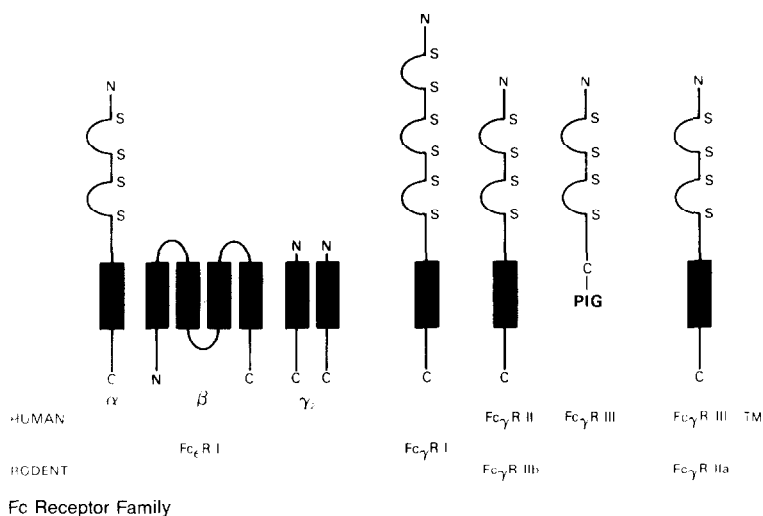
Despite being members of the same family, FcγRs exhibit rich structural diversity. In the mouse, FcγRII is encoded by two genes: a and b. Because of alternative

splicing the latter is expressed in two forms (b1 and b2; Ravetch et al., 1986). (I use Latin rather than Greek letters for these genes to avoid confusion with possible subunits associated with these receptors [see below].) In humans, one or two genes for FcγRI and a minimum of two genes for FcγRII (CDw32) have been identified. The FcγRIII (CD16) polypeptide was found to be anchored in the membrane of neutrophils through a phosphatidylinositol glycan linkage (FcγRIII-PIG; Simmons and Seed, 1988). This receptor is deficient on neutrophils in patients with paroxysmal nocturnal hemoglobinuria, an acquired disorder affecting PIG tail attachment. The deficiency of this receptor may be responsible for the susceptibility to infections associated with this disease (Selvaraj et al., 1988; Huizinga et al., 1988). Most recently, a transmembrane form of that receptor (FcγRIII-TM) was found in natural killer cells (NK cells; Scallon et al., 1989). The cDNAs corresponding to the two FcγRIII receptors differed by single nucleotide substitutions. One of these changes converts the termination codon UGA (seen in FcγRIII-PIG) to CGA, thereby extending the predicted cytoplasmic domain by 21 amino acids. This extension yields a transmembrane receptor, probably by preventing a specific protease from generating a PIG-anchored receptor.

A comparison of the predicted amino acid sequences suggests that the processed FcγRs from different species can be classified into three groups: Group I would contain human FcγRI (Allen and Seed, 1989) (the cloning of a corresponding mouse receptor has not yet been reported). Group II would contain the several forms of human FcγRII (CDw32) (Stuart et al., 1987; Hibbs et al., 1988; Stengelin et al., 1988) and mouse FcγRIIb. Group III would contain human FcγRIII-TM and mouse FcγRIIIa. Support for this classification comes from the finding that the two receptors classified as group III are the only ones expressed in human and mouse natural killer (NK) cells.

Within a group (i.e., between species), the receptors show strong homology in their extracellular portions, although not more than between groups. In addition, within a group the transmembrane segments show high homology (50%–76% identical residues), but between groups the sequence homology is very low (0%–33% identical residues). The cytoplasmic tails show unexpectedly low homology within a group (16%–27%) and virtually none between groups; one exception is a form of human FcγRII that, according to Ravetch, shows 60% identity with mouse FcγRIIb.

This pattern leads me to suggest the following hypothesis: In group III (and in FcεRI α subunit; see below) the sequences of the cytoplasmic tails may be relatively nonconstrained because they serve some generic function that can be satisfied by a variety of sequences. Correspondingly, the conserved cytoplasmic tails in group II could be functionally relevant to signal transduction. In addition, the transmembrane segments, which are the most highly conserved between species, may serve some specific



function—either by themselves or through interaction with other proteins yet to be defined. That different groups of FcγRs have different transmembrane segments would then mean that they mediate different functions. An attractive possibility is that the homologous transmembrane domains associate with homologous subunits. It is possible that subunits belonging to FcγRs have been missed so far because they dissociate during conventional isolation, as observed for the subunits of the high-affinity receptor for IgE (see below). The detection of a polypeptide equivalent to the γ subunit of FcεRI in macrophages—cells that do not express these receptors—supports this view (Ra et al., 1989).

FcγRs are found in numerous hematopoietic cells: monocytes, macrophages, all types of granulocytes, platelets, B and T lymphocytes, and NK cells. They are involved in many important immunological functions including phagocytosis, clearing of immune complexes, antibody-dependent cytotoxicity, secretion of mediators of inflammation, and the regulation of antibody production. Soluble forms of FcγRs have also been described that may be involved in the regulation of IgG production, but the structural relation of these factors to other FcγRs remains to be defined. Northern blot analysis now permits a precise assessment of the distribution of the different forms of FcγRs, and may help to define the roles of individual receptors. For example, because NK cells mediate antibody-dependent cytotoxicity and seem to express exclusively the group III receptors, one can relate these receptors with that particular function.

The genes for FcγRII and for the α subunit of FcεRI have been localized to the distal end of mouse chromosome 1 (Huppi et al., 1988). This implies a common evolutionary origin. Possibly duplication has occurred recently, preventing dispersion of these genes throughout the genome. Alternatively, selective pressures may maintain these genes within close proximity. The linkage of these genes with the gene for the unrelated γ subunit associated with the α subunit of FcεRI, and possibly with

other FcRs, may be relevant. More studies about the genomic organization of these genes, the exact distance between them, and their regulation and cellular expression will be necessary to resolve this question, and may clarify the functional significance of the structural heterogeneity of these receptors.

The High-Affinity Receptor for IgE (FcεRI)

The high-affinity receptor for IgE plays a central role in allergy. The allergic individual produces an abnormal amount of IgE in response to specific antigens, and this IgE is bound by high-affinity receptors that are found exclusively on the surfaces of mast cells and basophils. This attachment of monomeric IgE via its Fc region does not in itself stimulate the cells. However, when specific allergens react with the receptor-bound IgE, the cells explosively discharge their granular contents, synthesize leukotrienes, and release potent mediators responsible for many of the symptoms of allergic diseases.

FcεRI is a tetrameric complex of one IgE binding α subunit, one β subunit, and two disulfide-linked γ subunits (figure). The noncovalent association between the subunits is sensitive to mild detergent. However, the tetrameric structure of the receptor remains substantially stable when protective phospholipid or submicellar concentrations of detergent are used during purification. The sensitivity to detergent observed with this receptor may be applicable to other receptors and in particular to FcγRs.

We recently reported the complete structure of FcεRI and proposed a model for the tetrameric receptor αβγ₂ that posits seven transmembrane domains (Blank et al., 1989). The cDNAs for the rat and human α subunit (Kinet et al., 1987; Shimizu et al., 1988) encode a polypeptide that is structurally similar to the FcγRs described above. The extracellular portion contains two Ig-related domains that are homologous to each other but, interestingly, are more homologous to the corresponding domains of FcγRs, suggesting a common ancestor. The transmembrane domains of the α subunit of FcεRI and of the group III FcγRs contain eight consecutive residues that are iden-

tical, including a single aspartic acid. This may suggest a functional relationship between these receptors. The extracellular and transmembrane domains of the rat and human α subunits are clearly conserved, containing 49% and 67% identical residues, respectively. By contrast, the intracytoplasmic tails diverge substantially, both in the sequence of residues (only 23% identity) and in length (20 residues in rat α and 31 residues in human α).

The predicted amino acid sequence for the β subunit shows no leader peptide, and hydropathicity plots predict four transmembrane domains (Kinet et al., 1988). This would require both the amino and carboxyl termini to be on the same side of the membrane. Studies with monoclonal antibodies have demonstrated that both termini of the β subunit are in fact cytoplasmic.

From the cDNA sequence for the γ subunit, we recently detected a substantial homology between the γ subunit and the ζ chain of the T cell receptor (Miller et al., 1989): 59% identity between the first 29 residues of the rat γ subunit and the corresponding residues from the mouse ζ chain, including a cysteine residue located at the cell surface. There are other striking similarities: both exhibit short extracellular segments (5 and 8 residues), single transmembrane domains with a common aspartic acid, and long intracytoplasmic tails (42 and 113 residues), and they form disulfide-linked dimers. It will be interesting to see if a functional relationship is found between these two subunits.

Efficient expression of the rodent Fc ϵ RI receptor on the surface of COS cells was only achieved after cotransfection of the cDNA for all three subunits, and such transfectants exhibited the appropriate subunit composition and binding properties. These successful cotransfections suggest that only tetrameric receptors can reach the plasma membrane. However, the human α subunit was successfully expressed on transfected cells not only in the presence of rodent β and γ subunits but also in the presence of the γ subunit alone without apparent need for β (Miller et al., 1989). When analyzed in detail, this unexpected finding may provide further insight into how the subunits interact with each other.

So far, the high-affinity receptor has only been found on mast cells and basophils. Analysis by Northern blotting of a variety of cell lines confirmed the exclusive expression of α and β , but not the γ subunit, in mast cells, basophils, and related cell lines.

The Low-Affinity Receptor for IgE (Fc ϵ RII)

A receptor with low affinity for IgE (Fc ϵ RII or CD23) is found on B and T lymphocytes, monocytes, and eosinophils. Three groups isolated cDNA clones that code for this receptor from the human lymphoblastoid line RPMI-8866 (Kikutani et al., 1986; Lüdin et al., 1987; Ikuta et al., 1987). The encoded polypeptide contains 321 residues with no leader peptide and a single putative transmembrane domain. Like the transferrin receptor, the carboxyl terminus of this receptor is oriented on the outside of the cell. This receptor is substantially homologous to the human asialoglycoprotein receptor and to other animal lectins but shares no homology with other IgE binding proteins or other Fc receptors, particularly Fc ϵ RI. This is so

far the only Fc receptor whose Ig binding portion does not belong to the Ig superfamily and to the related Fc receptor family.

Recently an additional form, Fc ϵ RIIb, was isolated that differs from Fc ϵ RIIa only at seven residues in the amino-terminal cytoplasmic region but that shares identical carboxy-terminal extracellular domains (Yokota et al., 1988). These two forms are the apparent result of alternative RNA splicing and transcriptional initiation at different sites. The distribution of the two forms is different: Fc ϵ RIIa is expressed in resting B cells, whereas Fc ϵ RIIb is found on a variety of cells such as B cells, monocytes, and eosinophils, following interleukin-4 induction. Fc ϵ RIIb is also expressed on peripheral blood lymphocytes in atopic individuals, possibly because of abnormal regulation of interleukin-4 production.

The role of Fc ϵ RII in host defense mechanisms, particularly against IgE-inducing parasites, has been suggested because of its presence on the surfaces of monocytes and eosinophils. In addition, Fc ϵ RII has been thought to be involved in the regulation of growth and differentiation of B cells and also in the regulation of IgE synthesis, possibly via a soluble fragment released from the receptor (IgE binding factor). How the two forms of Fc ϵ RII relate to the proposed functions of the receptor remains to be established.

Mechanisms of Action of Fc Receptors

How does the Fc region of antibodies recruit a variety of cellular defense mechanisms? It is unlikely that attachment of antigen to antibody leads to some subtle allosteric activation of the Fc region that triggers effector functions. Rather, it appears that aggregation of antibodies by multivalent antigens induces aggregation of Fc receptors, which in turn is the critical signal for cellular activation. Many biochemical signals initiated by Fc receptors have been reported, including the activation of different phospholipases, sodium and calcium channels, and possibly serine proteases. Until these activities are mapped definitively onto appropriate molecules, it will be difficult to assess the degree of involvement of Fc receptors in triggering them. One group of investigators has reported isolation of channels that coaggregate with Fc ϵ RI upon stimulation by antigen to produce an influx of Ca²⁺ (Hemmerich and Pecht, 1988). When the cloning of these channels is accomplished, it will be interesting to see if cotransfection of Fc ϵ RI and of the channel will reproduce the phenomenon observed in artificial membrane bilayers.

Conclusions

Comparison of homologous Fc receptors from different species shows that the transmembrane domains exhibit substantial conservation and share long stretches of identical residues. This suggests that the transmembrane domains are functionally important. Upon aggregation of receptors, these domains could send an intracellular signal or, alternatively, could interact with transducing elements that have not yet been identified. It is striking that of the FcRs diagrammed in the figure, Fc ϵ RI is so far the only multisubunit receptor. Why should receptors whose mechanism of activation by aggregation and activities are so similar be structurally so different? Will molecules such

as the β and γ subunits from Fc ϵ RI be found to be associated with other Fc receptors? If so, the most interesting aspect of this association will be its functional implications.

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