Human Inflammatory Disease

Clinical Immunology Volume 1

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HUMAN INFLAMMATORY DISEASE

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PREFACE

In September 1982, two of us (GM and LML) were crossing the Straits of Messina on our way to Taormina. We had just attended an international meeting and were commenting upon the lack of creativity and real communication that characterizes so many of these meetings. By the time we had landed in Sicily, we had sketched the outlines of a new format for a meeting that would cover a variety of fields and that would be useful to scientists working in basic research as well as to clinically oriented scientists. It took four years for these ideas to materialize into the practical experiment that was the First Capri Conference on Clinical Immunology.

The present volume contains the lectures delivered at the conference that was held in Capri June 15 to 19, 1986. The discussions that followed each lecture were an integral part of the meeting and have been included in this volume. The meeting covered four aspects of clinical immunology, a field which continues to grow exponentially. The conference was opened by the Italian Minister of Health, Costante Degan, and started with a symposium on the immunology of pulmonary diseases. This section covered a wide spectrum of topics, ranging from biochemical studies of IgE receptors to the clinical aspects of allergic diseases. The session ended in Mario Condorelli's villa that overlooks the Faraglioni and the Marina Piccola. Paola Condorelli gave a splendid example of Italian hospitality that will be difficult to forget.

The second symposium covered what is now a rapidly emerging field—cardiac immunology. Practically all the experts in this area attended the session, and at the conclusion, the importance and the potential of cardiac immunology were clear to all.

Adenosine receptors have recently been found in the central nervous system, on inflammatory cells, in cardiac tissues, and in a variety of other tissues. The relevance of adenosine metabolism and purine receptors in many clinical situations was discussed in the third symposium.

The last symposium was dedicated to basic immunologic and biochemical mechanisms of activation and control of inflammatory cells that play a fundamental role in a variety of rheumatic disorders.

The pathophysiologic aspects of many immunologic diseases, such as asthma, acute respiratory distress syndrome, ischemic heart disease, myocardial infarction, vasculitis, myocarditis, congenital and acquired immunodeficiencies, and autoimmune and rheumatic disorders are discussed in different chapters of this book.

According to the premise, a major theme of the conference was the interaction between basic and clinically oriented scientists from different backgrounds. Immunologists, clinical immunologists, cardiologists, biochemists, pulmonologists, critical care physicians, rheumatologists, and pharmacologists presented overviews of their subjects as well as their most recent data.

The interaction between specialists from different disciplines in a pleasant, relaxing environment proved to be extremely fruitful both for the speakers and for those who attended. The original format of this meeting won the approval of the participants, and we consider the First Capri Conference on Clinical Immunology a successful experiment that should be repeated.

The vigor of the discussion with regard to areas of progress and uncertainty reflects the excellence of the presentations and the deft astuteness of the session chairmen. It is hoped that this volume will reflect the intellectual excitement that was generated at the conference.

We should like to thank our sponsors, Bayropharm Italiana, Cilag, Italfarmaco, Sandoz, and Sigma-Tau, for making the First Capri Conference on Clinical Immunology possible.

We gratefully express our appreciation to Doctors Massimo Triggiani and Raffaele Cirillo, who dedicated many hours of their time to the preparation of this meeting. In addition, we should like to express our special appreciation to Anna Rita Bonamici and Gabriella Agatensi (O.I.C., Florence) for the highly efficient and dedicated manner in which they organized the conference. Jean Gilder deserves a special mention for the untiring and skillful manner in which she guided all the editorial aspects of production of this volume.

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BIOCHEMICAL ASPECTS OF RHEUMATIC DISEASE

1 IgE Receptors

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The main characteristic of immunoglobulin E (IgE) is its capacity to bind a variety of serum and cellular components (e.g., IgE binding factors, IgE receptors). Table 1-1 presents a list of some of these IgE binding molecules.

In this review we shall dwell mainly on the high affinity IgE receptor present on the surface of peripheral blood basophils, tissue mast cells, and related tumor cells, which is able to bind with high affinity and specificity the Fc region of monomeric IgE ($Fc_\epsilon R$).

The binding of IgE to the $Fc_\epsilon R$ apparently does not cause any known cellular perturbation, even when all receptors are saturated; however, bridging of receptor bound IgE, in the presence of calcium, triggers cell degranulation and release of the contents of basophilic intracellular granules. In addition to preformed mediators, newly synthesized molecules (such as a variety of metabolites of arachidonic acid) are released by activated cells.

In the last few years the IgE receptor system has been extensively investigated for several reasons. First, the elucidation of the biochemical pathways leading to the release of chemical mediators would be of great importance in understanding the mechanisms of allergic and inflammatory reactions and in providing a firmer basis for therapeutic intervention.

Second, the IgE receptor system represents the simplest and most attractive model for the study of antigen induced, antibody mediated transmembrane signaling and cellular reactions, since cell triggering does not require other macromolecules, other than antigen and antibody. Thus, the interest in this system goes far beyond the fields of allergology and clinical immunology, as has been pointed out by Metzger,[11-13] Rossi,[14] and König et al.,[15] and the system can also be employed as a model to study ligand–cell receptor interactions.[16] Finally, the IgE receptor system represents a very good model to study the process of secretion.

Increases in our knowledge of the structure and properties of the high affinity IgE receptor have been regularly reviewed in the past 10 years.[11-15,17-23]

DESCRIPTION OF THE SYSTEM

The IgE Molecule

The structure and physicochemical and biologic properties of IgE have been described in great de-

TABLE 1-1 Molecules Interacting with IgE

	Cellular Source	*Reference*
High affinity receptor	Basophils, mast cells, and related tumor cells	See text
Low affinity receptor[a]	Eosinophils	1
Low affinity receptor[a]	Monocytes, macrophages	2, 3
Low affinity receptor[a]	Platelets	4
Low affinity receptor[a]	Lymphocytes (T- and B-)	2, 3
Low affinity receptor[a]	Mast cells	5, 6
IgE binding factors:		
IgE-TsF	Lymphocytes (Lyt-2^+)	
IgE potentiating factor[b]	Lymphocytes (Lyt-1^+, FcR$^+$)	7
IgE suppressor factor[b]	Lymphocytes (Lyt-1^+, FcR$^+$)	
Histamine releasing factors[c]	Macrophages	9, 10

[a] The affinity of these receptors is at least 100-fold less than that of the basophil–mast cell receptor.
[b] These factors share common antigenic determinants: they also share a determinant with the FcR on both T and B cells.[8]
[c] These factors, also called IgE dependent macrophage factors, induce histamine release from human basophils and lung mast cells, possibly by interacting with cell surface IgE. The relationship of these factors to the IgE receptor on macrophages is not known.

tail.[24-27] These studies have shown that IgE, like all other classes of immunoglobulins, is composed of two heavy (ϵ) and two light (κ/λ) polypeptide chains, connected by covalent (-S-S-) and noncovalent interactions. The domain structure of IgE is similar to that of IgM in that it has an extra domain ($C_\epsilon 2$) in place of the hinge region of IgG.

More recent work on IgE has focused mainly on the following aspects:

Generation of Active Fragments

One of the goals of these kinds of studies is to facilitate the design of synthetic analogues that inhibit IgE mediated hypersensitivity reactions by competing with endogenous IgE for the binding to the $Fc_\epsilon R$. The problem has been approached by proteolytic digestion and by recombinant DNA technology. The enzymatic approach has been rather disappointing with both human[26] and rodent[28] IgE. The only active fragments described so far are whole Fc fragments obtained from human IgE.

Recombinant DNA technology seems much more promising.[29,30] Unglycosylated polypeptides, which bind specifically to human basophils, were obtained by coupling the cDNA coding for all or most of the Fc region of the human ϵ chain to an expression plasmid and subcloning it in *Escherichia coli*. This approach has demonstrated that it is possible to construct fragments of cDNA coding for polypeptides that retain binding properties; it is therefore plausible that in the near future it will be possible to define more accurately the region involved in the binding to the IgE receptor.

The only conclusion we can draw at present from the studies just mentioned is that IgE interacts with its specific receptor at the level of the $C_\epsilon 2$-$C_\epsilon 3$ junction.[28] It appears that only the carboxy terminal two-thirds of the $C_\epsilon 2$ domain are involved in the binding.[30] Somewhat at variance with this conclusion are the results obtained by Stanworth et al.,[31] showing that synthetic oligopeptides reproducing a sequence in the $C_\epsilon 4$ domain of human IgE are able to trigger rat mast cells. It is noteworthy that these peptides are active only at rather high concentrations (10^{-5} to 10^{-4} M).

Conformation of Receptor Bound IgE

IgE molecules possess segmental flexibility.[32] Receptor bound IgE bends at the $C_\epsilon 2$-$C_\epsilon 3$ junction,[33-35] which other studies have shown to be also the site of interaction with the receptor (see foregoing). One can imagine the IgE as "sitting" in the receptor with the Fab fragments and the $C_\epsilon 4$ domain sticking out. The implications of this asymmetric interaction are not yet clear. However, it has been suggested that it is important for the interaction of receptors with each other or with other membrane components necessary for cell triggering.[35]

Mapping of Antigenic and Functional Sites with Monoclonal Antibodies

Conrad et al.[36] produced two mouse monoclonal antibodies (MAb) against rat IgE, one of which (MAb B5) reacts with the Fab region of the IgE molecule; the antigenic site recognized by the other MAb (A2) is in the Fc region, but it is not the same site that interacts with the $Fc_\epsilon R$.

By using different rat monoclonal antibodies against mouse IgE, Baniyash and Eshhar[37] have also demonstrated several different epitopes on IgE. One monoclonal antibody (MAb 51.3) recognizes three repetitive determinants on the Fc region, one of which is also involved in the binding to the $Fc_\epsilon R$.[38] Metzger et al.,[23] however, have suggested a different interpretation of these results.

In conclusion, the monoclonal antibody approach seems to be a very promising one for the identification and isolation of defined fragments that bind to $Fc_\epsilon R$.

The Cells

Three types of cells possess high affinity receptors for IgE: peripheral blood basophils, connective tissue mast cells (mainly located near blood vessels), and mucosal mast cells (which are particularly abundant in the gastrointestinal tract). The term "mucosal" mast cell, however, is a misnomer, since this type of cell is present also in nonmucosal tissues.[39]

Although basophils and mast cells are indistinguishable by light microscopy, they show a number of ultrastructural, histochemical, and functional differences.[40-43]

By electron microscopy and spectral analysis, for instance, it is possible to show differences in the structure and content of granules; moreover, the three types of cells differ from each other in their sensitivity to various secretagogues and pharmacologic agonists, such as disodium chromoglycate, which inhibits degranulation only in connective tissue mast cells.

Human as well as rat mast cells are heterogeneous.[39,44,45] Connective tissue mast cells are larger than mucosal mast cells, and their granules contain at least five times as much histamine. Mucosal mast cells have a shorter life span, and their growth and differentiation are dependent on T cell factors. In contrast to connective tissue mast cells, they are not sensitive to the inhibitory effect of disodium chromoglycate.

The exact relationships among these types of cells are not fully known, although there is some evidence for a common precursor.[46] An interesting observation is that connective tissue mast cells retain some proliferative potentiality. It has been suggested that when production of mast cells is required, connective tissue mast cells respond first, by dedifferentiating and then proliferating and differentiating again into mast cells in situ.[47]

Most of the studies described in this chapter have been carried out with the so-called rabbit basophilic

leukemia (RBL) cells. This cell line was derived from a transplantable basophilic leukemia induced in a Wistar rat by the oral administration of the carcinogen β-chloroethylamine[48] and was subsequently adapted to tissue culture.[49,50]

The general characteristics and properties of RBL cells have been described in detail.[49,51] RBL cells are morphologically similar to normal rat basophils and peritoneal mast cells and share with them many properties,[52] even though there are a number of interesting differences.[53] On the basis of similar histologic features and secretory granule biochemical characteristics, recent studies indicate a close relationship among RBL cells, rat culture derived mast cells, and rat mucosal mast cells.[54] These three types of cells contain a smaller amount of histamine than connective tissue mast cells, and in contrast with the latter cells, their predominant granule proteoglycan is not heparin.

An interesting characteristic of RBL cells is the inverse relationship between the growth rate and the number of IgE receptors per cell.[55] Cells in stationary cultures accumulate up to 0.9 to 1.7 $\times 10^6$ receptors per cell, whereas in forced exponential cultures the number of receptors per cell stabilizes at 4 to 6 $\times 10^5$.

Normal cells, by contrast, have a lower number of receptors per cell; estimates vary from 4 $\times 10^4$ in rat mucosal mast cells[56] to 0.4 to 2 $\times 10^5$ in human basophils[40,57] and 1 to 5 $\times 10^5$ in human lung mast cells[40] and rodent peritoneal mast cells.[56-60]

Uncloned RBL cells failed to release histamine following IgE mediated triggering.[48,61] Barsumian et al.,[62] however, were able to isolate several clones of RBL cells with a wide variation in histamine releasing capacity (from 10 to 100 percent). The molecular size of $Fc_\epsilon R$ and its tryptic map appear to be the same in normal mast cells and RBL cells,[63,64] and the parameters for IgE binding are also similar.[58] However, RBL cells and normal mast cells differ in their response to IgE oligomers,[65] to be discussed.

BINDING PARAMETERS OF CELL BOUND AND SOLUBILIZED RECEPTORS

The binding of IgE to $Fc_\epsilon R$ can be described by the simple reaction:

$$[\text{IgE}] + [\text{receptor}] \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [\text{receptor} \bullet \text{IgE}]$$

This is true for both intact RBL cells[66] and solubilized receptors.[67] The binding parameters, calculated from kinetic studies, are reported in Table 1-2. Similar values were obtained with normal rat mast cells.

One special feature of the system is the extremely high value of K_A, which depends on both a relatively high constant of the forward reaction and a very slow rate of dissociation. Indeed, once IgE is bound to its receptor on mast cells, IgE-receptor complexes survive different protocols of mast cell purification.[71] Moreover, IgE remains bound to the receptor throughout the purification procedures of the complexes to be described. The high affinity of the IgE for its receptor explains in part, at a molecular level, the clinical observation that once the skin has been sensitized with IgE, sensitization lasts several weeks, owing to the slow dissociation of IgE from cutaneous mast cells.[72]

The value of K_A for solubilized receptors is about 200-fold greater than that estimated for cell associated receptors, probably because of an underestimation of k_{-1} in studies performed with intact cells[73] or to a "gating" effect of cell surface glycoproteins.[16]

TABLE 1-2 Binding Parameters of IgE to the Receptor [a]

	°C	*Intact Cells* [b]	*Solubilized Receptors* [c]
K_1	4°	2.1×10^4 M^{-1} sec^{-1}	5×10^5 M^{-1} sec^{-1}
	37°	8.8×10^4 M^{-1} sec^{-1}	
K_{-1}	4°	1.4×10^{-5} sec^{-1}	2.6×10^{-7} sec^{-1}
	37°	1.7×10^{-5} sec^{-1}	
$K_A = K_1/K_{-1}$	4°	1.2×10^9 M^{-1}	2×10^{12} M^{-1}
	37°	6.0×10^9 M^{-1}	

[a] The data refer to the interaction of monomeric IgE and univalent receptors.
[b] Comparable values have been obtained with rodent[58,66] and human[69,70] cells. No differences in affinity were found between mucosal and connective mast cells,[56] even though the actual values of K_A were not reported in this study.
[c] Binding parameters at 37 °C are not available for solubilized receptors because of their marked temperature sensitivity ($\tau_{1/2}$ at 37 °C, 19 min).[67,68] An approximate value of $1 \times 10^8 M^{-1} sec^{-1}$ for the forward reaction was obtained from a few experiments in which it was possible to measure an initial rate more accurately.[67]

$Fc_\epsilon Rs$ display a high "class specificity" as do FcRs for other individual immunoglobulin classes and subclasses;[74] no other homologous monomeric Ig is able to compete with IgE.[49,67] In intact RBL cells (but not in plasma membranes from such cells), IgGa immune complexes could inhibit the binding of IgE;[75] these results, however, have not been confirmed.[76]

Earlier studies indicated also that the "species specificity" was very strict;[49,59,77] only IgE of closely related species (like mouse and rat) could be bound by the same receptor.[49] However, more recent studies seem to indicate that the species specificity of the IgE receptor—again like that of FcRs for other Ig classes[74]—is not as stringent as class specificity. In particular, and in contrast with earlier studies, human IgE has been found to bind to rodent cells,[78] and, vice versa, rodent IgE binds to human cells[69,79] and to isolated receptors from human cells,[80] even though the binding affinities are much lower, at least in neoplastic cells. Cultured human basophils, isolated from cord blood, by contrast seem to bind to both rodent and human IgE with comparable affinity.[70] This discrepancy was attributed to the pathologic state or immaturity of the leukemia cells. However, it is difficult to reconcile these contrasting results.

STRUCTURE OF THE RECEPTOR

Solubilization and Assay of Receptors

IgE receptors from either intact RBL cells or plasma membranes can be easily solubilized using nonionic detergents, such as triton X-100 and X-114 and Nonidet P40.[50,67,81] Solubilized receptors can be purified by affinity chromatography.

The purification steps can be monitored and the yields at each step calculated by using the assay developed by Rossi et al.[67] This assay is based on the differential precipitability in ammonium sulfate of free and receptor bound IgE. At a concentration of about 45 percent saturated ammonium sulfate, more than 80 percent of the bound IgE is precipitated, as compared with 10 percent or less of free IgE.

More recently polyethylene glycol (PEG) has been employed instead of ammonium sulfate.[82] It has been found that 85 to 95 percent of both free receptors and IgE receptor complexes precipitate at 13 percent PEG, whereas 95 percent of the free IgE remain soluble. PEG also can be used to separate free antigen, antigen–IgE-receptor complexes, and IgE-receptor complexes.

Purification Strategies

The purification strategy can be aimed at the purification of either free receptors or IgE-receptor complexes. In the former instance receptors are trapped on IgE-Sepharose columns and eluted with denaturants.[67,83,84] In the latter case receptors are previously saturated with either haptenated IgE (e.g., phenylarsonilated IgE[85,86]) or with hapten specific IgE (e.g., monoclonal anti-DNP IgE[87]). The $Fc_\epsilon R$-IgE complexes are then adsorbed on specific adsorbents (antihapten antibodies in the case of haptenated IgE, or hapten conjugates when hapten specific IgE is employed). In both instances the complexes are eluted with an excess of free hapten. IgE-receptor complexes can be finally dissociated by gel filtration in SDS.[86] A summary of this procedure is given in Table 1-3. It is important to point out that the procedure described in the table has been scaled up in order to yield nanomolar amounts of receptors.

For analytic purposes two other procedures have been employed: immunoprecipitation with an IgE–anti-IgE system[63,88,89] and immunoprecipitation with antireceptor antibodies.[89–91]

Subunit Structure

The material purified according to the procedures just outlined is composed of a single polypeptide chain, virtually free of contaminants, as shown by polyacrylamide gel electrophoresis in SDS (SDS-PAGE).[63,86,88] The molecular weight of this component, dubbed the α chain,[87] is about 45K, as determined by gel filtration in 6 M guanidine hydrochloride[92] or by extrapolating to infinite gel concentration values obtained by SDS-PAGE, which range between 52 and 62 kDa, depending on the degree of cross linking of the gel.[93] The α chains isolated from several RBL sublines maintained under different conditions present small differences in their molecular weights.[94]

The receptor on intact cells has a much more complex structure. Preliminary observations suggested that an additional polypeptide chain with an apparent M_r value equal to 30K was associated with the 45K component.[83,89] Conclusive evidence of the existence of this polypeptide has been provided by Holowka et al.[95] by using RBL cells biosynthetically labeled with ^{3}H-aminoacids. Labeled cells were reacted with IgE, and the IgE-

TABLE 1-3 Purification of IgE Receptors from RBL Cells [a]

Step	*Procedure*	*Yield (%) Overall*	*Yield (%) At Each Step*
1	Aceton powder extract	29	29
2	Polyethylene glycol precipitation of IgE receptor complexes	21	73
3	Affinity chromatography of complexes	12	57
4	Gel filtration in SDS of complexes	10	79

[a] From Kanellopoulos et al.[86] The starting material was obtained by homogenizing solid RBL tumors grown intraperitoneally in two week old WKY/N rats.