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EARLY MOLECULAR EVENTS IN ANTIGEN-ANTIBODY CELL ACTIVATION

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INTRODUCTION

Immunoglobulins are multichained glycoproteins synthesized by all vertebrate species, which serve as the fundamental recognition units in immune reactions. In such reactions they serve a dual role. One role is to serve as receptors, stimulation of which initiates the immune response to a particular antigen. This stimulation can result in both a proliferation of antigen-specific lymphocytes and differentiation of lymphocytes into plasma cells that secrete antigen-specific immunoglobulins ("antibodies").

A second role of immunoglobulins is to serve as the effector substances which, upon interaction with the antigen, ultimately lead to disposal of the latter. Antibodies have no capacity to degrade or alter irreversibly the offending antigen. A variety of indirect mechanisms are utilized instead. For example, antibodies directed to surface antigens on a cell may destroy the cell by initiating a complex interaction involving the set of proteins collectively called *complement* (1). Complement activation can directly damage cell plasma membranes leading to cytolysis. In other instances, particles coated with antibodies may become phagocytosed with subsequent degradation occurring in the phagocyte's lysozomes (2, 3). Still another mechanism (with which I deal in greater detail) involves adherence of antibodies to cells, exposure of which to the antigen initiates exocytosis of granules from the cells (4). The contents of these granules stimulate a variety of reactions that can be loosely grouped as inflammatory. These reactions are apparently directed to the removal of the antigen by nonspecific means.

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In this review, I describe what is known about how immunoglobulin molecules interact with antigens and how this interaction leads to the consequences discussed above. Much more is known about the former than about the latter. Several of the topics discussed have been the subjects of separate recent reviews. The literature cited is therefore meant to be illustrative rather than exhaustive.

GROSS STRUCTURE OF ANTIBODIES

Almost all the information we have about the structure of antibodies is derived from analyses of immunoglobulins secreted by cells. Historically the first molecules studied were those whose production was stimulated by immunization with specific antigens (5). The ability to perform detailed analyses was considerably enhanced as it became recognized that the products of neoplastic plasma cells—or plasma-like cells—were antibody-like (6). In diseases such as multiple myeloma or Waldenström's macroglobulinemia the serum may contain vast amounts of homogeneous immunoglobulins, which can be isolated readily, sequenced, and even subjected to crystallographic analysis (7–9). In this section I summarize those aspects of immunoglobulin structure necessary to understand how these proteins function. Detailed descriptions are available (e.g. 10, 11).

Chain Structure

As already mentioned, immunoglobulins are multichained. They can be described by the formula $(HL)_{2n}$. In most instances $n = 1$; that is, there are four chains. In two "classes" of immunoglobulins, higher polymers are formed: $n = 2$ or 3 in some IgA and $n = 5$ in most IgM proteins. As suggested by the formula, an individual immunoglobulin shows $2n$-fold symmetry.

LIGHT CHAINS L in the formula signifies light chains. These have a molecular weight of approximately 23,000. Two major classes are found in many species: κ and λ. There are no known functional correlates that can be assigned to these two classes. Moreover, since in several species one of the classes is practically absent (12) without an apparent deficiency of immune responsiveness, it is unlikely that the classes have contemporary significance. Light chains have an internal repeat structure consisting of two globular units ["domains" (13)] each about 110 amino acids long. While the amino acid sequences of the two units show only a distant relationship, the three-dimensional structure exhibits a fundamental similarity (14) referred to as the immunoglobulin fold. The 110-amino acid unit at the amino terminal end shows great sequence diversity and is termed the variable

domain of the light chain (V_L). The variability in V_L is not random; several short "hypervariable" stretches are interspersed among more invariant "framework" sequences (15, 16). While linearly separated in the primary sequence, the hypervariable stretches are brought together spatially by the three-dimensional folding of the V_L domain. The carboxy-terminal unit (C_L) is almost invariant among chains of the same class.

HEAVY CHAINS The H chains of immunoglobulins have a structure reminiscent of that of L chains. There is an internal repeating structure of four or five globular domains, each again about 110 amino acids in length. The unit at the amino-terminal end shows a distinctive sequence variability much like that of V_L and is termed V_H. The remaining domains show more obvious sequence homology to each other. Each is folded into the characteristic immunoglobulin fold pattern. These domains are referred to as C_H1, C_H2, C_H3, and where present C_H4.

In humans there are nine classes of heavy chains; seven ($\gamma1$,$\gamma2$,$\gamma3$,$\gamma4$, $\alpha1$,$\alpha2$, and δ) have three C_H domains, while two (μ and ϵ) have four. Other differences among heavy chain classes include class-specific constant region sequence differences and carbohydrate side chains. In addition, all except the μ and ϵ chains have an additional stretch of amino acids which is interposed between two C domains. The sequences of these extra segments bear no obvious relationship to the sequences in the domains. These segments are referred to as the "hinge" regions (17) because there is a variety of evidence suggesting that they serve as the principal site of flexibility in the immunoglobulin (18). The hinge regions of the different classes of immunoglobulins are distinctive (Table 1). There are no known functional correlates to these variations.

Table 1 Comparison of hinge regions of human immunoglobulins

Immunoglobulin class	Length of amino acid residues[a]	Number of cysteines	Number of prolines	Location of hinge or flexible interface
IgG1	18	3[b]	5	$C_H1:C_H2$
IgG2	15	4	4	$C_H1:C_H2$
IgG3	65	11	22	$C_H1:C_H2$
IgG4	15	2	5	$C_H1:C_H2$
IgA	25	3	11	$C_H1:C_H2$
IgM	0[c]	1	—	$C_H2:C_H3$[d]
IgE	0[c]	2	—	$C_H2:C_H3$

[a] See Reference (19). Residue 216 (20) was used as the starting point. Data on IgG2 and IgG3 from References (21, 22) respectively.
[b] One of these contributes to the heavy-light chain disulfide.
[c] No apparent hinge sequence.
[d] There is also evidence for flexibility at the $C_H1:C_H2$ interface (23).

Topology of Domains

Most immunoglobulins have at least one disulfide bond that links each light chain to a heavy chain and one disulfide that links the two heavy chains to each other. The exact sequence position and number of these interchain disulfides vary for the different classes. Nevertheless, the available information suggests that they are similarly arranged in space. While undoubtedly stabilizing the overall structure of the molecule, in many instances mild reduction, which selectively cleaves these interchain disulfide bonds (leaving the intrachain disulfides intact), causes no gross chain dissociation. The domains are topologically arranged as follows (proceeding from the amino terminal ends): $V_L : V_H$, $C_L : C_H1$, $C_H2 : C_H2$, $C_H3 : C_H3$, and when present $C_H4 : C_H4$. The structure is Y-shaped with the arms formed by the two "Fab" regions ($V_L : V_H$, $C_L : C_H1$) and the leg (Fc region) formed by the remaining heavy chain constant region domains (24).

Combining Sites

Since antibodies have no known intrinsic capacity to irreversibly affect the antigens to which they bind, it is clear that they must interact with two classes of substances: antigens and effector substances. The latter I refer to by the general term *receptors.* I discuss the justification of this term below.

COMBINING SITES FOR ANTIGENS The combining sites for antigens on antibodies have been defined in considerable detail (9). The stoichiometry is one site per heavy chain–light chain pair. In IgG, $(HL)_2$, there are two such identical sites; in IgM, $(HL)_{10}$, there are ten. Each site forms a depression at the tip of a Fab region. The walls of the cavity are largely formed by the hypervariable regions of V_L and V_H. It is the spatial arrangement and chemical nature of the side chains of the amino acid residues in the hypervariable regions that account for antibody specificity. Though in some instances charge-charge interactions may play a role, mostly cooperative weak interactions account for the free energy of binding (25, 26). In several instances, there is evidence that small conformational changes in either the antigen (27) or the combining site (28) enhance complementarity (induced fit). In favorable circumstances the ΔG of binding can be < -9 kcal though ~ -7 kcal is more commonly observed. The combining sites vary in size encompassing, e.g. 2–6 monosaccharide or amino acid residues (10). The kinetics of binding are unremarkable; the first-order dissociation rate constant usually determines the relative magnitude of the equilibrium constant (29, 30). To the extent that information is available there is nothing unique about antibody-combining sites for antigens compared to the combining sites for ligands on other proteins.

COMBINING SITES FOR RECEPTORS Immunoglobulins are known to interact physiologically with three substances other than those required for the biosynthesis, physiological transport, and degradation of the polypeptide chains. One of the three is J chain, an ~15,000 molecular weight polypeptide associated with the polymeric forms IgM and IgA (31). It is likely that J chain contributes to the polymerization process; other functions for it are more speculative (31). A second substance is secretory component—a 70,000 dalton glycoprotein which is associated with immunoglobulins found in secretions (32). Its likely role is to protect the immunoglobulin from premature degradation. Both J-chain and secretory component are associated with the Fc regions. Finally there is the class of substances referred to here as *receptors.* By this term I mean those entities that upon interaction with antigen-antibody complexes stimulate some form of effector system. The extent to which these receptors have been defined and characterized is quite variable. In one instance, the classical complement system, the receptor (Clq) has been visualized by electronmicroscopy (33, 34) and detailed structural analysis is proceeding rapidly (35). At the other extreme, e.g. the receptor *for* endogenous immunoglobulin on B-lymphocytes, the existence of a receptor can only be inferred and even that inference is not accepted by some (see section on B lymphocytes).

With only one well-documented exception, the evidence suggests that the receptors of various effector systems interact with the Fc region of immunoglobulins. The one documented exception is the "alternate" complement pathway, activation of which can be mediated by Fab regions (36).

The sites of interaction between receptors and the Fc regions of antibodies are as yet only poorly defined. There is considerable evidence that Clq interacts principally with the C_H2 regions on IgG molecules (37–39), and a 62 amino acid peptide from this region shows substantial Clq binding activity (40). Tryptophans have been implicated in the interaction (39, 41, 42). Active peptides derived from the C_H4 domain of IgM have been described (43). As discussed elsewhere (44), the significance of this finding is uncertain.

Mast cells have receptors for IgE and there is indirect evidence that the penultimate C_H3 domains of the ϵ-chains are involved (45). The evidence for a combining site on a pentapeptide sequence from the C_H2 region of the ϵ-chains (46) is very doubtful (47).

A variety of cells contain so-called Fc receptors, that is, plasma-membrane components that bind antigen-antibody complexes via the Fc of the antibodies. There is evidence for C_H2, C_H3, or both C_H2 and C_H3 being involved (48). The apparent discrepancies remain unresolved.

The cells (B-cells) from which the antibody secreting cells (plasma cells) are derived have immunoglobulins on their surfaces whose specificity is

identical with that of the antibody which the daughter cell(s) will produce. There is evidence that most of the Fc region of the surface immunoglobulin on B-cells is exposed [reviewed in reference (49)]. This suggests that only the carboxy-terminal region of the surface immunoglobulin reacts with the putative cell receptor.

COMBINING SITE INTERACTIONS

Structural Studies

DOMAIN INTERACTIONS It is clear from the previous discussion that the combining sites on antibodies for antigens are spatially removed from the combining sites for the receptors of effector systems. It is therefore appropriate to consider how these combining sites and the domains in which they reside may interact.

Trans interactions *Trans* interactions are the interfaces between domains lying on alternate sides of the axes of pseudosymmetry between the heavy and light chains and the axis of symmetry between the carboxy-terminal halves of the heavy chains. These interactions are with one exception strong; however, direct studies are still limited.

The V_L and V_H domains interact so strongly that noncovalently bound "F_V" fragments consisting of one V_L and one V_H can be isolated (50). The antigen-binding properties of such fragments are little different from those of the intact antibodies. This suggests that the three-dimensional structure of the two domains is well maintained.

The C_H1 and C_L domains face each other over a broad interface. There are numerous close interactions as determined directly in several X-ray analyses (7, 8). The interface between C_H3 domains is very similar to that between C_H1 and C_L (24, 51). Selective cleavage of the heavy chains amino-terminal to the C_H3 domains yields a dimeric fragment $(C_H3)_2$ whose noncovalently bound domains cannot be dissociated from one another without denaturants (52).

The only domains that are known to have weak or even mildly repulsive interactions are the C_H2 domains. There is evidence that in the absence of interchain disulfides these domains can, and perhaps prefer to, spread apart (52–54); however, the disulfide bond(s) can be reformed by deliberate oxidation.

Cis interactions While there is ample evidence for significant interactions between domains normal to the long axis of the molecule, longitudinal or *cis* interactions are much fewer. The X-ray data indicate few interactions

between V_L and C_L or V_H and C_H1 in the Fab regions (7, 8). Furthermore, the angle formed by the axes of pseudosymmetry between V_L and V_H and C_L and C_H1 is quite variable (24).

There is a striking lack of evidence for *cis* interactions between the carboxy-terminal domains of Fab (C_L and C_H1 and the amino terminal domains of Fc (C_H2). In the most intensively studied immunoglobulins—IgG—a hinge region separates these domains (Table 1). It is here that the well known ready cleavage of immunoglobulins by proteases occurs. By a variety of criteria the fine structure of the resulting fragments is unchanged by this cleavage. Moreover, the fragments produced, Fab and Fc, show no tendency to associate.

Some data suggest that C_H2 and C_H3 interact strongly (55); however, other data do not support this (56, 57). In any case, such interactions do not appear to perturb the basic structure of the individual domains substantially.

ANTIGEN-INDUCED CHANGES While the study of domain interactions in immunoglobulins can provide valuable clues about the likelihood of combining site interactions, the results are usually too imprecise to permit one to predict accurately whether such interactions actually occur. A more reliable approach is to search for ligand-induced changes directly. In this section I consider changes in the structure of immunoglobulins induced by ligands; in the following sections, I consider changes in the functional properties of the combining sites themselves. The older data as well as the results of more recent studies have been reviewed in detail (44, 58) and I limit my discussion here to a description of the methods used and a summary of the principal findings.

Direct studies, e.g. by X-ray diffraction analyses of myeloma proteins in the presence and absence of antigen-like ligands, have failed to detect changes in conformation (9). These studies have so far been limited to an investigation of Fab fragments. Nonetheless, if the Fab regions do not change it is hard to imagine that the Fc regions in the intact molecule would change *unless cleavage of the molecule into its Fab and Fc regions itself would produce a liganded conformation.* This was in fact proposed by Huber et al (59). These workers raised the possibility that antigen-induced changes in the Fab regions would lead to altered Fab:Fc interactions through a shortening of the hinge regions. The failure to observe the antigen-induced changes in the isolated Fab regions was explained by postulating that they were already in the liganded form as a result of the proteolytic cleavage used to produce the fragments. The authors made two predictions that posed critical tests of their theory. One was that the Fab (and the Fc regions) would be different in the intact versus the cleaved molecule. The

second prediction was that antigen should induce a change in the rate with which the hinge region can be cleaved by proteases or the rate at which hinge region disulfides can be cleaved by reducing agents. Both predictions appear to have been wrong (24, 60), and there is really no direct evidence for this theory. This theory potentially explained the unusual findings of Pilz et al (61, 62). These workers, using low angle X-ray scattering, described antigen-induced changes in the calculated radius of gyration of antibody but not in the radius of gyration of the isolated Fab fragments. The failure to find support for the theory of Huber et al leaves the results of the scattering studies unexplained.

Other indirect studies by a wide variety of physicochemical techniques (optical rotatory dispersion, circular dichroism, circular polarization of luminescence, depolarization of fluorescence, fluorescence, electron spin and nuclear magnetic resonance, neutron scattering, hydrogen exchange, immunochemical analysis) have failed to detect changes in Fc due to antigen binding, have detected changes that cannot be clearly assigned to the Fc regions, or have failed to document that the observed changes are stoichiometrically correlated with antigen combining site saturation (44, 58).

Functional Studies

ACTIVATION BY ANTIGENS The most direct way to study the effect of antigen binding on the interaction of antibody with receptors is to look for functional correlates. It has long been known that a variety of effector systems are optimally stimulated by antigen-antibody complexes consisting of more than one antibody molecule. What has been uncertain is whether the aggregation of antibody molecules (*a*) provides the critical signal per se, (*b*) is important only indirectly (aggregation being required to initiate or enhance conformational changes), or (*c*) is incidental.

An apparent exception to the rule that antibody aggregates are required for optimal responses is the observation that single molecules of IgM appear to be capable of initiating the classical complement cascade upon interaction with antigen (63). This is more of a semantic exception than a real one. Since effector system receptors interact with the Fc regions it is aggregation of the Fc regions with which we are concerned. IgM contains five Fc regions per molecule and in this case Fc aggregation may still be of importance. In this instance the aggregation would be an *intra*- rather than *inter*-molecular.

All of the older and much of the recent data are consistent with a failure of antibodies to activate effector systems unless the Fc regions of two or more antibody molecules are aggregated. The results of two recent investigations appear to be exceptions. In the first, an apparently monofunctional

antigen stimulated complement interaction with IgM antibodies (64). There are two unusual aspects to these and related experiments. First though the antigen may be monovalent it must be large—much larger than conceivably necessary to fill the antigen-combining site. Second, the stoichiometry does not seem appropriate. Optimal interaction with complement occurred under conditions where apparently only a minute fraction of the antigen-combining sites should have been saturated (44).

In the second study a bivalent antigen was used—an artificial dimer of the "loop" sequence of chicken lysozyme (65). Upon interaction with IgG antibodies several phenomena occurred that led to the proposal that a circular complex between the bivalent antigen and single bivalent antibodies occurred. Substantial additions of antigen did not appear to disrupt the postulated circular complexes. Nevertheless, the interaction with complement observed with such complexes was remarkably sensitive to excess antigen. Thus, in its functional properties this system behaves just like other systems where aggregation of antibodies appears to be critical. I can only conclude that the exceptional findings of Brown and Koshland (64) and Pecht et al (65) need further clarification before their more general significance can be evaluated.

ACTIVATION WITHOUT ANTIGENS The importance of aggregation is suggested by the finding that aggregation of antibody Fc regions by whatever means can stimulate effector systems. Aggregation induced by heating, cross-linking reagents, and antibodies to antibodies have all been found effective (66–68). Moreover, the same results can be obtained with isolated Fc regions. Thus neither antigens nor the antigen-combining sites of antibodies are required.

The principle of parsimony leads me to conclude that the aggregation is the critical event per se. I think it unlikely that in all the procedures used to aggregate the antibody, a similar specific conformational change occurs in the Fc regions. Others conclude differently (reviewed in 69).

MECHANISM OF ANTIBODY-MEDIATED ACTIVATION

In some systems (e.g. complement), receptor binding is promoted by complexing of antigen with antibody. In others (e.g. the IgE-mast cell system) the receptor interacts with the antibody in the absence of antigen, but the system is not activated unless the antibody becomes aggregated.

Regardless of whether the antibody is simply aggregated or aggregated and conformationally changed, several possibilities can be envisioned with regard to the role of the antibody in the subsequent events.

Alternative Mechanisms

One possibility is that the antibody is directly involved. It may be, for example, that the aggregated or aggregated and altered Fc regions become enzymatically active. Alternatively, the altered Fc regions might interact with a new component and changes in the latter might generate the signal.

A second possibility is that the antibody is only very indirectly involved, that it is the receptor that binds to the antibody which plays the critical role. A clear choice between these major alternatives can now be made in one antibody-mediated system: the IgE-mast cell system.

IgE-Mast Cell System

IgE is a four-chained immunoglobulin whose epsilon (ϵ) heavy chains have four constant region domains (70). Like all other immunoglobulins it is secreted by plasma cells. Its unique property is its ability to bind with exceedingly high affinity to mast cells or the related peripheral blood basophils (71). The binding is via the Fc region, and by itself is not known to perturb the cell to which it is bound. Since the Fc regions of IgE are the same regardless of the specificity of the antigen-combining sites in the Fab regions, a single mast cell may bind antibodies of a variety of specificities. As many as 10^6 IgE can bind specifically to the surface membrane (72). Any manipulation that leads to surface aggregation of the IgE Fc regions [cross-linking by antigens (73), bifunctional reagents (74), anti-IgE (75), and lectins (76)] stimulates the cells to undergo degranulation. Other, nonantibody reagents can also trigger the cells (77). In some of these studies it has been shown that the cells are compartmentalized in that local, partial, degranulation can occur (78, 79). It appears likely that IgE-mediated stimulation is similarly compartmentalized so that multiple stimulatory events over the cell surface may be necessary to achieve maximal release. It has recently been demonstrated unambiguously that dimers of IgE (prepared with bifunctional cross-linking reagents) are fully capable of initiating individual stimulatory events ("unit signals") (73). The cell component that binds IgE to the cell surface is being studied by several groups. It is a glycoprotein $5–10 \times 10^4$ daltons in mass and behaves like an integral membrane protein (reviewed in 80). Importantly, it is functionally univalent; one molecule of receptor binds only one molecule of IgE. Thus when IgE is aggregated, there is a stoichiometric aggregation of the receptors. Using antibodies directed to the receptor, it has recently been shown that mastocytoma cells *grown in the total absence of IgE can be triggered by (bivalent) antireceptor antibodies* (81). IgE blocks the antireceptor-induced release. Similar studies on normal cells have yielded similar results (82, 83). While the latter cells contain some IgE on their surfaces, control studies suggested no role for the IgE in the antireceptor-stimulated degranulation.

Thus in this system, the antibody appears to function exclusively as a mechanism by which specific antigens can aggregate (dimerize) receptors. No other role for the IgE must be invoked.

Complement

The importance of aggregation of antibodies in antibody-mediated complement activation has already been referred to. In the classical pathway, this involves the interaction of Clq with antibody. By unknown mechanisms Clr and Cls, components attached to Clq, are activated leading to proteolytic conversion of inactive Cls to active Cls by Clr. Activated Cls is itself a protease that reacts with subsequent components. It is uncertain whether the initial activation requires the participation of specific regions on the immunoglobulin other than those directly involved in binding Clq. There are some data that suggest that simple interaction of Clq is inadequate (42); however, in those experimental manipulations it is possible that changes in the topology of the combining sites were affected. These changes may have been responsible for the failure to observe complement activation in the face of unaltered Clq binding. It would be interesting to test whether cross-linking of the combining sites for antibody on Clq (e.g. with antibodies directed to those regions) would result in Cl activation.

B Lymphocyte

Another system of paramount interest to immunologists is the B-lymphocyte. As indicated previously these cells have surface-bound immunoglobulin which they themselves produce. (This is in contrast to the mast cells referred to above, in the section on the IgE-mast cell system, which do not synthesize the IgE bound to their surface membranes.) The surface immunoglobulin on the ontogenetically most primitive B-cells is IgMs—the four-chained subunit of IgM. Later, IgD may appear and still later other classes of antibody (84). On any particular cell all the surface immunoglobulins have identical combining sites for antigens (85).

The surface immunoglobulins have the properties of integral membrane proteins. The structures involved in the integration of the immunoglobulins in the membrane have not been identified. There is conflicting evidence for a hydrophobic "tail" or other constituent on the surface immunoglobulin that would explain its capacity to bind to the membrane of B-cells (86–89; see also 49). Consequently it is possible that a still unidentified immunoglobulin-binding component exists. It would be interesting to explore this possibility by the use of cross-linking reagents.

There is still considerable controversy about the role of the immunoglobulin of B lymphocytes. At one extreme is the proposal that the role of the antibody is exclusively to bind antigens (90). It is suggested that struc-

tures on the antigen itself or structures that have become attached to the antigen (e.g. by prior interaction of antigen with other cells or cell products) provide the stimulatory signal. Others have postulated a direct role for the immunoglobulin with or without invoking "second" signals (91).

Nevertheless, a variety of studies have shown that antibodies directed to this surface immunoglobulin are stimulatory (92–97). Bivalent antibody is required. Even in those cases where the signal appears to have been inhibitory (98), more than a passive role for the surface immunoglobulin (or the component that binds it to the cell surface) is implied.

Other Systems

IMMUNOLOGICAL SYSTEMS Recent results suggest that thymus-derived (T) lymphocytes have intrinsic antibodies on their surface membranes (99). The precise nature of these antibodies, and even their presence, has been in much dispute (100). Too little is known about this system to allow one to compare it to the others referred to above.

A variety of other cells have the capacity to bind extrinsic antibody to their surface membranes with or without the presence of complement [see Chapters 8, 10–13 in reference (101)]. In all of these, cell activation appears to require aggregated antibody (Fc regions). Undoubtedly the requirement for aggregated antibody is partially explained by the enhanced binding achieved by multivalent interactions. Whether in addition the aggregation is required to activate is uncertain. Experiments with antibodies directed *to* the Fc receptors on such cells would be of interest. Since bivalent fragments of antibodies can be prepared that lack Fc regions, the effect of aggregating the Fc receptors per se could be assessed.

NONIMMUNOLOGICAL SYSTEMS It has recently been observed that bivalent (i.e. cross-linking) antibodies directed to hormone receptors can in certain instances mimic the activity of the hormone [see Chapters 25–27 in reference (101)]. I mention these not as other examples of antibody-mediated cell activation; they are, but in a different sense than used in this review. The results with antibody-mediated triggering of hormone receptors do, however, raise the possibility that in other systems aggregation of receptors may be significant. In at least one of these systems—the insulin system—there is new evidence that the hormone is self-aggregating when bound to the surface membrane (102).

SEQUELAE OF ACTIVATION

Surface Phenomena

It is by now well known that many cell membrane components are more or less freely mobile in the plane of the membrane. It is not surprising

therefore that cross-linking of surface antibody, if extensive enough, can lead to substantial surface redistribution (reviewed in 49, 103).

In one well defined system, the IgE-mast cell system (see section on IgE-mast cell system), it has been possible to investigate directly the role of gross surface redistribution in the IgE-mediated cell activation (104, 105). There is good evidence that redistribution, though it can occur, does not contribute to cell stimulation. In studies in which IgE-coated cells were reacted with anti-IgE the dose of the latter could be titrated such that various amounts of IgE redistribution occurred. No correlation between exocytosis and gross redistribution was observed. Indeed, doses of anti-IgE that were high enough to induce gross redistribution during the time period in which exocytosis was anticipated were inhibitory (104). The mechanism of this inhibition is unknown.

Comparable studies on systems such as B-lymphocytes are difficult to perform. The problem is that most of the criteria used to study activation occur long after the addition of the stimulant. Discussions on the role of redistribution in the variety of B-lymphocyte responses are not conclusive (49, 10).

In addition to causing surface rearrangements, cross-linking reagents can induce endocytosis of membrane components. The possible role of internalization in antibody-mediated cell activation is uncertain (49, 103).

Metabolic Changes

In no instance are there data that define the immediate sequelae of antigen-antibody reactions on the surfaces of cells. In the IgE-mast cell system, receptor aggregation appears to provide the initial signal. What the signal consists of is unknown. In that system the only extrinsic substance required is free Ca^{2+} (106). Several studies suggest that a change in Ca^{2+} permeability occurs at an early stage but whether this is step two or twenty-two is unknown (107, 108). Recent discussions of other metabolic events in this system can be found in reference (77). In the IgE-mast cell system it is possible to isolate the receptor. Studies are proceeding in several laboratories to see whether isolated receptors (*a*) can produce detectable changes or (*b*) are altered during triggering.

There is a vast literature on metabolic changes in lymphocytes stimulated by antigen and other substances. There are recent reviews by Gomperts (107) dealing with the role of calcium, by Ferber & Resch (109) on changes in membrane lipids, by Kaplan (110) on Na+/K+ transport, and by Wedner & Parker (111) on cyclic nucleotides and other changes. The interested reader may, however, first want to read the review by Waksman & Wagshal (112). These authors while focusing on the role of "cytokines"—soluble mediators produced by or acting upon lymphocytes—emphasize the complexities that must be dealt with. There is enormous heterogeneity of cell

types and cell cycle stages in the usual systems studied. The frequent use of lectins that can react with a vast number of different cell-surface components complicates the interpretation of findings even more. It seems likely that definitive information will require the use of more well defined systems than are commonly used at present.

SUMMARY AND CONCLUSIONS

The importance of aggregation in antibody-mediated reactions has been widely accepted for decades. What has eluded definition are the reasons aggregation is so necessary. One simple possibility, that by permitting multiple cooperative interactions it enhances antigen binding to antibody, undoubtedly is part of the answer. The weight of the evidence suggests that this explanation is insufficient, however. Rather, aggregation per se is necessary and in particular, aggregation of those regions of the antibody molecule that react with the receptors of effector systems. Again one might postulate that this simply enhances the binding of antibody to receptors but again while this is undoubtedly true it is only a partial explanation. It could be that aggregation of the antibody creates changes in the latter that are required for activation of the receptor. To the extent that the changes are postulated to be topological, i.e. that they involve changes in the arrangement in space of the combining sites on antibodies for receptors, there is considerable evidence in support of this notion. There is little or no evidence in favor of the postulate that aggregation produces meaningful changes in the structure of the combining sites for receptors per se, and there is at least one system where this can be pretty well ruled out. In IgE-mediated stimulation of mast cells it is possible to eliminate the IgE altogether; aggregating the receptor itself is sufficient.

My own conclusion is that the receptors of the effector systems should now receive the intensive experimental attention that has heretofore been directed toward the antibody molecules. We need to understand the role of aggregation in triggering the receptors. In the case of the classical pathway of complement activation this means understanding how intramolecular changes in Clq activate Clr. In the case of the cell receptor for IgE this means understanding how dimerization of the surface receptor produces changes. Such knowledge could provide the basis for new approaches to therapeutic intervention.

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