

STRUCTURAL BASIS OF ANTIBODY FUNCTION

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INTRODUCTION

It is less than 20 years since the general architecture of antibodies was elucidated and even less time since the explicit molecular basis of antibody specificity

about the immune system, two basic principles have emerged: (*a*) Antibodies remain the only known structures whose diversity is sufficient to explain the fine specificity exhibited by the immune response; and, (*b*) antibody function is mediated by a molecule whose structure consists of two distinct regions—one that carries a recognition site for antigenic determinants, and a second by which the antibody reacts with receptors of a variety of effector systems.

In this review we examine the current information on the structure of antibodies. We do not describe again the basic four-chain structure of immunoglobulins nor the division into variable and constant regions, which are by now well known (e.g. 4, 90, 118). We instead concentrate on the higher resolution data, much of which is still in the course of refinement. We discuss the Fabs, in particular with reference to the combining site and the specificity

binding site of protein A of *Staphylococcus aureus* and of Clq; and the structure of the hinge with reference to its possible role in separating Fab and Fc.

Whereas the characterization of the structures of individual proteins and of their interactions with small molecules can now be carried out with some

sophistication, the interaction of two or more macromolecules still presents considerable difficulties. It is not surprising therefore that our understanding of how antibodies interact with the macromolecular receptors on effector systems (e.g. C1q in the complement pathway, Fc receptors on cell membranes) is much less advanced. Our review of this aspect of antibody structure and function therefore involves more questions than answers.

IMMUNOGLOBULIN STRUCTURE

General Comments

Our knowledge of the three-dimensional structure of antibodies at atomic resolution rests mainly on X-ray diffraction investigations of fragments. Intact proteins for which X-ray analyses have been carried out consist of Kol (106), an IgG1(λ) human myeloma protein, the protein Dob (161), an IgG1(κ) human cryoglobulin, and recently the human myeloma IgG1(λ) protein, Mcg (129). In Kol, and also apparently in another immunoglobulin, Zie (58a), the crystal structure contains an unusual feature: The Fc occupies a number of different positions in the crystal that are not crystallographically related, with the result that no significant electron density occurs in this region of the crystal, there being an abrupt drop in density at the end of the hinge. In both Dob and Mcg there is a 15-amino-acid-residue deletion in the hinge (63, 169), thus, presumably, reducing the flexibility of the molecule and enabling the Fc to be located in the electron density, although in the case of Dob the crystals are disordered and do not diffract to high resolution.

The structures of three Fabs have been published, Newm (148), Kol (106), and McPC603 (152), as well as the structures of a number of V_L dimers and L chain dimers (4). The structure of human IgG Fc has been determined, and also its complex with protein A of *Staphylococcus aureus* (43). These structures have been reviewed most recently by Amzel & Poljak (4) and are not covered comprehensively in this review.

Because of the deficiencies in the crystals of the intact molecules, our knowledge of the whole antibody molecule has to be a sum of its parts. The flexibility of the molecule, in particular in the region between the Fab and the Fc, may preclude for some time visualizing directly by X-ray diffraction an intact molecule with intact hinge at atomic resolution. However, the checks that can be made on this composite three-dimensional picture of the antibody molecule are reassuring. Thus, the Kol Fab in isolated form in the crystal is quite similar to the Fab of the whole molecule in its crystal form. Also, the Fc in Dob has, within the experimental error of the comparison, the same overall structure as does the Fc in the isolated Fc crystals.

Fab Structure and the Antibody Combining Site

McPC603 AND THE PHOSPHOCHOLINE BINDING SITE The structure of McPC603 Fab, a mouse myeloma IgA (κ) with phosphocholine (PC) binding capability, has been determined at 3.1-Å resolution (41, 42, 125, 152) and is being refined to 2.7 Å (Y. Satow, D. R. Davies, manuscript in preparation). The overall three-dimensional structure of the Fab is illustrated in Figure 1, which demonstrates the strong lateral association between domains of the light (L) and heavy (H) chains, together with the relatively weak longitudinal interactions along each chain. Figure 1 also shows the clustering of six of the seven hypervariable regions at the tip of the Fab, forming the complementarity-determining surface (89, 90). The variable domains have a very similar three-dimensional structure for both the L and H chains and across species (4, 42, 123). The constant domains C_L and C_{H1} are also very similar. Both the variable and the constant pairs of domains are related by approximately twofold (rotation about the V axis of 180° will superpose V_L on V_H) and the angle between the two axes has been referred to as the elbow bend of the Fab and has been observed to vary

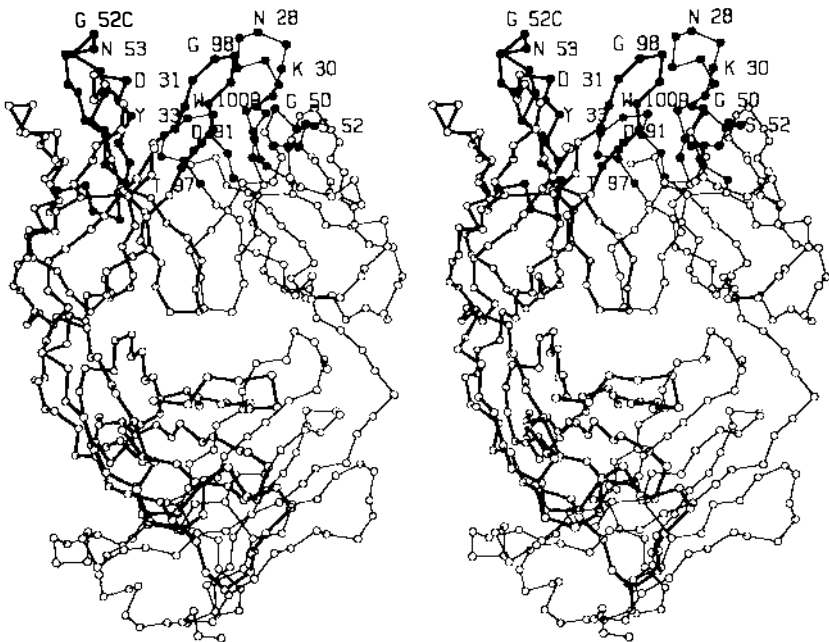


Figure 1 The α -carbon backbone of McPC603 Fab. The heavy chain is represented by the thick line. The two variable domains are at the top and the constant domains are at the bottom of the figure. The complementarity-determining residues (CDR) are shown as filled circles. Two residues in each CDR loop have been labeled.

between approximately 137° for Fab Newm (4), 135° for McPC603 (152), 147° for Dob (161), and approximately 170° for Kol (106).

Figure 2 shows the combining site of McPC603 with PC bound. (Y. Satow, E. A. Padlan, G. H. Cohen, D. R. Davies, manuscript in preparation). The choline is attached at the bottom of a pocket located principally between the hypervariable regions H3 and L3. The phosphate is on the surface and contacts residues from the heavy chain. It is apparent that PC is a small molecule and that the greater part of the hypervariable surface is not directly in contact with it. At the front of the pocket there are two hydrogen bond donors positioned within reasonable hydrogen bonding distance of the phosphate oxygens; these are the hydroxyl group of Tyr 33H and the guanidinium group of Arg 52H (152). The residues lining the inside of the pocket are Tyr 94L on the right side, Asp 91L on the left, Leu 96L at the back, (125, 146), and the side chain of Trp 100aH at the top left. In addition, the backbone of residues 92–94L form the lower rim of the front of the pocket.

CONFORMATIONAL CHANGE One of the mechanisms proposed for effector function activation involves an allosteric change upon antigen binding (110). Since crystals of immunoglobulins have large solvent channels and can bind to haptens soaked in through these channels, crystallographic investigation offers a direct way for observing conformational changes, when they occur.

When PC binds to McPC603 in the crystal, no significant conformational change occurs in the protein. There is a small movement of Trp 104a away

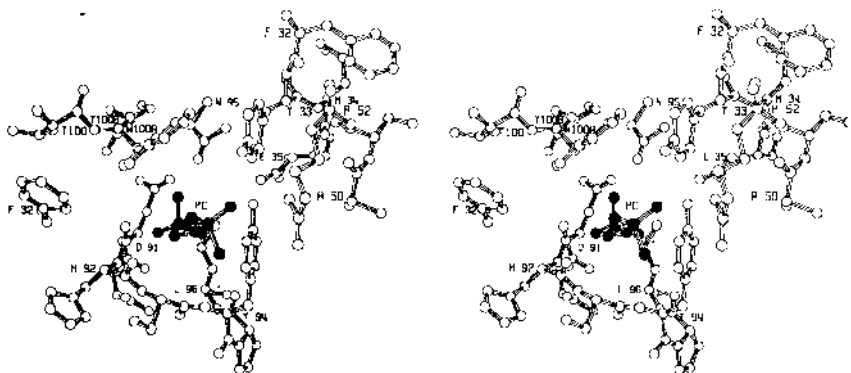


Figure 2 Stereo drawing of the combining site of McPC603 with phosphocholine bound. The lower residues (91–96 and F32) are from the light chain. The remaining residues belong to the three complementarity-determining regions of the heavy chain. The phosphocholine has the phosphate group in front with the choline moiety buried in a pocket.

from the pocket, but no other change of any significance. However, there are several reasons why it cannot be concluded from this observation that antigen-antibody interaction results in no conformation change:

1. The crystals are grown in a concentrated ammonium sulfate solution, and it has been observed that in the absence of PC there is a peak at the phosphate binding site interpreted to be a sulfate ion (126). A conformational change might have been triggered by the presence of this sulfate ion, so that no additional change would be observed upon PC binding. However, in this respect it should be noted that in Fab Newm, (148) no conformational change occurs upon binding of a neutral vitamin K₁ derivative.

2. PC is small and the association constant ($\sim 10^5 \text{ M}^{-1}$) with McPC603 might be insufficient to trigger a conformational change that could be induced by a larger, more tightly binding antigen. The same consideration applies to Fab Newm.

3. The only two structures at atomic resolution of immunoglobulins with known binding specificity rule out the possibility that changes that occur with the intact molecule might not occur with fragments (81).

Thus, although there is no support from X-ray diffraction for a conformational change associated with antigen binding, such a change cannot be rigorously excluded.

McPC603: THE CONTACTING RESIDUES AND THE EFFECT OF CHANGES IN THE COMBINING SITE The PC molecule is in direct contact with only a limited number of residues. They include side chains from all three heavy chain hypervariable regions and from one (L3) light chain region. The next most distant range of contacts contain many residues that play a role in positioning the directly contacting residues and changes in these might be expected to influence PC binding. An example of such a side chain is Glu 35H, a residue in the interface between V_H and V_L that makes a hydrogen bond with the hydroxyl of Tyr 94L, which is in turn a major contacting residue with hapten. A mutant of S107, a PC-binding myeloma protein, has been observed that has lost the ability to bind PC and also that fails to agglutinate PC-SRBC (144). Amino acid analysis showed that the mutation results in substitution of an alanine for glutamic acid in position 35H. Although a change of this magnitude is likely to produce a significant rearrangement of side chains in its vicinity simply because of the difference in volume of the two side chains, the loss of contact with Tyr 94L reduces an important constraint on a residue in direct contact with hapten.

Another mutant observed by Cook et al (38) is more puzzling. The mutant still bound PC, but it bound less well than S107 to PC coupled to

different carriers, and showed a decrease in affinity for a variety of PC-carrier conjugates. The only amino acid change observed was that of Asp → Ala in the fifth position of the heavy chain J region. Changes in the carboxy terminal half of V_L were not entirely ruled out, but they did not appear in a tryptic peptide analysis. The strange aspect of this mutation site is that it is spatially well removed from the PC pocket so that it might not have been expected to be involved in antigen binding. Another curious feature is that diverse carriers coupled to PC were all affected, which implies they all have some contact with this residue, or with a region influenced it.

SEQUENCE COMPARISON OF PC-BINDING ANTIBODIES Sequences and binding data are available for a variety of PC-binding myeloma proteins and monoclonal antibodies. They have recently been reviewed by Rudikoff (145) and are only discussed here in relation to the three-dimensional structure of McPC603.

The heavy chain The sequences of 19 heavy chains of BALB/c immunoglobulins that bind phosphocholine have been analyzed (74, 145). Ten of these are identical and employ the T15 sequence. The remaining nine differ by one to eight residues from the T15 sequence. Gene isolation and analysis have revealed that all 19 of the V_H regions must have arisen from the single germline T15 V_H gene segment (39). M167 is the most divergent protein with eight V_H substitutions. In both M167 and HPCG13 the same change (Thr at position 40) occurs, but all the other substitutions are unique, occurring in only one protein.

The PC-contacting residues Tyr33 and Arg52, together with Glu35, are present in all of these sequences. Similarly, all of the BALB/c sequences with the single exception of M167 contain a tryptophan at 100b. There is considerable variation in the D region for these proteins, accompanied by only relatively small changes in PC affinity, consistent with the fact that, with the exception of Trp100b, CDR3 of the heavy chain does not play a major role in defining the PC pocket.

The light chains The light chains of PC-binding antibodies can be represented by the three BALB/c myeloma proteins, T15, M603, and M167 (34, 35). These light chains differ considerably in sequence, despite the similarity of their corresponding heavy chain sequences. However, in the PC-binding region, in particular with the contacting residues Tyr94, Pro95, and Leu96, their sequences are the same. They also all employ the same J_L sequence (J5).

The invariant residues in these light and heavy chains provide strong support for the suggestion that these PC-binding antibodies all have the same overall combining site for PC (125), with differences in binding specificity being contributed by the amino acid substitutions.

Fab Newm The structure of the Fab of Newm, a human IgG (λ) myeloma protein, has been refined to a nominal 2-Å resolution (4, 148). The elbow bend is 137° and the L chain has a seven-residue deletion that includes residues 55 and 56 of CDR2, and 56 to 62 of the framework region FRIII.

The combining site is formed by the association of the remaining five hypervariable regions. The principal feature of the site is a shallow groove 15 × 6 × 6 Å deep, bordered by residues from the H and L chains. Fab Newm binds several haptens at this site with affinity constants ranging from 10³ to 10⁵ M⁻¹. A derivative of vitamin K₁ binds with the higher affinity and a crystallographic investigation has demonstrated that the menadione ring system binds in the shallow groove with the phytyl chain draped along the surface, making contact with a number of residues from the light and heavy chains (5). The number of contacts provided by the phytyl chain can probably account for the difference in binding between menadione and the vitamin K₁ derivative (10³ vs 1.7 × 10⁵ M⁻¹). As noted above, no conformational change is observed upon the hapten binding.

KoL The human IgG λ cryoglobulin Kol and its Fab both crystallize and both structures have been solved, the intact molecule at 3.5 Å and the Fab, for which the combining site specificity is unknown, at 1.9 Å resolution (106). The crystals of the intact molecule display an unusual form of disorder, described above, that prevents visualization of the Fc.

The Fab crystallizes well and has provided a detailed high resolution structure. The L chain conformation is quite similar to that of Fab Newm, except for the presence of the seven additional residues around CDR2. However, in the H chain, CDR3 is eight residues longer than Newm (and six longer than McPC603), and these additional residues fold into the combining site and fill

in Newm. This combining site is rich in aromatic side chains, being filled largely by Trp (47H, 52H, 90L and 108H), Tyr (35H, 35L and 97L), and His (59H).

In both the crystals of the intact molecule and the Fab, the same contact is made between the hypervariable surface and the hinge segment, Cys221-Cys230, the light chain C terminus Glu212-Ser214, and the residues 133–

138 and 196–199 of C_{H1} of a neighboring molecule. This contact involves considerable surface area and it has been estimated that $1314\text{\AA}^2$ of the Kol hypervariable surface is excluded from solvent. The contact is tight and closely packed and involves hydrophobic interactions as well as salt links and hydrogen bonds. Marquart et al (105) suggest that this interaction could be a prototype antibody-antigen interaction and might be responsible for the cryo properties of this molecule.

ANTIBODY COMBINING SITES Data on antibody combining sites have been comprehensively reviewed by Givol (76). Here, we only highlight a few structural topics.

What common features, if any, will the three-dimensional structures of the combining sites share? Givol (76) notes that the sizes of these sites are comparable to those of some enzymes. Lysozyme, for example, has a groove that will accommodate a hexasaccharide, and this is believed to represent about the upper limit in size for this kind of antigen (88, 102). The binding of antidextran can be divided into two classes: end-binders like W3129, which bind only the nonreducing end of the dextran; and middle-binders like QUPC52, which bind in the middle of the chain to runs of six glucose units. It has been suggested that the former type of antibody might have a pocketlike site, whereas the latter might be more likely to have a lengthy groove (33, 89).

Since three-dimensional structures are known for but two Fabs with known binding specificities, only a limited picture can be obtained from direct observation. It is nevertheless suggestive that one of these structures, McPC603, has a pocket for binding PC, whereas the other, Newm, has a shallow groove where the menadione binding site is located. Again, if we were to argue from analogy with enzymes we might expect a pocket or groove in most antibodies to provide specificity. However, specificity can also be produced by complementarity between two interacting surfaces without necessarily invoking grooves or pockets, and significant contributions to the free energy of interaction can come from exclusion of hydrophobic groups from contact with water. This kind of specificity, like the interactions between subunits in a multisubunit protein, can be quite precise and can be destroyed by single amino acid changes. For instance, not only is it necessary to maintain complementarity of two interacting surfaces, but there also needs to be a suitable juxtaposition of oppositely charged groups forming salt bridges as well as appropriate disposition of hydrogen bond donors and receptors. Accordingly, although antibodies specific for small antigenic determinants will probably have a groove or pocket, other antibodies specific for an array of amino acids such as epitopes on the surface

of a protein need not necessarily have these features. The Kol protein combining site, although similar in some respects to Newm, has its groove partially filled

this site interacts strongly with the C-terminal portion of another Fab in what could be a prototype antibody/protein antigen complex.

The availability of monoclonal antibodies to specific hybridoma technology should provide a significant increase in the diversity of antibodies studies by X-ray diffraction. In particular, they offer the opportunity to study interactions with ligands that are larger than simple haptens and that could completely fill the combining site. One example is an anti-influenza virus neuraminidase that has already been crystallized (37). More of these anti-protein antibodies need to be studied, both alone and complexed with antigen, to obtain structural comparisons with other studies (12, 57, 164). Other monoclonal antibodies to polysaccharides such as $\alpha 1 \rightarrow 6$ linked dextran should clarify the mechanism of binding for linear antigens (90) and the effects of amino acid changes on specificity.

MODEL BUILDING STUDIES OF Fv Since in the last 10 years high resolution structures have been determined for only three different Fab's, and only two (M603 and Newm) have known binding specificities, it appears that X-ray crystallography can only provide a small fraction of three-dimensional structures for interesting antibody combining sites. There is also the difficulty that only some Fab's can be induced to crystallize. An alternative approach to direct X-ray analysis is to utilize the knowledge available from crystallography together with known amino acid sequences to construct models of the Fv's of interesting antibodies. The general problem of protein folding, i.e. how a polypeptide chain several hundred amino acid residues long folds into its final globular form, is being extensively investigated. However, it is most unlikely that it will soon be possible to predict correctly the final

ertheless, the forces that contribute to the stability of a protein continue to be studied and are being refined. There is also an increased appreciation of the dynamic aspects of protein structure.

The problem of predicting antibody combining site structures is a special case with some features that make it an attractive candidate for investigation by molecular modeling. The similarity of variable domain structures is quite remarkable (4, 120-122), which indicates a strong conservation of the three-dimensional structure of the framework part of the variable domains. Also, the most variable parts of the V domains occur largely at one end of the domain in the hypervariable loops. As a result, a comparison of the sequence with that of V domains of known structure could, in optimal

cases, directly lead to a preliminary model that could then be refined by a suitable energy minimization technique. The problem becomes more complicated when there are amino acid insertions and deletions that change the length of the hypervariable loops. The new loops could be copied wherever possible from similar size loops in proteins whose structures have been determined. The $V_L:V_L$ dimers Rei and Au (60) provide an example of very similar three-dimensional structures associated with loops of the same length, although there are 18 amino acid differences in each domain. A different point of view would be inferred from the L chain dimer of Mcg (58) where corresponding hypervariable loops do not preserve the local twofold symmetry of the V domains but have different conformations as a result of interaction with neighboring molecules in the crystal.

The earliest modeling studies involved the 2,4-dinitrophenol-binding mouse myeloma protein MOPC315. The combining site of MOPC315 has been discussed (135) relative to the structure of Newm. Padlan et al (124) constructed a molecular model for MOPC315 that utilizes the framework structure of M603 and the hypervariable loops derived from other V regions that have CDR's of similar length whose structure had been determined by X-ray diffraction. An extensive nuclear magnetic resonance investigation of MOPC315 (54, 55, 186) and its interaction with ligands has led to a refined model that is similar to the original model, but has a different orientation for the DNP binding site (89). The crystal structure of the Fv of MOPC315 is being investigated and should ultimately provide a basis for evaluating these models (6). Subsequently, Davies & Padlan (40) constructed a model for the homogeneous rabbit antibody (BS5) to type III pneumococcal polysaccharide. Potter et al (136) presented a model for the inulin-binding myeloma protein EPC109. More recently, Stanford & Wu (167) have constructed a backbone model for MOPC325, and Feldmann et al (62) have proposed models for J539 and included a proposal for the binding of hexasaccharide. The crystal structure for J539 has been determined at 4.5 Å and the atomic resolution structure is under investigation so that it should soon be possible to test this model.

None of the models described above has been subjected to any form of energy minimization. They may give an approximate general, low resolution picture of the combining sites particularly where they illustrate some striking insertion or deletion, as in EPC109. However, they are unlikely to be accurate to better than several angstroms for the backbone atoms, and could be quite incorrect in positioning the amino acid side chains. Until, for at least a few cases, they are compared with the results of X-ray diffraction these models should be regarded as being quite hypothetical and should be treated with caution. In the case of MOPC315, the structure investigation by Padlan et al (124) was consistent with the known chemical data from

affinity labeling and did lead to the discovery of an error in the sequence determination, but these correlations derive from coarse rather than fine detail in the model. Since single amino acid changes can produce large effects on structure, it is perhaps optimistic at this stage to expect to define an antibody site with reasonable precision. Certainly, some powerful form of energy minimization will be necessary to ensure that the models produced do at least satisfy the basic requirement of stereo-chemistry. However, satisfactory prediction also requires a larger library of known structures of antibodies to a greater variety of antigens.

THE H:L ASSOCIATION For the combinatorial mechanism for generating antibody diversity to be reasonably effective, most light chains should have the ability to combine with most heavy chains. This requirement has been examined both *in vitro* and *in vivo*.

The γ -L interaction has been shown to obey second-order kinetics (7, 13, 22, 69) and has a high affinity with $K_a > 10^{10} \text{ M}^{-1}$ (13). When the competitive association of autologous and heterologous pairs of chains (i.e. pairs derived or not from the same myeloma) was examined, it was discovered that there was a preferred association with the autologous chain (46, 78, 104, 170). When heterologous V_k was added to Fd' , no significant association took place except in the presence of C_k . This was not true for the autologous V_k , which did not require the presence of C_k (96). Isolated V_k recombined with the autologous V_H , but heterologous pairs did not associate by the criterion of UV difference spectra (80). It is clear that the association of the two complete chains must be significantly aided by the presence of the constant domain of the light chain. Nevertheless, the preferential association for the autologous pair observed in these *in vitro* experiments does imply some form of prior selection. Whether or not any additional selection is needed other than that for antigen binding *per se* is not clear. Clearly, if the V_H and V_L do not associate at all or associate very weakly, then their effectiveness in forming a combining site will be greatly reduced.

The source of the variability in the $V_H:V_L$ association comes from the involvement of hypervariable residues in the $V_H:V_L$ interface, as noted previously (41, 42, 148). Figure 3 shows the residues that make contact across the interface between V_H and V_L in McPC603 (Y. Satow, D.R. Davies, manuscript in preparation). There are many highly conserved residues that interact, particularly those in FRII, such as Glu39. However, at one end of the interface a variety of interactions can be seen to occur between hypervariable and framework residues and between pairs of hypervariable residues. These must contribute to the forces governing the exact positioning of V_L relative to V_H . That this is not always the same even in related pairs has been demonstrated by Marquart et al (106) for the proteins

Kol (IgG λ) and Newm (IgG1 λ) where small differences ($\sim 9^\circ$) occur in the position of V_H relative to V_L that are outside the limit of experimental error.

In vivo results from cell fusion experiments demonstrated the existence of most of the possible "artificial" most, if not all, H and L chain can randomly recombine to produce new immunoglobulins that do not interfere with cell viability (114). This apparent contradiction raises the possibility that the preferential association is an in vitro artifact, but this would appear to be ruled out by the numerous very carefully controlled experiments. Alternatively, the difference in the affinities between autologous and heterologous pairs of chains may be so small as to be unobservable under cellular conditions.

When a hybrid H/L recombinant is formed, then there is the additional question of how its binding properties will relate to those of the parent molecules. This would appear to depend on how similar the parent chains are, and Sher et al (157) observed that with reconstituted H/L hybrids from

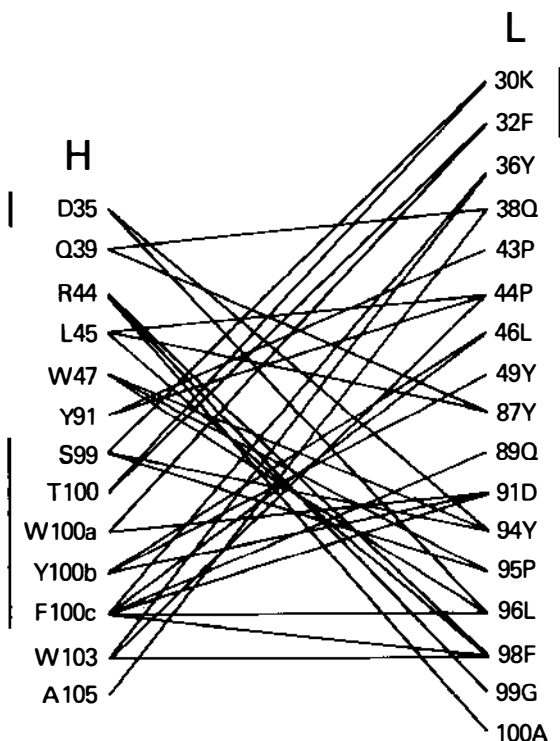


Figure 3 The residues that form the interface between V_H and V_L in McPC603. The vertical bars indicate the complementarity-determining residues. The connecting lines join those residues that have atoms within a distance of 4 Å of one another.

three anti-PC myeloma proteins, those with common idiotypes retained specificity upon recombination, whereas those without common idiotypes did not. Similarly, Manjula et al (103) showed that all the recombinants of anti-galactan myeloma proteins they examined showed anti-galactan activity comparable to the parents. However, as reviewed by Rudikoff (145), these anti-galactan heavy and light chains are so similar to one another that it is not surprising they exhibit the same binding properties. Recently, Kranz & Voss (97) examined the fluorescein binding of all the possible recombinants from six monoclonal antibodies to fluorescein and found that no hybrid has anti-fluorescein activity; the sequence and idio type of these antibodies was not analyzed.

The Fc

The human IgG Fc has been crystallized and the structure has been determined at 2.9-Å resolution, Figure 4 (43, 44). The structure differs from that of a Fab in that there is no protein/protein contact between the two C_{H2} domains. Instead, the complex carbohydrate attached to Asn297 occupies the interface region between the two domains with weak interactions between the two carbohydrate chains. The two C_{H3} domains associate in a manner similar to the $C_{H1}:C_L$ domains of the Fab. The two C_{H2} domains would seem to be positioned by the contacts made between C_{H2} and C_{H3} of the same domain and there is evidence that some flexibility can occur in this region. In the protein Dob, the Fc has a very similar structure to the isolated Fc (161), despite the absence of the hinge disulfide bridge due to a deletion of 15 residues in the heavy chain at this point.

The structure of Fc with a fragment of protein A from *Staphylococcus aureus* attached to it has been determined (43). Two contacts are made between the fragment B of protein A and two neighboring, symmetry-related Fc molecules. One of these, involving primarily hydrophobic interactions and believed to be the interaction occurring in solution, involves residues 251–254, 310–315 of C_{H2} , and 430–437 of C_{H3} . Since binding of protein A does not inhibit complement activation (192) and since the binding site of C1q has been reported to be on C_{H2} (195), the C1q-Fc interaction site must be located elsewhere on C_{H2} (see below and Figure 4).

The Hinge

The hinge region of the heavy chain is encoded for by a separate minigene (147) and varies in length (91) from five residues in IgG to 62 residues in human IgG3, where there appears to be a clear example of gene quadruplication. Marquart et al (106) in the intact Kol crystal observe the structure of the hinge to be double-stranded, disulfide-linked parallel chains, with each chain adopting a three-fold polypyrrolone-like helical structure.

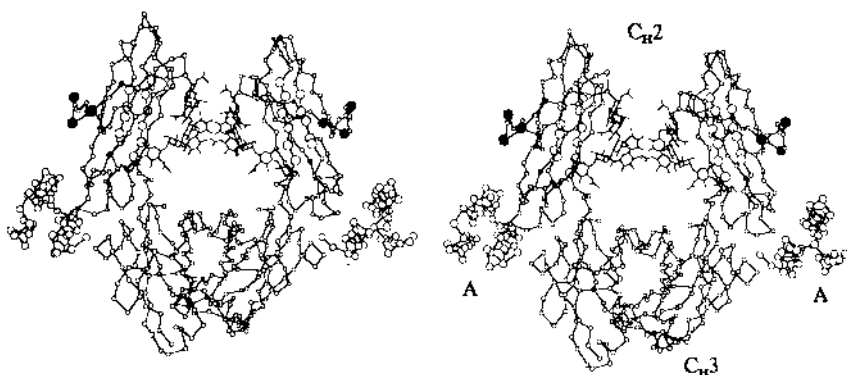


Figure 4 Stereo drawing of the α -carbon backbone of the Fc of human IgG bound to fragment B of protein A from *Staphylococcus aureus*. Based on coordinates obtained from the Brookhaven Protein Data Bank (43). The complex carbohydrate occupies the space between the two C_{H2} domains. The protein A is shown with clusters of open circles in the region between C_{H2} and C_{H3} . ●, ◐, and ○ on the Fc are the α -carbons of those residues, respectively, that have been proposed to bind to C1q in 138, 16, and 24.

Klein et al (91) have investigated the effect of major deletions in the hinge on the expression of biological effector functions. They found that proteins Dob and Lec, both of which have 15 amino acid deletions, leaving them with effectively no hinge peptide, could not express several effector functions. In particular, they showed no ability to bind C1q or to activate complement. However, neither displayed any difference with normal IgG1 in binding to protein A from *Staphylococcus aureus*. The three-dimensional structure for the Dob protein is approximately T shaped (161). In this protein the overall structure of the Fc region is very similar to that observed by Deisenhofer (43) in the crystals of the isolated Fc. Thus, there is no evidence that the lack of ability to perform effector functions is due to a major quaternary change (although minor changes would not be detected). The protein A binding indicates that the junction between $C_{\gamma 2}$ and $C_{\gamma 3}$ is not significantly changed. However, in the Dob protein the C_L and $C_{\gamma 2}$ domains are quite close to each other, and in the conformation observed in the crystal, they would block binding of a large molecule such as the head of C1q to the end of $C_{\gamma 2}$ proximal to the Fab. The presence of a somewhat rigid two-stranded hinge, with the strands linked by disulfide bridges and with each strand adopting a polyproline-like conformation (95, 106), would produce a greater separation of the Fab and Fc. Indeed, in IgG3, if the total hinge region adopted an extended rigid polyproline conformation (106), it would be more than 150 Å long.

Membrane Immunoglobulins on B-Lymphocytes

The unique characteristic of B-lymphocytes is the presence of membrane-bound immunoglobulin on their surface. The special nature of this immuno-

Table 1 Extra sequences of membrane immunoglobulin^a

Segment	Length	Net charge	Homology	Presumed disposition
I	12 (μ) 18 (γ)	-6 to -7	0.08 ^b (0.78) ^c	extracellular
II	26	0	0.65 (0.96)	intramembranous
III	3 (μ) 28 (γ)	+2 to +3	1.0 (0.71)	intracellular

^aData from IgG1, 2a, 2b, and IgM (142, 182, 194).^bFraction of identical residues at positions shared by μ and γ .^cFraction of identical residues at positions shared by γ .

globulin has recently been elucidated by sequencing the cloned DNA from Balb/c mice. In each case studied so far, the molecules differ from secreted immunoglobulins having a membrane-specific portion coded for by two exons, M1 and M2. M1 is separated from the exon coding for the carboxy terminus of the secreted immunoglobulin by 1.4–1.8 kbases and M2 in turn is separated from M1 by an intron from ~ 0.1 –0.8 kbases. The amino acid sequences show features common to other membrane proteins that have a transmembrane portion: Proceeding in the direction of C-terminus, several negatively charged residues are followed by a hydrophobic stretch that is followed by several positively charged residues (Table 1). It is likely that these represent regions disposed on the outer surface of the membrane, within the bilayer and on the cytoplasmic surface of the membrane, respectively. Tyler et al (182) have suggested that the presence of as many as six negatively charged residues in the stretch of 18 amino acids in the external segment of mIgG may preclude much secondary structure and thus lead to membrane hinge. It contains a cysteine in the middle of the sequence, which may form an interchain disulfide bond (182). In mIgM this region is five residues shorter and contains no cysteine.

A notable feature is the conservation of the hydrophobic sequence of 26 amino acids in the transmembrane portion. The three γ chains show only a single amino acid difference (albeit two base changes) in this segment and nine differences when compared to the membrane μ chains. This is much less divergence than is observed for the other constant region domains of these heavy chains. The conservation is also striking in view of the variability seen both in the hydrophobic leader sequences of various proteins as well as for the intramembranous segments of other (even related) integral membrane proteins (182, 194). That is, the mere requirement that the 26 residue segments are intramembranous would not necessarily lead to the prediction of the marked sequence homology observed. If the 26 hydrophobic segment is an α -helix with 1.5 Å per residue, its length would be ~ 4 nm, adequate to span the membrane bilayer. Moreover, this would cluster those residues

that have diverged on one "side," leaving the other aspect virtually invariant for the four isotypes for which information is available (23, 75). This raises the possibility that these mIg are interacting with another membrane protein (23, 75). It would be interesting to test this possibility by the use of cross-linking reagents or by isolating the mIg under conditions that appear to stabilize interactions between the subunits of other membrane receptors (141).

The hydrophilic segment of mIg presumed to be on the cytoplasmic aspect of the membrane is variable: For mIgM it appears to consist of only three residues (142), whereas for $\gamma 1$, $\gamma 2a$, and $\gamma 2b$ it is 28 residues. Among the latter, the variability increases towards the C-terminus; seven of the final 14 residues show exchanges.

FUNCTIONAL ASPECTS OF IMMUNOGLOBULINS

General Comments

CONSEQUENCES OF ANTIGEN BINDING TO ANTIBODY The combining sites of most functional proteins such as enzymes reflect a pervasive evolutionary trend. Once a structure capable of effectively cleaving the substrate has evolved, subsequent selective pressures promote its stability. The residues that contact the ligand are most preserved, whereas those that provide simply the scaffolding are less constrained from varying (reviewed in 189). With immunoglobulins the situation is virtually the opposite. It is those residues capable of contacting the antigen that show the greatest diversity, whereas the supporting "framework" residues tend to be more invariant (68, 193).

What is gained with such a system is a diversity of combining sites so great that at least some members of the repertoire are capable of binding to any given cluster of atoms, including epitopes that neither the individual nor even its species are likely to have encountered previously. What is lost is the capacity to catalyze a covalent change in the ligand in the way that the sites on enzymes, finely honed by evolution, can. Instead, the binding of antigen is made productive by other means. In some instances, this occurs simply by the antibody preventing the antigen from adhering to and penetrating vulnerable tissues (107).

In other instances, it is the ability of the antibody complexed with antigen to activate an effector system that is critical. For the most part, the effector systems recruited are cells. The number of different such systems corresponds much more closely to the number of distinctive Fc regions with which they interact than to the number of combining site specificities, but there is no simple one-to-one correspondence of heavy chain isotype to the

effector systems it can stimulate. Several different Ig isotypes may activate the same system and any single isotype can initiate several diverse effector reactions.

The effector systems are usefully divided into clonal and nonclonal classes. In clonal systems, the immunoglobulins on a single cell that play a critical role in its activation all bear the same antigen combining site. Within this class are the central elements of the immune response; the antigen receptors (membrane immunoglobulin) on B lymphocytes are a suitable example. In nonclonal systems, an activation event can be mediated by a mixture of immunoglobulins bearing different combining sites. These nonclonal systems constitute the principal mechanisms by which the organism actively rids itself of the offending antigen.

MECHANISM OF ANTIBODY ACTION The biochemical perturbations activated by the antigen-antibody complexes are many. Nevertheless, the preponderance of evidence suggests some common features. Just as the evolutionary mechanism that generates the diversity of combining sites precludes these sites from having enzymic activity, it seems equally unlikely that in each case the antigen would be capable of acting like a heterotropic allosteric modifier that would change the conformation of the Fc region reproducibly. Similarly, if only those antigens would be effective whose epitopes happened to be just so spaced that a reproducible distortion of the semi-rigid hinge region of the immunoglobulin would ensue, this would substantially limit the number of productive antigen-antibody encounters. These theoretical arguments are supported by considerable experimental data. The latter provide strong evidence against either an allosteric or distortive mechanism of antibody action. The data instead suggest that clustering of two or more Fc regions is the initiating event in most, if not all, of the effector systems activated by antigen-antibody complexes. The older data have been analyzed previously (109, 110, 190); some newer results are discussed in the review of specific

Clonal Systems

B-LYMPHOCYTES The consequences of antigen binding to the immunoglobulins displayed on the surface of B-lymphocytes are varied. They range from making the cells unresponsive to subsequent challenge (118a) to promoting proliferation, and differentiation into cells that secrete antibodies of different isotypes (80a). Elucidating those factors that are decisive in regulating the outcome is one of the central problems in immunology. It has become apparent that interaction with exogenous molecules and cells, as well as the state of maturation of the B cell itself, critically affect the

pathway chosen. Despite this complexity, it is clear that perturbation of the surface immunoglobulin can provide an essential signal without which nothing happens. The most persuasive studies are those in which the perturbation is induced by anti-immunoglobulin. The latter experimental tool permits examination of a population of B-lymphocytes that bears immunoglobulins of diverse specificity (115). With few exceptions (155, 156), those studies show that the critical common feature is the clustering of the surface immunoglobulin by bivalent antibodies or antibody fragments [$F(ab'_2)$]. Such protocols may be somewhat artificial [though see Rajewsky (139a)], but investigations with hapten conjugates have led to similar conclusions (49, 61). The latter more physiological experiments have other complexities associated with them, however.

At present, it is unclear how the size or number of immunoglobulin clusters on the cell affect the eventual outcome. It is also not clear whether there are other events associated with the clustering that are common to all the pathways the stimulated B-lymphocyte can eventually follow. The answers will not come quickly. Current available assessments of the outcome involve measurements temporally distant from the stimulus—often several days. What is sorely needed is to identify early events that presage the ultimate result. Acute changes, apparently resulting from the clustering of the surface immunoglobulin in and of itself, have been reported, e.g. mobilization of bound Ca^{2+} (18), association of the clustered immunoglobulin with the cytoskeletal matrix (17), and changes in the surface distribution of the mIg (177). These cannot yet be correlated with the final fate of the stimulated cells. Other perturbations reported [methylation of phospholipids, activation of a serine esterase, and generation of cytoplasmic factors (94)] appear to require additional exogenous factors (B-cell growth factor and interleukin 2) that are known to affect the cell's future activity. The quantitative role of the clustering of the membrane immunoglobulin has not yet been analyzed in those cases.

Although the immune system as a whole is unlikely to play a significant role in general homeostasis of an organism's internal environment, it obviously does have a variety of regulatory elements within the context of immune responses themselves. The mechanisms by which hormone receptors are controlled (down regulation, desensitization, amplification) are therefore likely to have their counterparts in the control of events mediated by the immunoglobulins on B-lymphocytes also, but appropriate systems for study of the latter are only now emerging.

Two other aspects of the activation of B-lymphocytes mediated by mIg are of interest. Since the mIg are mobile in the plane of the membrane [albeit not as mobile as might be expected from theoretical considerations (56)], it is reasonable to ask why spontaneous clustering of the mIg does not activate the cell if the association is a critical event. One answer is that

whereas it may be a critical event, it is likely to be inadequate in the absence of accessory factors, as already discussed. Nevertheless, in systems where such accessory factors are not required, a similar question arises (below). A second question of interest is the following apparent paradox (86). On the one hand, B cells bearing Ig with higher affinities for the antigen are activated preferentially when antigen is limiting (affinity selection) (162); on the other hand, multipoint attachment of antigen to mIg-bearing lymphocytes can be expected on theoretical grounds, to make the amount of antigen bound be quite insensitive to the intrinsic binding affinity of the combining sites on the mIg (86).

The two questions may in fact be related. What is likely to be the significant factor is the size and persistence of the clusters of mIg, whether they arise spontaneously or result from the interaction of antigen with mIg of low or high affinity (111). This subject is discussed further in the context of a simpler system (see below).

T-LYMPHOCYTES Thymus-processed lymphocytes are capable of mediating a variety of antigen-specific effects apparently without the use of exogenous immunoglobulins. The uncertain nature of the T-cell antigen-specific receptor that must be involved continues to be a major challenge with which immunologists are wrestling (85). Although these receptors may share common features with secreted or membrane-bound Ig, not enough is known to make them an appropriate subject for discussion in a review of antibody structure and function.

Nonclonal Systems: Cells Bearing Fc Receptors

Many cells interact with the Fc region of immunoglobulins (183). In those cases where we have the most information, the interaction is mediated by a plasma membrane glycoprotein usually of molecular weight 50,000–70,000. The binding may be more or less firm to the monomeric Ig and more or less specific for certain heavy chain isotypes and the species of origin. The nature of these “Fc receptors” (130) to which the Ig binds is outside the subject area of this survey; reviews on one or more of them have been published recently (70, 112, 183). Here we focus on two aspects only: (a) the sites on the Ig that interact with the Fc receptors, and (b) the role of the immunoglobulin in mediating the functions in which the receptors participate.

SITES ON Ig TO WHICH THE Fc RECEPTORS BIND

IgG The literature about the sites in the Fc region that interact with cellular receptors is confusing (reviewed in 2). In part, this arises because various investigators have studied immunoglobulins and cells from different

animals. The relationship between isotypes from different species is by no means a simple one nor can one assume that the receptors on alveolar macrophages, peritoneal macrophages, and peripheral blood monocytes will be similar even within a species, let alone from different species. The assays employed may also be important. Those in which multipoint attachment can amplify weak intrinsic association constants can be expected to yield results different from those in which direct (monomeric) binding is examined. Because certain types of fragments of Fc are more readily prepared from the Ig of some species than of others, numerous reports deal with the binding of Ig fragments to cells of a heterologous species. Data derived from such studies may be useful for providing specificity criteria for distinguishing different receptors, but it may confuse more than clarify physiologically significant interactions. We, therefore, focus principally on those studies involving reagents from the same or closely related species. The use of fragments, peptides, or mutant proteins even from homologous species may present additional problems of interpretation because of sequence homologies in the domains of immunoglobulins and related proteins. To cite just two examples, Ciccimarra et al (32) observed binding activity in a decapeptide from C γ 3 that, however, is likely to be buried in the native structure (51). Furthermore, β 2-microglobulin, the light chain of the class I major histocompatibility antigens, was found by Painter et al (128) to bind to macrophage Fc receptors.

In some instances binding of isolated C γ 3 modules to cell receptors can be observed. Human pFc' and C γ 3 fragments interact weakly with human monocytes, although not to human granulocytes (65, 66). A weak interaction has also been seen in an analogous combination with reagents prepared from rabbits (73) and mice (50) but not from guinea pigs (3).

Isolated C γ 2 domains are inactive but many studies demonstrate the involvement of these regions. Cleavage of the interheavy chain disulfides in the hinge region, which increases the segmental flexibility of C γ 2, reduces the binding activity of human IgG1 to human granulocytes (66, 73). Masking the interdomain cleft between C γ 2 and C γ 3 with a fragment of protein A from *Staphylococcus aureus* also interferes with the binding of human IgG1 to human granulocytes, but not to human monocytes (67).

Using variants of the mouse MPC 11 myeloma, Diamond et al (48) showed that the receptor for IgG2b on mouse macrophages recognized a segment in the C γ 2 domain and the receptor for IgG2a, sequences from both C γ 2 and C γ 3 domains. The complexity of these interactions is strikingly illustrated in a report on IgG produced by another variant of this myeloma. It fails to bind to the mouse macrophage receptors for IgG2a even though the variant has completely intact C γ 2 and C γ 3 regions of the 2a isotype (14). This mutant has hybrid heavy chains with the first 220 residues of the 2b isotype and the remaining residues (221 to the carboxy terminus)

of the 2a class. Cleavage by papain of the hinge region of this protein occurs one residue away from the normal site of cleavage, after residue 240 versus 239 for both native IgG2a and IgG2b. This shows that this region is conformationally different from that of the normal proteins. The fragment derived from such cleavage of the variant protein binds to the receptors perfectly well. These unusual results indicate that although the principal contacts between the receptors and IgG may be in the C γ 2 and C γ 3 domains, the hinge regions may indirectly affect binding by affecting the relationship of the Fab regions to the C γ 2 domains. Although the human myeloma proteins that have a deleted hinge have so far been tested only on murine macrophages, the results of those studies suggest an analogous phenomenon (95). A study of Facb fragments ((Fab-C γ 2)₂) in a homologous system has been reported by Johanson et al (87). They found that rabbit Facb bound very well to the Fc receptors on membranes of the yolk-sac. Notably, the reduced molecules, which dissociate into monomers of Fab-C γ 2, bound equally well, whereas neither the Fab nor the pFc' (C γ 3) fragments were active. Activity of Facb fragments albeit with Ig and macrophages from heterologous species has also been observed in certain instances, but not in others (cf. 8, 119).

IgE Several types of cells have one or more different surface receptors capable of binding IgE (26, 165). However, one of the receptors on mast cells and basophils is unique: Its affinity for monomeric IgE is at least 100-fold greater than that observed for any other Fc receptor for any other immunoglobulin. In the homologous human system, the Fc fragments ((C ϵ 2-C ϵ 3-C ϵ 4)₂) bind well, but fragments comparable to Facb, i.e. (Fab-C ϵ 2)₂, or pFc', i.e. (C ϵ 4)₂, do not (53). A report that a pentapeptide from the C ϵ 2 domain inhibited binding of the intact IgE (79) could not be corroborated in a detailed study (11). That in the rodent system such a peptide could displace bound IgE (185) is hard to reconcile with data showing that even intact IgE cannot displace bound molecules (187). By exclusion, it has been postulated that C ϵ 3 plays a major role in the interaction (53). This was supported by indirect data concerning the location of irreversible physicochemical changes induced by conditions that lead to irreversible changes in binding. Recently, the rates of tryptic cleavage of unbound IgE and IgE bound to solubilized receptors were compared in the rodent system (132). Whereas cleavage at all sites was inhibited, the most striking (>40-fold) inhibition was observed at a site likely to be close to the C ϵ 3: C ϵ 2 interdomain region. These results also point to the importance of the C ϵ 3 domains. It is interesting that by several criteria the latter domains may be homologous to the C γ 2 domains of IgG (53). There is evidence against the carbohydrate on the IgE contributing significantly to the binding affinity (98).

Overall, the results with IgG and IgE suggest that in most instances the penultimate carboxyterminal C_H domains play a significant, perhaps even the most important, role in the interaction between immunoglobulins and the Fc receptors on cells.

MECHANISM BY WHICH CELLS BEARING Fc RECEPTORS ARE ACTIVATED The Fc receptors on cells are implicated in a wide variety of immune mechanisms. Some of these can be meaningfully grouped together as involving immunoregulatory functions. Despite the apparent importance of this group, we do not consider it since the mechanism by which Ig mediates these functions is still largely unknown. A useful series of articles on the role of such Fc receptors in regulating the function of T cells has recently been compiled (116).

A second functional group that can be distinguished includes those receptors by which the cell directly or indirectly disposes of antigen. We discuss two of such functions for which the role of the Ig and its mechanism of action have been actively investigated.

Endocytosis It is likely that engulfment by macrophages of antigens that have successfully penetrated the organisms external barriers is quantitatively the most important mechanism for the disposal of antigens. This engulfment is markedly enhanced if the antigen has antibody bound to it (reviewed in 160). It has been known virtually from the time Fc receptors were discovered that antigen-antibody complexes are readily distinguished by the cell from uncomplexed IgG. This can be observed even when the concentration of the latter exceeds that of the former by four or more orders of magnitude (150). The evidence is overwhelming that the clustering of Fc regions engendered by the antigen-antibody interaction is necessary and sufficient to explain the selectivity. This has been rigorously analyzed theoretically and experimentally by Segal and his colleagues (150, 151, 154). These workers provide evidence that the Fc receptors cycle between the plasma membrane and the interior. They propose that the multipoint attachment that aggregation of Fc promotes can account for the preferential internalization of the complexed immunoglobulin (151, 154). That is, given a moving chain of cycling Fc receptors, the complexes of Ig simply hop aboard more readily. The important corollary is that the complexes need not generate any signal.

Secretion: macrophages Though endocytosis of immune complexes by macrophages can apparently be accounted for by a simple mechanism, this process seems inadequate to explain the stimulation of synthesis and secretion of various mediators by macrophages when challenged with antigen-antibody complexes (15, 27, 134, 139, 143, 176). In these instances, the cell

clearly has been activated to do something new. That the activation occurs on the surface of the cell is indicated by the results of Ragsdale & Arend (139) and Rouzer et al (143). The latter stimulated prostaglandin synthesis and secretion by beads coated with antibodies in the presence of cytochalasin D, which prevents phagocytosis. That immune complexes of guinea pig IgG2 induce superoxide anion production by homologous macrophages threefold more efficiently than IgG1 despite their being equivalently phagocytosed (176) could be similarly interpreted. In this and other studies (134), the greater efficacy of larger complexes was noted. Macrophages also have Fc receptors more or less specific for homologous IgE. Artificially dimerized IgE was sufficient to trigger release of lysosomal enzymes from the cells (47).

Secretion: mast cells Mast cells have receptors that bind IgE with extraordinarily high affinity. Moreover, triggering such cells via the IgE bound to their surface requires nothing more than something that will react appropriately with the IgE and (in most studies) a 1 mM pinch of Ca^{2+} . These factors have permitted a more complete investigation of this system than has been possible with any other cellular system activated by antibodies (113).

The current consensus is that IgE-mediated triggering of mast cells occurs when antigen, reacting with the cell-bound IgE, clusters the previously unassociated receptors for IgE. Three principal observations form the basis for this model: (a) the receptors in situ are univalent (108, 149); (b) the receptors are mobile (9, 28, 100, 149, 171); and (c) multivalent antibodies to the receptor mimic completely the action of the antigen (83, 84) in the total absence of IgE. The first observation indicates that the process is intermolecular rather than intramolecular; the second shows that the molecules that mediate the reaction need not be closely adjacent prior to triggering; and the third demonstrates that the important molecules are likely to be the receptors and not the IgE.

The mechanism by which clustering of these Fc receptors mediates degranulation is reviewed elsewhere (113). Here we indicate a few salient points only. In this system also, evidence shows that larger and more numerous clusters provoke more complete or rapid activation (64, 92) but that even dimers can provide a unit signal (64, 92, 153). In this system also (see above), the question arises of why, if clustering is all that is required, is spontaneous release not observed?¹ There is as yet no answer to this

¹It could be argued that since these cells have a desensitization mechanism that is also triggered by clustering (92), low levels of clustering are insufficient to cause release because of an unfavorable balance between the activation and deactivation. If this were true, one would then anticipate spontaneous desensitization. Since this has not been observed, the dilemma remains the same.

question based upon experimental data. It is possible that effective clustering cannot occur because of an energy barrier that is only overcome by the free energy provided by the binding of antigen to IgE. It is also possible that the lifetime of spontaneous aggregates is too short to induce a signal; even a ligand that binds relatively weakly to an individual IgE could dramatically increase the time the receptors remain clustered, thereby permitting the necessary molecular changes to take place (45). The interaction of mobile ligands (e.g. in a planar layer of lipids) at a limited interface with the cell-bound IgE is sufficient to cluster receptors (10, 188) and cause mediator release in this system (188), which demonstrates that rather indirect clustering is all that is required.

Other Although its functional role is undefined, isolation from human leukemic cells of an Fc receptor has been reported by Suzuki and his colleagues (173). On sodium dodecyl sulfate gels, this has a size similar to that reported by Thoenes & Stein (178), but in many of its characteristics it appears to be very different from the Fc receptors isolated by others (183). It is the first receptor for which an intrinsic activity has been claimed (phospholipase A2 activity), an activity stimulated by interaction with IgG in solution, particularly with aggregates of IgG (172, 175). The mechanism by which IgG stimulates this activity remains to be clarified. The same group has reported recently that similar results can be obtained for the receptor specific for IgG2b on the P388D₁ line of mouse macrophages (174).

Nonclonal Systems: Complement

CLASSICAL PATHWAY The activation of the classical pathway of the complement cascade provides a unique opportunity to study an effector system activated by antigen-antibody complexes. The receptor of this system, C1, can be readily isolated in amounts adequate to perform rigorous chemical analyses (140), electron micrographs are yielding detailed pictures of its complex structure (168), and sensitive molecular assays are available to assess its activation rapidly in solution (29). Nevertheless, it has been extraordinarily difficult to define precisely what is going on—a sobering spectacle to those interested in even the simplest cellular system!

Sites on Ig with which C1 interacts Defining the site of interaction of the globular heads of the C1q subunit of C1 with Ig is complicated by the observation that there may be several peptides derived from or related to immunoglobulins, which in their isolated form can interact with C1q (127, 128). Therefore, studies with fragments generated by procedures that involve even transient exposure to denaturants (e.g. those used to generate an

Fab₂ fragment) must be interpreted cautiously. Nevertheless, the weight of evidence favors interaction of native IgG via the C_H2 domains, although only one such domain in intact IgG appears to be bound by C1q at a time (127). One of the most convincing pieces of evidence for the importance of this region is that mutant IgG's, such as the human proteins Dob and Lec, fail to bind and activate C1 (95). These proteins are missing the hinge region but seem otherwise intact (see above). It appears likely that changes in the disposition of the Fab regions with respect to the C_γ2 domains is responsible (95)—an explanation virtually the same as that used to rationalize the failure of certain mouse IgG mutants to bind to Fc receptors (above), as well as for a variety of observations on the effect of reduction of the hinge disulfides in intact and fragmented IgG (reviewed in 52).

Burton et al investigated the site on rabbit IgG that interacts with C1q by using specific inhibitors, chemical modification, and an analysis of sequence conservation and of accessibility (24). Based on these results, as well as the work of others, they proposed that the site principally involves charged residues in the two carboxyterminal β -strands of the C_γ2 domains (residues 318–322; 332–337). A group in Madrid (16) correlated controlled conjugation of carboxylate groups on human IgG with inactivation and implicated primarily glutamic acid residues 258 and 293. The latter is in a region proposed by Brunhouse and Cebra from analysis of sequence data (20). Work with synthetic peptides led to the proposal that His 285, Lys 288, and Lys 290 were important in human IgG (138). On the other hand, deglycosylation of rabbit IgG also has been reported to decrease C1q binding (191). Results with concanavalin A also suggested that exposure of the carbohydrate might be important in one instance (99) but not in another (184). None of the approaches used distinguishes between direct and indirect effects. The results are depicted in figure 4, which shows the α carbons of the implicated residues. Notably, all these residues are well removed from the binding site for protein A of *Staphylococcus aureus*. Although the residues proposed by the three groups differ, they could all be correct. Firstly, the terminal atoms of the side chains may be topologically considerably closer than the positions of the α carbons of the same residues. Secondly, the site of interaction with C1q could be extensive, involving many amino acid side chains (cf Figure 3). Only procedures giving direct data will yield more precise information: either crystallographic visualization, or the use of cross-linking reagents and isolation of peptides that contain residues from IgG and C1q cross-linked to each other.

Because of some unusual aspects of the activation of C1 by IgM (below), the site(s) on the latter that interact with C1q are of interest. The C_μ4 domain is stated to fix C1 efficiently (21, 71), which supports earlier results with fragments of this domain (82). Nevertheless, as noted above with

respect to IgG, these results could be quite misleading. Although the findings of Siegel & Cathou (159) do not rule out the possibility that the C μ 4 domains are active, these workers interpreted their results on the effect of heating IgM as implicating the C μ 2 domains.

Mechanism of activation C1 by Ig Whereas some data in support of the need for aggregation of IgG (36) have been reinterpreted (166), sufficient other results indicate that Fc clustering is critically important for efficient activation (109, 110, 190). New observations on the lack of effect of antigen alone on the binding IgG to C1q (101, 181) and C1 activation (180) provide further evidence against allosteric effects being critical. That univalent rabbit Fab/c fragments (Fab-(C γ 2, C γ 3)₂) efficiently induced complement-mediated lysis of the lymphocytes to which they were bound (77) is a strong argument against a distortive mechanism.

Tschopp has observed that the relative efficacy of artificial oligomers to activate C1 parallels closely their relative affinity for C1q (179). A preparation of monomers of IgG fit the same pattern, but Tschopp could not determine whether or not this was due to contamination with traces of aggregates. It remains possible then that monomeric IgG could induce a signal either solely by its interaction with the globular portion of C1q or perhaps by reacting with the nearby (168) C1r. The role of aggregation could then simply be that of enhancing the binding by multipoint attachment (such as has been proposed for endocytosis by macrophages; see above). It would be useful to know whether or not with monomeric IgG more rigorously freed of aggregates activation was nevertheless observed.

Most studies with IgM suggest that only antigens that engage more than one subunit are capable of stimulating its ability to activate C1 (93, 137, 158), but the work of Koshland's group suggests otherwise (19, 30, 31). Although haptens were ineffective, they found that larger univalent antigens initiated IgM-mediated activation of C1. The mechanism is unclear by which these antigens, which are substantially bigger than what can be accommodated in a combining site, produce this effect. The authors propose several alternatives (31).

A new role for IgG in its interaction with the classical pathway has been described (25). It appears that C4 forms a covalent bond with the C γ 1 domain during the activation of the cascade. Whether or not the IgG plays more than a passive role in this effect is not known.

ALTERNATIVE PATHWAY Activation of the alternative pathway of complement fixation remains as the single exception to the rule that antibodies stimulate effector mechanisms via their Fc regions. It has been repeatedly demonstrated that F(ab')₂ fragments are active—as active as

intact IgG—but since this pathway can be initiated in the total absence of immunoglobulins (117), interpretations of the role of the antibody become more uncertain. Several studies suggest that the monovalent Fab' or Fab are inactive or much less active (59, 72, 133, 163), but a recent study reports otherwise (1). Different assays were used and the experts in this field will have to sort this matter out. One aspect about which there does seem to be agreement is that activation requires multiple molecules of antibody attached to the antigen.

ACKNOWLEDGMENT

We wish to thank Enid Silverton, who prepared Figures 1, 2, and 4.

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