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Regulation of Antibody Production Mediated by Fcγ Receptors, IgG Binding Factors, and IgG Fc-Binding Autoantibodies

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ABSTRACT: Fc receptors (FcRs) are immunoglobulin-binding structures that enable antibodies to perform a variety of functions by forming connections between specific recognition and effector cells. Besides eliciting cytotoxicity, inducing secretion of mediators and endocytosis of opsonized particles, FcRs are involved in the regulation of antibody production, both as integral membrane proteins and as soluble molecules released from the cell surface. Most FcRs belong to the same family of proteins as their ligands (immunoglobulin superfamily). This review contains recent data obtained by use of monoclonal antibodies and cloning studies on FcRs and FcR-like molecules. The importance of fine specificity of receptor binding site(s) — that of the conformation of FcRs and their ligands in triggering signaling mechanisms — is analyzed. The regulatory function of membrane-bound and -released FcRs; the correlation between cell cycle, FcR expression, and release; as well as the possible mechanisms of these phenomena are discussed.

KEY WORDS: Fc receptors, IgG binding factor, rheumatoid factor, B cell regulation.

I. INTRODUCTION

Immune complexes have been shown to be involved in the complex regulatory system controlling immune responses. Simultaneous passive administration of antibody and antigen results in suppression of antibody production. The feedback suppression can be determinant-specific[1] and may be caused by blocking of the corresponding epitopes.[1,2] In such regulation, the antigen-binding site bearing part of the antibody molecule may play a decisive role. However, it was shown that various forms of antibody feedback suppression are Fc-dependent, i.e., the intact Fc part of IgG antibodies is essential for induction of the down-regulation of humoral responses.[3–5] All isotypes of IgG are suppressive, and the down-regulatory mechanism operates only at low concentrations of antibody. The Fc-dependent suppression is not determinant-specific, affects both primary IgM and IgG responses, and does not depend on the presence of T cells. Concerning the mechanism of Fc-dependent feedback regulation, several possibilities were raised, and most probably various pathways may lead to identical results. However, the most important target of regulatory factors seems to be the B cell itself.

The Fc-dependent regulation of humoral immune responses is based on the interaction of the Fc portion of IgG and various Fc-binding structures, including membrane-bound Fcγ receptors (FcγRs), soluble immunoglobulin-binding factors (IBFs), rheumatoid factors (RFs), and Clq. Based on these interactions, a formal and functional basis of an isotypic regulatory network was proposed. Regarding the dual function of antibodies, its link to the idiotypic network, and in particular to components outside the immune system, was suggested.[6,7] The networks formed by functional interactions of idiotopes and paratopes on the one hand, and IgG Fc and Fc-binding

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structures on the other, maintain a steady state that returns to equilibrium after external or internal perturbations.

The Fc-dependent regulation of antibody production and in part the regulation of isotypes are linked to the interaction of IgG Fc and membrane-bound or soluble Fc-binding structures. It is remarkable that the binding of structurally different regulatory molecules of various origins to the Fc part of IgG might affect antibody production similarly. These interactions are highly influenced by the isotype and actual conformations of IgG molecules as ligands on the one hand and by some structural features of Fc-binding structures on the other. The actual phase of the humoral response at the time when the interaction of the partner molecules takes place is also decisive in the outcome of the regulation.

In this review, structural features of IgG Fc and those of FcγRs, IgG BFs, and RFs related to their regulatory function are discussed, the role of their interaction in antibody regulation is analyzed, and a hypothesis concerning the mechanism(s) of their regulatory function is raised.

II. STRUCTURAL FEATURES OF IgG AFFECTING THE INTERACTION WITH Fc-BINDING MOLECULES

A. Antigen-Induced Structural Alterations in the IgG Molecule

Antibodies of the IgG isotype play an important role as down-regulators of the humoral immune response. Although IgG antibodies can suppress the antibody response to specific antigen, the suppression is non-epitope-specific[8,9] and is Fc-dependent. The importance of IgGFc is supported by data showing that $F(ab')_2$ fragments are unable to induce suppression.[10,11] Moreover, monoclonal IgG antibodies with Fc parts lacking carbohydrate chains and unable to bind to FcRs also do not suppress antibody response. Finally, FcR-specific antibodies also reverse the ability of IgG to down-regulate antibody production. As this review deals with regulatory molecules interacting with IgG Fc, the following section discusses the structural features of IgG that may be decisive in the binding of regulatory molecules such as membrane-bound or soluble FcRs and Fc-specific antibodies (RFs).

One of the oldest dogmas in immunology is that the native antibody molecule is ready to bind the corresponding antigen but does not perform other biological functions. Several functions of antibodies depend on the structural units of the Fc part as well as on structural alterations in this part of the molecule as secondary manifestations of antigen-antibody interactions. It has also been suggested that this conformational reorganization is responsible for the expression of hidden determinants in the Fc part of the immunoglobulin molecules, and, consequently, responsible for activation of effector mechanisms. The nature of these steric alterations, as well as the characterization of the interdomain and intermolecular interactions involved in the induction of effector functions, has been studied intensively but is still not understood entirely.

The supposition that the conformation of complexed antibodies differs from that of native counterparts was based, in the beginning, mainly on the observation that anti-immunoglobulin antibodies raised by immune complexes might react with hidden determinants of the native molecules as well. Clear evidence for one type of conformational change in the immunoglobulin molecule following antigen binding was provided by electron microscopy showing that two Fab fragments of IgG can vary from 10 to 180° on the antigen binding.[12,13] Although the introduction of other sophisticated methods (such as high-resolution crystallography, small-angle X-ray scattering, NMR, etc.) allowed the measurement of some hapten-induced structural changes, the nature of the antigen-elicited alterations in the antibody molecules is still much discussed. Three alternative models were postulated to explain the molecular mechanisms, leading to the well-known functional consequences of antigen binding.[14]

The *allosteric model* assumes that the antigen, owing to its chemical structure, induces conformational changes within the antibody-combining site, leading to the expression of secondary binding sites.

The *distortive model* suggests that antigen binding induces distortion in the relative position of the domains, resulting in effects similar to those postulated for the allosteric model.

The *associative model* proposes that the polymerization of the antibody molecules by the multideterminant antigen is a necessary condition for triggering the antibody-mediated functions.

The allosteric model is an attractive hypothesis without sufficient experimental support. Based on X-ray data, it was suggested that as the consequence of interdomain contacts the antibody molecule becomes more rigid following antigen binding. It was also supposed that the allosteric change involves a folding back of the hinge peptides between the CH2 domains and the formation of longitudinal contacts between the CH1 and CH2 domains, and/or the hinge region transfers the conformational alteration to the Fc.[15,16] Other data also support (or at least do not exclude) the possibility that longitudinal interdomain interactions following antigen binding pave the way for signal transfer. Based on observations using circular polarization of luminescence, it was suggested that antigen-produced conformational alterations in the Fab trigger conformational changes in the Fc region as well.[17,18] The influence of antigen binding on the longitudinal interdomain interactions was supported by hydrogen-deuterium exchange data showing that the observable exchange rates were affected by the binding of hapten.[19] The results of several other studies, however, are not consistent with these possibilities.[20–22]

The term *distortion* refers to alterations in topological distribution of molecular segments, the change of the intramolecular geometry of the antibody (e.g., that of the angle between the Fab and *Fc regions*). The main point in both the allosteric and the distortive models is that due to alterations in the molecule, a single antibody molecule without association is also able to trigger different functions. This possibility has been more or less neglected, because the greater efficiency of highly associated antibody molecules in triggering effector functions is unquestionable when compared with oligomers and cyclic monomers. Nevertheless, one has to consider the importance of the triggering capacity of single molecules as the consequence of their distortion; especially in the case of molecules inserted into the plasma membrane, where even the slightest structural alterations may have striking effects on the surroundings. Several findings support the view that the size of complexes and the number of associated antibody molecules are not the only factors determining the ability of complexes to trigger the effector functions. The intramolecular geometry of the single IgG molecule might also affect triggering.

The investigation of the consumption of total complement in guinea pig serum by interaction with complexes formed with type III oligosaccharides of different length has shown that smaller haptens than 28-saccharide did not influence complement fixation. Moreover, circular polarization of luminescence was limited to the Fab domains only when complexes were formed with short oligosaccharides, whereas spectral changes attributed to the Fc region were observed when larger haptens were used.[23,24] Comparing the complement-activating capacity of monomeric complexes, it was also shown that complexes containing the smaller bivalent hapten (16-sugar oligosaccharide) were inactive in contrast to the 26-sugar-containing complexes, which exhibited significant complement-activating capacity. These monomeric complexes proved to be cyclic monomers. The main difference between the closed monomers was in the length of the spacer separating the determinants reacting with the single binding sites of the antibody molecule.[25,26] These data are in good agreement with Pecht et al.'s[27] observations showing that the cyclic monomers formed with the lysozyme bis-loop antigen and the corresponding antibody activate complement.

We have to stress here, however, that, according to Metzger,[14,28] the data demonstrating antigen-induced allosteric or distortive alterations in the antibody molecule are not convincing. Simply the approximation of two or more otherwise unmodified Fc regions might be a sufficient stimulus.[22,29] Recent X-ray diffraction studies comparing uncomplexed antibody with antigen-antibody complexes also could not prove conformational changes transmitted into the Fc part of the antibody molecule by the binding of antigen.[30,31]

As structures interacting with the Fc region of IgG differ in their affinity and mono- or polyvalent binding capacity, and also in the nature and localization of interaction sites, the abovementioned conformational alterations are not equally restrictive in these intermolecular con-

tacts. Another important aspect of this kind of interaction is the possibility for simultaneous binding, resulting in limitations for molecular rearrangements.

B. The Flexibility of IgG Molecules

Their great flexibility enables antibodies to form productive links between specific antigen and effector sites. The flexibility of antibodies can be characterized as segmental flexibility, which in the case of IgG molecules in solution may be localized to the V-C dimer (elbow flexibility), to the Fab parts $F(ab')_2$ flexibility, and to the region between the Fc and $F(ab')_2$ [$Fc(F(ab')_2$ flexibility]. The domains themselves are not flexible.[32] Recent data point to the rotation of Fab arms[33] and wagging the Fc part.[34] The segmental motion within the IgG molecule is mediated mainly by the hinge region, which allows the Fab parts to alter their positions relative to the Fc part. The structural features of the hinge may influence the access to functionally important binding sites. Recently it was also shown that the FcγRI-binding site is located in the lower hinge region itself. This is why some structural and functional aspects of the IgG hinge need to be discussed here.

The area of the IgG molecule located between the CH1 and CH2 domains is called the hinge region. From the study of monoclonal immunoglobulins with structural defects involving a deletion of residues corresponding to the entire hinge region, it was concluded that the rigid hinge region, stabilized by its constituent disulfide bonds, acts as a spacer limiting the degree to which the Fab regions can approach the Fc region and cause steric blockade of effector sites located in the CH2 domains.[35,36]

The hinge region can be divided into three parts.[37] The core hinge (Lys 226 to Lys 229) has a relatively stable conformation hardly influenced by the presence or absence of the Fab and Fc regions. The lower hinge (Pro 230 to Leu 234) is flexible; its conformation seems to be independent of the Fab region but dependent on the intact core. The upper hinge (Lys 222 to Thr 225) is the most flexible part. A correlation was found between the length of the upper hinge, complement-activating capacity, and segmental flexibility of the molecule.[38,39]

In sequence terms the hinge in human IgG3 molecules shows remarkable differences when compared with that of the other IgG subclasses. IgG3 has a very long hinge of about 70 amino acids, including 21 proline and 11 cysteine residues.[40] The hinge of IgG3 is extended in spatial terms as well (about 14 nm).[41] The hinges of the other subclasses resemble each other more closely; although, according to Burton,[37] that of IgG1 is somewhat more extended than those of IgG2 and IgG4.

It is now generally accepted that the hinge modulates the functional activity of IgG antibodies. There is a well-defined correlation between the shape of IgG molecules of various isotypes and their complement-activating capacity. More compact antibodies such as IgG2 and IgG4 are less efficient in this respect than IgG3 and IgG1. Probably the different movement of Fab arms influences differently the accessibility of the Fc domains. However, it is emphasized by Burton[42] that flexibility alone cannot be responsible for hinge modulation of antibody functions. It seems that the CH1 domain also plays a specific role in modulating the functional activities of the Fc region.[43]

C. The Possible Role of the Carbohydrate Moieties

IgG molecules possess associated carbohydrate chains. It is remarkable that the glycosylation of the CH2 domain has been conserved throughout evolution. Although no obvious function could be attributed to the sugar components of IgG, one cannot exclude their possible influence on the conformation of the molecule, and, consequently, on the accessibility of functionally important binding sites within the Fc domains. The two carbohydrate chains of IgG, consisting of a set of about 20 structures based on a mannosylchitobiose core, are linked to the Asn 297 residue of each CH2 domain.[44] Remarkable structural variations of the carbohydrate moiety of IgG were described, but no correlation between this heterogeneity and IgG isotype was found.[45] The spatial structures of the two car-

bohydrate moieties linked to the CH2 domains have shown some differences in composition and mobility. The structural function of these chains is most probably their contribution to the typical spacing of the CH2 domains.[46,47] Nonglycosylated IgG retains antigen-binding capacity but is unable to bind some FcRs and activate the complement.[11] The interaction of mouse IgG2a with human monocyte FcR was also shown to be markedly affected by the loss of the CH2-domain oligosaccharides. It was concluded that the decreased FcR affinity shown by aglycosylated IgG2a is compatible with an effect on overall Fc structure, which perturbs the FcR binding site.[48]

Because unglycosylated IgG molecules are unable to suppress the antibody response as well, one cannot exclude the functional importance of the carbohydrate moieties of IgG in conferring the molecular conformation necessary for the induction of these Fc-dependent regulatory functions.

III. INTERACTING SITES IN IgG FOR Fc-BINDING MOLECULES

The regions that serve as binding sites for molecules interacting with IgG and play a role in triggering effector functions are located mainly in the CH3 and CH2 domains. Some of them are accessible in the native molecules, whereas others are in hidden positions and become available only after conformational alterations. Therefore, the mapping of these binding sites is a fairly complicated task, and some of the results are conflicting. For this reason, before reviewing experimental data on mapping of the binding sites on Fc, it is necessary to summarize data that shed light on the contact between various parts of the IgG Fc region.[49]

It is well known that CH3 and CH2 domains exhibit a bilayer structure.[50,51] There are two antiparallel beta pleated sheets in each domain that are linked by intradomain disulfide bonds. The two CH3 domains interact strongly through their first sheets, and due to this close association a relatively large surface area is in a buried position. The first sheets of the CH2 domains are covered by the sugar moieties of the molecule (i.e., parts of the surface of the CH2 domains are hidden). There is no direct contact between the two CH2 domains, although the carbohydrate components are in weak interaction with each other. The longitudinal association between the two Fc domains is significant, as this type of interaction also results in parts of the surface areas of the domains being buried.

The members of the immunoglobulin superfamily can be characterized by very similar, preserved three-dimensional structures.[52] In this respect the IgG isotypes, including their Fc parts, show a high degree of similarity.

A. Binding Sites for C1q

Although the activation of the complement system by complexed IgG is not the subject of this review, the localization of binding sites that interact with the first component of the complement cascade should be mentioned here. Namely, the mapping of binding sites for FcR and RF very often demands a comparison with the location of groups interacting with C1q, which is present in body fluids at relatively high concentration. C1q not only is found in body fluids, but also is able to bind to specific receptors expressed on immune competent cells.

Human C1q is a hexavalent molecule with a very atypical structure. The complex (composed of 18 polypeptide chains) resembles a bouquet of six tulips, with the helical regions representing the stalks and the globular regions the flowers (the latter containing the IgG binding site).[53] Two binding sites per C1q head are supposed.[54] Monomeric IgG3 and IgG1 are able to fix the complement; however, only the serum concentration of IgG1 is sufficient to interact with C1q (and occupy about 30% of C1q binding sites). Native IgG4 does not fix complement. The structures accountable for C1q binding seem to be located entirely within a single domain.[55,56]

The aggregation of IgG results in increased C1q binding.[57] However, the reasons for this are not clear and the explanations are conflicting. One could expect that conformational alterations due to aggregation of IgG molecules could promote multivalent interactions with C1q. However, the critical analyses of Metzger[14,22,28] and recent data of X-ray diffraction studies compar-

ing uncomplexed antibody with antigen-antibody complexes rule out the role of conformational alterations in this respect.[30,31] Studying the binding of C1q to antibody-coated cells showed that C1q binds preferentially to the region of highest antibody concentrations and that the increased affinity of binding is based on the multivalent character of C1q.[58] Experiments showing that antigen binding to the antibody does not influence C1q fixation[59] speak also against the importance of conformational alterations in IgG.

Regarding C1q binding sites, even the early studies showed that C1q interacts with the CH2 domain of IgG.[60,61] Concerning the localization of binding sites for C1q in the CH2 domain, the importance of Trp 277 and the flanking residues (Lys 274–Gly 281) was raised by Boackle et al.,[62] describing this as a "cryptic" site that might be exposed only when antibody interacts with the antigen. Other observations,[63] however, did not confirm this suggestion.

Several other C1q binding sites were later suggested, including Lys 290 through Glu 295[64] and His 285 through Arg 292.[65] The latter region contains the positively charged residues His 285, Lys 288, Lys 290, and Arg 292 in human IgG1. Because the comparison of this region in IgGs that interact with C1q raised the problem that C1q binding would require either species specificity or the capability of C1q to interact with various structures, Lukas et al.[65] suggested that complement-binding IgGs with different sequences compensate by use of proximate residues. According to Burton et al.,[56] the C1q binding site involves residues in the last two antiparallel β-strands of the CH2 domain, containing several charged residues that may have importance in IgG-C1q interaction. Recently, by altering residues that are expected to be expressed on the surface, Duncan and Winter[66] suggested residues Glu 318, Lys 320, and Lys 322 as the minimal residues that are essential for C1q binding in human IgG1. These residues are located on the outside face of the CH2 domain, allowing direct contact with the C1q. A peptide mimic of this region was able to inhibit complement lysis. The region was suggested as a possible conservative binding site by Burton.[42] This group of residues can be found close to the hinge region and might become obstructed in hinge-restricted immunoglobulin isotypes that do not fix C1q.

The removal of the carbohydrate resulted in decreased C1q binding.[67] Aglycosylated mouse monoclonal IgG2b antibody lost its complement-activating capacity,[68] whereas in the case of IgG2a slight reduction in C1q binding affinity was found as a result of aglycosylation.[48]

All of these partly conflicting data show that the unequivocal localization of the C1q binding site(s) in the CH2 domain of IgG has not been accomplished yet.

B. Binding Sites for Membrane-Bound Fcγ Receptors

1. Human Fcγ Receptors

IgG binding to macrophages was first described by Berken and Benacerraf,[69] and the term *Fc receptor (FcR)* has been generally accepted since the early 1970s. According to the definition of the IUIS Subcommittee on Nomenclature, FcRs are "a group of surface membrane molecules that specifically bind homologous immunoglobulins via the Fc portion and which putatively mediate biological functions."

A wide range of cells express FcRs, most of them belonging to the immune system (including monocytes, macrophages, granulocytes, K cells, B cells, and some T cells), but FcRs are present in membranes of other cell types as well. Molecules belonging to the family of FcRs show amazing heterogeneity in molecular weight, binding affinity, and cellular distribution. The functional versatility of FcRs is partly the consequence of this structural heterogeneity and partly that of the cooperation between FcRs and other cell surface components. The activities mediated by these receptors are to a certain extent effector functions. From this point of view, the immunoglobulin-binding receptor represents an important tie between the antibodies and the effector cells such as macrophages, K cells, and granulocytes. The antibody complexes with the antigen and triggers the effector cells for phagocytosis or killing via their Fc receptors. The cross-linking of FcRs with each other and/or with other mem-

brane constituents induces not only effector activities in the strict sense, but secondary events as well, including release of enzymes, prostaglandins, and leukotriens. Among the latter, one may find highly active molecules that are involved in the regulation of immune phenomena. This leads to another level of FcR function, namely, their role in the regulation of immune responses.

The structural heterogeneity, especially the differences in membrane anchoring and transmembrane and cytoplasmic parts, explains some functional differences as a consequence of the ability of FcRs to open various pathways of transmembrane signaling. According to recent data, three groups of immunoglobulin-binding receptors can be recognized (summarized in Table 1).[70]

In a wider sense, soluble FcRs (immunoglobulin-binding factors), C1q and Fc-binding structures on microorganisms (such as FcRs on parasites, bacterial FcRs, virally encoded FcRs) belong also to the family of immunoglobulin-binding structures. In this review we concentrate on human receptors for IgG. Concerning their structure and function, their binding properties and role in regulation of antibody responses of membrane-bound and soluble IgG FcRs will be discussed first.

Three classes of human IgG-binding FcRs have been defined by Anderson and Looney[71] by using monoclonal antibodies recognizing FcR epitopes. The three types of receptors are expressed on various and overlapping populations of cells (Table 2).

FcγRs consist of one polypeptide chain having the same general organization and are encoded by a cluster of closely related genes, situated in the long arm of chromosome 1. With the exception of the phosphatidyl-inositol- (PI-) linked FcγRIII, the FcγRs consist of one extracellular part containing several disulfide-bonded immunoglobulinlike domains, a single transmembrane segment, and an intracytoplasmic hydrophilic tail.

The high-affinity FcγRI (CD64) is a 72-kDa glycoprotein[72] that is expressed mainly on mononuclear phagocytes; the receptor is inducible in human neutrophils by interferon-γ.[73] The rank order of affinity of FcγRI for IgG isotypes is IgG1 = IgG3 > IgG4; it does not interact with IgG2. The receptor is trypsin resistant, and the core protein (after removal of N-linked carbohydrates) has an M_r of 40,000.[74]

Recently, molecular cloning studies have shown that the extracellular domains of FcγRI comprised three immunoglobulinlike domains.[75,76] The first two bear homology with the low-affinity FcγRs (which possess only two extracellular domains). The third domain is structurally unique and may be responsible for the high affinity of the receptor. Three different transcripts have been cloned, two of them representing polymorphisms, while the third shows

TABLE 1
Immunoglobulin Binding Receptors

Receptor	Cell
Immunoglobulin superfamily	
FcγRI	Macrophages, IFN-γ-activated neutrophils
MHC class I-like FcRs	Intestinal epithelial cells
FcεRI	Mast cells, basophils
Poly-Ig FcR	Epithelial cells
FcγRII	Monocytes, platelets, neutrophils, B cells, K562
FcγRIII	Neutrophils, NK, K cells, mononuclear phagocytes
Lectin family	
Asyaloglycoprotein-receptor-binding IgA	Hepatocytes
MAC-2, binds IgE	Macrophages
FcεRII	Platelets, macrophages, eosinophils, B cells, some T cells
Structure unknown	
Low-affinity IgA receptor	B lineage, activated T cells
Low-affinity IgM receptor	B lineage
IgD receptor	T cells
IgE binding factor homologous to retroviral product	

TABLE 2
Human Fcγ Receptors

FcR	Mr	Characteristics	Expression	Affinity for human isotypes	Binding of murine IgG
FcγRI CD64	72,000	High affinity	Mononuclear phagocytes	IgG1 = IgG3 > IgG4, does not bind IgG2	IgG2a and IgG3
FcγRII CD32	40,000	Low affinity	Monocytes, platelets, neutrophils, B cells, K562	IgG1 = IgG3, less readily IgG2 and IgG4	IgG1
FcγRIII CD16	50,000–70,000	Low affinity	Neutrophils, NK, K cells, mononuclear phagocytes	IgG1 = IgG3	

diversity in the cytoplasmic domain. It seems that more than one gene is responsible for encoding the FcγRI.

FcγRII (CD32) is a protein of 40,000 M_r[77] and is widely distributed on a variety of cells such as monocytes, platelets, neutrophils, and B cells. FcγRII has low affinity[78] (the equilibrium affinity constants [K_a] are less than 10^7 M^{-1}); hence, it requires multiple Fc interaction (aggregated IgG) and binds IgG1 and IgG3 equally well, while less readily binding the other subclasses. The receptor is structurally polymorphic, and monocytes from different donors bind either strongly or weakly murine IgG1.[79]

Recently, several reports dealt with the genomic organization of genes encoding for the FcγRII in humans,[75,80–86] which seem to be more complex than those of the murine system, and elucidated the structural heterogeneity of the human FcγRII.

Two distinct but similar cDNA clones encoding isoforms of the FcγRII differentially expressed either by human B cells or activated T cells and monocytes were described. The nucleotide sequence of the cDNA clone from B cells (βFcγRII) contains an open reading frame that encodes a protein with an extracellular domain of 179 amino acids. It has four evenly spaced Cys residues, suggesting the existence of two domains that may contain three potential N-linked glycosylation sites. The presumed transmembrane domain consists of 26 residues and is followed by four positively charged amino acids that begin a cytoplasmic domain of 44 amino acids. The deduced amino acid sequence of the protein encoded by clones from peripheral T cells and monocytes (αFcγRII) shares an extracellular domain of 179 amino acids with two presumptive sites for N-linked glycosylation and a transmembrane domain of 26 residues, whereas the cytoplasmic domain consists of 76 amino acids.

The predicted relative mass of both molecules is consistent with the observed M_r of 40,000. The protein encoded by βFcγRII has a deduced M_r of 27,000 with three, whereas the one encoded by αFcγRII has a deduced M_r of 31,000 with two, N-linked glycosylated sites. The human receptors have almost identical extracellular and transmembrane domains that are very similar to those of the mouse β2FcγRII product. On the other hand, the human clones code for very different signal peptides and cytoplasmic domains.

The low-affinity FcγRIII (CD16), expressed primarily on neutrophils, NK, and K cells and to some extent on monocytes and macrophages,[87] has an M_r of 50,000 to 70,000. FcγRIII expressed on neutrophils is different from that present on tissue macrophages.[88,89] The receptor shows preferential binding of IgG1 and IgG3. In comparison with the other classes of FcγRs, the number of copies of FcγRIII expressed on the cell membrane is relatively high; for example, 10^3 FcγRII are present on platelets,[90] while a few hundred thousand copies of FcγRIII are expressed on neutrophils.[91]

Two distinct forms of the low-affinity FcγRIII were described. FcγRIII expressed on neutrophils was shown to be anchored to the outer lea-

flet of the plasma membrane by a glycosylphosphatidyl-inositol moiety (FcγRIII.PIG).[92,93] On the other hand, FcγRIIIs present on NK cells, macrophages, and cultured monocytes are transmembrane proteins (FcγRIII.TM).[92,94]

The cDNA clone for FcγRIII isolated from a human leukocyte library was found to encode a 46-kDa phosphatidyl-inositol-glycan-linked membrane protein (FcγRIII.PIG).[92,95–98] The receptor had an extracellular domain consisting of two immunoglobulinlike loops that were most homologous to human FcγRII and mouse FcγRIIa, yet it contained six sites for N-linked glycosylation. The difference between cDNAs corresponding to FcγRIII.PIG and FcγRIII.TM was characterized by single-nucleotide substitutions. Due to one's converting the termination codon UGA (PIG) to CGA, the predicted cytoplasmic domain was extended by 21 amino acids.[95]

An alloantigen (NA) recognized by autoantibodies was shown on human FcγRIII.[99] NA1 and NA2 antigens were reported to be neutrophil specific. The monoclonal antibody CLBgran11 recognized one polymorphic form of the NA system (NA1) expressed on neutrophils, whereas the same antibody did not detect FcγRIII on NK cells.[100]

A molecular basis for the NA1/NA2 allelism has been defined by Ravetch and Perussia.[94] Nucleotide differences between NA1 and NA2 were found only in III-1 genes and their transcripts, and the allelic differences were assigned to Ser 65 and Val 106.

Two genes (III-1 and III-2) encode FcγRIII in humans,[94] differing by 10 single nucleotide substitutions in the coding regions. III-1 is expressed exclusively in neutrophils, whereas III-2 is expressed both in NK cells and in macrophages. The III-1 transcript generates the GPI-linked molecule, while the III-2 transcript is translated to the transmembrane form. Remarkable functional consequences result from these differences: III-2 products are able to mediate ADCC and phagocytosis[88]; III-1 products are not. FcγRIIIs expressed on polymorphonuclear and NK cells differ at the protein level (distinct molecular weight, different epitopes in the extracellular domains, differential expression of the NA allelic protein polymorphism), which may explain the different functional capacities of FcγRIIIs found both in individuals of different NA types and in various cell types.[89,101]

2. Factors Affecting Ligand Binding to FcγRs

The IgG-binding FcRs belong to the same superfamily of molecules as their ligands, i.e., their extracellular parts are closely related or identical and show considerable sequence homology with Ig domains. Because most probably the contact residues on the Ig molecules and the binding site(s) on the FcRs are likely exposed on the surface of the corresponding molecules, the receptor-ligand interactions may be similar to the interdomain interactions of Ig molecules.[102] The homology in the extracellular domains containing the binding site(s) on the one hand, and the striking differences in the structure of transmembrane parts and especially in the cytoplasmic tails on the other, explain why the interaction of identical/similar ligands with FcRs results in activation of different signaling mechanisms.

It seems that the expression of FcγRs and FcγR isoforms on various cell types is highly regulated, suggesting that each receptor species is specialized for well-defined functions associated with the corresponding cells. The functional consequences of the structural differences described in IgG binding FcγRs were already mentioned in relation to the III-1 and III-2 gene products. The importance of type-specific variations among cytoplasmic domains and that of the regulated pattern of cell type expression can be illustrated by the two isoforms of FcγRII.[103] By transfecting FcγRII DNAs into receptor negative cells, Miettinen et al.[103] could clarify which properties are associated with the various isoforms. It was already mentioned that in mouse the macrophage isoform (FcγRII-B2) is identical to the lymphocyte isoform (FcγRII-B1), except for an in-frame insertion in the cytoplasmic tail of FcγRII-B1 that increases its length from 47 to 94 amino acids. It was found that while FcγRII-B2 mediated efficient ligand uptake and delivery to lysosomes via internalization in coated pits, the cytoplasmic tail insertion characteristic for the lymphocyte isoform (FcγRII-B1) reduced the

ability of the receptor to mediate ligand uptake and degradation, i.e., the 47 amino acid insertion in the FcγRII-B1 cytoplasmic tail disrupted the capability to localize in clathrin-coated pits. This cytoplasmic domain variation may explain why macrophages and not B cells are capable of efficient FcγR-mediated endocytosis (the functional importance of which will be discussed later).

The actual conformation of the receptor molecule may also modulate the receptor function by influencing receptor association, binding capacity, and receptor release (see later). Here we would like to mention only two factors that (although not proven yet) may play a role in alteration of the conformation of FcγRs.

Glycosylation of FcγRs has been clearly established; however, little is known about differential glycosylation between individual FcγR families or about the functional importance of receptor glycosylation. The possible role of carbohydrates on human FcγRs in ligand binding was raised by Kimberly et al.[104] and will be discussed below. Here we would like to call attention to the different glycosylations of FcγRII and FcγRIII, which may have some functional consequences. It was shown that an epitope recognized by the monovalent Fab fragment of antibody 3G8 on FcγRIII expressed on PMN cells was conformationally dependent on the integrity of the *endo* H-sensitive moiety of the receptor.[105] Moreover, the immunoglobulin-ligand binding site was also dependent on the integrity of the same *endo* H-sensitive oligosaccharide. Regarding the influence of the carbohydrate moieties on the overall structure of IgGFc, one has to consider the importance of differential glycosylation in the actual conformation of FcγR molecules, also.

Here we have to mention briefly (and discuss later in detail) the possible effects of receptor phosphorylation. Phosphorylation has been shown to alter the ligand-binding capacity of some receptors and to induce release or endocytosis of others.[106,107] One of the early consequences of cell activation is the phosphorylation of FcγRII molecules expressed both on murine and human B cells.[108,109] It is most likely that the activation-induced phosphorylation of FcγRII results in conformational alteration of the receptor and consequent decrease in ligand-binding capacity. The phosphorylation-induced changes, on the other hand, might create suitable molecular conditions for proteolytic cleavage and release of the FcγRII.[109,110]

3. Mapping of Interacting Sites for FcγRs

It should be emphasized that despite their similar extracellular domains, the various forms of human FcγRs might differ in fine specificity and binding affinity. Moreover, the ligand binding and signal transfer might be influenced by the actual conformation of the receptor. Finally, it should be noted that the density of the ligand and the actual conformation of the immunoglobulin molecules are also important in the interaction of FcRs and their ligands. In this respect, the identification and topographical mapping of groups on the IgG recognized by the FcγR molecules are very critical.

a. FcγRI

Earlier studies attributed cytophilic activity to the CH3 domains of IgG, based on findings showing that pFc′ fragments of human IgG inhibited monocyte binding of IgG.[111] This result, however, is disputable because little or no ability of human pFc′ fragment to inhibit IgG binding to human monocytes was found under more exact experimental conditions.[112–114]

The results of studies in which CH3 domain peptides were used to inhibit IgG binding to monocytes are also conflicting. While Ciccimara et al.[115] found the peptide corresponding to residues 407 through 416 inhibitory, Ratcliffe and Stanworth[116] could not confirm this finding. Studying the binding of pFc′ fragment, domain- and hinge-deleted IgGs, and aglycosylated IgG to monocytes, Woof et al.[48,114] could not find evidence to implicate the CH3 domain in monocyte binding, whereas there was indirect evidence for the involvement of the CH2 domain. It was suggested that the CH3 domain does not

express the contact residues forming the human FcγRI interaction site, but is involved in a composite CH2/CH3 interaction site. By using monoclonal antibodies specific for isotypic, allotypic, or isoallotypic epitopes, the topographical distribution of groups recognized by the human FcγRI was determined.[117–120] The results obtained by using monoclonal antibodies as well as those found in comparative studies on the interaction of human, mouse, and rabbit IgG isotypes with human FcγRI, in addition to the investigations with chymeric antibodies and aglycoforms of human IgG1 and IgG3, allowed the localization of FcγRI-binding region on human IgG. These results, together with the observations that following the binding of IgG to human FcγRI the CH3 domain epitopes were still available for monoclonal antibodies,[121] unambiguously excluded the direct role for the CH3 domain.

Two regions of sequence that may form a continuous protein surface on the CH2 domain seem to be important in the interaction with FcγRI expressed on monocytes. These regions are close to the hinge (Leu 234–Ser 239) and near the C terminal end of the domain (Gly 316–Lys 338). Their average position is close to the two β-strands and joining bend formed by the residues Gly 316 to Lys 338.[122] It is of great importance that although IgG1 and IgG3 have identical sequences (Glu-Leu-Leu-Gly) at residues 233 to 236, IgG2 is different (Pro-Val-Ala and a deletion at residue 236). This explains the lack of binding of IgG2 to FcγRI. However, due to the extended rigid structure of the hinge region in IgG3,[42] the accessibility of its interacting site compared with that of IgG1 is better. It was shown that the two immunoglobulinlike extracellular domains of FcγRI form one active binding site interacting with a region in IgG1 or IgG3, located at the N-proximal end of their CH2 domain. The high-affinity binding of these isotypes may depend on the rigid ligand-binding site interaction, supported by the steric effect of the non-Ig-like third extracellular domain of the receptor.

Results clarifying the topography of the region of Fc interacting with human FcγRI point to the expression of one active binding site on the receptor. A different pattern was obtained, however, in the case of FcγRIII and FcγRII.

b. FcγRIII

Regarding the localization of submolecular sites on IgG interacting with FcγRs on K cells, and to understand the mechanism of cell-mediated killing of IgG-sensitized target cells, we have to mention the early work of Spiegelberg, Perlmann, and Perlmann.[123] They suggested that the CH3 domain might be responsible for the high-affinity binding, whereas the CH2 domain could play the role of triggering during ADCC. Recent observations proved this suggestion to be correct[124] and, moreover, that ADCC-mediating FcγRIIIs expressed on K cells possess two active binding sites. The interacting groups on the IgGFc domains were also localized, and data were provided that pointed to the importance of the actual conformation of the IgG ligand in triggering antibody-dependent lysis of IgG-opsonized target cells.

The fine specificity and signal-inducing capacity of FcγRIII were studied in experiments monitoring the lytic activity of K cells.[125,126] It was shown that FcγRIIIs mediating the lytic process of K cells possess two active binding sites, and efficient lysis depends on the simultaneous interaction of them with the CH2 and CH3 domains. The groups that react with the CH2 domain-specific binding site may involve the region of residues Lys 274 to Arg 301 and the lower hinge as well, whereas the interacting groups within the CH3 domain have been localized to the region of Ser 408 to Arg 416. In an "associative recognition" system,[127] in which the firm binding of the interacting effector and target cells was mediated additionally to IgG by C3b bridges, an enhanced rate of killing was observed. In such systems, by using CH2 or CH3 domain-specific monoclonal antibodies to inhibit ADCC,[126] and in experiments applying CH2 or CH3 domain-deleted paraproteins,[128] it was demonstrated that lytic signals are mediated only by the interaction of the CH2 domain of the sensitizing antibody with the corresponding binding site of FcR; moreover, that the interaction between the CH3 domain and CH3-specific binding site is responsible only for increasing the binding affinity.

Comparing the ADCC activity of monocytes (mediated mainly by FcγRI) and K cells (me-

diated by FcγRIII), it was shown that IgG1- and IgG3-sensitized erythrocytes were equally efficient in inducing monocyte-mediated ADCC.[129] Although both isotypes bind to the same receptor (FcγRIII), IgG1 was more active than IgG3 in K-cell-mediated lysis. It was remarkable, however, that the increase in the density of the target-cell-sensitizing IgG3 molecules resulted in effective induction of killing.

These differences can be explained by the structural and conformational dissimilarities of these isotypes and, consequently, by their differing interactions with the binding sites of the receptor. IgG1 and IgG3 differ in the lengths of their hinge regions. Furthermore, there is a characteristic variance in the hydrophilicity of the CH2 domain residues, which include the potential contact residues interacting with the CH2 domain-specific binding site of FcγRIII.[129] The unique primary amino acid sequences of the IgG1 and IgG3 domains, i.e., Lys/Gln 274 and Asn/Lys 276, may result in differences of the tertiary structure of these isotypes. Therefore, the dissimilarity in lysis-triggering capacity of these two isotypes can be explained by the differing accessibility of the CH2 and CH3 domains. Presumably, both the CH2 and the CH3 domains of IgG1 are able to interact with the corresponding binding sites of FcγRIII, whereas, as a consequence of the differences in amino acid residues at critical positions (contact residues), only the CH3 domain of the IgG3 molecule is accessible for effective binding. However, above a critical density of the target cell-sensitizing IgG3 antibody molecules, probably due to inter-Fc interactions, the disadvantages of IgG3 in lysis induction might be overcome by changes in conformation. These events lead to the accessibility of the triggering site on the CH2 domain. All these observations emphasize the importance of the actual conformation and structure of the ligand in induction of functionally significant signals mediated by the receptor.

c. FcγRII

Although the interacting groups on the Fc domains have not yet been localized, it was shown that FcγRII molecules express one specific binding site for CH2 and a second for CH3 domains.[130] It was reported that a subset of FcRs expressing human peripheral lymphocytes released their FcRs when incubated at 37°C in serum-free medium; furthermore, functionally active, soluble FcRs could be isolated from the supernatants of the cells.[131,132] The released receptors were found to be monomeric and interacted with the CH3 domain of IgG.[133] Another population of FcR^+ cells did not release their receptors under similar conditions: FcγRIII expressed on K/NK cells and FcγRII expressed on resting B cells[130] were characterized as stable types being maintained in a cell-attached state.

Within PMBC, several subsets (including B and T cells, monocytes, and K cells) express FcγR. Because the possibility that K cells or monocytes might be responsible for FcγR release was excluded[134] and because the $Fc\gamma R^+$ T cell population was relatively small, B lymphocytes were anticipated to release FcγR under the given conditions. It was proven that the *in vivo*-activated B cells are identical to the subset of human PMBCs that were previously found to shed FcγR[135,136] and that the released monomeric, CH3 domain-specific FcγRs were shown to be FcγRII[130,137] (and/or proteolytic fragments of this receptor). Because the released and isolated receptors cannot inhibit completely the EA rosette formation, it was suggested that the FcγRII molecules expressed on the resting B cells possessed, similarly to FcγRIII, two binding sites.

Chimeric antibodies were also used to define the interaction site of human FcγRs.[119] The IgG1 and IgG3 specificity had been confirmed for human FcγRII with the chimeric antibodies as well; therefore, the attention was focused on the sequence 233 to 237 to explain this specificity. Using the cell lines Daudi and K562, rosette formation with the IgG3 mutant was investigated.[138] The Leu/Ala-234 and Leu/Ala-237 mutants gave only 10 to 20% of the values obtained for the natural IgG3 sequence. Rosette formation for the Leu/Ala-235 and Leu/Glu-235 mutants was also reduced. These observations suggest that human FcγRI and FcγRII possess overlapping nonidentical specificities for human IgG.

Finally, we have to mention the possible role of carbohydrate chains of FcγRs in ligand binding. Based on the observation that aggregated

human IgG inhibits the phagocytosis by PMN of structures containing mannose-specific adhesins, Kimberly et al.[104,105,139] suggested that FcγRs expressed on these cells would have carbohydrate chains with mannosyl groups available for specific lectin binding. It was shown that $Fc\gamma RIII_{PMN}$ might have this oligosaccharide chain. It was also found that ligands other than IgG opsonins can bind to human $Fc\gamma RIII_{PMN}$ through lectin-carbohydrate interactions, i.e., the carbohydrate moieties, besides influencing the function of the "classic" IgG Fc binding site, bind specific lectins within the receptor directly.

C. Binding Sites for Soluble FcγRs (IgG BFs)

Soluble immunoglobulin-binding substance found in the cell free supernatants of activated lymphocytes was first described by Fridman and Golstein.[140] Since then, IBFs specific for each immunoglobulin isotype have been described.[141] Immunoglobulin-binding factors modulate the *in vitro* synthesis of immunoglobulins and regulate antibody production in an isotype-specific manner.

T cells expressing FcγRs for a given isotype spontaneously produce IBF for the same isotype. A more efficient production of IBF could be achieved when activated T cells were used.[142] Incubation of the activated T lymphocytes in serum-free medium resulted in the disappearance of FcγRs from the FcγR-bearing T cells and in the appearance of IgG BF in the supernatants of the cells.[143] The latter phenomenon draws attention to the possibility that IgG BF might be the soluble form of T cell FcγRs.

It is now clear that besides T lymphocytes, other FcγR positive cells, including B cells,[144] macrophages,[145] and granulocytes,[146] can produce IBF; their production is regulated by various cytokines[147] and by interaction of FcR positive cells with immunoglobulin.[148] In patients or mice with immunoglobulin-secreting tumors, a higher number of T cells bearing FcRs for the same isotypes as the paraprotein was observed.[149] Moreover, IBF in the serum of the mouse[150] and human[151,152] was detected.

It is not known which mechanism could account for the presence of cell free Fc receptors in normal serum, and it is also remarkable that in spite of the abundant opportunity to bind to IgG molecules, the Fc receptors could be isolated as noncomplexed, free molecules (most probably due to their higher binding affinity to IgG in the solid phase). This means that FcR release appears *in vivo* as well, and one cannot exclude the possibility that, especially in the microenvironment of the cells acting as "Fc receptor sources," the released soluble FcRs might inhibit (regulate) Fc-dependent functions.

In mouse, IBFs produced by T cells were identified as glycoproteins showing size and charge heterogeneity.[153,154] The relationship between IgG BF and FcγRII now seems to be revealed. It was shown that the mouse FcγRII-specific monoclonal antibody 2.4G2 recognizes IgG BF produced by FcγRII positive cells[155]; moreover, L cells transfected with a cDNA coding for FcγRII express FcγR (56 kDa) and produce a glycoprotein (38 kDa) that binds to monoclonal antibody 2.4G2.[156] How IgG BF is generated is still under discussion. One of the possibilities is alternative splicing of mRNA coding for FcγR. On the other hand, IgG BF can be formed by proteolytic cleavage of the membrane-bound FcγRII.[110,130,137,156] (The role of proteolysis in receptor release will be discussed in detail later.)

Independently of its origin, the IgG BF is most probably a fragment of one of the membrane-bound FcγRs. This soluble fragment preserves its IgG-binding capacity. One may suppose, therefore, that IgG BFs interact with the same groups on IgGFc as their membrane-bound counterparts.

D. Binding Sites for Rheumatoid Factors (RFs)

1. Rheumatoid Factors

Autoantibodies recognizing the Fc region of autologous IgG have been proven to be the product of activated B cells, the precursors of which are natural constituents of the normal B cell repertoire.[157,158] As these types of antibodies originally were found in sera of patients with rheu-

matoid arthritis (RA),[159] they were termed *rheumatoid factors* (RFs). However, this definition at the present covers the entire population of autoantibodies having IgGFc reactivity.

Depending on the nature of the stimulus involved in the induction of RF production, these types of autoantibodies can be classified as

- Spontaneously occurring anti-Fc autoantibodies comprising a substantial fraction of the normal immunoglobulin repertoire
- Anti-Fc autoantibodies produced as a result of polyclonal B cell activation delivered by different stimuli
- Anti-Fc autoantibodies induced by antigen-antibody immune complexes formed in the course of the memory antibody response or administered as preformed immune complexes
- Anti-Fc autoantibodies generated as a result of autoimmune processes

According to our present knowledge, autoantibodies recognizing the Fc region of IgG can differ in their structural and functional properties, which show strong correlation with the mode of their induction. On the basis of their binding characteristics, at least two main categories of anti-Fc autoantibodies can be distinguished: multireactive — mostly IgM-type anti-Fc autoantibodies — and oligo- or monoreactive, IgM-, IgG-, or IgA-type anti-Fc autoantibodies.

Fc-reactive autoantibodies characterized by broad polyspecificity possess binding activity not only for IgG but also for different self- and non-self-antigenic structures, including nuclear components (DNA, histones), cytoskeletal proteins (myosin, actin, vimentin), bromelain-treated red blood cells, peptidoglycans, and various haptens (nitrophenyl, dinitrophenyl, phenyl-oxazolone).[160–162] Most of these polyspecific autoantibodies belong to the IgM class, although IgG-type antibodies with similar polyreactive binding properties also have been described.[163] Many data support the suggestion that these antibodies are products of activated B cells maintaining their V gene segments in germ line configuration.[164–166] The $CD5^+$ ($Ly1^+$) B cell population seems to play an important role in the production of this type of autoantibody.[167–170] The presence of polyspecific IgM-type RFs has been shown in normal human and murine individuals, and their level seems to be under the control of IgH-C, MHC, and other genes.[171–174] The production of polyspecific IgM RFs can be enhanced by different B cell activators, such as lipopolysaccharides, neutral proteinases, or peptidoglycans in mice[175–178] and Epstein-Barr virus, peptidoglycans, or *Staphylococcus* protein A in humans.[179,180] This type of anti-Fc autoantibody is also produced at elevated level in autoimmune situations, as has been shown both in humans and mice.[167, 181–184]

The other population of anti-Fc autoantibodies involves IgM- as well as IgG- and IgA-type antibodies with restricted reactivity for certain isotypes of IgG molecules. These types of autoantibodies are induced primarily by antigen-antibody complexes, and their isotype specificity reflects the dominant subclass of the corresponding antigen-specific antibody response, as it was shown in rabbits and mice.[185–190] These anti-Fc autoantibodies are generated in the course of the anamnestic immune response or as a consequence of immune complex injection under the control of regulatory T cells.[191–193] These RFs are also found in autoimmune conditions.[167,194,195]

The spontaneous lupuslike syndrome of MLR-lpr/lpr mice was characterized by IgM- and IgG-type autoantibody production.[184,196] Both kinds of RFs are found in RA and can be detected at elevated levels in many other rheumatic and non-rheumatic disorders associated with chronic infection and inflammation.[182,183] The latter population of RFs is most likely the product of plasma cells bearing the $CD5^-$ ($Ly\text{-}1^-$) phenotype, although the lineage origin of these types of autoantibodies is still questionable.[162,167,195,197,198]

In contrast to polyspecific IgM-type RFs induced by polyclonal B cell activation, antigen-driven rigorously controlled expansion of self-reactive B cell clones can be assumed during the generation of monoreactive RFs characterized by the features of isotype switching, affinity maturation and somatic mutations.[162,164,194,199,200,201]

2. Mapping of Interacting Sites for RFs

Similarly to other protein antigens, the Fc region of IgG can be recognized by antibodies

as a mosaic of overlapping epitopes.[202] Identification of binding sites involved in RF-IgG interaction raises special problems in the case of polyspecific autoantibodies.[163,203–207] The regions of IgG molecules involved in the binding of oligo- or monospecific RFs, however, can be localized by conventional epitope mapping strategies. These studies have revealed that human monoclonal IgMs having RF activity react with IgG1, IgG2, and IgG4 subclasses and recognize the CH2-CH3 interdomain region, which is also involved in SpA binding.[208–210] This RF specificity shows strong correlation with SpA binding, which also depends on the presence of amino acid residues His 435 and Tyr 436, located in the CH3 domain and responsible for allotypic variations of the IgG3 subclass.[211] Recent data from chimeric domain-shuffled IgGs, however, have revealed that the specificities of polyclonal RFs and monoclonal cryoglobulins with RF-like activity are not identical.[212]

As decreased terminal galactosylation of IgG was demonstrated in RA patients,[213,214] the role of carbohydrates in RF binding became the subject of intensive investigations.[212,215] These studies revealed that the binding of human RFs to IgG3 could be modified by the absence or nature of the carbohydrate moiety of the CH2 domain.[212]

Despite the high number of RF-type autoantibodies isolated during different phases of the immune response of different inbred strains of mice, the precise localization of epitopes has not been determined. Detailed fine specificity analysis has focused on the characterization of their isotype and allotype restriction or cross-reactivity with other homologous or heterologous IgGs. These data have led to some interesting conclusions concerning the specificity features of mouse RFs.

The IgG2a isotype seems to play a predominant role in normal RF induction and also in MRL-lpr/lpr and 129/Sv mice.[216,217] These IgG2a-reactive RFs showed allotype specificity, and the determinants could be localized to the CH3 domain of IgG1, IgG2a, and IgG3 isotypes or to the C-terminal end of the CH2 domain.[217,218] LPS induces mostly IgG1- and IgG2a-reactive RFs,[219,220] whereas application of LPS-TNP-IgG complexes results in the production of IgG2b- and IgG3-reactive RFs.[199]

Immune complex-induced RFs show preferential binding to the isotype included in the immune complex, and, in good correlation with this finding, the specificity of antigen-induced RFs reflects the isotypic pattern of the antigen-specific response.[50,186–188,191,204] In other words, this isotype restriction means that protein antigens eliciting mainly IgG1-type antibody production preferentially induce IgG1-reactive RFs in the course of the secondary response, whereas other types of antigen (e.g., carbohydrates) generate RFs of different isotype specificity.[189,190]

As has been shown by Coutelier,[221,222] viral infections in the mouse result predominantly in IgG2a-type antiviral antibodies. It was also shown that repeated influenza virus infection of Balb/c mice induces predominantly the production of IgG2a-type antibodies, and the appearance of IgG2a-reactive autoantibodies could also be shown. Hybridomas isolated from the spleen of influenza virus-infected Balb/c mice represent IgM, IgG, and IgA RF-producing clones that can be characterized by similar functional properties.[197] These autoantibodies show strong specificity for IgG2a molecules of the parental allotype, do not cross-react with immunoglobulins of other species or various self- or nonself-antigenic structures, and preferentially bind aggregated or complexed IgG2a compared with the native counterpart.

The reactivity pattern of these autoantibodies with IgG2a fragments and IgG2a and IgG2b allotypes revealed that they very likely recognize regions of the CH2 domain. The interaction of the IgA-type monoclonal antibody was dependent on the amino acid residue Ala at position 305, whereas the recognition of IgG2a by IgM-type RFs was dependent on the amino acid residue at position 258 characteristic only for IgG2a of "a" allotype.[223]

Besides their differing epitope specificity, RFs have other important functional properties that definitely distinguish them from conventional anti-immunoglobulin reagents and may have interesting implications for their possible biological or regulatory functions. Recent data categorize RFs as low- or high-affinity antibodies by terms of their poly- or monospecificity and the relative amounts of ligands required for inhibition of binding.[167,194] Relatively high functional avidity

of IgM-, IgA-, and IgG-type anti-Fc monoclonal antibodies for aggregated IgG2a could be demonstrated.[197]

The symmetrical Fc region participates as a bivalent ligand in its interaction with most conventional antibodies.[202] However, depending on the localization of the recognized region, the Fc portion can be involved in RF binding as a functionally monovalent ligand, as was shown in the case of certain human RFs.[224] A similar sterically restricted interaction was suggested in the case of ↓ IgG2a-reactive ↓ mouse ↓ monoclonal antibodies.[223]

The steric restriction of the binding of polyvalent IgM-type RFs was proven by their sensitivity for mild reduction[186,223] and by the molecular weight determination of IgG-IgM immune complexes.[225] Comparative studies with IgM, IgG2b, and IgA RFs having similar functional properties and fine specificity demonstrated that polyvalent IgM and IgA RFs are extremely sensitive for the appropriate steric orientation of IgG2a Fc portion, whereas IgG2b RF and its reduced and alkylated forms readily bind to IgG2a adsorbed to the solid phase even at lower concentrations. This distinction can be explained by the high flexibility of the hinge region in IgG compared with IgM lacking a functional hinge.[202] The sterically restricted interaction of IgG2a and these types of RFs might have an important influence on the structure of immune complexes formed by antigen-antibody-RF interactions and can provide an explanation for the preferential binding to solid-phase bound, complexed, or aggregated IgG2a.

3. RF-Binding and Fc-Mediated Functions

Secreted IgG molecules in body fluids occur in the presence of soluble IgG-binding structures (such as Clq and IgG BFs and RFs) and also have the chance to interact with different cells expressing FcγRs. As most Fc-mediated interactions prefer antibodies complexed with the corresponding antigen, unbound IgG can avoid these interactions, whereas complexed IgG will interact with one or more of these structures. The possibility of these interactions occurring is highly influenced by the actual composition of immune complexes, depending on the fine specificity, affinity, valency, and isotype of the antibodies and the relative concentrations of the antigen. Regarding this possibility, the interesting question arises of whether the binding of the antigen, resulting in the local redistribution of soluble or cell-bound antibodies, enables the simultaneous interaction with various Fc-binding structures. This naturally occurring, and potentially competitive, situation is presumably highly influenced by various factors actually present in the microenvironment.

The simple way to study these possibilities is to test the competitive nature as well as the localization of interaction sites on the IgG Fc, as has been discussed before. Mapping the CH2- and CH3-domain-related binding sites revealed that different FcγRs interact with distinct regions that are not identical with respect to C1q or SpA binding sites. In contrast, the binding of certain RFs was proven to be inhibited by SpA-Fc interaction.

However, recent studies with mouse IgG2a-reactive monoclonal antibodies proved that binding of naturally occurring IgM-, IgA-, and IgG-type anti-Fc autoantibodies does not inhibit C1q, C3b, or SpA binding to aggregated or complexed IgG2a and does not influence the ability of complexed IgG2a to interact with high- or low-affinity FcγRI or FcγRII.[223] These data strongly suggest that the Fc region of IgG is ready for simultaneous interactions with distinct Fc-binding structures and show that RF binding does not eliminate the potency of IgG2a to participate in other Fc-mediated functions. One may assume, however, that these types of interactions are not only limited by the direct competition for interaction sites, but also are influenced by steric hindrance and altered structural properties of the complexed immunoglobulins. The multivalent interaction with C1q or polyvalent RFs might stabilize or reorganize IgG-IgG interactions maintained by antigen bridges, which may result in restricted flexibility of IgG or in stabilized Fc-Fc interactions proven to be essential for many Fc-mediated interactions.[226–228]

An example of this type of effect was provided by experiments showing that the binding of IgM- and IgA-type monoclonal RFs to aggre-

gated IgG2a did not interfere with C1q or C3b binding, but still resulted in diminished complement activation mediated by both the classic and alternative pathways.[223] Similar results were explained by an altered molecular microenvironment for C3b deposition or C1 activation.[229,230]

Binding of anti-Fc autoantibodies to IgG aggregates results in structurally more complicated complexes than the interaction of other Fc-binding structures with complexed immunoglobulins. Immune complexes containing anti-Fc autoantibodies (similarly to idiotype-anti-idiotype immune complexes) expose two heterologous Fc parts, potentially active in Fc-mediated interactions. The anti-Fc-containing aggregates, depending on the fine specificity and isotype of the participating antibodies (as with idiotype-anti-idiotype immune complexes) might be involved in various Fc-mediated functions.[231]

According to early observations, IgM-type RFs are able to fix C1 and mediate complement activation;[232,233] however, their capability to do so is highly dependent on the size of immune complexes.[234] These studies also revealed the relatively low activity of IgM RFs in this respect, explained by the inappropriate conformational change to acquire the "staple"-like orientation of the subunits instead of the original "star"-like arrangement.[235] These results were confirmed by studies showing that immune complexes formed by aggregated IgG2a and IgM or IgA RFs mediate complement activation, via the classic or alternative pathways, exclusively through the IgG2a component. This was demonstrated by the reduction and alkylation of aggregated IgG2a, which does not influence RF binding but abolishes complement activation via both pathways.[223] It is remarkable that, while the IgA-type RF behaved similarly to the IgM type, anti-IgG2a autoantibody of IgG2b isotype did not inhibit (but rather enhanced) complement activation via the classic pathway. This supports the importance of increased efficiency of IgG-IgG immune complexes in this respect.

The multiple potential of RF-bound immune complexes manifests itself in important functional consequences. Attachment and *in vivo* clearance of immune complexes is enhanced in the presence of IgM RFs, probably due to elevated internalization by FcγR. However, the simultaneous complement-mediated opsonization via C3b receptors was decreased in the presence of RFs, in good correlation with data pointing to decreased complement activation by RF-bound IgG aggregates.[160,223]

IV. REGULATION OF ANTIBODY PRODUCTION BY IgG Fc-BINDING MOLECULES

A. Antigen-Induced Events in B Cells

The specific humoral immune response to an immunogen is dictated by the continuously changing repertoire of B cells. The so-called naive repertoire of B lymphocytes develops independently of antigen stimulation as the result of genetic recombination events. During the actual immune response, due to the antigenic challenge, only a fraction of the naive repertoire is activated. The outcome of the B cell activation is the clonal expansion of the antigen-specific B cells. Immunogens, interacting with antigen-binding receptors expressed on the B cell membrane (mIgM, mIgD), may induce cell activation in two different ways. B lymphocytes expressing mIg and MHC class II molecules are among the most efficient antigen-presenting cells, binding and focusing relatively low concentrations of protein antigens by means of their antigen receptors.[236,237] The processed antigen is presented to T helper cells, i.e., the antigen-specific B cells induce helper T cells to produce cytokines that promote B cell activation. On the other hand, antigens bound to membrane immunoglobulins on B cells induce transmembrane signaling, leading to B cell activation.[238]

The various signals induced via antigen, cytokine, and mitogen receptors drive the resting B cells from the G_0 to the S phase of the cell cycle, activating different signaling pathways. The cross-linking of mIg triggers inositol lipid metabolism, and this generates two second messengers, inositol 1,4,5-triphosphate (IP_3) and 1,2-diacylglycerol. The former induces increased intracellular Ca^{2+} levels by releasing Ca^{2+} from intracellular stores, whereas diacylglycerol activates PKC. In addition, changes of membrane potential can be observed.[239–241] Efflux of K^+

ions is also induced by cross-linking antigen receptors,[242] and it has been shown that functional voltage-gated K^+ channels are required at a precise stage of the G_1 phase of the B cell cycle.[243]

As a consequence of B cell activation, certain modifications in cell surface marker expression have been described. The enhancement of MHC class II antigen expression appears late in the G_0 phase, before the activated B cells enter G_1.[239] The early G_1 phase is characterized by mRNA synthesis and enhanced expression of FcγRII. In the late G_1 stage, the expression of transferrin receptor is also increased. It is remarkable that, depending on the degree of activation, B cells differ in their responsiveness to anti-Ig, i.e., resting and activated B lymphocytes differ in the signaling pathway through mIg and exhibit differential sensitivity to the stimulatory effects of PMA and calcium ionophore.[244] The modification in cell-surface receptor expression makes the B cells sensitive to various signals that can drive the cell to the next activation phase.[245] According to Melchers and Lenhardt,[246] the B cells are blocked at three restriction points in the cell cycle until the proper growth signal is provided. One of the restriction points is identified at the G_0 phase, when the B cells have already rearranged their immunoglobulin genes and expressed mIgM. In this stage, low levels of MHC class II and FcγRII molecules are expressed, and the cells contain low amounts of cytoplasmic RNA; also, the cells are ready to interact with antigens.[178] The significant increase of MHC class II antigens before entering the G_1 phase favors the interaction of B cells with T helper cells.[247] Another restriction point seems to be in the G_1 phase, during which various factors, including C3b[248] and IL-1,[249] can promote the B cells into S phase.

Various factors may affect these antigen-induced events in B cells. Transforming growth factor β (TFG-β) is inhibitory on B cells: human B cell proliferation induced by cross-linking mIgs can be blocked by TGF-β, acting during the $G_0 \rightarrow G_1$ transition.[250] Negative regulatory signals are mediated by cross-linking the major pan-β cell-specific glycoprotein CD19 as well, inhibiting the replication and the transition from $G_0 \rightarrow G_1$ of dense B cells activated by anti-Ig antibodies.[250,251] The most important cell membrane structure in this respect, however, seems to be the FcγRII.

B. Regulation of Antibody Production by Membrane-Bound FcγRs

Early *in vivo* studies called attention to the importance of the intact Fc portion of IgG in the IgG-mediated feedback inhibition of antibody response,[252] and to the immunoglobulin-binding receptors in transferring inhibitory signals to the B cells. The latter statement was based on the observation that antigen-antibody complexes are efficient regulators of immune responses (reviewed in Reference 253), and it turned out that the inhibition of humoral responses by immune complexes requires an intact Fc part of the antibody molecule.[254] Several models explaining the mechanism of suppression of antibody formation were suggested, but most data support the assumption that immune complexes directly affect the B cells and that their interaction with both the mIg and FcγRs represent the blocking signal.

It was found that immobilized immune complexes inhibit the action of the B cell mitogen LPS on murine spleen cells;[255] moreover, soluble complexes do not mediate this inhibition. The possible role of suppressor T cells was excluded. Therefore, it was concluded that the immobilized complexes bind to B lymphocytes via their FcRs as well, and trigger a central "off" signal that blocks proliferation and consequent antibody production. Other observations[256] also confirmed that the linkage of antigen receptors and FcγRs directly block B cells; moreover, precondition of this "off" signal is the simultaneous occupation of both receptors. Recent data indicate that resting B cells that have not encountered antigen are most susceptible to the down-regulatory signal induced by cross-linking of mIg and FcγRs.[257]

Experiments showing that immunoglobulins are efficient B cell tolerogens led Waldschmidt and Vitetta[258] to control the possible Fc dependency of B cell tolerance induced by haptenated immunoglobulin molecules. It was shown that the ability of haptenized immunoglobulins to suppress or tolerize B cell responses was dependent on their FcR binding capacity. Because such hap-

tenized immunoglobulin molecules are able to cross-link the mIg and FcγRs on the same and different B cells, it was suggested that such unresponsiveness represents a model of antibody feedback inhibition and not tolerance to self antigens. This view was supported by data showing that monoclonal anti-FcR antibody was able to reverse the inhibitory effect of a whole anti-μ antibody.[259]

Recently, the biochemical basis of the inhibitory effect of rabbit anti-immunoglobulin has been elucidated. It was shown that $F(ab')_2$ fragments of anti-immunoglobulin provoke prolonged release of IP_3 in B cells, whereas intact antiimmunoglobulin causes only a transient increase in polyphosphoinositide-specific phosphodiesterase (PPI-PDE), which hydrolyzes PIP_2, generating IP_3 and DAG. This response involves a guanine-nucleotide regulatory protein (G_p) that couples mIg to the PPI-PDE.[260]

Rigley et al.[261] have shown that co-cross-linkage of mIg and FcγRs with intact antibody on B cells uncouples the antigen receptors from G_p but does not affect G_p/PPI-PDE coupling. These findings suggest that control of the level of PPI-PDE activity may play a role in antigen-induced B cell regulation. Moreover, one has to emphasize that one important function of FcγRII is the "fine tuning" of B cell responses to antigen.

Here we have to mention briefly the possible role of complement receptors (CRs) in B cell regulation, because besides mIg-FcγRII cross-linking, the immune complex-mediated regulation of immune response might be modulated by mIg-CR interaction as well. Cross-linking of CR2 by immobilized C3b and C3d stimulates, whereas the occupancy of the receptors without cross-linking inhibits, the entry of activated B cells into the S phase of the cell cycle.[249] A synergistic interaction between CR2 and mIgM was shown by Tsokos et al.[262] and Carter et al.[263] as well.

It is remarkable that IgG-containing immune complexes are able to enhance immune responses. TNP-specific monoclonal IgG antibodies were found to suppress antibody response when antigen was applied in particulate form (TNP-SRBC) but enhanced the response to soluble antigens (TNP-KLH).[264] This enhancing effect of IgG was linked with the complement-activating capacity of the antibody. Heyman[265] raised the possibility that this enhancing effect of IgG depends on its capacity to interact with both FcRs and CRs; the latter might result in direct stimulation of B cells via their complement receptors. These observations call attention to the possibility that both FcγRIIs and CRs participate in the regulation of antibody production at the B cell level.

C. The Role of Soluble FcγRs in B Cell Regulation

Much evidence was presented in the previous sections of the regulatory function of membrane-bound FcγRs, and it seems obvious that IgGFc and FcγRs are two sides of the same coin in this respect. The question is whether FcγR fragments released from the cell membrane and still possessing their Fc-binding capacity are also regulatory molecules, modulating the antibody production of B cells.

It is well known from the early studies on IBF that these factors, which are released from $FcγR^+$ T cells and that bind specifically to the Fc portion of immunoglobulins, modulate the production of the corresponding isotype.[140,142] It has already been mentioned that IBFs are released from cells other than T cells; moreover, that the latter have similar modulatory effects on antibody production. The mechanism of IBF-mediated modulation is not clarified yet, and most of the studies concerning the regulatory role of IgG BFs are related to T cell products. One may suppose, however, that the regulatory pathways opened by IgG BFs of various origin, due to their similar binding properties, may be alike. Factors influencing IBF production and the possible mechanisms of IBF action will now be briefly discussed.

Because FcγR expression is modified by factors affecting cell activation, one may suppose that the production of IBF (a split product of the same receptor) is also induced and modified by the same components. The effect of cytokines on IgE BF production has been thoroughly studied.[266] IL-4 and IFN-γ are the two major cytokines influencing both FcεRII (CD23) expression and IgE BF release from B cells.[267] Other factors,

such as IgE BF itself[268] and low-molecular-weight B cell growth factor,[268,269] play similar roles in IgE BF release. IL-2 and IL-4 influence the release of IgD BF,[270] and IgG BF production is affected by IFNs that inhibit IL-4-induced IgG BF release as well.[271]

Here we need to mention that the capacity of immunoglobulins to modify the expression of the appropriate FcRs is an important component in the complex regulatory mechanism mediated by membrane-bound and soluble FcRs. A great bulk of data prove that FcRs, and IBFs for various isotypes, can be induced by corresponding immunoglobulins.[272] It was shown both in humans and mice that gammopathies often go together with the development of large numbers of T cells that express FcγRs for isotypes of corresponding myeloma proteins. The myeloma-bearing patients, or mice, also produce IBF specific for the dominating isotype.[273,274]

The mechanism of FcR expression and IBF release induced by immunoglobulins has not been elucidated yet. It seems that receptor aggregation is the prerequisite of IgG BF production. Because no change in mRNA content in immunoglobulin-treated cells has been found, it has been suggested that immunoglobulin up-regulates FcR and IBF at the posttranscriptional level.[275]

IgG BF inhibits the production of IgG in secondary *in vitro* antibody responses and of IgM in primary responses. The precise molecular mechanisms of this suppression have not yet been elucidated. Studying the *in vitro* inhibitory effect of FcγRII$^+$ IgG BF-producing T hybridomas on the production of IgG in B hybridomas,[276] it was found that the production of IgG was inhibited through noncognate interactions, indicating that soluble factor(s) mediated this inhibitory effect. The inhibition seems to affect H- and L-chain synthesis as well as the H- and L-chain-encoding mRNA steady state. Because a complete IgG molecule was required on the B hybridoma to demonstrate the blocking effect by the IgG BF-producing T hybridoma, it is likely that the inhibition may be mediated by interactions between the released IgG BF and mIgG. One cannot exclude, however, the effect of other soluble mediators able to induce a blockade of immunoglobulin gene expression.

Although the possibility exists that besides IgG BF, other soluble factors, acting synergistically with it, may also mediate the suppression of antibody production, such an effect of IgG BF itself is indisputable. Recent studies have shown that recombinant soluble FcγRII inhibits antibody production *in vitro*.[277] In these investigations soluble recombinant FcγRIIs containing only the two extracytoplasmic domains of the molecule were used. L cells were transfected with the mutated cDNA inserted into an expression vector. The soluble FcγRIIs isolated from the culture medium of the resultant cell line inhibited secondary and primary *in vitro* antibody responses.

D. Correlation between FcγRII Expression, Release, and B Cell Cycle

Aggregation of antigen receptors (mIgs) promotes activation of matured B cells for clonal expansion and antibody production against antigens. In the course of their response to antigen, B cells are exposed to numerous positive and negative regulatory signals modifying the net outcome of the humoral response. Membrane-bound and soluble FcγRII molecules play a key role in these regulatory processes.

Several factors may alter the FcγRIIs and the signals mediated by these receptors. Expression and release affect the actual number of the receptors on the B cell membrane (up- and down-regulation). Their fine specificity and ligand-binding affinity might be influenced by the actual conformation of the receptor. Conformational alterations, on the other hand, might be decisive with respect to the proteolytic cleavage and receptor release as well. Finally, the relation of FcγRIIs to other B cell membrane constituents may also affect the signals mediated by the receptors. Therefore, the expression and release of FcγRIIs are integral components of mechanisms regulating antibody production of B cells. From this point of view, FcγRII expression and release, such as the actual conformation of the receptors, can be regarded as regulatory factors. To understand the regulatory events, the time course of these phenomena should also be taken into consideration.

The differential expression of FcγRs on various cells is regulated by genetic and environ-

mental factors. The developmental expression of FcγRs partly depends on selective unmethylation of DNA sequences in both the α and the β genes.[278,279] Besides genetic factors, the differential expression of FcRs is regulated by cytokines. Thus, recombinant IFNγ has antagonistic effects on macrophage αFcγR and βFcγR transcripts.[280] An up-regulatory effect of rIL-2 and rIFN on αFcγRII in various cells was also observed,[279] and it is well known that IL-4 up-regulates mRNA-encoding FcεRIIb but not FcεRIIa.[281]

The differential expression of FcγR subtypes has functional importance to cells on which several subtypes of these receptors are simultaneously expressed. B cells express only FcγRIIB1; therefore, the genetic and environmental factors might affect first of all the number of FcγRIIs available in functionally active form on the B cell membrane. In this respect IL-4 seems to be important, as it was shown that this cytokine induces loss of murine B cell FcγRII ligand-binding capacity.[282] IL-4 can reverse FcγRII-mediated inhibition of B lymphocyte activation as well.[283]

Concerning the time course of FcγRII expression and release, it was shown that LPS activation of murine B cells induces both an elevated level of B1FcγRII mRNA and the expression of FcγRII in the G_{1a} phase of the cell cycle;[284] moreover, the appearance of a new epitope on FcγRs was also observed that correlated with the ability to release soluble FcγRs.[144]

Following the activation of resting human B lymphocytes, the expression of FcγRIIs showed a biphasic time course. As early as 10 min after stimulation, a transient increase of FcγRII expression was shown and simultaneously decreased ligand-binding capacity of the receptors was observed. Later, after 3 to 24 h, a decrease in the number of FcγRII-bearing cells was seen. However, on the second day of activation, a significant increase in FcγRII expression, mainly on enlarged blast cells, was found. At the same time, soluble fragments (33 kDa) of FcγRIIs, with the ability to bind to human IgG Fc, were released.[130,137] The release of receptors occurred in the early G_1 phase of the cell cycle; later receptor release was accompanied by the enhancement of the FcγRII expression before the B cells reached the S phase.

The regulation of B cells by the low-affinity FcεRII and IgE BF has been thoroughly studied in the last few years.[285] Because there are several remarkable similarities between the mechanisms resulting in the production of IgG BF and IgE BF, we touch briefly on the release process of FcεRIIs.

CD23 is a type II integral membrane protein of M_r 45 kDa, originally defined as a differentiation antigen of B lymphocytes. The cDNA for the human FcεRII has been cloned, and the predicted length of the receptor is 321 amino acids. Although a significant portion of its extracellular domain bears a marked homology to C-type animal lectins and contains an inverse "RGD" sequence, neither of these appears to be involved in the low-affinity IgE binding.[285] CD23 is expressed at a very low level on resting B cells; its expression can be up-regulated by a number of agents such as IL-4, phorbol esters, and IL-2.[286,287] Activated B cells release soluble CD23 by proteolysis of the membrane-bound protein. Initially 35 kDa soluble degradation fragments are produced that rapidly degrade to the more stable 25-kDa form; these fragments are capable of binding to IgE.[288] (The long-lived 14-kDa product has no IgE binding capacity.) It seems that the enzyme involved in this proteolytic process is a thiolesterase (SH-protease), although it is possible that more than one enzyme is responsible for the generation of IgE BF of different size. It is also supposed that the enzyme cleaving the 33.000 to 37.000. IgE BF precursors is cell bound and is expressed on all FcεRII-positive cells.

The possibility that murine IgG BF can be formed by proteolytic cleavage was raised by Sautes et al.[156] and such a mechanism was suggested in case of human FcγRIIs as well.[130,137] It was shown that the ability of activated, but not of resting, human B cells to release FcγRIIs correlates with the appearance of a trypsinlike serine protease activity in the early G_1 phase of the cell cycle. It was also shown that specific serine esterase blockers inhibit the release of FcγRII from B cells. Analysis of the predicted amino acid sequence of FcγRII, and hydrophilicity of its residues, showed that at least two sites could be the targets of trypsinlike serine proteases. Following the expected proteolytic cleavage at these sites, the disulfide bridges can still hold the molecule

together, which explains why the ligand-binding capacity of the released 33-kDa fragment is still preserved. Thus, the proteolytic cleavage of FcγRIIs and the release of IgG BF seem to be similar phenomena to those of FcεRII degradation and IgE BF release, respectively.

E. Regulation of Antibody Production by RFs

This part of the review focuses on the potential regulatory role of Fc-reactive RF-like autoantibodies. On the basis of our own observations, we support the suggestion of Monestier et al.[289] that two distinct populations of Fc-reactive autoantibodies may play different functional roles in immune processes.

The functional role of polyspecific autoantibodies possessing a low degree of selectivity in their binding was suggested to be to clear the cell debris from the body[290] or to build the first line of defense against microorganisms.[168] In contrast, mono- or oligoreactive RFs produced as a result of selective B cell expansion and maturation give rise to regulatory autoantibodies with highly restricted binding properties directing them to well-defined IgG determinants.

The proportion of Fc-binding polyspecific autoantibodies in the normal immunoglobulin repertoire is relatively high and was estimated to compose about 10 to 15%.[186] Polyclonal B cell activation, as well as secondary antigen challenge, increases the frequency of Fc-reactive B cells. However, under normal conditions due to suppressive mechanisms, the expansion and maturation of only a few clones may take place, resulting in the production of high-affinity immunoglobulin-reactive autoantibodies. These antibodies might have different roles in the regulation of immune processes than those continuously present in the circulation.

Polyspecific RF-like autoantibodies, in both B cell-bound and secreted forms, have the potential to interact with various self-structures, depending on their cross-reactivity pattern. In contrast, high-affinity, monospecific, isotype, and immune complex-restricted recognition of IgG by certain RFs is able to focus these autoantibodies to soluble or cell-bound immune complexes having the appropriate composition and structure.

Regulatory Fc-binding autoantibodies may act at different levels. B cells, as professional antigen-presenting cells, are able to take up antigens via their mIgs but not by their FcγRIIs, ensuring the antigen specificity of B cell activation. B cells bearing Fc-binding mIg molecules, however, are able to focus any antigen-antibody complexes, providing a possibility for efficient mIg-mediated internalization of the antigen. The specificity of this process is restricted by the isotype of the antigen-binding antibodies. Due to the comparatively high proportion of polyspecific autoreactive and Fc-binding specificities in the normal B cell repertoire, relatively high numbers of B cells might be activated in this manner. Such activation of B cells is followed by further T cell-mediated selection and maturation.

Fc-reactive autoantibodies can potentially react with soluble or cell membrane-bound IgG. It was clearly shown, however,[197] that the direct interaction of RFs with B cells via mIg can be limited by the inability of monoreactive RFs to interact with membrane-bound immunoglobulin in its unliganded form. The possibility, however, that appropriate cross-linking of mIg resulting in clusters will be recognized by these RFs (which do not bind to native, but only to aggregated, immunoglobulins) remains to be elucidated.

Another possibility regarding the direct interaction of RFs with B cells would be an immune complex-mediated binding. Rheumatoid factors as effective cross-linkers of antigen-complexed immunoglobulin molecules may have a particular role in delivering positive or negative signals for B cells, as suggested by Heyman.[265] Both the direct and immune complex-mediated interaction of RFs with mIg of B cells might provide a flexible possibility for FcγRII-mediated negative signaling by forming a bridge between mIg and FcγRIIs.

Many hypotheses have been published on the regulatory potential of RFs, but only few experimental data are available. A depressed anti-hapten response affecting all isotypes was shown in MRL-lpr/lpr mice characterized by a high level of RFs reacting with all IgG isotypes, whereas the selective suppression of IgG2a-type auto-

antibodies was observed in 129/Sv mice.[289,291] It was suggested that, depending on their regulatory potential, two categories of RFs exist. The first type of RF includes those that appear during the conventional antibody response, do not possess inhibitory capacity, and are induced by both T-independent and T-dependent antigens.[291] The other group represents high-affinity, isotype-specific RFs occurring in disease-prone mice and exhibiting inhibition of the IgG response. In contrast to these observations, Sinclair and Panoskaltsis[292] discussed the possibility of an up-regulation mediated by RFs acting as inhibitors for negative signaling via FcγRs.

Recent data demonstrated that the high-affinity, isotype-specific, immune complex-restricted RFs can also be observed in the course of the ongoing immune response against viral infections.[197] *In vivo* studies revealed that RFs representing the IgM class can mediate regulatory function in normal Balb/c mice activated by different antigens.[293] In these studies, IgM-type IgG2a-reactive autoantibodies were passively administered to virus-infected or oxazolone-boosted animals, and the titer of antigen-specific isotypes was tested after secondary antigen challenge. Injection of micro- to nanogram amounts of RFs resulted in decreased IgG2a-type antibody production during the secondary response, demonstrating an isotype-specific activity. The time kinetics of the antigen-specific response in antibody-treated animals, as well as the slight effect on total IgG2a levels in accordance with the low concentration of effective doses, argue against the simple chance of promoted clearance of IgG2a antibodies and support the possibility of regulatory processes. Although the mechanism of this isotype-specific regulatory effect of RFs has to be clarified, the protocol employed in these *in vivo* studies points to a direct B cell effect mediated by antigen-antibody-RF immune complexes.

V. REGULATORY MECHANISMS MEDIATED BY Fc-BINDING MOLECULES

The aim of the previous sections was to show that IgGFc-binding molecules, including membrane-bound FcγRIIs, IgG BFs, and RFs, play important roles in the regulation of antibody production. These structurally diverse molecules, which are of different origins, share some common features. All three interact with IgG-Fc, belong to the immunoglobulin superfamily of proteins, are components of the isotype-regulatory network, and regulate antibody production (at least partly) on the B cell level.

On the other hand, however, there are characteristic differences among these structures concerning origin, fine specificity, binding property, and way of action. To understand the complexity of the various regulatory pathways of antibody production, it seems worthwhile to compare the mechanisms of their action and to define their places in the hierarchy of molecules modifying the humoral immune responses.

Resting B cells express both mIg and FcγRII. Constitutive phosphorylation of the low-affinity FcγRII on resting B cells is negligible; neither activation of trypsinlike serine esterases nor release of FcγRII (IgG BF production) could be observed in the G_0 phase of the B cell cycle. In the very early stage of B cell activation, the type II FcγRs are phosphorylated, and this may result in conformational alteration of the molecules. Several functional consequences can be attributed to such conformational reorganization. First of all, the decreased binding capacity observed in this early phase of B cell activation can be explained by the phosphorylation of FcγRIIs. If the receptors are expressed but their binding capacity is decreased, one can speak about "functional down-regulation." This means that despite the presence of FcγRIIs, due to functional insufficiency, their cross-linking with mIg mediated by antigen-complexed IgG antibody is hindered. This excludes the induction of inhibitory signals via FcγRII, i.e., this mechanism is favorable to increasing antibody production in the early phase of B cell activation.

Conformational alteration might be favorable to the proteolytic cleavage of FcγRIIs as well. As activation of proteolytic enzymes can be seen as early as phosphorylation, one might suppose that proteolytic fragmentation of FcγRII (IgG BF production) and functional down-regulation of the receptor begin simultaneously.

After a transient decrease, a significant increase of FcγRII expression and release was found

in the early G_1 phase of the B cell cycle. This "functional up-regulation" (increased expression of functionally intact receptors) allows the cross-linking of mIg and FcγRII by antigen-IgG complexes. The "off" signal induced by this interaction of receptors might be an important step in regulatory events resulting in the gradual suspension of antibody production. At the same time another pathway of antibody regulation is opened: due to the increased proteolytic cleavage of FcγRII, the release of IgG BF is enhanced.

The mechanism of IgG BF's inhibiting the production of the given immunoglobulin isotype is not known. Because following activation the B cell can be regarded as the IgG BF source as well (according to our observations), we suggest that at this level of regulation IgG BF can be considered as an autocrine regulator of B cells. Whether IgG BF (a truncated FcγRII) can interact with native or aggregated membrane-bound IgG molecules should be elucidated; however, in this respect the IgG BF may behave like RF. On the other hand, it was shown that IgG BFs released from B cells inhibit antibody production under similar experimental conditions to T cell-derived IBFs, i.e., the involvement of T cells in this regulatory mechanism cannot be excluded.

Regulatory RFs, due to their functional restriction, cannot bind to nonaggregated mIg. However, it is most likely that cross-linked mIgs are able to interact with RFs that recognize merely complexed IgG. Therefore, in the early phase of B cell activation, the binding of RFs may provide an additional activation signal by further cross-linking mIg molecules. At the same time, the functional down-regulation of FcγRII excludes the induction of off signals, which could be otherwise elicited by IgG-containing RF complexes. In the early G_1 phase of B cell activation, due to the functional up-regulation of FcγRII, the binding of complexes containing both IgG and RF might promote the cross-linking of mIg and FcγRII. Consequently, in this stage of B cell activation the FcγR-mediated suppression of antibody production will be more pronounced.

In conclusion, the three different IgG Fc-binding molecules discussed in this review — the membrane-bound FcγRII, IgG BF, and RF — regulate antibody production of B cells by different mechanisms but with the same net result. In the early phase of B cell activation, they promote antibody production, whereas their inhibitory effect is dominant in later stages.

Finally, antigen presentation via mIg is an important function of B cells. In contrast to the FcεRII molecules expressed on the same B cell, FcγRIIB1, due to its structural properties, is not involved in antigen internalization but, consequently, in antigen presentation. On the other hand, RFs may influence the antigen-presenting function as well by promoting mIg-mediated antigen uptake.

REFERENCES

1. **Möller, G.,** Antibody mediated suppression of the immune response is determinant specific, *Eur. J. Immunol.*, 15, 409, 1985.
2. **Zalcberg, I., Silveira, A. V., and Möller, G.,** Regulation of the antibody response to sheep erythrocytes by monoclonal IgG antibodies, *Eur. J. Immunol.*, 17, 1343, 1987.
3. **Uhr, J. W. and Möller, G.,** Regulatory effect of antibody on the immune response, *Adv. Immunol.*, 8, 81, 1968.
4. **Voisin, G. A.,** Role of antibody classes in the regulatory facilitation reaction, *Immunol. Rev.*, 49, 3, 1980.
5. **Morgan, E. L. and Weigle, W. O.,** Biological activities residing in the Fc region of immunoglobulin, *Adv. Immunol.*, 40, 61, 1987.
6. **Jerne, N. K.,** Idiotypic networks and other preconceived ideas, *Immunol. Rev.*, 79, 5, 1984.
7. **Fridman, W. H., Daeron, M., Amigorena, S., Rabourdin-Combe, C., and Neauport-Sautes, C.,** Bases for an isotypic network, *Mol. Immunol.*, 23, 1141, 1986.
8. **Brüggemann, M. and Rajewsky, K.,** Regulation of the antibody response against hapten-coupled erythrocytes by monoclonal anti-hapten antibodies of various isotypes, *Cell. Immunol.*, 71, 165, 1982.
9. **Heyman, B. and Wigzell, H.,** Immunoregulation by monoclonal sheep erythrocyte specific IgG-antibodies: suppression is correlated to level of antigen binding and not to isotype, *J. Immunol.*, 132, 1136, 1984.
10. **Sinclair, N. K. St. C.,** Regulation of the immune response. I. Reduction in ability to inhibit longlasting IgG immunological priming after removal of the Fc fragment, *J. Exp. Med.*, 129, 1183, 1969.
11. **Heyman, B., Nose, M., and Weigle, W. O.,** Carbohydrate chains on IgG2b: a requirement for efficient feedback immunosuppression, *J. Immunol.*, 134, 4018, 1985.

12. **Feinstein, A. and Rowe, A. J.,** Molecular mechanism of formation of an antigen-antibody complex, *Nature*, 205, 147, 1965.
13. **Valentine, R. C. and Green, M. M.,** Electron microscopy of and antibody-hapten complex, *J. Mol. Biol.*, 27, 615, 197.
14. **Metzger, H.,** Effect of antigen binding on the properties of antibody, *Adv. Immunol.*, 18, 169, 1974.
15. **Huber, R., Deisenhofer, J., Colman, P. M., Matsushima, M., and Palm, W.,** Crystallographic structure studies of an IgG molecule and an Fc fragment, *Nature*, 264, 415, 1976.
16. **Huber, R., Deisenhofer, J., Colman, P. M., Matsushima, M., and Palm, W.,** X-ray diffraction analysis of immunoglobulin structure, in *Das Immunsystem*, Melchers, F. and Rajewsky, K., Eds., Springer-Verlag, Berlin, 1976.
17. **Schlessinger, J., Steiberg, I. Z., Givol, D., Hochman, J., and Pecht, I.,** Antigen-induced conformational changes in antibodies and in their Fab fragments studied by circular polarisation of fluorescence, *Proc. Natl. Acad. Sci. U.S.A.*, 72, 2775, 1975.
18. **Pecht, I.,** Recognition and allostery in the mechanism of antibody action, in *Das Immunsystem*, Melchers, F. and Rajewsky, K., Eds., Springer-Verlag, Berlin, 1976, 41.
19. **Zavodszky, P., Jaton, J. C., Venyaminov, S. Yu., and Medgyesi, G. A.,** Increase of conformational stability of two homogenous rabbit immunoglobulin G after hapten binding, *Mol. Immunol.*, 18, 39, 1981.
20. **Willan, K. J., Golding, B., Givol, D., and Dweck, R. A.,** Specific spin labeling of the Fc region of immunoglobulins, *FEBS Lett.*, 80, 133, 1977.
21. **Jaton, J. C., Wright, J. K., Schmidt-Kessen, A., and Engel, J.,** Interactions of monovalent and multivalent oligosaccharide ligands with homogenous anti-polysaccharide antibody and its possible effects on the conformation of the IgG molecule, in *Immunology 1978*, Gergely, J., Medgyesi, G. A., and Hollan, S. R., Eds., Akademiai Kiado, Budapest, 1978, 277.
22. **Metzger, H.,** The effect of antigen on antibodies: recent studies, *Contemp. Top. Mol. Immunol.*, 1, 119, 1978.
23. **Jaton, J. C., Huser, H., Braun, D. G., Givol, D., Pecht, I., and Schlessinger, J.,** Conformational changes induced in homogenous anti-Type III pneumococcal antibody by oligosaccharides in increasing size, *Biochemistry*, 14, 5312, 1975.
24. **Jaton, J. C., Huser, H., Reisen, W. F., Schlessinger, J., and Givol, D.,** The binding of complement by complexes formed between a rabbit antibody and oligosaccharides of increasing size, *J. Immunol.*, 116, 1363, 1976.
25. **Gergely, J., Medgyesi, G. A., Füst, G., and Rajnavölgyi, E.,** Constitution of immune complexes and their interaction with the complement system, in *Immunology 1978*, Gergely, J., Medgyesi, G. A., and Hollan, R. S., Eds., Akademiai Kiado, Budapest, 1978, 293.
26. **Medgyesi, G. A., Füst, G., Gergely, J., and Jaton, J. C.,** Activation of the classical complement pathway by homogenous anti-SIII antibody bound to bivalent or trivalent oligosaccharide antigens, *Mol. Immunol.*, 16, 949, 1979.
27. **Pecht, I., Ehrenberg, B., Calef, E., and Arnon, R.,** Conformational changes and complement activation induced upon antigen binding to antibodies, *Biochem. Biophys. Res. Comm.*, 74, 1302, 1977.
28. **Metzger, H.,** General aspects of antibody structure and function, in *Fc Receptors and the Action of Antibodies*, Metzger, H., Ed., Telford Press, Caldwell, NJ, 1990, 7.
29. **Metzger, H.,** Antibodies as effector molecules, *Harvey Lect. Ser.*, 80, 40, 1986.
30. **Sheriff, S., Silverton, E. W., Padlan, E. A., Cohen, G. H., Smith-Gill, S. J., Finzel, B. C., and Davis, D. R.,** Three-dimensional structure of an antibody-antigen complex, *Proc. Natl. Acad. Sci. U.S.A.*, 84, 8075, 1987.
31. **Fischmann, T., Souchon, H., Riottot, M. M., Tello, D., and Poljak, R. J.,** Crystallization and preliminary X-ray diffraction studies of two new antigen-antibody (lysozyme-Fab) complexes, *J. Mol. Biol.*, 203, 527, 1988.
32. **Wrigley, N. G., Brown, E. B., and Skehel, J. J.,** Electron microscopic evidence for the axial rotation and inter-domain flexibility of the Fab regions of IgG, *J. Mol. Biol.*, 169, 771, 1983.
33. **Wade, R. H., Taveau, J. C., and Lamy, J. N.,** Concerning the axial rotation flexibility of the Fab regions of immunoglobulin G, *J. Mol. Biol.*, 206, 349, 1989.
34. **Burton, R. D.,** Antibody: the flexible adaptor molecule, *TIBS*, 15, 64, 1990.
35. **Klein, M., Haeffner-Cavaillon, N., Isenman, D. E., Rivat, C., Navia, M. A., Davies, D. R., and Dorrington, K. J.,** Expression of biological effector functions by immunoglobulin G molecules lacking the hinge region, *Proc. Natl. Acad. Sci. U.S.A.*, 78, 524, 1981.
36. **Dorrington, K. J. and Klein, M.,** Aspects of immunoglobulin G structure relevant to its interaction with Fc receptors, *Mol. Immunol.*, 19, 1215, 1982.
37. **Burton, D. R.,** The conformation of antibodies, in *Fc Receptors and the Action of Antibodies*, Metzger, H., Ed., Telford Press, Caldwell, NJ, 1990, 31.
38. **Oi, V. T., Vuong, T. M., Hardy, R., Reidler, J., Dangl, J., Herzenberg, L. A., and Stryer, L.,** Correlation between segmental flexibility and effector function of antibodies, *Nature (London)*, 307, 136, 1984.
39. **Dangl, J. L., Wensel, T. G., Morrison, S. L., Stryer, L., Herzenberg, L. A., and Oi, V. T.,** Segmental flexibility and complement fixation of genetically engineered chimeric human, rabbit and mouse antibodies, *EMBO J.*, 7, 1989, 1988.
40. **Kabat, E. A., Wu, T. T., Bilofsky, H., Reid-Miller, M., and Perry, H.,** Sequences of proteins of im-

munological interest, U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, 1983.
41. **Marquart, M., Deisenhofer, J., Huber, R., and Palm, W.,** Crystallographic refinement and atomic models of the intact immunoglobulin molecule Kol and its antigen-binding fragment at 3.0 and 1.9 Å resolution, *J. Molec. Biol.*, 141, 369, 1980.
42. **Burton, D. R.,** Immunoglobulin G: functional sites, *Mol. Immunol.*, 22, 161, 1985.
43. **Birshtein, B. K., Campbell, R., and Diamond, B.,** Effects of immunoglobulin structure on Fc receptor binding: a mouse myeloma variant immunoglobulin with a γ2b-γ2a hybrid heavy chain having a complete γ2a Fc region fails to bind to γ2a Fc receptors on mouse macrophages, *J. Immunol.*, 129, 610, 1982.
44. **Rademacher, T. W. and Dwek, R. A.,** Structural, functional and conformation analysis of immunoglobulin G-derived asparagine-linked oligosaccharides, *Prog. Immun.*, 5, 95, 1984.
45. **Mizouchi, T., Taniguchi, T., Shimizu, A., and Kobata, A.,** Structural and numerical variations of the carbohydrate moiety of immunoglobulin G, *J. Immunol.*, 129, 2016, 1982.
46. **Deisenhofer, J.,** Crystallographic refinement and atomic models of a human Fc fragment and its complex with fragment B protein A from *Staphylococcus aureus* at 2.9 and 2.8 Å resolution, *Biochemistry*, 20, 2361, 1981.
47. **Sutton, B. J. and Phillips, D. C.,** The three-dimensional structure of the carbohydrate within the Fc fragment of immunoglobulin G, *Biochem. Soc. Trans.*, 11, 130, 1983.
48. **Leatherbarrow, R. J., Rademacher, T. W., Dwek, R. A., Woof, J. M., Clark, A., Burton, D. R., Richardson, N., and Feinstein, A.,** Effector functions of a monoclonal aglycosylated mouse IgG2a: binding and activation of complement component C1 and interaction with human monocyte Fc receptor, *Molec. Immunol.*, 22, 407, 1985.
49. **Padlan, E. A.,** X-ray diffraction studies of antibody constant regions, in *Fc Receptors and the Action of Antibodies*, Metzger, H., Ed., Telford Press, Caldwell, NJ, 1990, 12.
50. **Poljak, R. J., Amzel, L. M., Avey, H. P., Chen, B. L., Phizackerley, R. P., and Saul, F.,** Three-dimensional structure of the Fab′ fragment of a human immunoglobulin at 2.8 Å resolution, *Proc. Natl. Acad. Sci. U.S.A.*, 70, 3305, 1973.
51. **Davies, D. R. and Metzger, H.,** Structural basis of antibody function, *Annu. Rev. Immunol.*, 1, 87, 1983.
52. **Williams, A. F. and Barclay, A. N.,** The immunoglobulin superfamily — domains for cell surface recognition, *Annu. Rev. Immunol.*, 6, 381, 1988.
53. **Reid, K. B. M.,** Proteins involved in the activation and control of the two pathways of human complement, *Biochem. Soc. Trans.*, 11, 1, 1983.
54. **Schumaker, V. N., Calcott, M. A., Spiegelberg, H. L., and Müller-Eberhard, H. J.,** Ultracentrifuge studies of the binding of IgG of different subclasses to the C1q subunit of the first component of complement, *Biochemistry*, 16, 5175, 1976.
55. **Painter, R. H., Foster, D. B., Gardner, B., Hughes-Jones, N. C.,** Functional affinity constants of subfragments of immunoglobulin G for C1q, *Molec. Immunol.*, 19, 127, 1982.
56. **Burton, D. R., Boyd, J., Brampton, A. D., Easterbrook-Smith, S. B., Emanuel, E. J., Novotny, J., Rademacher, T. W., van Schravendijk, M. R., Sternberg, M. J. E., and Dwek, R. A.,** The C1q receptor site on immunoglobulin G, *Nature (London)*, 288, 338, 1980.
57. **Wright, K. J., Tschopp, J., Jaton, J.-C., and Engel, J.,** Dimeric, trimeric and tetrameric complexes of immunoglobulin G fix complement, *Biochem. J.*, 187, 775, 1980.
58. **Dower, S. K. and Segal, D. M.,** C1q binding to antibody-coated cells: predictions from a simple multivalent model, *Mol. Immunol.*, 18, 823, 1981.
59. **Liberti, P. A., Bausch, D. M., and Schoenberg, L. M.,** On the mechanism of C1q binding to antibody. I. Aggregation and/or distortion of IgG vs. combining site transmitted effects, *Mol. Immunol.*, 19, 143, 1982.
60. **Colomb, M. and Porter, R. R.,** Characterization of a plasmin-digest fragment of rabbit immunoglobulin gamma that binds antigen and complement, *Biochem. J.*, 145, 177, 1975.
61. **Yasmen, D., Ellerson, J. R., Dorrington, K. J., and Painter, R. H.,** The structure and function of immunoglobulin domains. IV. The distribution of some effector functions among the Cγ2 and Cγ3 homology regions of human immunoglobulin G, *J. Immunol.*, 116, 518, 1976.
62. **Boackle, R. J., Johnson, B. J., and Caughman, G. B.,** An IgG primary sequence exposure theory for complement activation using synthetic peptides, *Nature (London)*, 282, 742, 1979.
63. **Pristowsky, M. B., Kehoe, J. M., and Erickson, B. W.,** Inhibition of the classical complement pathway by synthetic peptides from the second constant domain of the heavy chain of IgG, *Biochemistry*, 20, 6349, 1981.
64. **Brunhouse, R. and Cebra, J. J.,** Isotypes of IgG: comparison of the primary structures of isotypes which differ in their ability to activate complement, *Molec. Immunol.*, 16, 907, 1979.
65. **Lukas, T. J., Munoz, H., and Erickson, B. W.,** Inhibition of C1-mediated immune hemolysis by monomeric and dimeric peptides from the second constant domain of human immunoglobulin G, *J. Immunol.*, 127, 2555, 1981.
66. **Duncan, A. R. and Winter, G.,** The binding site for C1q on IgG, *Nature (London)*, 332, 563, 1988.
67. **Winkelhake, J. L., Kunicki, T. J., Elcomebe, B. M., and Asrerm, R. H.,** Effects of pH treatments and deglycosylation of rabbit immunoglobulin G on the binding of C1q, *J. Biol. Chem.*, 255, 2822, 1980.
68. **Nose, M. and Wigzell, H.,** Biological significance

of carbohydrate chains on monoclonal antibodies, *Proc. Natl. Acad. Sci. U.S.A.*, 80, 6632, 1983.
69. **Berken, A. and Benacerraf, B.,** Properties of antibodies cytophilic for macrophages, *J. Exp. Med.*, 146, 241, 1966.
70. **Lynch, R. G., Sandor, M., Waldschmidt, T. J., Mathur, A., Schaiff, W. T., Berg, D. J., Snapp, K., Mueller, A., Robinson, M. G., Noben, N., and Rosenberg, M.G.,** Lymphocyte Fc receptors: expression, regulation and function, *Mol. Immunol.*, 27, 1167, 1990.
71. **Anderson, C. L. and Looney, R. J.,** Human leukocyte IgG Fc receptors, *Immunol. Today*, 7, 264, 1986.
72. **Anderson, C. L.,** Isolation of the receptor for human IgG from a human monocyte cell line (U937) and from human peripheral blood monocytes, *J. Exp. Med.*, 156, 1794, 1982.
73. **Perussia, B., Dayton, E. T., Lazarus, R., Fanning, V., and Trinchieri, G.,** Immune interferon induces the receptor for IgG1 on human monocytic and myeloid cells, *J. Exp. Med.*, 158, 1092, 1983.
74. **Frey, J. and Engelhardt, W.,** Characterization and structural analysis of Fc receptors of human monocytes, a monoblast cell line (U937) and a myeloblast cell line (HL-60) by monoclonal antibody, *Eur. J. Immunol.*, 17, 583, 1987.
75. **Allen, J. M. and Seed, B.,** Nucleotide sequence of three cDNAs for the human high affinity Fc receptor (FcRI), *Nucleic Acid Res.*, 16, 11824, 1988.
76. **Allen, J. M. and Seed, B.,** Isolation and expression of functional high-affinity Fc receptor complementary DNAs, *Science*, 243, 378, 1989.
77. **Rosenfeld, S. I., Looney, R. J., Leddy, J. P., Abraham, D. C., and Anderson, C. L.,** Human platelet Fc receptor for immunoglobulin G. Identification as a 40,000-molecular-weight membrane protein shared by monocytes, *J. Clin. Invest.*, 76, 2317, 1985.
78. **Looney, R. J., Abraham, G. N., and Anderson, C. L.,** Human monocytes and U937 cells bear two distinct Fc receptors for IgG, *J. Immunol.*, 136, 1641, 1986.
79. **Tax, W. J. M., Willems, H. W., Reekers, P. P. M., Capel, P. J. A., and Koene, R. A. P.,** Polymorphism in mitogenic effect of IgG1 monoclonal antibodies against T3 antigen on human T cells, *Nature*, 304, 445, 1983.
80. **Lewis, V. A., Koch, T., Plutner, H., and Mellman, I.,** A complementary cDNA clone for a macrophage-lymphocyte Fc receptor, *Nature*, 324, 372, 1986.
81. **Seki, T.,** Identification of multiple isoforms of the low-affinity human IgG Fc receptor, *Immunogenetics*, 30, 5, 1989.
82. **Brooks, D. G., Qiu, W. Q., Luster, A. D., and Ravetch, J. V.,** Structure and expression of human IgG FcRII (CD32). Functional heterogeneity is encoded by the alternatively spliced products of multiple genes, *J. Exp. Med.*, 170, 1369, 1989.
83. **Stuart, S. G., Trounstine, M. L., Vaux, D. J. T., Koch, T., Martens, C. L., Mellman, I., and Moore, K. W.,** Isolation and expression of cDNA clones encoding a human receptor for IgG (FcγRII), *J. Exp. Med.*, 166, 1668, 1987.
84. **Stengelin, S., Stamenkovic, I., and Seed, B.,** Isolation of cDNAs for two distinct human Fc receptors by ligand affinity cloning, *EMBO J.*, 7, 1053, 1988.
85. **Engelhardt, W., Geerds, C., and Frey, J.,** Distribution, inducibility and biological function of the cloned and expressed human βFc receptor II, *Eur. J. Immunol.*, 20, 1367, 1990.
86. **Ravetch, J. V. and Anderson, C. L.,** FcγR family: proteins, transcripts, and genes, in *Fc Receptors and the Action of Antibodies*, Metzger, H., Ed., Telford Press, Caldwell, NJ, 1990, 211.
87. **Perussia, B., Acuto, O., Terhorst, C., Faust, J., Lazarus, R., Fanning, V., and Trinchieri, G.,** Human natural killer cells analyzed by B73.1, a monoclonal antibody blocking Fc receptor function. II. Studies of B73.1 antibody-antigen interaction on the lymphocyte membrane, *J. Immunol.*, 130, 2142, 1983.
88. **Clarkson, S. B. and Ory, P. A.,** CD16. Developmentally regulated IgG Fc receptors on cultured human monocytes, *J. Exp. Med.*, 167, 408, 1988.
89. **Lanier, L. L., Ruitenberg, J. J., and Phillips, J. H.,** Functional and biochemical analysis of CD16 antigen on natural killer cells and granulocytes, *J. Immunol.*, 141, 3478, 1988.
90. **Karas, S. P., Rosse, W. F., and Kurlander, R. J.,** Characterization of the IgG-Fc receptor on human platelets, *Blood*, 60, 1277, 1982.
91. **Fleit, H. B., Wright, S. D., and Unkeless, J. C.,** Human neutrophil Fc receptor distribution and structure, *Proc. Natl. Acad. Sci. U.S.A.*, 79, 3275, 1982.
92. **Huizinga, T. W. J., van der Schoot, C. E., Jost, C., Klaassen, R., Kleijer, M., von dem Borne, E. G. Kr., Roos, D., and Tetteroo, P. A. T.,** The PI-linked receptor FcRIII is released on stimulation of neutrophils, *Nature*, 333, 667, 1988.
93. **Selvaraj, P., Rossed, W. F., Silber, R., and Springer, T. A.,** The major Fc receptor in blood has a phosphatidylinositol anchor and is deficient in paroxysmal nocturnal hemoglobinuria, *Nature*, 333, 565, 1988.
94. **Ravetch, J. V. and Perussia, B.,** Alternative membrane forms of FcγRIII(CD16) on human NK cells and neutrophils: cell-type specific expression of two genes which differ in single nucleotide substitutions, *J. Exp. Med.*, 170, 481, 1989.
95. **Simmons, and Seed, B.,** The Fc receptor of natural killer cells is a phospholipid-linked membrane protein, *Nature*, 333, 568, 1988.
96. **Peltz, A. G., Grundy, H. O., Lebo, R. V., Yssel, H., Barsh, G. S., and Moore, K. W.,** Human FcγRIII: cloning, expression, and identification of the chromosomal locus of two Fc receptors for IgG, *Proc. Natl. Acad. Sci. U.S.A.*, 86, 1013, 1989.
97. **Perussia, B., Tutt, M. M., Qiu, W. Q., Kuziel, W. A., Tucker, P. W., Trinchieri, G., Bennett,**

M., Ravetch, J. V., and Kumar, V., Murine natural killer cells express functional Fc receptor II encoded by the FcγRα gene, *J. Exp. Med.*, 170, 73, 1989.
98. **Scallon, J. B., Scigliano, E., Freedman, V. H., Miedel, M. C., Pan, Y.-C. E., Unkeless, J. C., and Kochan, J. P.,** A human immunoglobulin G receptor exists in both polypeptide-anchored and phosphatidylinositol-glycan-anchored forms, *Proc. Natl. Acad. Sci. U.S.A.*, 86, 5079, 1989.
99. **Werner, G., von dem Borne, A. E. G. K., Bos, M. J. E., Tromp, J. F., van der Plas-van Dalen, C. M., Visser, F. J., Engelfriet, C. P., and Tetteroo, P. A. T.,** Localization of the human NA1 alloantigen on neutrophil Fc-gamma receptors, in *Leukocyte Typing III*, Vol. 3, *Human Myeloid and Hematopoietic Cells*, Reihertz, E. L., Haynes, B. F., Nadler, L. M., and Bernstein, I. D., Eds., Springer-Verlag, New York, 1988, 109.
100. **Tetteroo, P. A. T., van der Schoot, C. E., Visser, F. J., Bos, M. J. E., and von dem Borne, A. E. G. K.,** Three different types of Fcγ receptors on human leukocytes defined by workshop antibodies: FcγRlow on neutrophils, FcγRlow of K/NK lymphocytes, and FcγRII, in *Leukocyte Typing III. White Cell Differentiation Antigens*, McMichael, A. J., Ed., Oxford University Press, Oxford, UK, 1987, 702.
101. **Fanger, M. W., Shen, L., Graziano, R. F., and Guyre, P. M.,** Cytotoxicity mediated by human Fc receptors for IgG, *Immunol. Today*, 10, 92, 1989.
102. **Anderson, P., Morimoto, C., Breitmeyer, J. B., and Schlossman, S. F.,** Regulatory interactions between members of the immunoglobulin superfamily, *Immunol. Today*, 9, 199, 1988.
103. **Miettinen, H. M., Rose, J. K., and Mellman, I.,** Fc receptor isoforms exhibit distinct abilities for coated pit localization as a result of cytoplasmic domain heterogeneity, *Cell*, 58, 317, 1989.
104. **Kimberly, R. P., Tappe, N. J., Merriam, L. T., Redecha, P. B., Edberg, J. C., Schwartzman, S., and Valinsky, J. E.,** Carbohydrates on human Fcγ receptors. Independence of the classical IgG and non-classical lectin-binding sites on human FcγRIII expressed on neutrophils, *J. Immunol.*, 142, 3923, 1989.
105. **Salmon, J. E., Kapur, S., and Kimberly, R. P.,** Opsonin-independent ligation of Fc receptors: the 3G8-bearing receptors on neutrophils mediate the phagocytosis of concanavalin A-treated erythrocytes and non-opsonized *Escherichia coli*, *J. Exp. Med.*, 166, 1798, 1987.
106. **Hoxie, J. A., Mathews, D. M., Callahan, J., Cassel, D. L., and Cooper, R. A.,** Transient modulation and internalization of T4 antigen induced by phorbol esters, *J. Immunol.*, 137, 1194, 1986.
107. **Alcantra, O., Phillips, J. L., and Bolt, D. H.,** Phorbol diesters and transferrin modulate lymphoblastoid cell transferrin receptor expression by two different mechanisms, *J. Cell. Physiol.*, 129, 329, 1986.
108. **Hunziker, W., Koch, T., Whitney, J. A., and Mellman, I.,** Fc receptor phosphorylation during receptor-mediated B-cell activation, *Nature (London)*, 345, 628, 1990.
109. **Sarmay, G., Pecht, I., and Gergely, J.,** Phosphorylation of type II Fcγ receptor on activated human B lymphocytes, *Int. Immunol.*, 2, 1235, 1990.
110. **Gergely, J. and Sarmay, G.,** Production of soluble FcγR by activated B lymphocytes, *Pathobiology*, in press.
111. **Okafor, G. O., Turner, M. W., and Hay, F. C.,** Localization of monocyte binding site of human immunoglobulin G, *Nature (London)*, 248, 228, 1974.
112. **Alexander, M. D., Andrews, J. A., Leslie, R. G. Q., and Wood, H. W.,** The binding of human and guinea pig IgG subclasses to homologous macrophages and monocyte receptors, *Immunology*, 35, 115, 1978.
113. **Barnett-Foster, D. E., Dorrington, K. J., and Painter, R. H.,** Structure and function of immunoglobulin domains. VIII. An analysis of the structural requirements in human IgG1 for binding to the Fc receptor of human monocytes, *J. Immunol.*, 124, 2186, 1980.
114. **Woof, J. M., Nik Jaafar, M. I., Jefferis, R., and Burton, D. R.,** The monocyte binding domain(s) on human immunoglobulin G, *Mol. Immunol.*, 21, 523, 1984.
115. **Ciccimara, F., Rosen, F. S., and Merler, E.,** Localisation of the IgG effector site for monocyte receptors, *Proc. Natl. Acad. Sci. U.S.A.*, 72, 2081, 1975.
116. **Ratcliffe, A. and Stanworth, D. R.,** The use of synthetic gamma chain peptides in the localization of the binding site(s) on human IgG1 for the Fc receptors of homologous monocytes and heterologous mouse macrophages, *Immunol. Lett.*, 4, 215, 1982.
117. **Nik Jaafar, M. I., Lowe, J. A., Ling, N. R., and Jefferis, R.,** Immunogenic and antigenic epitopes of immunoglobulins. V. Reactivity of a panel of monoclonal antibodies with subfragments of human Fc and abnormal paraproteins having deletions, *Mol. Immunol.*, 20, 679, 1982.
118. **Nik Jaafar, M. I., Lowe, J. A., Ling, N. R., and Jefferis, R.,** Immunogenic and antigenic epitopes of immunoglobulins. VII. The topographical distribution of Fc epitopes and the relationship of an isoallotypic specificity to the presence of histidine 435, *Mol. Immunol.*, 21, 137, 1984.
119. **Brüggemann, M., Williams, G. T., Bindon, C. I., Clark, M. R., Walker, M. R., Jefferis, R., Waldmann, H., and Neuberger, M. S.,** Comparison of the effector functions of human immunoglobulins using a matched set of chimeric antibodies, *J. Exp. Med.*, 166, 1351, 1987.
120. **Jefferis, R., Lund, J., and Pound, J.,** Molecular definition of interaction sites on human IgG for Fc receptors (huFcγR), *Mol. Immun.*, 27, 1237, 1990.
121. **Partridge, L. J., Woof, J. M., Jefferis, R., and Burton, D. R.,** The use of anti-IgG monoclonal antibodies in mapping the monocyte receptor site for IgG, *Mol. Immun.*, 23, 1365, 1986.

122. **Woof, J. M., Partridge, L. J., Jefferis, R., and Burton, D. R.,** Localization of the monocyte-binding region on human immunoglobulin G, *Mol. Immunol.*, 23, 319, 1986.
123. **Spiegelberg, H. L., Perlmann, H., and Perlmann, P.,** Interaction of K lymphocytes with myeloma proteins of different IgG subclasses, *J. Immunol.*, 116, 1464, 1976.
124. **Gergely, J. and Sarmay, G.,** IgG-Fc-receptors: ligand binding and lysis induction, *Immunol. Lett.*, 20, 1, 1989.
125. **Sarmay, G., Benczur, M., Petranyi, Gy., Klein, E., Kahn, M., Stanworth, D. R., and Gergely, J.,** Ligand inhibition studies on the role of Fc receptors in antibody-dependent cell-mediated cytotoxicity, *Mol. Immunol.*, 21, 43, 1984.
126. **Sarmay, G., Jefferis, R., Klein, E., Benczur, M., and Gergely, J.,** Mapping of the functional topography of Fc with monoclonal antibodies: localization of epitopes interacting with the binding sites of Fc receptors on human K cells, *Eur. J. Immunol.*, 15, 1037, 1985.
127. **Erdei, A., Benczur, M., Fabry, Zs., Dierich, M. P., and Gergely, J.,** C3 cleaved by, membrane proteases binds to C3b acceptors expressed on Con A-stimulated human lymphocytes and enhances antibody dependent cellular cytotoxicity, *Scand. J. Immunol.*, 20, 125, 1984.
128. **Sarmay, G., Jefferis, R., and Gergely, J.,** CH2 and CH3 domain deleted IgG1 paraproteins inhibit differently Fc receptor mediated binding and lysis, *Immunol. Lett.*, 12, 307, 1986.
129. **Rozsnyai, Z., Sarmay, G., Walker, M., Maslanka, K., Valasek, Z., Jefferis, R., and Gergely, J.,** Distinctive role of IgG1 and IgG3 isotypes in Fc R-mediated functions, *Immunology*, 66, 491, 1989.
130. **Sarmay, G., Rozsnyay, Z., and Gergely, J.,** FcγRII expression and release on resting and activated human B lymphocytes, *Mol. Immunol.*, 27, 1195, 1990.
131. **Sarmay, G., Istvan, L., and Gergely, J.,** Shedding and reappearance of Fc, C3 and SRBC receptors on peripheral lymphocytes from normal donors and chronic lymphatic leukaemia (CLL) patients, *Immunology*, 34, 315, 1978.
132. **Sandor, M., Füst, G., Medgyesi, G. A., and Gergely, J.,** Isolation and characterization of Fc receptor shed from human peripheral mononuclear blood cells, *Immunology*, 35, 559, 1978.
133. **Uher, F., Jancso, A., Sandor, M., Pinter, K., Biro, E., and Gergely, J.,** Interaction between actomyosin complexes and Fc receptors on human peripheral mononuclear cells, *Immunol. Lett.*, 2, 213, 1981.
134. **Sarmay, G., Benczur, M., Petranyi, Gy., Klein, E., Kahn, M., Stanworth, D. R., and Gergely, J.,** Ligand inhibition studies on the role of Fc receptors in antibody-dependent cell-mediated cytotoxicity, *Mol. Immunol.*, 21, 43, 1984.
135. **Sandor, M., Füst, G., Medgyesi, G. A., Erdei, A., and Gergely, J.,** The heterogeneity of Fc receptors of human peripheral mononuclear cells, *Immunology*, 38, 353, 1978.
136. **Sandor, M., Füst, G., Medgyesi, G. A., and Gergely, J.,** Isolation and characterization of Fc receptor shed from human peripheral mononuclear blood cells, *Immunology*, 35, 559, 1978.
137. **Sarmay, G., Rozsnyai, Z., Szabo, I., Biro, A., and Gergely, J.,** Modulation of type II Fcγ receptor expression on activated human B lymphocytes, *Eur. J. Immunol.*, 21, 241, 1991.
138. **Walker, M. R., Woof, J. M., Brüggemann, M., Jefferis, R., and Burton, D. R.,** Interaction of human chimeric antibodies with the human FcRI and FcRII receptors: requirements for antibody-mediated host-target cell interaction, *Mol. Immunol.*, 26, 403, 1989.
139. **Salmon, J. E. and Kimberly, R. P.,** Phagocytosis of concanavalin A-treated erythrocytes is mediated by the Fcγ receptor, *J. Immunol.*, 137, 456, 1986.
140. **Fridman, W. H. and Golstein, P.,** Immunoglobulin-binding factor present on and produced by thymus-processed lymphocytes (T cells), *Cell. Immunol.*, 11, 424, 1974.
141. **Fridman, W. H. and Sautes, C.,** Immunoglobulin-binding factors, in *Fc Receptors and the Action of Antibodies*, Metzger, H., Ed., Telford Press, Caldwell, NJ, 1990, 335.
142. **Le Thi Bich Thui, Samarut, C., Brochier, J., Fridman, W. H., and Revillard, J. P.,** Suppression of mitogen induced peripheral B cell differentiation by soluble receptors for Fc IgG released from lymphocytes, *Eur. J. Immunol.*, 10, 894, 1980.
143. **Neauport-Sautes, C., Dupuis, D., and Fridman, W. H.,** Specificity of Fc receptors on activated T cells. Relation with released immunoglobulin binding factor, *Eur. J. Immunol.*, 5, 849, 1975.
144. **Pure, E., Durie, C. J., Summerill, C. K., and Unkeless, J. C.,** Identification of soluble Fc receptors in mouse serum and the conditioned medium of stimulated B cells, *J. Exp. Med.*, 160, 1836, 1984.
145. **Calvo, C., Watanabe, F., Metivier, D., and Senik, A.,** Human monocyte-cell line U937 releases suppressive IgG BF, *Eur. J. Immunol.*, 16, 25, 1986.
146. **Le Thi Bich Thui, Samarut, C., Rabourdin-Combe, C., and Revillard, J. P.,** The suppressive activity of FcγRs is not related to their T cell origin, *Cell. Immunol.*, 68, 252, 1982.
147. **Fridman, W. H., Ed.,** Structures and functions of low affinity Fc receptors, *Chem. Immunol.*, 47, 1989.
148. **Daeron, M., Neauport-Sautes, C., Yodoi, J., Moncuit, J., and Fridman, W. H.,** Receptors for immunoglobulin isotypes (FcR) on murine T cells. II. Multiple FcR induction on hybridoma T cell clones, *Eur. J. Immunol.*, 15, 668, 1985.
149. **Hoover, R. G., Gebel, H. M., Dieckgraefe, B. K., Hickman, S., Rebbe, N. F., Hirayama, N., Ovary, Z., and Lynch, R. G.,** Occurrence and potential significance of increased number of T cells with Fc receptors in myeloma, *Immunol. Rev.*, 56, 115, 1981.
150. **Khayat, D., Dux, Z., Anavi, R., Shlomo, Y., Witz,**

I. P , and Ran, M., Circulating cell free Fcγ2b/1 receptor in normal mouse serum: its detection and specificity, *J. Immunol.*, 132, 2496, 1984.

151. **Khayat, D., Geffrier, C., Yoon, S., Scigliano, E., Soubran, C., Weil, M., and Unkeless, J. C.,** Soluble circulating Fcγ receptor in human serum, a new ELISA assay for specific and quantitative detection, *J. Immunol.*, 100, 235, 1987.
152. **Sarfati, M., Bron, D., Lagneaux, L., Fonteyn, C., Frost, H., and Delespesse, G.,** Elevation of Ige-binding factors in serum of patients with B-cell derived chronic lymphoid leukemia, *Blood*, 71, 94, 1988.
153. **Blank, U., Daeron, M., Galinha, A., Fridman, W. H., and Sautes, C.,** The carbohydrate moieties of suppressor IgG binding factor released by murine T cells, *Mol. Immunol.*, 24, 1061, 1987.
154. **Blank, U., Daeron, M., Galinha, A., Malard, V., Fridman, W. H., and Sautes, C.,** Identification of the FcγRII related component of murine IgG-BF, *Mol. Immunol.*, 26, 107, 1989.
155. **Daeron, M., Neauport-Sautes, C., Blank, U., and Fridman, W. H.,** 2.4G2 a monoclonal antibody to macrophage Fcγ receptors reacts with murine T cell Fcγ receptors and IgG-binding factors, *Eur. J. Immunol.*, 16, 1545, 1986.
156. **Sautes, C., Varin, N., Hogarth, P. M., Unkeless, J. C., Teillaud, C., Even, J., Lynch, A., and Fridman, W. H.,** Molecular and functional studies of recombinant soluble Fcγ receptors, *Mol. Immunol.*, 27, 1201, 1990.
157. **Levinson, A. I. and Martin, J.,** Rheumatoid factor: Dr. Jekyll or Mr. Hyde, *Br. J. Rheum.*, 27, 83, 1988.
158. **Poncet, P., Matthes, T., Billecoco, A., and Dighiero, G.,** Immunochemical studies of polyspecific natural autoantibodies: charge, lipid reactivity, $F(ab')_2$ fragment activity and complement fixation, *Mol. Immunol.*, 25, 981, 1988.
159. **Heller, G., Kolodny, M. H., Lepow, I. H., Jacobson, A. S., Rivera, M. E., and Marks, G. H.,** The haemagglutination test for rheumatoid arthritis. IV. Characterization of the rheumatoid agglutinating factors by analysis of serum fractions prepared by ethanol fractionation, *J. Immunol.*, 74, 340, 1955.
160. **Hartman, A. B., Mallett, C. P., Srinivasappa, J., Prabhakar, B. S., Notkins, A. L., and Smith-Gill, S. J.,** Organ reactive autoantibodies from non-immunized adult Balb/c mice are polyreactive and express non-biased V_H gene usage, *Mol. Immunol.*, 26, 359, 1989.
161. **Rousseau, P. G., Mallett, C. P., and Smith-Gill, S. J.,** A substantial proportion of the adult Balb/c available B cell repertoire consists of multireactive B cells, *Mol. Immunol.*, 26, 003, 1989.
162. **Lydyard, P. M., Quartey-Papafio, R., Bröker, B., Mackenzie, L., Jouquan, J., Blaschek, M. A., Steele, J., Petrou, M., Collins, P., Isenberg, D., and Youinou, P. Y.,** The antibody repertoire of early human B cells. I. High frequency of autoreactivity and polyreactivity, *Scand. J. Immunol.*, 31, 33, 1990.
163. **Fernandez, P.-A., Ternyck, T., and Avrameas, S.,** Immunochemical studies of a murine polyreactive IgG2b autoantibody with rheumatoid factor activity, *Mol. Immunol.*, 26, 539, 1989.
164. **Shlomshik, M. J., Nemazee, D. A., Sato, V. I., Van Snick, J., Carson, D. A., and Weigert, M.,** Variable region sequences of murine IgM anti-IgG monoclonal autoantibodies (rheumatoid factors). A structural explanation for the high frequency of IgM anti-IgG B cells, *J. Exp. Med.*, 164, 407, 1986.
165. **Manheimer-Lory, A. J., Monestier, M., Bellon, B., Alt, F. W., and Bona, C. A.,** Fine specificity, idiotypy, and nature of cloned heavy-chain variable region genes of murine monoclonal rheumatoid factor antibodies, *Proc. Natl. Acad. Sci. U.S.A.*, 83, 8293, 1986.
166. **Monestier, M., Manheimer-Lory, A., Bellon, B., Painter, C., Dang, H., Talal, N., Zanetti, M., Schwartz, R., Pisetsky, D., Kuppers, R., Rose, N., Brochler, J., Klareskog, L., Holmdahl, R., Erlanger, B., Alt, F., and Bona, C.,** Shared idiotypes and restricted immunoglobulin variable region heavy chain genes characterize murine autoantibodies of various specificities, *J. Clin. Invest.*, 78, 753, 1986.
167. **Burastero, S. E., Casali, P., Wilder, R., and Notkins, A. L.,** Monoreactive high affinity and polyreactive low affinity rheumatoid factors are produced by $CD5^+$ B cells from patients with rheumatoid arthritis, *J. Exp. Med.*, 168, 1979, 1988.
168. **Nakamura, M., Burastero, S. E., Notkins, A. L., and Casali, P.,** Human monoclonal rheumatoid factor-like antibodies from $CD5^+$ $(Leu\text{-}1)^+$ B cells are polyreactive, *J. Immunol.*, 140, 4180, 1988.
169. **Hayakawa, K., Hardy, R. R., Honda, M., Herzenberg, L., Steinberg, A. D., and Herzenberg, L. A.,** Ly-1 B cells: functionally distinct lymphocytes that secrete IgM autoantibodies, *Proc. Natl. Acad. Sci. U.S.A.*, 81, 2494, 1984.
170. **Hardy, R. R., Hayakawa, K., Shimizu, M., Yamasaki, K., and Kishimoto, T.,** Rheumatoid factor secretion from human $Leu\text{-}1^+$ B cells, *Science*, 236, 81, 1987.
171. **Pereira, P. and Coutinho, A.,** I-E-linked control of spontaneous rheumatoid factor production in normal mice, *J. Exp. Med.*, 170, 1825, 1989.
172. **Van Snick, J. L. and Masson, P. L.,** Age-dependent production of IgA and IgM autoantibodies against IgG2a in a colony of 129/Sv mice, *J. Exp. Med.*, 149, 1519, 1979.
173. **Van Snick, J. L. and Masson, P. L.,** Incidence and specificities of IgA and IgM anti-IgG autoantibodies in various mouse strains and colonies, *J. Exp. Med.*, 151, 45, 1980.
174. **Van Snick, J.,** A gene linked to the IgG-C locus controls the production of rheumatoid factor in the mouse, *J. Exp. Med.*, 153, 738, 1981.
175. **Dresser, D. W.,** Most IgM-producing cells in the mouse secrete autoantibodies (rheumatoid factor), *Nature*, 274, 480, 1978.

176. **Vischer, T. L.,** Neutral proteinases induce rheumatoid factor production in mouse spleen cell cultures, *Clin. Exp. Immunol.*, 55, 99, 1984.
177. **Dziarski, R.,** Preferential induction of autoantibody secretion in polyclonal activation by peptidoglycan and lipopolysaccharide, *J. Immunol.*, 128, 1018, 1982.
178. **Dziarski, E.,** Anti-immunoglobulin autoantibodies are not preferentially induced in polyclonal activation of human and mouse lymphocytes, and more anti-DNA and anti-erythrocyte autoantibodies are induced in polyclonal activation of mouse than human lymphocytes, *J. Immunol.*, 133, 2537, 1984.
179. **Levinson, A. I.,** Nature of stimulus for rheumatoid factor production, in *Autoantibodies to Immunoglobulins,* Shakib, F., Ed., *Monogr. Allergy,* S. Karger, Basel, 1989, 26.
180. **Shokri, F., Mageed, R. A., Maziak, B. R., and Jefferis, R.,** Expression of V_HIII-associated cross-reactive idiotype on human B lymphocytes. Association with staphylococcal protein A binding and *Staphylococcus aureus* Cowan I stimulation, *J. Immunol.*, 146, 936, 1991.
181. **Izui, S., Kelley, V. E., Masuda, K., Yoshida, H., Roths, J. B., and Murphy, E. D.,** Induction of various autoantibodies by mutant gene lpr in several strains of mice, *J. Immunol.*, 133, 227, 1984.
182. **Carson, D. A.,** Rheumatoid factor, in Kelly, W. N., Harris, E. D., and Ruddy, S., Eds., *Textbook of Rheumatology,* W. B. Saunders, Philadelphia, 1985, 664.
183. **Carson, D. A., Chen, P. P., Fox, R. I., Kipps, T. J., Jirik, F., Godfien, R. D., Silverman, G., Radoux, V., and Fong, S.,** Rheumatoid factor and immune networks, *Ann. Rev. Immunol.*, 5, 109, 1987.
184. **Hang, L. M., Theofilopoulos, A. N., and Dixon, F. J.,** A spontaneous rheumatoid arthritis-like disease in MRL/l mice, *J. Exp. Med.*, 155, 1690, 1982.
185. **Aho, K. and Wager, O.,** Production of "anti-antibodies" in rabbits. Appearance in rabbit serum of "anti-antibodies" reacting with autogenous and isogenous antibody, following autostimulation with protein antigens, *Ann. Med. Exp. Fenn.*, 39, 79, 1961.
186. **Van Snick, J. and Coulie, P.,** Rheumatoid factors and secondary immune responses in the mouse, *Eur. J. Immunol.*, 13, 890, 1983.
187. **Coulie, P. and Van Snick, J.,** Rheumatoid factors and secondary immune responses in the mouse. II. Incidence, kinetics and induction mechanisms, *Eur. J. Immunol.*, 13, 895, 1983.
188. **Nemazee, D. A. and Sato, V. L.,** Induction of rheumatoid antibodies in the mouse. Regulated production of autoantibody in the secondary humoral response, *J. Exp. Med.*, 158, 529, 1983.
189. **Stanley, S. I., Bishoff, J., and Davie, J. M.,** Antigen induced rheumatoid factors: protein and carbohydrate antigens induce different rheumatoid factor responses, *J. Immunol.*, 139, 2936, 1987.
190. **Stanley, S. L., Jr., Li, E., and Davie, J. M.,** Antigen induced rheumatoid factors: characteristics of monoclonal rheumatoid factors produced after protein and carbohydrate immunization, *Mol. Immunol.*, 25, 285, 1988.
191. **Nemazee, D. A.,** Immune complexes can trigger specific, T cell-dependent autoanti-IgG antibody production in mice, *J. Exp. Med.*, 161, 242, 1985.
192. **Coulie, P. and Van Snick, J.,** Rheumatoid factor (RF) production during anamnestic immune response in the mouse. III. Activation of RF precursor cells is induced by their interaction with immune complexes and carrier-specific helper T cells, *J. Exp. Med.*, 161, 88, 1985.
193. **Holmdahl, R., Klageskog, L., Anderson, M., and Hansen, C.,** High antibody response to autologous type II collagen is restricted to H-2^q, *Immunogenetics,* 24, 84, 1986.
194. **Casali, P., Burastero, S. E., Balow, J. E., and Notkins, A. L.,** High affinity antibodies to ssDNA are produced by CD^- B cells in systemic lupus erythematosus patients, *J. Immunol.*, 143, 3476, 1989.
195. **Tarkowski, A., Czerkinsky, C., and Nilsson, L.-A.,** Detection of IgG rheumatoid factor secreting cells in autoimmune MRL/l mice: a kinetic study, *Clin. Exp. Immunol.*, 58, 7, 1984.
196. **Izui, S. and Eisenberg, R. A.,** Circulating anti-DNA-rheumatoid factor complexes in MRL/l mice, *Clin. Immunol. Immunopathol.*, 15, 536, 1980.
197. **Fazekas, Gy., Rajnavölgyi, E., Kurucz, I., Sintar, E., Kiss, K., Laszlo, G., and Gergely, J.,** Isolation and characterization of IgG2a-reactive autoantibodies from influenza virus-infected BALB/c mice, *Eur. J. Immunol.*, 20, 2719, 1990.
198. **Paavonen, T., Quartey-Papalio, R., Delyes, P. J., Mackenzie, L., Lund, T., Youinou, P., and Lydyard, P. M.,** CD5 mRNA expression and autoantibodies in early human B cells immortalized by EBV, *Scand. J. Immunol.*, 31, 269, 1990.
199. **Taguchi, H., Kanoh, M., Takubo, N., Kadota, S., Kanazawa, K., Hitsumoto, V., Shirata, T., and Utsumi, S.,** IgG isotype and isotype specificity of murine monoclonal IgG rheumatoid factors, *Clin. Exp. Immunol.*, 80, 136, 1990.
200. **Shlomshik, M., Nemazee, D., Van Snick, J., and Weigert, M.,** Variable region sequence of murine IgM anti-IgG monoclonal autoantibodies (rheumatoid factors): II. Comparison of hybridomas derived by lipopolysaccharide stimulation and secondary protein immunization, *J. Exp. Med.*, 165, 970, 1987.
201. **Shlomshik, M. J., Marshak-Rothstein, A., Wolfowicz, C. B., Rothstein, T. L., and Weigert, M. G.,** The role of clonal selection and somatic mutation in autoimmunity, *Nature,* 328, 805, 1987.
202. **Jefferis, R. and Mageed, R. A.,** The specificity and reactivity of rheumatoid factors with human IgG, in *Autoantibodies to Immunoglobulins,* Shakib, F., Ed., Monogr. Allergy, S. Karger, Basel, 1989, 26.
203. **Pisetsky, D. S., Darwin, B. S., and Reich, C. F.,** IgG binding of monoclonal anti-nuclear antibodies from MLR-1pr mice, *Immunology,* 71, 586, 1990.

204. **Holmdahl, R., Nordling, C., Rubin, K., Tarkowski, A., and Klareskog, L.,** Generation of rheumatoid factors after immunization with collagen type II-anti-collagen II immune complexes. An anti-idiotypic antibody to anti-collagen II is also rheumatoid factor, *Scand. J. Immunol.*, 24, 197, 1986.
205. **Bona, C. A.,** Epibodies. A particular set of anti-idiotypes specific for autoantibodies, *Clin. Immunol. Newsl.*, 6, 87, 1985.
206. **Chen, P. P., Fong, S., Houghten, R. A., and Carson, D. A.,** Characterization of an epibody. An anti-idiotype that reacts with both the idiotype of rheumatoid factors (RF) and the antigen recognized by RF, *J. Exp. Med.*, 161, 323, 1985.
207. **Johnson, P. M., Phua, K. K., and Evans, H. B.,** An idiotypic complementary between rheumatoid factor and antipeptidoglycan antibodies?, *Clin. Exp. Immunol.*, 61, 373, 1985.
208. **Nardella, F. A., Teller, D. C., Barber, C. B., and Mannik, M.,** IgG rheumatoid factors and staphylococcal protein A bind to common molecular region on IgG, *J. Exp. Med.*, 162, 1811, 1985.
209. **Nardella, F. A., Schröder, A. K., Svensson, M.-L., Sjöquist, J., Barber, C., and Christensen, P.,** T15 group A streptococcal Fc receptor binds to the same location on IgG as staphylococcal protein A and IgG rheumatoid factors, *J. Immunol.*, 140, 922, 1987.
210. **Sasso, E. H., Barber, C. V., Nardella, F. A., Yount, W. J., and Mannik, M.,** Antigenic specificities of human monoclonal and polyclonal IgM rheumatoid factors, *J. Immunol.*, 140, 3098, 1988.
211. **Huck, S., Fort, P., Crawford, D. H., Lefranc, M.-P., and Lefranc, G.,** Sequence of a human immunoglobulin gamma 3 heavy chain constant region gene: comparison with the other human C genes, *Nucl. Acids Res.*, 14, 1779, 1986.
212. **Artandi, S. E., Canifield, S. M., Tao, M.-H., Calame, K. L., Morrison, S. L., and Bonagura, V. R.,** Molecular analysis of IgM rheumatoid factor binding to chimeric IgG, *J. Immunol.*, 146, 603, 1991.
213. **Parekh, R. B., Dwek, R. A., Sutton, B. J., Fernandes, D. L., Leung, A., Stanworth, D. R., Rademacher, T. W., Mizouchi, T., Taniguchi, T., Matsuta, K., Takeuchi, F., Nagano, Y., Miyamoto, T., and Kobata, A.,** Association of rheumatoid arthritis and primary osteoarthritis with changes in the glycosylation pattern of total serum IgG, *Nature*, 316, 452, 1985.
214. **Axford, J. S., Mackanzie, L., Lydyard, P. M., Hay, F. C., Isenberg, D. A., and Roitt, I. M.,** Reduced B cell galactosyltransferase activity in rheumatoid arthritis, *J. Immunol.*, 140, 3098, 1988.
215. **Newkirk, M. M., Lemmo, A., and Rauch, J.,** Importance of the IgG isotype, not the state of glycosylation, in determining human rheumatoid factor binding, *Arth. Rheum.*, 33, 800, 1990.
216. **Van Snick, J., Stassin, V., and de Lestre, B.,** Isotypic and allotypic specificity of mouse rheumatoid factors, *J. Exp. Med.*, 157, 1006, 1983.
217. **Aguado, M. T., Balderas, R. S., Rubin, R. L., Duchosal, M. A., Kofler, R., Birshtein, B. K., Secher, D. S., Dixon, F. J., and Theofilopoulos, A. N.,** Specificity and molecular characteristics of monoclonal IgM rheumatoid factors from arthritic and non-arthritic mice, *J. Immunol.*, 139, 1080, 1987.
218. **Stassin, V., Coulie, P., Birshtein, B., Secher, D., and Van Snick, J.,** Determinants recognized by murine rheumatoid factors: molecular localization using a panel of mouse myeloma variant immunoglobulins, *J. Exp. Med.*, 158, 1763, 1983.
219. **Izui, S., Eisenberg, R. A., and Dixon, F. J.,** IgM rheumatoid factors in mice injected with bacterial lipopolysaccharides, *J. Immunol.*, 122, 2096, 1979.
220. **Tarkowski, A., Holmdahl, R., and Klageskog, L.,** Rheumatoid factors in mice, in *Autoantibodies to Immunoglobulins*, Shakib, F., Ed., Monogr. Allergy, S. Karger, Basel, 1989, 26.
221. **Coutelier, J.-P., Van der Logt, J. T. M., Hessen, F. W. A., Vink, A., Warnier, G., and Van Snick, J.,** IgG2a restriction of murine antibodies elicited by viral infections, *J. Exp. Med.*, 165, 64, 1987.
222. **Coutelier, J.-P., Van der Logt, J. T. M., Hessen, F. W. A., Vink, A., and Van Snick, J.,** Virally induced modulation of murine IgG subclasses, *J. Exp. Med.*, 168, 2373, 1988.
223. **Rajnavölgyi, E., Fazekas, Gy., Daeron, M., Teillaud, J.-L., Fridman, W. H., Jefferis, R., and Gergely, J.,** Fine specificity and functional properties of rheumatoid factor-like autoantibodies isolated from influenza virus infected Balb/c mice, unpublished manuscript.
224. **Nardella, F. A., Teller, D. C., and Mannik, M.,** Studies on the antigenic determinants in the self-association of IgG rheumatoid factor, *J. Exp. Med.*, 154, 112, 1981.
225. **Robbins, D. L., Skilling, J., Benisek, W. F., and Wistar, R.,** Estimation of the relative avidity of 19S IgM rheumatoid factor secreted by rheumatic synovial cells for human IgG subclasses, *Arth. Rheum.*, 29, 722, 1986.
226. **Easterbrook-Smith, S. B., Vandenberg, R. J., and Alden, J. R.,** The role of Fc:Fc interactions in insoluble immune complex formation and complement activation, *Mol. Immunol.*, 25, 1331, 1988.
227. **Klaasen, R. J. L., Goldschmeding, R., Tetteroo, P. A. T., and von dem Borne, A. E. G. K.,** The Fc valency of an immune complex is the decisive factor for binding to low-affinity Fc receptors, *Eur. J. Immunol.*, 18, 1373, 1988.
228. **Jungi, T. W., Lerch, P. G., Cachelin, A. B., and Morell, A.,** Monomeric and dimeric IgG1 as probes for assessing high-affinity and low-affinity receptors for IgG on human monocyte derived macrophages and on activated macrophages, *Mol. Immunol.*, 55, 555, 1985.
229. **Doekes, G., Schouten, J., Cats, A., and Daha,**

M. R., Reduction of the complement activation capacity of soluble IgG aggregates and immune complexes by IgM-rheumatoid factor, *Immunology*, 55, 555, 1985.

230. **Doekes, G., and Hiemstra, P. S.,** Rheumatoid factor and complement activation, in *Autoantibodies to Immunoglobulins*, Shakib, F., Ed., *Monogr. Allergy*, S. Karger, Basel, 1989, 26.
231. **Rajnavölgyi, E., Reth, M., Uher, F., Miklos, K., Gergely, J., and Rajewsky, K.,** Fc-dependent effector functions of idiotype-anti-idiotype immune complexes, *Mol. Immunol.*, 21, 127, 1984.
232. **Tesar, J. T. and Schmid, F. R.,** Conversion of soluble immune complexes into complement-fixing aggregates by IgM-rheumatoid factor, *J. Immunol.*, 105, 1206, 1970.
233. **Tanimoto, K., Cooper, N. R., Johnson, J. S., and Vaughan, J. H.,** Complement fixation by rheumatoid factor, *J. Clin. Invest.*, 55, 437, 1975.
234. **Doekes, G., Van Es, L. A., and Daha, M. R.,** Activation of C1q by soluble IgG aggregates as detected by a novel one-step hemolytic assay that specifically measures proenzyme form of C1s, *J. Immunol.*, 131, 1924, 1983.
235. **Feinstein, A., Richardson, N. E., Gorick, B. D., and Hugh-Jones, N. C.,** Immunoglobulin M conformational change is a signal for complement activation, in *Protein Conformation as an Immunological Signal*, Celada, F., Schumaker, V. N., and Sercarz, E. E., Eds., Plenum Press, New York, 1983, 47.
236. **Chestnut, R. W., Colon, S. M., and Grey, H. M.,** Requirements for the processing of antigens by antigen-presenting B cells. I. Functional comparison of B cell tumors and macrophages, *J. Immunol.*, 129, 2382, 1982.
237. **Lanzavecchia, A.,** Antigen uptake and accumulation in antigen-specific B cells, *Immunol. Rev.*, 99, 39, 1987.
238. **DeFranco, A. L., Gold, M. R., and Jakway, J. P.,** B-lymphocyte signal transduction in response to anti-immunoglobulin and bacterial lipopolysaccharide, *Immunol. Rev.*, 95, 161, 1987.
239. **Cambier, J. C. and Ransom, J. T.,** Molecular mechanisms of transmembrane signalling in B lymphocytes, *Annu. Rev. Immunol.*, 5, 175, 1987.
240. **Klaus, G. G. B., Bijsterbosch, M. K., O'Garra, A., Harnett, M. H., and Rigley, K. P.,** Receptor signalling and crosstalk in B lymphocytes, *Immunol. Rev.*, 99, 19, 1987.
241. **McDougall, S. L., Grinstein, S., Gelfand, E. W.,** Activation of Ca^{++} dependent K^+ channels in human B lymphocytes by anti-immunoglobulins, *J. Clin. Invest.*, 81, 449, 1988.
242. **Ransom, J. T. and Cambier, J. C.,** The dynamics of K^+ efflux and Ca^{2+} influx in B lymphocytes following antigen receptor crosslinking, *Proc. Soc. Gen. Physiol.*, in press.
243. **Amigorena, S., Choquet, D., Teillaud, J.-L., Korn, H., and Fridman, W. H.,** Ion channels and B cell mitogenesis, *Mol. Immunol.*, 27, 1259, 1990.
244. **Ingles, J., Engel, P., De La Calle, O., and Gallart, T.,** Differential responsiveness of human B lymphocytes to phorbol ester and calcium ionophore based on their state of activation, *Immunology*, 67, 359, 1989.
245. **Lenhardt, W., Karasuyama, H., Rolink, A., and Melchers, F.,** Control of the cycle of murine B 11 of α- and β-growth factors and of B cell maturation factors, *Immunol. Rev.*, 99, 241, 1987.
246. **Melchers, F. and Lenhardt, W.,** Three restriction points in the cell cycle of activated murine B lymphocytes, *Proc. Natl. Acad. Sci. U.S.A.*, 82, 7681, 1985.
247. **Janeway, C. A., Jr., Bottomly, K., Babich, J., Conrad, P., Conzen, S., Jones, B., Kaye, J., Katz, M., McVay, L., Murphy, D. B., and Tite, J.,** Quantitative variation in Ia antigen expression plays a central role in immune regulation, *Immunol. Today*, 5, 99, 1984.
248. **Melchers, F., Erdei, A., Schultz, T., and Dierich, M. P.,** Growth control of activated, synchronized murine B cells by the C3d fragment of human complement, *Nature (London)*, 317, 264, 1985.
249. **Howard, M. and Paul, W. E.,** Regulation of B cell growth and differentiation by soluble factors, *Ann. Rev. Immunol.*, 1, 307, 1983.
250. **Barrett, T. B., Shu, G. L., Draves, K. E., Pezutto, A., and Clark, E. A.,** Signaling through CD19, Fc receptors or transforming growth factor-β: each inhibits the activation of resting human B cells differently, *Eur. J. Immunol.*, 20, 1053, 1990.
251. **Pezzutto, A., Dörken, B., Rabinovich, P. S., Ledbetter, J. A., Moldenhauer, G., and Clark, E. A.,** CD19 monoclonal antibody HD37 inhibits anti-immunoglobulin induced B cell activation and proliferation, *J. Immunol.*, 138, 2793, 1987.
252. **Sinclair, N. R. S., and Chan, P. L.,** Regulation of the immune response. IV. The role of the Fc-fragment in feedback inhibition by antibody, *Adv. Exp. Biol.*, 12, 609, 1971.
253. **Uhr, J. W. and Möller, G.,** Regulatory effect of antibody on the immune response, *Adv. Immunol.*, 8, 81, 1968.
254. **Abrahams, S., Phillips, R. S., and Miner, R. A.,** Inhibition of the immune response by tS antibody. Mechanism and site of action, *J. Exp. Med.*, 137, 870, 1973.
255. **Ryan, J. L. and Henkart, P. A.,** Fc receptor mediated inhibition of murine B lymphocyte activation, *J. Exp. Med.*, 144, 768, 1976.
256. **Kölsch, E., Oberbarnscheidt, J., Bruner, K., and Heuer, J.,** The Fc-receptor: its role in the transmission of differentiation signals, *Immunol. Rev.*, 49, 61, 1980.
257. **Uher, F. and Dickler, H. B.,** Independent ligand occupancy and cross-linking of surface Ig and Fc receptors downregulates B-lymphocyte function.

Evaluation in various B-lymphocyte populations, *Mol. Immunol.*, 23, 1177, 1985.
258. **Waldschmidt, T. J. and Vitetta, E. S.,** Fc receptors on B cells mediate down regulation of antibody secretion, *Ann. Immunol.*, 1985.
259. **Phillips, N. E. and Parker, D. C.,** Crosslinking of B lymphocytes, Fcγ receptors and membrane immunoglobulin inhibits anti-immunoglobulin-induced blastogenesis, *J. Immunol.*, 132, 627, 1984.
260. **Bijsterbosch, M. K. and Klaus, G. G.,** Crosslinking of surface immunoglobulin and Fc receptors on B lymphocytes inhibits stimulation of inositol phospholipid breakdown via the antigen receptors, *J. Exp. Med.*, 162, 1825, 1985.
261. **Rigley, K. P., Harnett, M. M., and Klaus, G. G. B.,** Co-cross-linking of surface immunoglobulin Fcγ receptors on B lymphocytes uncouples the antigen receptors from their associated G protein, *Eur. J. Immunol.*, 19, 481, 1989.
262. **Tsokos, G. C., Kinoshita, T., Thyphronitis, G., Patel, A. D., Dickler, H. B., and Finkelman, F. D.,** *J. Immunol.*, 144, 239, 1990.
263. **Carter, R. H., Spycher, M. O., Yin, C. N. G., Hoffman, R., and Fearon, D. T.,** Synergistic interaction between complement receptors type 2 and membrane IgM on B lymphocytes, *J. Immunol.*, 141, 457, 1988.
264. **Wiersma, E. J., Coulie, P. G., and Heyman, B.,** Dual immunoregulatory effects of monoclonal IgG-antibodies: suppression and enhancement of the antibody response, *Scand. J. Immunol.*, 29, 439, 1989.
265. **Heyman, B.,** Fc-dependent IgG-mediated suppression of the antibody response: fact or artifact?, *Immunol. Today*, 11, 310, 1990.
266. **Marcelletti, J. F. and Katz, D. H.,** FcεR$^+$ lymphocytes and the regulation of the IgE antibody system. I. A new class of molecules termed IgE-induced regulants (EIR) which modulate FcεR expression by lymphocytes, *J. Immunol.*, 133, 2821, 1984.
267. **Bonnefoy, J. Y., Defrance, T., Peronne, C., Menetrier, C., Pene, J., de Vries, J.-E., and Bancherau, J.,** Human recombinant interleukin 4 induces normal B cells to produce soluble CD23/IgR binding factor analogous to that spontaneously released by lymphoblastoid B cell lines, *Eur. J. Immunol.*, 18, 117, 1988.
268. **Delespesse, G. M., Sarfati, M., and Hofstetter, H.,** Human IgE-binding factors, *Immunol. Today*, 10, 159, 1989.
269. **Gordon, J., Flores-Romo, L., Cairns, J. A., Millsum, M. J., Lane, P. J., Johnson, G. D., and MacLennan, I. C. M.,** CD23: a multifunctional receptor/lymphokine?, *Immunol. Today*, 10, 153, 1989.
270. **Amin, A. R., Coico, R. F., Finkelman, F., Siskind, G. W., and Thorbecke, G. J.,** Release of IgD-binding factor by T cells under the influence of interleukin 2, interleukin 4, or cross-linked IgD, *Proc. Natl. Acad. Sci. U.S.A.*, 85, 9179, 1988.
271. **Fridman, W. H., Gresser, I., Bandu, M. T., Aguet, M., and Neauport-Sautes, C.,** Interferon enhances the expression of Fcγ receptors, *J. Immunol.*, 124, 2436, 1980.
272. **Daeron, M., Yodoi, J., Neauport-Sautes, C., Moncuit, J., and Fridman, W. H.,** Receptors for immunoglobulin isotypes (FcR) on murine T cells. I. Multiple FcR expression on T lymphocytes and hybridoma T cell clones, *Eur. J. Immunol.*, 15, 662, 1985.
273. **Hoover, R. G., Dieckgraefe, B. H., and Lynch, R. G.,** T cells with Fc receptors for IgA: induction of T cells in vivo by purified IgA, *J. Immunol.*, 127, 1560, 1981.
274. **Mathur, A. and Lynch, R. G.,** Increased Tγ and Tμ cells in Balb/c mice with IgG and IgM plasmacytomas and hybridomas, *J. Immunol.*, 136, 521, 1986.
275. **Daeron, M., Bonnerot, C., Sandor, M., Varin, N., Hogarth, P. M., Even, J., and Fridman, W. H.,** Molecular mechanisms regulating the expression of murine T cell Fcγ receptor II, *Mol. Immunol.*, 25, 1143, 1988.
276. **Brunati, S., Moncuit, J., Fridman, W. H., and Teillaud, J.-L.,** Regulation of IgG production by suppressor FcγRII$^+$ T hybridomas, *Eur. J. Immunol.*, 20, 55, 1990.
277. **Varin, N., Sautes, C., Galinha, A., Even, J., Hogarth, P. M., and Fridman, W. H.,** Recombinant soluble receptors for the Fcγ portion inhibit antibody production *in vitro*, *Eur. J. Immunol.*, 19, 2263, 1989.
278. **Bonnerot, C., Daeron, M., Varin, N., Amigorena, S., Hogarth, P. M., Even, J., and Fridman, W. H.,** Methylation in the 5′ region of the murine βFcγR gene regulates the expression of the Fcγ receptor II, *J. Immunol.*, 141, 1026, 1988.
279. **Daeron, M., Bonnerot, C., Latour, S., Benhamou, M., and Fridman, W. H.,** The murine αFcγR gene product: identification, expression, and regulation, *Mol. Immunol.*, 27, 1181, 1990.
280. **Weinshank, R. L., Luster, A. D., and Ravetch, J. V.,** Function and regulation of a murine macrophage-specific IgG Fc receptor, FcγR-α, *J. Exp. Med.*, 167, 1909, 1988.
281. **Yokota, A., Kikutani, H., Tanaka, T., Sato, R., Barsumian, E. L., Suemura, M., and Kishimoto, T.,** Two species of human FcεR II (FcεRII/CD23): tissue specific and interleukin-4 specific regulation of the gene expression, *Cell*, 55, 611, 1988.
282. **Laszlo, G. and Dickler, H. B.,** IL-4 induces loss of B lymphocyte FcγRII ligand binding capacity, *J. Immunol.*, 141, 3416, 1988.
283. **O'Garra, A., Rigley, K. P., Holman, M., McLaughlin, J. B., and Klaus, G. G.,** B cell stimulatory factor 1 reverses Fc receptor-mediated inhibition of B lymphocyte activation, *Proc. Natl. Acad. Sci. U.S.A.*, 84, 6254, 1987.
284. **Amigorena, S., Bonnerot, C., Choquet, D., Fridman, W. H., and Teillaud, J.-L.,** FcγRII expression in resting and activated B lymphocytes, *Eur. J. Immunol.*, 19, 1379, 1989.

285. **Gordon, J., Flores-Romo, L., Cairns, J. A., Millsum, M. J., Lane, P. J., Johanson, G. D., and MacLennan, I. C.,** CD23: a multifunctional receptor/lymphokine?, *Immunol. Today,* 10, 153, 1989.

286. **Gordon, J., Webb, A., Walker, L., Guy, G. R., and Rowe, M.,** Evidence for an association between CD23 and the low molecular weight B cell growth factor, *Eur. J. Immunol.,* 16, 1627, 1986.

287. **Hivroz, C., Valle, A., Brouet, J. C., Banchereau, J., and Grillot-Courvalin, C.,** Regulation by interleukin-2 of CD23 expression of leukemic and normal B cells: comparison with interleukin-4, *Eur. J. Immunol.,* 19, 1025, 1989.

288. **Letellier, M., Sarfati, M., and Delespesse, G.,** Mechanisms of formation of IgE-binding factors (soluble CD23). I. FcεRII bearing B cells generate IgE-binding factors of different molecular weights, *Mol. Immunol.,* 26, 1105, 1989.

289. **Monestier, M., Bellon, B., Manheimer, A. J., and Bona, C. A.,** Rheumatoid factors. Immunochemical, molecular and regulatory properties, *Ann. N.Y. Acad. Sci.,* 475, 106, 1986.

290. **Grabar, P.,** Autoantibodies and physiological role of immunoglobulins, *Immunol. Today,* 14, 337, 1983.

291. **Manheimer, A. and Bona, C.,** Age-dependent isotype variation during secondary immune response in MRL-lpr mice producing auto-anti-γ-globulin, *J. Exp. Med.,* 103, 105, 1985.

292. **Sinclair, N. R. St. C. and Panoskaltsis, A.,** Rheumatoid factor and Fc signaling: a tale of two Cinderellas, *Clin. Immunol. Immunopathol.,* 52, 133, 1989.

293. **Rajnavölgyi, E., Kurucz, I., Fazakas, Gy., Sintar, E., Laszlo, G., and Gergely, J.,** In vivo manipulation of IgG2a production by isotype specific autoantibodies, *Mol. Immunol.,* 27, 1241, 1990.