ADVANCES IN IMMUNOLOGY, VOL. 82

Interactions of Immunoglobulins Outside the Antigen-Combining Site

ROALD NEZLIN* AND VICTOR GHETIE†

*Department of Immunology, Weizmann Institute of Science, Rehovot 76100, Israel
†Cancer Immunobiology Center, University of Texas Southwestern Medical Center
Dallas, Texas 75390

I. Introduction

Immunoglobulin (Ig) molecules possess two main types of interactions. First, they can form specific immune complexes (IC) with antigens using the antigen-combining site located on the tips of Fab regions. Just as important is the second type of interactions, with sites localized on Ig constant domains. Some of these interactions relate to the effector functions of antibodies, which are the essential part of the immune response. They include such well-known reactions as the activation of the complement cascade and the activation of cells after binding the cell Fc receptors. Both these processes, which are stimulated by the formation of antigen–antibody complexes, enhance significantly the response against infections. Other effector functions are related to the transportation of Ig molecules through cell membranes and IgG homeostasis, both operating independently of antigen binding. Also important are Ig interactions with proteins of bacterial and virus origin, which can significantly influence the course of infection diseases. Many past methodological advances were based on studies on binding bacterial proteins to Ig molecules.

The main structural unit of immunoglobulins is an Ig fold or domain, a compact globule, which is formed by antiparallel strands arranged in two β-pleated sheets. The sheets are packed face to face and linked by a disulfide bond (Fig. 1). The amino acid residues that comprised interactions sites belong to the β-strands, as well as to the loops connecting the strands and the interdomain linkers.

In this chapter, we discuss structural and functional aspects of interactions of Ig molecules with various ligands of animal, plant, bacterial, viral, or synthetic origin that may occur in the circulation or on the cell surfaces.

II. Fc Receptor-Binding Sites

A. Fc Cell Receptors Involved in Effector Functions

1. Fc_γ Receptors

The Fc_γ receptor ($Fc_\gamma R$) family consists of three representatives, $Fc_\gamma RI$ (CD64), $Fc_\gamma II$ (CD32), and $Fc_\gamma RIII$ (CD16) expressed on almost all cells of the immune system (Hulett and Hogarth, 1994). $Fc_\gamma RI$ has a high affinity for

0065-2776/04 $35.00

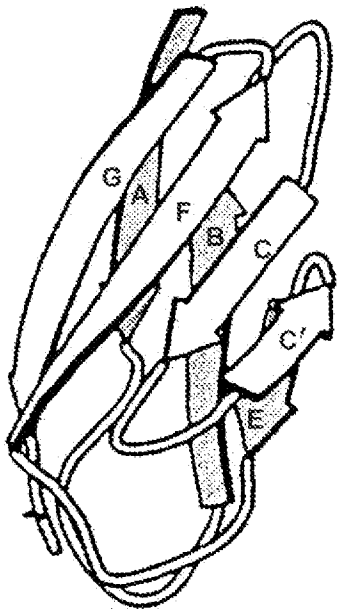

FIG 1 Structure of the C2 immunoglobulin domain. Reprinted from Barclay *et al.* (1997) with permission from Elsevier.

monomeric IgG ($10^8\ M^{-1}$), whereas the $Fc_\gamma RII$ and $Fc_\gamma RIII$ have a low affinity for monomeric IgG ($10^6\ M^{-1}$) but strongly bind IgG-containing IC. These $Fc_\gamma Rs$ exist in two or three isoforms, denoted a, b, and c. All $Fc_\gamma R$ are transmembrane glycoproteins belonging to the Ig superfamily, with the IgG-binding subunit consisting of two ($Fc_\gamma RIIb$ and c and IIIa and b) and three ($Fc_\gamma RIa$, b, and c) domains. There are activating ($Fc_\gamma RI$, $Fc_\gamma RIIa$, and $Fc_\gamma RIIIa$ and b) and inhibitory ($Fc_\gamma RIIb$) receptors coexpressed on the same effector cells. All $Fc_\gamma R$ show a high degree of sequence homology in their extracellular portion but differ in their cytoplasmic portion, carrying either the tyrosine-based activator (ITAM) ($Fc_\gamma RI$, $Fc_\gamma RIIa$, and $Fc_\gamma RIII$) or inhibitor (ITIM) ($Fc_\gamma RIIb$) motif (Ravetch and Bolland, 2001). The cross-linking of IgG antibodies bound to $Fc_\gamma RI$ by multivalent antigens or the interaction of IC with $Fc_\gamma RII$ and $Fc_\gamma RIII$ induces clustering of these receptors. As a result, the activation of cells and the initiation of a variety of effector mechanisms such as antibody-dependent cellular cytotoxicity, phagocytosis of IC, or release of inflammatory mediators are followed (Hogarth, 2002; Takai, 2002).

a. The Localization of the Fc-Binding Site on $Fc_\gamma R$. The crystal structures of the two Ig-like domains of Fc_γRII/III (D1 and D2) have been solved (Maxwell *et al.*, 1999; Sondermann *et al.*, 1999; Zhang *et al.*, 2000). It showed an acute hinge angle of about 50° between the two domains. The amino acid residues of the D2 domain are directly involved in the binding of the Fc_γ region, while the D1 domain is important for maintaining the D2 domain conformation (Radaev and Sun, 2001). The Fc_γRI also contains the D1 and D2 domains, which are highly homologous to their Fc_γRII/III counterparts, plus the third domain (D3) attached to the C-terminal end of domain D2 (Hulett and Hogarth, 1998). The significantly higher affinity of Fc_γRI for monomeric IgG is considered to be mediated by its third domain since both D1 and D2 displayed an affinity as low as that of the Fc_γRII/III (Harrison and Allen, 1998). Crystallographic data on the structure of Fc_γRI alone or in complex with Fc are absent, and the D2 domain of Fc_γRI may also be the key domain involved in the direct binding of IgG. The D3 domain may play a critical role in conferring high affinity and specificity by allowing receptor dimerization (Harrison and Allen, 1998).

The D2 domain consists of two β sheets arranged like a sandwich, one containing three antiparallel β strands (A, B, and E) and the other containing five (C′, C, F, G, and A′), a feature showed by the V domains of immunoglobulins. The Fc-binding sites are localized in the C′ strand and in the three main loops joining the BC, C′E, and FG strands. These loops and the C′ strand are adjacent and accessible to the Fc region. The replacement of most residues residing in the BC (Trp-113 to Ala-117), C′E (Asp-130 to His-134), and FG (Arg-155 to Lys-161) loops (alignment for Fc_γRIIIb) by alanine results in a decrease of the affinities for IgG (Radaev and Sun, 2001).

b. $Fc_\gamma R$ Interaction Sites of IgG. Earlier mutational analyses of the Fc residues involved in the binding of domain D2 pointed out that the main interaction site is localized in the lower hinge region and in the adjacent sites of the $C_\gamma 2$ domain (Tamm and Schmidt, 1997). The results of these experiments were confirmed by crystallographic studies of the human Fc_γRIII complexed with the Fc fragment of human IgG_1 (Radaev *et al.*, 2001; Sondermann *et al.*, 2000). The residues present in the interface between Fc_γRIII and both chains of the Fc fragment (denoted A and B) are shown in Table I (Radaev *et al.*, 2001). The differences in the amino acid sequence of the lower hinge region (234–239) of various isotypes of human and mouse IgGs may explain the hierarchy of their binding to FcR. Thus human IgG_3 and IgG_1 are binding to all FcRs with higher affinities than the IgG_4 and IgG_2, the latter devoid of any binding capacity to FcRI (Hulett and Hogarth, 1994).

The position of the D1 and D2 domains of Fc_γRIII into the opening of both Fc chains, which resembles a horseshoe, is shown in Fig. 2. The Fc_γRIII/Fc

TABLE I
INTERFACE CONTACTS BETWEEN HUMAN Fc_γRIII AND Fc_γ FRAGMENT[a]

Fc_γ	Location	Chain	Fc_γRIII	Loop/strand	Contacts
Leu-235	LH[b]	B	Thr-116	BC	H-S[c]
Leu-235	LH	A	His-135	C′E	H-S
Leu-235	LH	B	Val-158	FG	HC
Leu-235	LH	B	Gly-159	FG	HC
Gly-236	LH	A	His-134	C′E	H-S
Gly-236	LH	B	Val-156	FG	H-S
Gly-236	LH	B	Lys-161	FG	HC
Gly-236	LH	B	Tyr-90	A	HC
Gly-237	LH	A	Lys-120	C′	H-S
Gly-237	LH	A	His-134	C′E	H-S
Leu-238	LH	B	Gly-159	FG	HC
Ser-239	LH	A	Lys-120	C′	H-S
Asp-265	C_H2	A	Lys-120	C′	H-S
Pro-329	C_H2	B	Trp-90	A	HC
Pro-329	C_H2	B	Trp-113	BC	HC
Ala-330	C_H2	B	Ile-88	A	HC

[a]Radaev *et al.* (2001).
[b]LH, low hinge.
[c]H-S, hydrogen and salt bridges; HC, hydrophobic contact.

complex burried 1450 $Å^2$ of solvent-accessible area, from which approximately 60% is contributed by chains A and B of the lower hinge region (Radaev and Sun, 2001). This region is not visualized in the unbound Fc fragment, but becomes visible after cocrystallization with Fc_γRIII, indicating that the binding of the receptor stabilizes the conformation of the lower hinge region. The interface between Fc_γRIII and chain A is dominated by hydrogen bonding interactions, whereas chain B is involved in hydrophobic interactions (Table I). A hydrophobic proline "sandwich" is formed between Trp-90 and Trp-113 of Fc_γRIII and Pro-329 (chain B), which is extended, including Val-158 and Lys-161 (the aliphatic portion) from the receptor and Leu-235 (chain B) of Fc (Radaev *et al.*, 2001). The side chain of the Leu-235 residue also interacts with Gly-159 of the receptor so tightly that no other residue larger than glycine can be accommodated in this space. The steric constraint imposed by the Gly-159 and Leu-235 tight contact is reflected in the complete disruption of the interaction of Fc_γR with Fc by mutations of either one of these two residues (Hulett *et al.*, 1994). The mutation of Trp-113→Phe resulted in the loss of the binding of Fc, not only by disrupting the interaction with Pro-329 (Table I), but also by altering the orientation between D1 and D2 (Radaev and Sun, 2001).

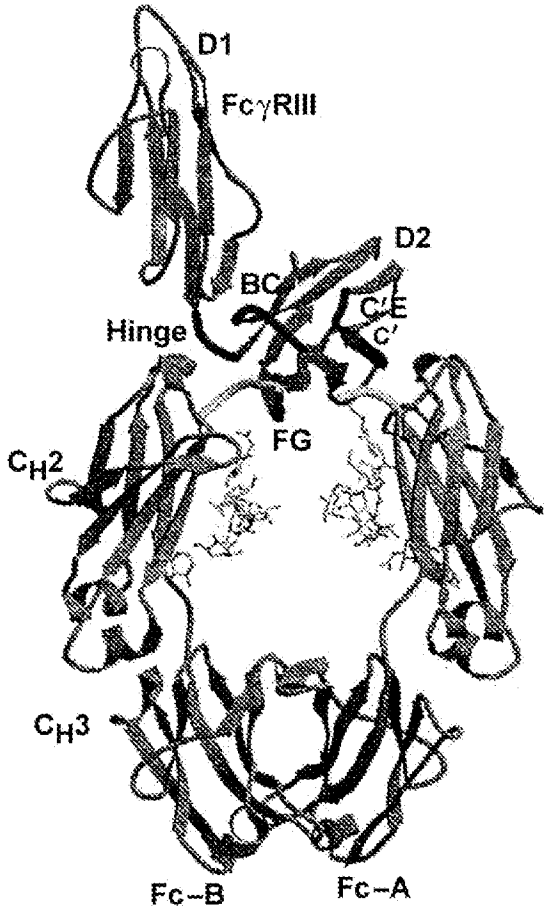

FIG 2 Model of the $Fc_\gamma R$ III complexed with IgG_1. Reprinted from Radaev and Sun (2001) with permission from Elsevier.

The presence of an N-linked oligosaccharide at Asp-297 in $C_\gamma 2$ is critical for binding to all $Fc_\gamma Rs$. The mutation of Asn-297 to Ala results in a considerably reduced affinity of the $Fc_\gamma Rs$ binding, as the carbohydrate located between the $C_\gamma 2$ domains is critical for the conformation of the region. The replacement of the amino acid residues interacting with the *N*-acetylglucosamine residues (e.g., Asp-265) altered both the composition of the carbohydrate and the binding to $Fc_\gamma RI$ (Jefferis and Lund, 2002).

In addition to these amino acid residues of the Fc region directly or indirectly involved in the binding to $Fc_\gamma Rs$, some other residues of the $C_\gamma 2$ domain

(Asp-270, Arg-292, Ala-327, Pro-331, and Lys-338) and $C_\gamma 3$ domain (Lys-414) are important for binding to $Fc_\gamma Rs$ (Canfield and Morrison, 1991; Shields *et al.*, 2001) (Fig. 3). Some mutations induced decreased binding to $Fc_\gamma RII$ and $Fc_\gamma RIII$, but not to $Fc_\gamma RI$ (e.g., Asp-270, Ala-327), or reduced binding to $Fc_\gamma RI$, but not $Fc_\gamma RIII$ (e.g., Arg-292, Lys-414). This was also true for a residue from the lower hinge (Ser-239), which, after mutation to alanine, affected only the binding to $Fc_\gamma RIII$, but not to $Fc_\gamma RI$ and $Fc_\gamma RII$. The fact that some mutations did not affect equally well the binding of IgG to all $Fc_\gamma Rs$ suggests that the contact interface between Fc_γ and the three $Fc_\gamma Rs$ may consist of some common (overlapping) residues, as well as a few distinct ones (Shields *et al.*, 2001).

c. The Stoichiometry of the $Fc_\gamma R$/Fc Interaction. Stoichiometry values of 1:1 for the $Fc_\gamma R$/Fc interaction have been obtained by ultracentrifugation and equilibrium size exclusion chromatography. X-ray structural studies of $Fc_\gamma RIII$ in the complex with Fc have shown that identical residues from the lower hinge A and B interact with different unrelated regions of the receptor. This excludes the possibility of the interaction of another receptor molecule with the same Fc_γ, thus confirming the 1:1 stoichiometry (Radaev *et al.*, 2001). Using nuclear magnetic resonance (NMR) spectroscopy, it was shown that mouse $Fc_\gamma RII$ binds to one of the two lower hinge regions of Fc, inducing a conformational change in the other site that precludes the binding of the second receptor. Precluding the $Fc_\gamma R$ aggregation by a divalent IgG molecule avoids permanent stimulation of the immune system by IgG monomers present in high concentration in serum (Kato *et al.*, 2000a, 2000b).

d. Functional Implications. $Fc_\gamma Rs$ link the humoral and cellular function of the immune system, being the key player in the activation and inhibition of the immune response (Hogarth, 2002; Takai, 2002). The engagement of the activating type of $Fc_\gamma Rs$ to targets coated by IgG antibodies triggers phagocytosis, cytolysis, and release of inflammatory cytokines. This activity can be downregulated by inhibitory $Fc_\gamma RIIb$, thus keeping the immune response within its normal limits (Ravetch and Bolland, 2001). Another important function of $Fc_\gamma R$ is the uptake of the IgG containing IC followed by intracellular degradation of antigen. The resulting peptides are directed either to the MHC antigen-presenting pathway or are completely destroyed. It is believed that defects in the activating/inhibitory function of $Fc_\gamma Rs$ or in the IC handling are linked to many autoimmune diseases (e.g., Goodpasture's syndrome, SLE, etc.) (Takai, 2002).

2. *Fc_α and Fc_μ Receptors*

Receptors for IgA, the second abundant Ig class, are expressed by different myeloid cells, including eosinophils, neutrophils, and monocytes (Monteiro and van de Winkel, 2003). The most studied receptor, $Fc_\alpha RI$ (CD89), is a

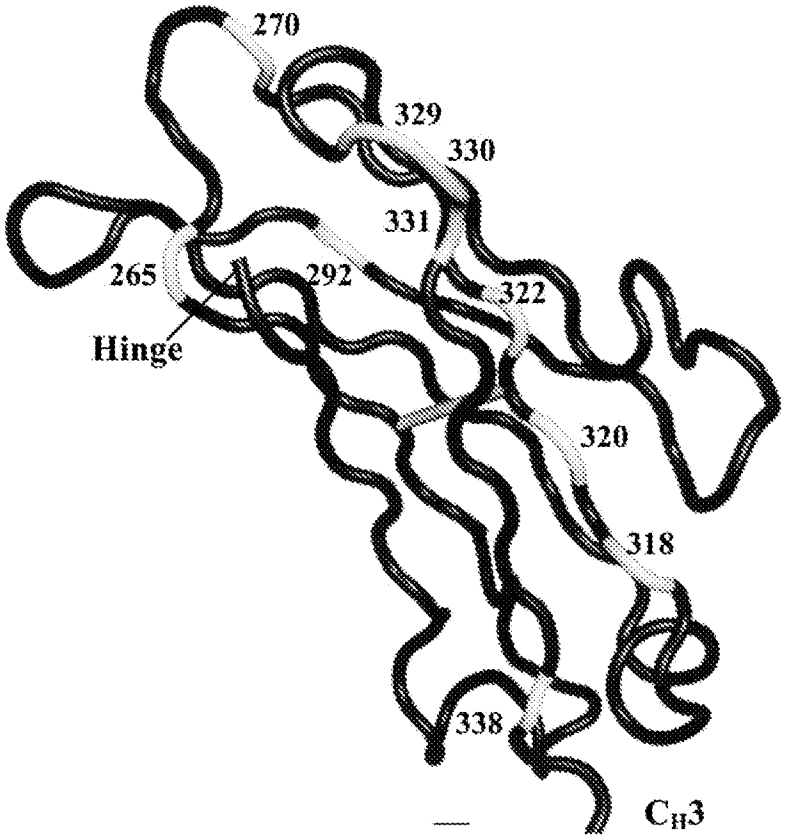

FIG 3 Amino acid positions in the $C_\gamma2$ domain involved in the binding of $Fc_\gamma R$ and C1q (prepared by J. Smallshaw). The participation of the amino acid residues from the lower hinge region in binding IgG to $Fc_\gamma R$ is presented in Table I.

Ligands	$C_\gamma2$ residues
All $Fc_\gamma Rs$ (human IgG_1)	265
$Fc_\gamma RII$, $Fc_\gamma RIII$ (human IgG_1); C1q (human IgG_1)	270
$Fc_\gamma RII$ (human IgG_1)	292
C1q (mouse IgG_{2b}); $Fc_\gamma RII$ (mouse IgG_{2b})	318
C1q (mouse IgG_{2b})	320
C1q (mouse IgG_{2b}, human IgG_1)	322
C1q (human IgG_1)	329
C1q (mouse $IgG_{2a,b}$); $Fc_\gamma RIII$ (human IgG_1)	330
C1q (human IgG_1); $Fc_\gamma RI$ (human IgG_3)	331
$Fc_\gamma RIII$ (human IgG_1)	338

heavily glycosylated transmembrane protein, the extracellular part of which comprises two Ig–like domains that are homologous to the proteins coded by the leukocyte receptor cluster, including the killer-inhibitory receptors. It is able to interact with both IgA subclasses with low affinity ($K_a \sim 10^6 M^{-1}$). Poly-IgA and IgA-IC are bound more effectively than monomeric IgA. Secretory IgA is able to bind $Fc_\alpha RI$ only in the presence of the integrin coreceptor Mac-1. The affinity of interaction is increased significantly after the immobilization of $Fc_\alpha RI$ (Wines *et al.*, 2001).

The localization of the $Fc_\alpha RI$ interaction site involved in binding IgA is different from the interaction sites of the other FcRs, as it locates in the membrane-proximal D1 domain. Studies of mutant $Fc_\alpha RI$ molecules and X-ray crystallographic studies of the $Fc_\alpha RI$–IgA_1 complex indicated that residues of four regions of D1, which build a single continuous region, participate in the interaction with IgA (Herr *et al.*, 2003; Wines *et al.*, 2001).

The IgA residues essential for interactions with human $Fc_\alpha RI$ were located at the $C_\alpha 2$–$C_\alpha 3$ interface. Using site-directed mutants, it was shown that two loops participate in the formation of the binding site (Pleass *et al.*, 1999, 2001). The first one belongs to the $C_\alpha 2$ AB helix/loop and comprises residues 257–259, which are conserved among human, mouse, and bovine IgA. The second region comprises residues 440–443 from the $C_\alpha 3$ FG loop. The critical role of these residues in binding $Fc_\alpha RI$ is supported by comparison of the reactivity of human IgA with bovine and mouse IgA. In bovine IgA, which is able to bind human $Fc_\alpha RI$, residues 440–443 are identical to those of human IgA. Mouse IgA with substitutions in positions 441 and 442 have no binding affinity. X-Ray crystallographic studies reveal that six residues of $Fc_\alpha RI$ and seven residues of Fc_α comprise a central hydrophobic core of the interface, which is flanked by several charged residues. Totally 19 conserved residues of Fc_α comprise the $Fc_\alpha RI$-binding site. The $Fc_\alpha RI$–IgA_1 interface buries 1656 $Å^2$ (Herr *et al.*, 2003).

The position of the IgA-binding site is markedly different from the receptor-binding sites of IgG and IgE, which are located correspondingly at $C_\gamma 2$ near the hinge region or at the top of the $C_\varepsilon 3$ domain. As a result, the IgA molecule bound to $Fc_\alpha RI$ located on the cell surface is in an "upright" position. IgG and IgE molecules bound to their receptors are in an opposite orientation (Herr *et al.*, 2003). A single Fc_α is able to bind two $Fc_\alpha RI$ molecules. The bivalent interaction of IgA with two $Fc_\alpha RI$ on the cell surface would lead to significantly higher binding activity and more effective stimulation of cell activities.

Several IgM-binding proteins were identified on B cells. One of them is anchored to the cell surface via a glycosylphosphatidylinositol linkage (Ohno *et al.*, 1990). Another one with both IgM- and IgA-binding activity ($Fc_{\alpha/\mu}R$) is expressed on human and mouse mature B cells and monocytes (Shibuya *et al.*, 2000). Its extracellular part consists of only one Ig-like domain, in contrast with

other Ig cell receptors. $Fc_{\alpha/\mu}R$ mediates endocytosis of IC composed of IgM antibody and staphylococcal cells.

3. *Fc_ε Receptors*

For the development of allergic reactions, the most important step is the interaction of IgE antibodies with the high-affinity IgE receptor, $Fc_\varepsilon RI$, expressed on mast cells, basophils, and some other cells. Multivalent allergens reacting with the bound IgE antibodies aggregate the IgE–$Fc_\varepsilon RI$ complex. The interaction results in signal transduction, leading to cellular activation and release of pharmacologically active substances mediating immediate hypersensitivity, thus triggering allergic reactions (Gould *et al.*, 2003; Metzger, 2002; Turner and Kinet, 1999).

$Fc_\varepsilon RI$ is a highly glycosylated heterodimer and contains an α chain, a β chain, and two γ chains. In a trimeric $Fc_\varepsilon RI$ molecule, the β chain is absent. The β and γ chains contain tyrosine activation motive (ITAM) and participate in intracellular signaling. The α chain is responsible for the interaction with IgE molecules. Its extracellular part is composed of two closely packed small Ig-like domains, α-1 (D1) and α-2 (D2), forming a convex surface adapted for interaction with a complimentary arch of Fc_ε. Mutational and structural studies had indicated that the α-2 domain and the linker between both domains are responsible for the contacts with Fc_ε fragment (Garman *et al.*, 2000).

The general structure of IgE is different from that of IgG. According to earlier fluorescent polarization studies, IgE molecules are less flexible and more compact (Nezlin, 1990). Fluorescence energy transfer experiments have shown that the IgE molecules are bent (Baird *et al.*, 1993), which could be explained by the bending of Fc domains in the domain linker regions. The results of X-ray structural studies correspond to a compact structure of Fc_ε. According to a suggested model, the $C_\varepsilon 3$–$C_\varepsilon 4$ domain two-fold axis is nearly perpendicular to that of the $C_\varepsilon 2$ domain and Fc_ε has an acutely bent structure (Wan *et al.*, 2002).

IgE reacts with $Fc_\varepsilon RI$ with high affinity ($K_d \sim 10^9$–10^{10} M^{-1}). Because the binding stoichiometry of the interaction is 1:1, monomeric IgE molecules cannot cross-link two receptors and induce cell activation. The receptor binds across the Fc_ε two-fold symmetry axis between the two Fc chains (Fig. 4) and therefore the interaction with the second receptor molecule is blocked. By genetic manipulation it was shown that the $C_\varepsilon 3$ domains of IgE molecule are involved in interaction with the receptor (Nissim *et al.*, 1993). According to X-ray crystallographic studies, residues of both $C_\varepsilon 3$ domains and of both $C_\varepsilon 2$–$C_\varepsilon 3$ linkers participate in contact with two binding sites on the $Fc_\varepsilon RI$ surface (Garman *et al.*, 1998, 2001).

Each of the $C_\varepsilon 3$ domains interacts with a distinct binding site on the surface of the $Fc_\varepsilon RI$ molecule. The first one is related to the C′C region of the α-2

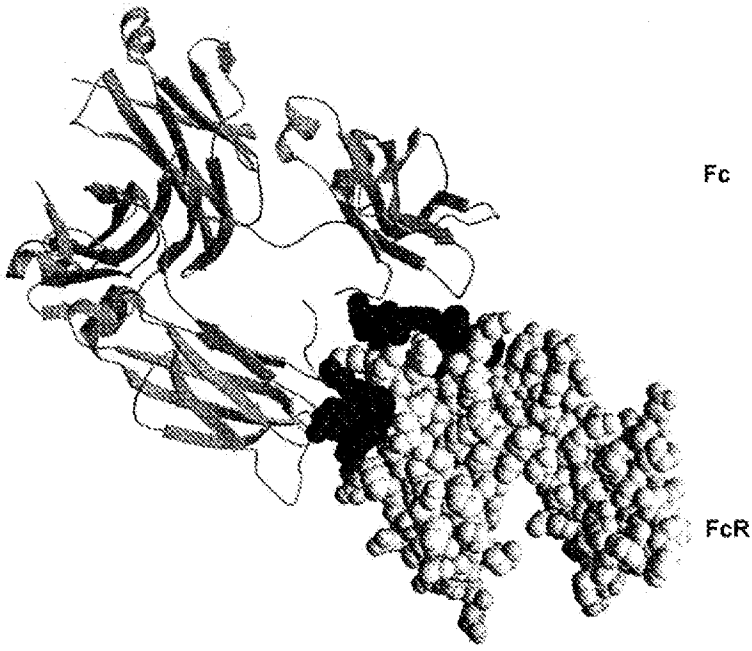

FIG 4 Model of the $Fc_{\varepsilon}RI\alpha$–Fc_{ε} complex. Reprinted from Garman *et al.* (2001) with permission from Elsevier.

domain, and Tyr-131 is a central residue of the site, which projects into a shallow pocket of one Fc_{ε} chain. The cluster of four exposed tryptophan residues located on the top of both receptor α domains is an important part of the second site. Two of these residues form a hydrophobic "sandwich" with Pro-426 of the second Fc_{ε} chain. Two other tryptophan residues interact with the $C_{\varepsilon}2$–$C_{\varepsilon}3$ linker. Site 1 is formed by 12 IgE residues and eight receptor residues burying about 860 $Å^2$ of surface area, and site 2 is formed by 10 IgE residues and eight receptor residues burying about 970 $Å^2$. Of the 15 receptor residues, seven are aromatic, which may contribute to the high stability of the IgE–receptor complex. Of 18 IgE residues interacting with $Fc_{\varepsilon}RI$, none is aromatic (Garman *et al.*, 2000; Wurzburg and Jardetzky, 2001).

These structural studies are important for understanding the mechanisms of allergy. The 1:1 stoichiometry of the $Fc_{\varepsilon}RI$–IgE interaction indicates that the essential step in the initiation of allergic reactions is the cross-linking of the receptor-bound IgE antibodies by multivalent allergens. X-ray crystallographic studies help indicate why IgE molecules react with the receptor with such a high affinity. Structural data are also very important for designing new types of inhibitory drugs useful for treatment of allergic diseases.

The second Fc_ε receptor, the low-affinity $Fc_\varepsilon RII$ (CD23), belongs to the C-type (calcium-dependent) lectins. It is expressed on a variety of cells in humans and reacts with IgE with an affinity 10^6–10^7 M^{-1}, as well as with several other proteins (Delespesse *et al.*, 1992). The binding stoichiometry of the CD23–IgE interaction is 2:1. It has been proposed that the receptor in an oligomeric form regulates IgE production (Kilmon *et al.*, 2001).

B. Fc Receptors Involved in Transcytosis and Catabolism

1. Neonatal Fc Receptor

The *neonatal Fc receptor* (FcRn) delivers IgG across the maternofetal barrier during gestation and is responsible for the maintenance of serum IgG levels by controlling its catabolism (Ghetie and Ward, 2000). The transfer of IgG from mother to young provides the newborn with humoral immunity. For maternal IgG to be transferred, several cellular barriers containing FcRn have to be crossed, first from the maternal blood across the placenta's trophoblasts (humans) or yolk sac cells (rodents) to the fetus, and second from the maternal blood into milk and then across the neonatal intestine following ingestion (rodents and ruminants) (Simister, 1998).

The persistence of IgG in the circulation is also regulated by FcRn present in the endothelial cells of adults (Borvak *et al.*, 1998; Ward *et al.*, 2003). FcRn as an IgG homeostat binds to IgG that is taken up by endothelial cells by nonspecific pinocytosis. The IgG molecules bound to FcRn are salvaged from degradation and recycle into the circulation. This process maintains the constant serum levels of IgG (Ghetie and Ward, 2000). A model of transcytosis and catabolism of IgG mediated by FcRn is presented in Fig. 5.

The key mechanism controlling both transport and recycling of IgG is its ability to react with FcRn in a pH-dependent manner. The receptor binds IgG at slightly acidic pH 6.0–6.5 and releases it at pH 7.0–7.4 (Martin *et al.*, 2001). After internalization, the binding of IgG to FcRn takes place mainly in the endosomal compartment, while the release takes place on the plasma membrane in contact with the slightly alkaline pH of the environment (blood) (Ghetie and Ward, 2000). IgG molecules in excess of the FcRn or unable to bind to FcRn are directed toward lysosomes and destroyed.

a. The Amino Acid Residues of FcRn Involved in the Binding of IgG. FcRn is a heterodimer of an α chain (homologous to MHC class I α chain) and β_2-microglobulin (Simister and Mostov, 1989). FcRn α-chain genes have been isolated and characterized. The FcRns of various species have a high degree of homology in the α-2 domain, which is involved in binding Fc_γ (Kacskovics *et al.*, 2000). The contact residues have been determined by X-ray crystallography and site-directed mutagenesis (Burmeister *et al.*, 1994a, 1994b; Martin

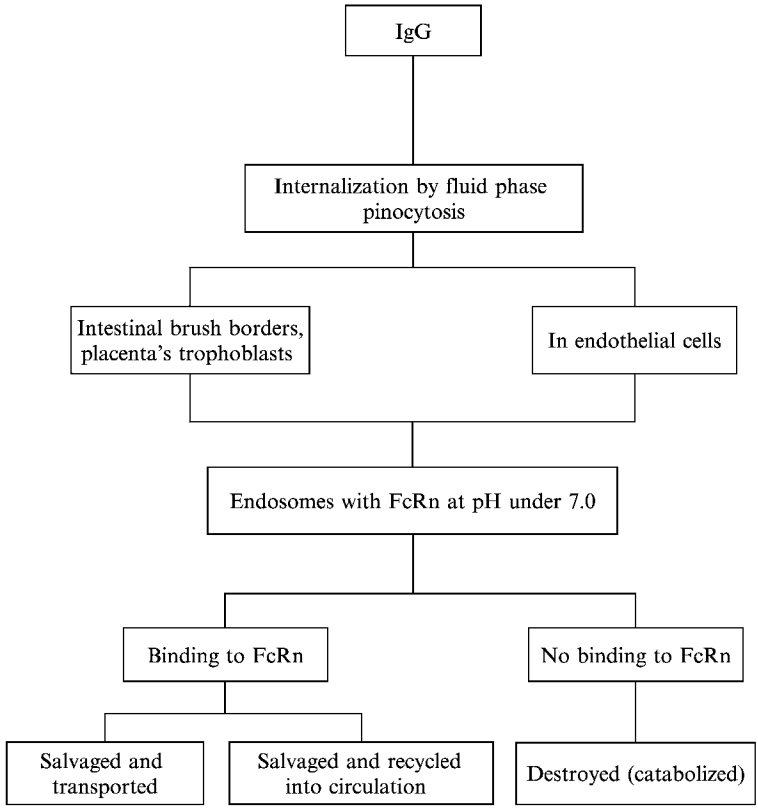

FIG 5 A model of transcytosis and catabolism of IgG mediated by FcRn.

et al., 2001; Vaughn *et al.*, 1997; West and Bjorkman, 2000). The residues that play a direct role in the interaction between IgG and FcRn are located in close proximity at the C end of the α-2 domain and are conserved in both human and mouse with the exception of residues 132 and 137 (Table II).

b. The Amino Acid Residues of IgG Involved in the Binding to FcRn. The delineation of IgG residues involved in regulation of transcytosis and catabolism was determined by site-directed mutagenesis (Kim *et al.*, 1994a; Medesan *et al.*, 1997) and confirmed by X-ray crystallography (Burmeister *et al.*, 1994b; Martin *et al.*, 2001). The resulting mutants were tested for binding recombinant FcRn and the ability to be transported through the neonatal intestine, yolk sac, placenta, and mammary gland (Cianga *et al.*, 1999; Firan *et al.*, 2001; Kim *et al.*, 1994b; Medesan *et al.*, 1996, 1997). The results demonstrated that

TABLE II
AMINO ACID RESIDUES INVOLVED IN THE FcRn–IgG INTERACTION[a]

Mouse FcRn[b]	Mouse IgG	Human FcRn[b]	Human IgG
Glu-117	His-310	Glu-117	His-310
Glu-118	Gln-311	Glu-118	Gln-311
Asp-132	His-435[c]	Glu-132	His-435[d]
Trp-133	Ile-253	Trp-133	Ile-253
Glu-137[e]	His-436[c]	Leu-137	Tyr-436[f]

[a]Ober *et al.* (2001).
[b]Homology alignment of mouse and human FcRn.
[c]In all IgGs except IgG_{2b}, where the residue is Tyr.
[d]In all IgGs except the IgG_3 allotype, where the residue is Arg.
[e]Asp in rat FcRn.
[f]In all IgGs except the IgG_3 allotype, where the residue is Phe.

TABLE III
DELINEATION OF THE AMINO ACID RESIDUES OF IgG INVOLVED IN THE BINDING OF FcRn, TRANSCYTOSIS AND CATABOLISM IN MOUSE[a]

			Transcytosis through	
Residue[b]	Affinity for FcRn	Catabolism	Intestine	Yolk sac
Wild type[c]	100	100	100	100
Ile-253	18	22	10	22
His-285[d]	98	89	98	83
His-310	13	14	12	17
His-433	75	97	71	78
His-435	5	15	25	11
His-436	20	41	51	83

[a]Medesan *et al.* (1997).
[b]All residues were mutated to Ala.
[c]The activities are expressed as percentage of the activity of the wild type.
[d]The His-285 is located in a loop on the external surface of the C_H2 domain, which is distal to the C_H2–C_H3 cleft (negative control).

residues located at the $C_\gamma2$ (Ile-253, His-310) and $C_\gamma3$ (His-435) domain interface are essential for transcytosis and regulation of IgG catabolism (Table III and Fig. 6). His-436 plays a minor but significant role in the mouse FcRn–IgG interaction.

Titration of His with a pK_a identical to the pH of the Fc–FcRn interaction (pH 6.5) accounts for the acid pH dependence of the binding. Thus there are

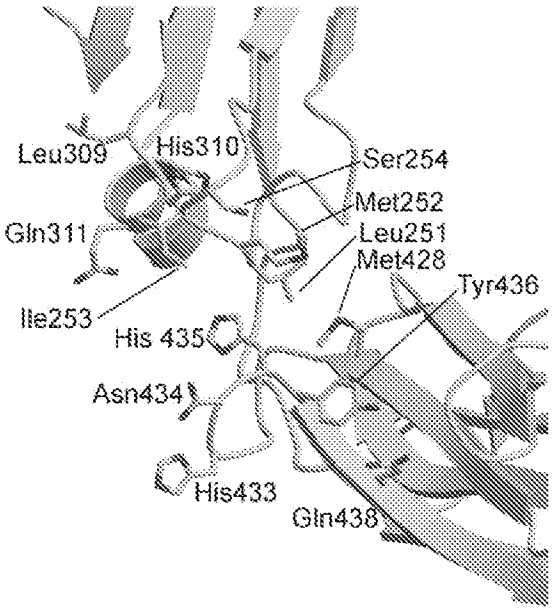

FIG 6 Structure of the $C_\gamma2$–$C_\gamma3$ cleft (prepared by P. Sondermann). The amino acid residues are those of human IgG_1. The following positions are involved in the binding: FcRn (253, 254, 309, 310, 433, 435, 436); RF (252, 253, 254, 433, 434, 435, 436); SpA (251, 252, 253, 254, 310, 311, 434, 435); SpG (251, 252, 253, 254, 311, 433, 434, 436, 438); and HSV-1 (435).

three pairs of salt bridges between rat Fc and FcRn, namely, His-310–Glu-117, His-435–Glu-132, and His-436–Asp-137 (Martin *et al.*, 2001). FcRn binds to Fc at pH < 6.5 (when the His residues are partially charged) and releases Fc after deprotonization at pH > 7.0. The acidic nature of the residues in FcRn and the presence of the His residues in IgG (Table II) strongly suggest that electrostatic forces may play a predominant role in mediating the FcRn–IgG interaction. Hydrophobic interactions between the Trp-133 in FcRn and Ile-253 of IgG are also certainly involved (Table II). In addition to these residues, others, at least in human IgG_1, are important for binding human FcRn. These include Ser-254, Lys-288 (in $C_\gamma2$) and Ser-415, His-433 (in $C_\gamma3$) (Shields *et al.*, 2001). However, in the absence of data on transcytosis and catabolism, it is difficult to estimate how IgG_1 mutants with these residues will be handled *in vivo*.

The close correlation between the effect of a given mutation of the mouse or human Fc on catabolism, transcytosis, and affinity for FcRn supports the concept that FcRn is involved in all three processes in mice and humans (Kim *et al.*, 1999; Medesan *et al.*, 1997). However, the ultimate proof that

FcRn plays a key role in both transcytosis and catabolism of IgG was provided by experiments using mice that do not express functional FcRn due to homozygous deletion of the gene encoding β_2-microglobulin. Thus the intestinal transfer of IgG was ablated in neonate mice with β_2 deficiency (Israel *et al.*, 1995). Similarly, the persistence (half-life) of IgG was considerably decreased in β_2-microglobulin knockout mice relative to wild-type mice (Ghetie *et al.*, 1996; Junghans and Anderson, 1996; Israel *et al.*, 1996) (Table IV). These results were recently confirmed by using mice, with a defective FcRn gene. Similar to β_2-microglobulin-deficient mice, these animals have a low perinatal IgG transport and a very short persistence of IgG in circulation (Roopenian *et al.*, 2003).

Human FcRn binds to all human IgG isotypes, but not to rat and mouse IgG, while mouse FcRn binds equally well to human, mouse, and rat IgG (Ober *et al.*, 2001). The lack of affinity of human FcRn for murine IgG explains the short persistence of mouse monoclonal IgG antibodies in humans as compared with human IgG. Conversely in mice, both human and mouse IgG have the same half-life, since they have a comparable affinity for mouse FcRn (Ghetie *et al.*, 2003).

c. The Conformational Dependence of the FcRn Interaction Site. The IgG residues that are critical for FcRn binding are located on three proximal loops of the $C_\gamma2$–$C_\gamma3$ cleft that are noncontiguous in the primary sequence (Table III and Fig. 6). This suggests that the relative position of residues might be dependent on the conformation of the β strands that support them and also on the topology of the $C_\gamma2$ and $C_\gamma3$ domain relative to each other. Thus the detrimental effect of mutating Pro to Ala at position 257 on the serum half-life and affinity for FcRn is most likely due to perturbation of the conformation of the loop encompassing Ile-253. The same sequence is most probably responsible for the shorter half-life of rat IgG_{2b} (Ala-257) relative to rat IgG_1 and IgG_{2a} (Pro-257) (Medesan *et al.*, 1998). The FcRn–IgG interaction can be also modulated by conformational effects of residues distal to the $C_\gamma2$–$C_\gamma3$ cleft

TABLE IV
INTESTINAL TRANSFER[a] AND CATABOLISM[b] OF IgG IN FcRn-DEFICIENT MICE

Mice	Intestinal transfer of mouse IgG_{2a} (mg equivalents/ml)	Catabolism of mouse IgG_1 (half-life, h)
Wild type	1.28	97.7
Deficient	0	17.6

[a]Israel *et al.* (1995).
[b]Ghetie *et al.* (1996).

(Zuckier *et al.*, 1998). Thus the removal of the hinge region from Fc results in a shorter half-life, indicating that this region determines the relative spatial orientation of $C_\gamma2$ and $C_\gamma3$ and the configuration of their interface. In fact, the replacement of the hinge sequence with a synthetic hinge containing an S–S bridge in the context of a different sequence generates an Fc fragment with a half-life similar to that of the wild type (Kim *et al.*, 1995).

d. The Stoichiometry of IgG–FcRn Interaction. The symmetry of the IgG molecule with two potential FcRn interaction sites suggests that one IgG molecule may bind two FcRn molecules simultaneously. The observation that a hybrid Fc fragment with only one functional FcRn interaction site per molecule is not transcytosed across the neonatal gut and has a considerably shorter half-life than the Fc fragment with two virtual FcRn interaction sites clearly suggested that two functional sites per IgG molecule are necessary for transcytosis and catabolism (Kim *et al.*, 1994b, 1994c).

Although the FcRn–Fc_γ complexes with 2:1 stoichiometry have been reported to form in solution (Martin and Bjorkman, 1999; Sanchez *et al.*, 1999), other studies indicated that the stoichiometry is 1:1 (Popov *et al.*, 1996). This observation suggests that the Fc or IgG molecules may be asymmetric with respect to FcRn binding. Sedimentation equilibrium clearly showed the existence of a high and low interaction site of the mouse Fc fragment for the mouse FcRn. A high-affinity 1:1 complex is first formed (binding constant $<0.13\,\mu M$), and subsequently a second FcRn molecule binds with lower affinity (binding constant $6\,\mu M$) (Schuck *et al.*, 1999). Recently it was shown that although soluble human FcRn does not dimerize, the membrane-anchored receptor can form dimers even in the absence of its IgG ligand (Praetor *et al.*, 2002). The reaction of the FcRn dimer with IgG may involve a symmetric (stand up) and asymmetric (lay down) configuration as suggested by the "ribbon" model of interaction (Raghavan and Bjorkman, 1996) (Fig. 7).

e. Engineering of the FcRn-Binding Site of IgG. The good correlation between the affinity of IgG for FcRn and IgG persistence in the circulation (Table III) has been exploited by engineering IgG molecules with increased affinity for FcRn and better pharmacokinetics. This was carried out by randomly mutating a recombinant murine Fc_γ at three residues located in proximity to the FcRn interaction site. Residues Thr-252, Thr-254, and Thr-256 were chosen because they are exposed and not highly conserved across species. One of these Fc_γ fragments with mutations to Leu-252, Ser-254, and Phe-256 had an affinity for FcRn three times higher than the wild type and a half-life of 153 h versus 93 h for the wild type (Ghetie *et al.*, 1997). Analysis of the affinity of human IgG_1 with multiple mutation in the $C_\gamma2$–$C_\gamma3$

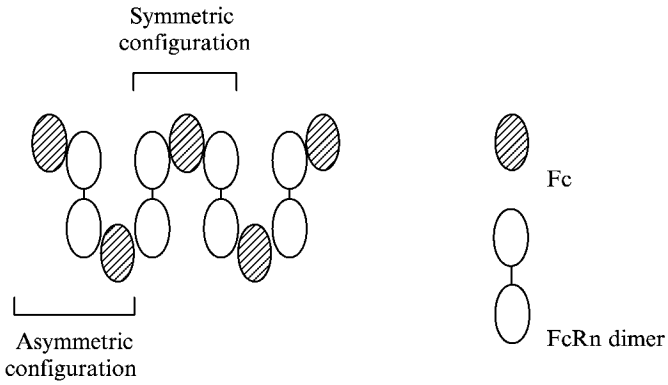

FIG 7 Schematic representation of the FcRn–Fc oligomeric "ribbon" model (according to Raghavan and Bjorkman, 1996). Both symmetric and asymmetric complexes have a 2:1 ratio between the FcRn dimer and the Fc fragment.

interface for human FcRn has shown that the affinity can be increased even higher (over 10 times) (Shields *et al.*, 2001). However, if the increase in affinity for FcRn at pH 6 is accompanied by a simultaneous increase at pH 7.4, this affects the release of IgG into the serum and offsets the benefits of the enhanced binding at pH 6. Consequently, human Fc_γ mutants with a 10- to 20-fold increase of their affinity at pH 6 for mouse FcRn and with a parallel increase at pH 7.4 have a significantly *shorter* half-life than the wild type. However, since these Fc_γ mutants do not have a parallel increase in their affinity for human FcRn at pH 7.4, it is possible that in humans their half-life would be longer than that of the wild type (Dall'Acqua *et al.*, 2002). Engineered humanized monoclonal antibody with a longer persistence in the circulation might have a better antitumor effect. Thus a long half-life results in a higher concentration gradient across cellular barriers with better penetration into the targeted tumor tissue (Ghetie *et al.*, 2004).

f. Therapeutic Implications. The administration of high doses of intravenous immunoglobulins (IVIG) leads to the enhanced catabolism of endogenous IgG (including autoantibodies). This is the result of saturation of FcRn in patients and leads to the destruction of all IgG present in excess (Masson, 1993; Yu and Lennon, 1999). The contribution of enhanced IgG catabolism to the beneficial effects of IVIG in the treatment of some autoimmune diseases is also suggested by the observation that the severity of experimental systemic lupus erythematosus (SLE) is greatly attenuated in β_2-microglobulin-deficient mice with a nonfunctional FcRn. The very low levels of IgG and antibodies in

these mice are the cause of the protection from the disease. Hence, since the mice lack a functional FcRn, they rapidly catabolize normal IgG and pathogenic autoantibody (Christianson *et al.*, 1997). It was also shown that β_2-microglobulin knockout mice are protected against some other antibody-mediated diseases (e.g., bullous pemphigus), since the amount of IgG antibodies reaching the epithelial target is greatly reduced due to their hypercatabolism (Liu *et al.*, 1997). Accordingly, the development of SLE in MLR-1pr mice can be blocked in the majority of cases by treatment with a synthetic peptide interacting with the FcRn-binding site of IgG (Marino *et al.*, 2000).

2. *Polymeric Immunoglobulin Receptor*

The transport of dimeric IgA (dIgA) and pentameric IgM (both associated with the J chain) to the mucosal lumen is mediated by the polymeric immunoglobulin receptor (pIgR). The receptor is localized on the basolateral face of the epithelial cells that have to be crossed over by pIg to reach the mucosal surface (e.g., gastrointestinal or respiratory tract). At the basolateral surface pIgR binds noncovalently polymeric immunoglobulins (pIgs) through the Fc regions, and after endocytosis it subsequently translocates pIgs to the apical surface of the cells. During this translocation, some pIgs (e.g., human dimeric IgA) are also covalently bound through their Fc region to pIgR (Norderhaug *et al.*, 1999). The release of the transcytosed pIgs from the plasma membrane takes place by proteolytic cleavage of the extracellular portion of pIgR (the secretory component) that remains attached to pIgs.

The majority of investigations focused on the interaction of dIgA with pIgR or with the secretory component (SC), because the secretory IgA is the main immunoglobulin that mediates the humoral defense of mucosal surfaces. The secretory IgA consists of two molecules of IgA dimerized tail to tail with one J chain molecule inserted between the penultimate cysteine residue of the two α-chain tail pieces (Corthesy, 2002). To the Fc region of both IgA molecules, the SC is attached by noncovalent (rabbit) or noncovalent and covalent (human) bonds (Fallgreen-Gebauer *et al.*, 1993; Frutiger *et al.*, 1986).

SC contains all five Ig-like homologous extracellular domains of pIgR (I–V) (Norderhaug *et al.*, 1999). The first four are structurally similar to the V region of Ig molecules (with all three CDR-like loops), while the fifth is similar to the C region (Coyne *et al.*, 1994). Each domain consists of ~110 residues and is stabilized by one (domain II) and two (domains I, III, IV, and V) disulfide bonds. Only two domains of pIgR/SC are directly involved in binding the Fc region of dIgA. Domain I carries the site for the noncovalent interaction with both dIgA and IgM (Bakos *et al.*, 1994). The mapping of the dIgA-binding site has revealed that a highly conserved sequence comprising residues 15–37 (in a CDR1-like loop) may be one of the binding site of domain I (Bakos *et al.*, 1991). Other sites located in CDR2-like and CDR3-like loops of domain I may

also be involved in the noncovalent binding to dIgA (Coyne *et al.*, 1994). The binding of domain I of pIgR/SC to dIgA involves the $C_\alpha3$ domain. The residues between positions 402 and 410 (Gln-Glu-Pro-Ser-Gln-Gly-Thr-Thr-Thr), located in an exposed loop of $C_\alpha3$, are considered to be a potential binding site (Hexham *et al.*, 1999).

Domain V carries the site for the covalent interaction with dIgA belonging to some species (human, murine, bovine, etc.). SC is bound to the dIgA–J complex by a single disulfide bond between Cys-463 of domain V and Cys-311 of $C_\alpha2$ (Fallgreen-Gebauer *et al.*, 1993). A putative model of the interaction between dIgA and SC involves noncovalent binding of domain I with the $C_\alpha3$ domain of one IgA molecule and a disulfide bond between domain V and the $C_\alpha2$ domain of the other IgA molecule. According to this model, only one of the α-chains of both IgA molecules is involved in the binding of SC, as the other pairs remain unoccupied (Norderhaug *et al.*, 1999). Domains II and III of SC do not interact directly with Fc of dIgA, but they are necessary for the covalent binding of domain V to $C_\alpha3$ of IgA (Crottet and Corthesy, 1999).

The fact that dIgA and IgM, devoid of the J chain, do not bind SC (Vaerman *et al.*, 1998) suggests that pIgR/SC needs to bind not only to the Fc region of IgA, but also to the J chain to form a stable IgA2–SC–J complex. It was speculated that such J chain-binding sites might be located on domain I of pIgR/SC (Norderhaug *et al.*, 1999). Thus the formation of the SC/dIgA complex is the result of the simultaneous noncovalent binding of pIgR/SC not only to $C_\alpha3$ of IgA, but also to the J chain via binding sites located on domain I.

The local humoral immune response is mainly mediated by secretory IgA, which plays a major role in protecting the mucosal surface against the invasion of pathogenic agents. SC present in the molecules of secretory IgA antibodies has a double role. First, it enhances the stability of the antibody by conferring resistance to the proteolytic attack of bacteria or local proteases (Crottet and Corthesy, 1998), and second, it ensures, through its multiple carbohydrate residues, appropriate tissue localization by anchoring the antibody to mucus lining the epithelial surface (Phalipon *et al.*, 2002).

III. Complement-Binding Sites

The complement system is an important mediator of innate immunity, linking it to the adaptive immune system. An array of complement proteins plays a significant role in host defense against infections and in inflammation (Reid, 1996). For the activation and function of the complement system, one of the most important steps is interaction of Ig molecules and IC with complement components. Several complement proteins interact with immunoglobulins (Miletic and Frank, 1995). The major Ig molecules, IgG and IgM, bind the C1q complement component, which initiates the classic complement pathway.

The C3b and C4b complement components form a covalent linkage with IgG, and IgG molecules can noncovalently bind C3a, C4a, and C5a anaphylatoxins.

A. C1q Binding

The efficient activation of the complement pathway begins by the interaction of Ig molecules with the C1q glycoprotein, which is one of three components of the first complement component C1 (Kishore and Reid, 1999). C1q is a large molecule (460 kDa) composed of 18 polypeptide chains (six A, six B, and six C), each built from about 220 residues. A pair of A–B dimers and one C–C dimer linked by covalent and noncovalent bonds compose a subunit, and three such subunits associate into a hexameric molecule resembling a "bunch of tulips" with six globular regions, which contain the binding site for Ig molecules. Such a specific C1q structure with multiple binding sites allows multivalent high-affinity interactions with Ig molecules to be realized, leading to complement activation.

Only IgG and IgM are capable of activating the classical complement pathway in humans. Monomers of these molecules have weak affinity to C1q, and they coexist in the circulation together with C1q without activating the complement cascade. The low affinity for C1q binding to monomeric IgG ($K_a = 5 \times 10^4$ M^{-1}) increases dramatically upon IgG aggregation to $K_a = 10^8$ M^{-1} due to polyvalent interactions. Human IgG_3 and IgG_1 have significantly higher binding activity than IgG_2, while IgG_4 cannot fix complement. The C1q binding hierarchy in mice is $IgG_{2a} > IgG_{2b} > IgG_1$ and in rats $IgG_{2b} > IgG_{2c} > IgG_1 > IgG_{2a}$ (Brüggemann *et al.*, 1989).

1. The Amino Acid Residues of IgG and IgM Involved in Binding C1q

The C1q binding to IgG is determined primarily by the structure of the $C_\gamma 2$ domain. Multiple amino acid residues build the C1q binding site. Residues Glu-318, Lys-320, and Lys-322 containing charged side chains compose the mouse IgG_{2b}-binding motif (Duncan and Winter, 1988). Glu-318 can be replaced by Thr or by Asp and Lys by Arg without loss of the lytic capacity of IgG_{2b}. The residues of this motif are conserved in all subclasses of human IgG and in most IgG subclasses of other mammals, which varied in their reactivity with C1q. These differences in C1q binding are determined by other $C_\gamma 2$ residues (Table V and Fig. 3). The substitution Leu-235→Glu in human IgG_1 abolishes human complement lysis, whereas the same mutation has no effect on the human C1q affinity of murine IgG_{2b} (Morgan *et al.*, 1995). Furthermore, Ala substitutions at positions 318 and 320 of human IgG_1 have little or no effect on C1q binding, whereas Ala substitutions at positions Asp-270, Lys-322, Pro-329, and Pro-331 considerably reduced C1q binding and activation of complement. The Lys-322→Arg mutation does not alter C1q binding, and probably a positive charge in this position is required for

TABLE V
AMINO ACID RESIDUES OF IgG AND IgM IMPORTANT FOR THE BINDING OF C1q COMPLEMENT COMPONENT

Immunoglobulin	Ig domain	Residues	References
Mouse IgG_{2b}	$C_\gamma2$	Glu-318	Duncan and Winter (1988)
		Lys-320	
		Lys-322	
Human IgG_1	$C_\gamma2$	Leu-235	Morgan *et al.* (1995)
		Asp-270	Idusogie *et al.* (2000)
		Lys-322	
		Pro-329	
		Pro-331	
Human IgG_3	$C_\gamma2$	Gly-233	Sensel *et al.* (1997)
		Leu-234	
		Leu-235	
		Gly-236	
		Lys-322	Thommesen *et al.* (2000)
Human IgM	$C_\mu3$	Asn-432	Arya *et al.* (1994)
		Pro-434	
		Pro-436	

the IgG_1 – C1q interaction (Idusogie *et al.*, 2000). The substitutions of prolines could change the configuration of the site. The binding site of human IgG_3 for C1q is also different from that of murine IgG_{2b} as only IgG_3 mutants that lacked Lys-322 show strong reduction in antibody-dependent complement lysis (Thommesen *et al.*, 2000). It seems likely that the C1q-binding sites of murine and human IgG are structurally different.

A position 331, which locates near the murine IgG_{2b} key binding motif, was identified as a critical element responsible for differences between the human IgG subclasses to activate complement (Tao *et al.*, 1993). Human IgG_4, which is unable to fix complement, has Ser-331, but molecules of other subclasses have proline at the same position. Substitution of Ser-331 in IgG_4 with Pro partly restores complement activity, and the Pro-331→Ser substitution decreases or even abolishes the capacity to activate complement of other IgG subclasses. According to studies of human IgG_2 and IgG_3, mutants for optimal C1q binding the presence of residues Glu-233, Leu-234, Leu-235, and Gly-236 in the N-terminus of $C_\gamma2$ (low hinge) is of importance and determines the relative capacity of IgG_2 and IgG_3 to bind C1q (Sensel *et al.*, 1997). All these data suggest that C1q-binding activity and complement lysis are dependent on multiple residues, which are not conserved. Some of the residues can support the specific conformation of the interaction site(s) at a distance.

The absence of the oligosaccharide at Asn-297 in $C_\gamma 2$ or alteration of its structure changes the ability of IgG to activate complement. Aglycosylated IgG_{2b} with mutation Asn-297→Ala has reduced the C1 activation capacity (Duncan and Winter, 1988), and IgG with truncated $C_\gamma 2$ oligosaccharides are deficient in C1q binding (Wright and Morrison, 1994). However, the polymerization of aglycosylated IgG_1 and IgG_3 significantly increases the affinity for C1q, and such polymers are able to bind C1q, although less efficiently than corresponding wild-type polymers (Coloma *et al.*, 2000). The C1q-binding site on the lower hinge and $C_\gamma 2$ overlap that of FcR (Fig. 3), and both ligands show a diminished ability to attach aglycosylated IgG. This suggests that C1q fixation and antibody-dependent cellular cytotoxicity (ADCC) are two mutually exclusive functions, and an antibody molecule bound to the target can bind either C1q or FcR-bearing cells, but not both simultaneously.

The IgM monomers do not activate complement, and their affinity to C1q is low ($K_a = 2.5 \times 10^4$ to $5 \times 10^5\ M^{-1}$). However, after interaction of IgM antibodies with antigen, the affinity for C1q increases 10^3- to 10^4-fold. The binding site for C1q localizes on $C_\mu 3$. The mutations of $C_\mu 3$ residues Asn-432, Pro-434, and Pro-436 decrease the complement-dependent cytolytic activity of mouse IgM (Arya *et al.*, 1994). Pro-436 of $C_\mu 3$ is homologous to Pro-331 of $C_\gamma 2$, which is involved in C1q binding by IgG. However, most probably the IgG and IgM sites for C1q binding are not identical. The intact interheavy S–S bonds and oligosaccharides at $C_\mu 3$ are important for the interactions with C1q (Wright *et al.*, 1990). After binding to an antigen with multiple epitopes, IgM molecules become five-legged table-like structures exposing sites for complement binding (Perkins *et al.*, 1991).

B. C3b and C4b, Binding

C3 and C4 complement components become covalently bound to antibody after activation by IC (Law and Dodds, 1997). These interactions play an important role in the elimination of antigens from the circulation. The C3- and C4-binding sites are formed after enzymatic removal of the N-terminal parts of chains of C3 and C4. Due to a major conformational rearrangement resulting from proteolytic cleavage, the internal thioester is exposed to the solvent and its carbonyl group forms an ester bond with hydroxyl groups on receptive molecules.

After incubation of C3 with heat-denatured IgG, C3b is covalently linked to the $C_\gamma 1$ domain of aggregated IgG in the region composed of residues 123–156 (Shohet *et al.*, 1993). However, in experiments with IC of ovalbumin and antiovalbumin rabbit antibodies, it was found that C3b binds not only to Fab, but also to Fc and even with similar efficiency (Antón *et al.*, 1994). Deleting the $C_\gamma 1$ domain does not alter the ability of IgG either to bind C3 or to activate the alternative pathway (Muñoz *et al.*, 1998a). SpG inhibits the

C3b covalent binding to the Fc region of human IgG_1. As the Fc-binding site for the B fragment of SpG is located in the cleft between $C_\gamma2$ and $C_\gamma3$, it is highly probable that the C3b interaction site is also positioned there. The SpG B domain does not interfere with the C3b binding to the Fab fragment (Muñoz *et al.*, 1998b), which could be explained by the fact that the Fab binding sites for SpG (residues 209–216) and C3b (residues 123–156) are placed on opposite faces of $C_\gamma1$ (Vivanco *et al.*, 1999). All the previously-mentioned data support the view that C3b can attach to multiple sites on IgG, which favors the important functional role of this complement component. C3b bound to IgG retains its capacity to react with the C3 cell receptors. Therefore IC can react with cells not only through their Fc receptors, but also through the C3 receptors, which facilitates the interactions of IC with cells.

C. Anaphylatoxin Binding

Upon activation of the complement cascade, the C3a, C4a, and C5a anaphylatoxins are released from the C3, C4, and C5 components, respectively. These small molecules are responsible for many inflammatory and anaphylactoid reactions, even at very low concentrations, contributing to various pathological conditions (Hugly, 1984). Anaphylatoxins form noncovalent complexes with human IgG in serum at an approximately 1:1 molar ratio. They can be separated from IgG in strong denaturing conditions. The separated C3a and C4a purified by high-performance liquid chromatography (HPLC) are able to reassociate with intact IgG molecules (Nezlin and Freywald, 1992). The binding site for C3a was localized by immunoblotting at the Fab region (Nezlin *et al.*, 1993). The reaction of IgG with C3a is specific, as C3a complexes with serum albumin were not found. C3a are cationic molecules with p*I* above 8.5 (Hugly and Müller-Eberhard, 1978), and electrostatic interactions with negatively charged groups have to play an important role for the formation of complexes with Fab. As anaphylatoxins are very stable molecules, it is quite possible that they retain their biological activity after combining with IgG. The complexes could probably react simultaneously with cells by the Fc region (with FcR), as well as by anaphylatoxin molecules bound to the Fab region (with corresponding cell surface receptors). Such bivalent interactions would augment the cell responses. It was proposed that IgG molecules could serve as scavengers of free anaphylatoxins by eliminating them from the circulation, especially when the level of these highly active complement fragments increases in serum significantly due to complement activation (Nezlin *et al.*, 1993). This view was recently supported by Basta *et al.* (2003). C3a bound to IgG was also found in commercial γ-globulin preparations (Nezlin, 1993).

IV. Proteins and Peptides Reacting with Fc

Many functionally important ligands interact with the Fc region of Ig molecules. Some of these interactions were studied in detail, such as binding Fc receptors and bacterial proteins, and are covered in corresponding sections of the review. Several other interactions are discussed below.

A. Clusterin

A conserved, highly glycosylated protein, *clusterin* (75–80 kDa) is constitutively synthesized in various tissues and presents in the circulation in concentration of about 0.1 mg/ml (Jones and Jomary, 2002). Precise functions of clusterin are still disputable. Clusterin interacts with many native or partly unfolded proteins using different binding sites (Lakins *et al.*, 2002). Clusterin binds to all isotypes of human IgG and to other Ig classes. It reacts with both Fab and Fc fragments. Fc-binding site(s) do not overlap with C1q- or SpA-binding sites, as these proteins do not inhibit clusterin binding. Clusterin can promote the formation of insoluble IC probably by cross-linking soluble complexes (Wilson and Easterbrook-Smith, 1992; Wilson *et al.*, 1991).

B. Fibronectin

Fibronectin (Fn), a large glycoprotein molecule, is found in soluble form in blood and other body fluids. Fn molecules are deposited as insoluble fibrils in the extracellular matrix and on the surface of many cells. Aggregated human immunoglobulins of all major classes bind Fn with the following hierarchy of affinity: IgG > IgM > IgA and $IgG_1 > IgG_3 = IgG_4 > IgG_2$ (Rostagno *et al.*, 1991, 1996). Fn–Ig complexes can be detected in normal plasma (Bray *et al.*, 1994). The Fc region of IgG is responsible for the interactions with Fn, and the affinity of the Fn binding to Fc ($K_d = 3.69 \times 10^9\ M^1$) is nearly identical to that of its binding to intact IgG.

Fn, as a constituent of the extracellular matrix, can associate with soluble circulating IC that are present in the blood of patients with autoimmune, rheumatic, and myeloproliferative disorders. It also interacts with Ig aggregates such as heat-denatured IgG or cold aggregated cryoglobulins, but not with monomeric IgG. The reaction is specific, as the blocking of Fn by anti-Fn antibodies inhibits the reaction with aggregated IgG. The matrix-associated Fn may participate in removing IC and aggregates of cryoglobulins from the circulation, causing their deposition in basement membranes of different organs. Such localization of IC could lead to the development of fibrotic diseases such as pulmonary fibrosis and glomerulonephritis, as well as occlusive vasculopathies and other pathology. Peptides from the Ig-binding site of Fn could be effective in blocking deposition of IC in tissues (Rostagno *et al.*, 2002).

C. Fc-Binding Peptides

A number of *polypeptides* of various length and affinity are bound to the Fc region of IgG. Information on peptide–Fc_γ complexes was obtained by fluorescence polarization experiments with IgG and Fc_γ labeled with fluorescent dye (Dudich *et al.*, 1978). It was observed that Fc-binding peptides could be separated either by dilution of IgG or Fc solutions or by decreasing their pH below pH 6. The dissociation of the labeled peptides from Fc_γ is responsible for the concentration and pH dependence of the rotational relaxation time (ρ_h) of IgG or the isolated Fc_γ. The ρ_h value is dropped after acidification of the IgG or Fc_γ solutions or after their dilution below 2 μM. As the molecular mass of the peptides is about 2 kDa, they have a small ρ_h value. This dependence of ρ_h on concentration was not found for Fab_γ. The separated peptides rotate independently and decrease the experimentally obtained mean values of the ρ_h of IgG or Fc_γ. Nearly half of the peptides separated from Fc_γ can reassociate with the fragment. The peptide-binding site is probably located in the $C_\gamma 2$ domain (Dudich and Dudich, 1983).

D. Rheumatoid Factor

Rheumatoid factors (RF) are IgM or IgG autoantibodies directed toward the Fc region of IgG and are present in the sera and synovia of patients with rheumatoid arthritis (RA) (Levinson, 1989).

There are several hypotheses for the origin of RF explaining some features of RF from RA patients. (1) RF-producing B cells are activated by T helper cells following the binding and processing of IC with IgG (Sutton *et al.*, 2000). In the activation of B cells, Toll-like receptors also participate (Leadbetter *et al.*, 2002). The activation of B cells through the Fc_γ region would be expected to determine all known features of RF, such as monoclonality, heterogeneous V-gene usage, idiotypical and isotypical diversity, and antigen-driven somatic mutation (Youngblood *et al.*, 1994). (2) The Fc oligosaccharides are altered, producing new epitopes that could elicit RF production (Newkirk, 1996). (3) There is antiidiotypic mimicking with some Fc-binding protein (Oppliger *et al.*, 1987; Tsuchiya *et al.*, 1990).

1. Contact Amino Acid Residues between Fc_γ and RF

The residues involved in binding RF are localized at the $C_\gamma 2$–$C_\gamma 3$ interface as determined by site-directed mutagenesis and confirmed by X-ray crystallography. The residues making contact with the RF-binding site of IgG were localized by X-ray crystallography using the Fab fragment of an IgM RF complexed with the Fc fragment of human IgG_4 (Corper *et al.*, 1997). The region of Fc_γ that is recognized by Fab of RF involves two segments of the $C_\gamma 2$–$C_\gamma 3$ cleft, namely, residues 251–255 in $C_\gamma 2$ and 422–440 in $C_\gamma 3$. Both

segments are also involved in binding FcRn, SpA, and SpG, for which the crystal structure of their complexes with Fc_γ was also determined (Sections II.B and VII.A). As with SpA and FcRn, the essential residues of these segments are Ile-253 (in $C_\gamma2$) and His-435 (in $C_\gamma3$) (Bonagura *et al.*, 1992, 1993; Corper *et al.*, 1997). If His-435 is substituted by Arg (as in an IgG_3 allotype), RF, as well as SpA and FcRn, fail to react with this IgG allotype (Kim *et al.*, 1999; Matsumoto *et al.*, 1983). The loop containing residues 309–311 involved in binding FcRn to IgG does not seem to play a significant role in the binding of RF.

The heterogeneity of RF from various patients was reflected in the ability of some polyclonal RF to react with the allotype of IgG_3 with Arg-435, as well as the inability of SpA to inhibit the RF–IgG interaction for some RFs (Artandi *et al.*, 1991). Although some of the residues recognized by RF are also involved in the binding of SpA (or SpG), there is only partial identity. Each of the three Fc_γ residues, which form hydrogen bonds to RF (Ile-253, Ser-254, and Asp-434), also forms hydrogen bonds to SpA and SpG, but the orientation of these bonds cannot be spatially superimposed. Furthermore, the salt bridge formed between Arg-255 (in $C_\gamma2$) and Asp-31 (in RF) has no correspondence in the SpA/SpG–IgG complexes (Corper *et al.*, 1997).

2. The Topology of the Fab RF–Fc IgG_4 Interaction

The residues involved in the binding of Fab RF to human Fc IgG_4 are completely different from those of SpA/SpG or FcRn and support the idea that there is little if any correspondence at the atomic level between the interactions of these Fc_γ ligands (Corper *et al.*, 1997). The Fab RF fragment is bound symmetrically on both side of Fc_γ, revealing a 2:1 stoichiometry. The bivalency of Fc_γ suggests that the mechanism of activation of RF-producing B cells may occur by cross-linking their surface antigen receptors by IgG and driving the autoimmune response (Sutton *et al.*, 2000). The topology of this particular Fab RF–Fc_γ interaction is significantly different from all other familiar Fab–antigen complexes. In the Fab RF–Fc_γ complex, only one side of the combining site surface participates in the interaction, and the number of contacts are fewer than in other antigen–antibody complexes due to the lack of contribution from L1 and L3 CDRs. The principal contact region of RF is only H3 CDR, which protrudes into the $C_\gamma2$–$C_\gamma3$ cleft. The consequence of this particular topology is that the conventional binding area remains unoccupied and is therefore accessible to other unidentified antigens, which may in fact be responsible for the induction of RF (Corper *et al.*, 1997).

The identification of the residues of Fab RF that are in contact with those of the $C_\gamma2$–$C_\gamma3$ interface of IgG_4 may not be relevant for other RFs due to their well-known idiotypic heterogeneity (Chen and Carson, 1994). However, as far as the Fc_γ residues involved in the binding of various RFs are concerned, the

available data clearly point to the participation of the residues from the two main loops of the $C_\gamma2$–$C_\gamma3$ cleft centered around Ile-253 and Ser-254 in the $C_\gamma2$ domain and Asp-434 and His-435 in the $C_\gamma3$ domain.

V. Proteins Reacting with Fab

Proteins that are able to react with the Fab portion of Ig molecules in a nonantigen way can activate cells bearing a particular V-domain family of the B cell Ig receptors. A requirement for cell activation is the functional multivalency of Fab-reacting molecules, which is essential for cross-linking of cell-bound Ig molecules. The B cell activation could lead to serious pathological events, especially if the stimulated B cells are producers of autoantibodies (Silverman, 1997). The Fab-reactive proteins can also bind to Ig molecules, which are complexed with the Fc cell receptors, and stimulate cell activities.

A. Prolactin

Prolactin (Prl), a stimulator of lactation, is a polypeptide hormone secreted by the anterior pituitary gland. In humans it presents as a 23-kDa monomer and also as dimers and polymers. Prl receptors are located mainly on cells of the mammary gland, but also on human B and T lymphocytes, monocytes, and NK cells. There is evidence that Prl has an immunomodulatory role. Prl can form complexes with IgG, interacting with the Fd part of all four human IgG subclasses (Walker *et al.*, 1995). The IgG–Prl complex has about 1 mol of N^ε(γ-glutamyl)lysine cross-links per mole of the complex, which indicated a possible role of enzyme transglutaminase in the formation of linkages between IgG and Prl. About 0.8% of all IgG molecules in the circulation are complexed with one or two Prl molecules. The Prl–IgG complexes can stimulate lymphocytes of some patients with chronic lymphocytic leukemia. However, Prl alone is not active in this respect, and the proliferating activity of the complex involves the engagement of both Prl and IgG. The effect is probably due to the coligation of the receptors specific to both these proteins.

B. Protein Fv

Protein Fv (PrFv), a 175-kDa hexavalent sialoprotein, is synthesized in the liver and released into the digestive tract during liver diseases. This protein is also present in healthy persons in complexes with luminal immunoglobulins (Bouvet *et al.*, 1996). PrFv is absent in the gut of axenic rats, but it appears after colonization by human gut microflora, which seems to be a major factor in the release of PrFv (Andrieux *et al.*, 1998). In feces, two types of PrFv complexes with $F(ab')_2$ of secretory IgA were found with molecular masses of 1800 and 800 kDa. The last complex consists of six $F(ab')_2$ and one PrFv dimer. PrFv binds to V_H domains of human immunoglobulins ($K_a = 6.7 \times 10^8\ M^{-1}$), as well

as of Ig molecules of most animal species studied at sites that are highly conserved throughout evolution. It preferentially interacts with the H chains of the V_H3 Ig family in areas of FR1 and 3 exposed to solvents and additionally can recognize some V_H6 and V_H4 immunoglobulins. PrFv competes with SpA for the same binding site on V_H3 (Silverman *et al.*, 1996). However, the binding sites for these two proteins are not identical. There are V_H3 molecules, which do not react with SpA, but bind PrFv. V_H6 and V_H4 immunoglobulins with PrFv-binding activity have no binding specificity for SpA. CDR sequences do not correlate with the PrFv-binding activity, and the interaction with PrFv does not interfere with the reaction of antibodies with antigen.

PrFv is capable of inducing or enhancing several important effector functions of immunoglobulins. This functional activity of PrFv could be explained by its ability to bridge Ig molecules and thus to form nonimmune complexes. Due to its constant presence in the intestinal tract, PrFv is an important factor in mucosal immunity. Large complexes of secretory IgA or its $F(ab')_2$ fragments are formed by PrFv in the digestive lumen, which greatly increases the protective capacity of antibacterial and antiviral agglutinating IgA antibodies and improves the excretion of bacteria from the gut. The Ig–PrFv complexes activate the classical complement pathway by mimicking IC, which could induce local intestinal lesions (Ruffet *et al.*, 1994). PrFv also acts as an activator of human basophils and mast cells by interacting with the V_H domain of IgE molecules bound to the cell Fc_ε receptors. Such reactions stimulate the synthesis and release of proinflammatory mediators such as histamine and leukotrienes, as well as cytokines interleukin (IL)-4 and IL-13 from these cells. It is likely that this PrFv activity could desensitize mast cells by continued interactions and prevent hypersensitivity reactions induced by allergens (Patella *et al.*, 1998). The V_H3–PrFv interactions could contribute to the development of malignancy. Nearly all studied V_H genes of mucosal-associated lymphoid tissue lymphoma cells are the V_H3 type (Hashimoto *et al.*, 2001). It was hypothesized that PrFv could react with B lymphocytes, which possess V_H3 Ig receptors and enhance the replication of these cells. The increased number of cell divisions of the V_H3 memory B cells could stimulate their transformation into lymphoma cells.

C. T Cell Protein CD4

CD4 is a 55-kDa membrane glycoprotein expressed on helper T lymphocytes and composed of four Ig-like domains (Brady and Barclay, 1996). It binds to the nonpolymorphic regions of MHC II molecules, to the human immunodeficiency virus (HIV) gp 120 protein, and also interacts with Ig molecules of nearly all classes with relatively low affinity. Two distinct sites on the CD4 first

V-set Ig-like domain (residues 21–28 and 35–38) are involved in the reaction with immunoglobulins, and one CD4 molecule is able to cross-link two Ig molecules (Lenert *et al.*, 1990, 1995). The similar CD4 sites on two juxtaposed antiparallel C′C″ loops are responsible for HIV gp 120 binding. This fact explains the inhibition of the CD4–Ig interaction by gp 120. Polianionic substances such as sulfated dextrans and heparin inhibit CD4 binding, which indicates participation of charged residues of CD4 in the reaction. The V_H FR residues participate in the interaction with soluble recombinant CD4. The interaction with CD4 enhances the antigen–antibody reaction, which could explain the antibody-mediated enhancement of the HIV infection.

D. Histidine-Rich Glycoprotein

Histidine-rich glycoprotein (HRG) is a 75-kDa protein composed of four domains. It is synthesized in liver and is contained in plasma at a relatively high concentration (0.11 mg/ml). The precise functions of HRG are still unknown, but its modular structure suggests the presence of several binding sites with the ability to interact with various ligands simultaneously. Indeed, HRG could bind to heparin, fibrinogen, complement components, immunoglobulins, and some other ligands. The N-terminal HRG domain (30 kDa) is involved in binding C1q and immunoglobulins. HRG binds to IgG of all subclasses with relatively higher affinity compared to its other ligands (Gorgani *et al.*, 1999a). The type of L chains affects the kinetics of the reaction. The on rate for HRG binding to $IgG_{1\kappa}$ and $IgG_{2\kappa}$ is significantly faster than that for the binding to $IgG_{1\lambda}$ and $IgG_{2\lambda}$. Surprisingly, the on rate for HRG binding to $IgG_{3\kappa}$ and $IgG_{4\kappa}$ is slower than that for $IgG_{3\lambda}$ and $IgG_{4\lambda}$. HRG is also able to bind to Bence–Jones proteins with an affinity higher for the κ type of these proteins. The binding site for HRG locates on Fab regions, as HRG binds to $F(ab')_2$ with the same affinity as to the whole IgG molecule (Gorgani *et al.*, 1997). The interaction of HRG with IgM_κ is much weaker than with IgG_κ.

HRG or its N-terminal domain can inhibit *in vitro* the formation of insoluble IC between ovalbumin and antiovalbumin IgG or between human IgG and rheumatoid factor. The addition of whole human plasma prevents the formation of insoluble IC, whereas plasma with reduced HRG concentration even enhances the formation of such complexes. The addition of HRG to plasma with reduced HRG concentrations restores its inhibitory activity. These data suggest that inhibition of the formation of IC by HRG is physiologically significant and could be relevant to the process of clearance of circulating IC. HRG also promotes the solubilization of preformed IC in a dose-dependent manner and inhibits interactions between monomeric IgG, as well as IC with mononuclear cells (Gorgani *et al.*, 1999b, 1999c).

VI. Lectins

Lectins are widely distributed proteins that interact noncovalently with carbohydrates, both simple and complex, as glycoproteins and glycolipids (Sharon and Lis, 2003). Each lectin has two or more combining sites and could cause cross-linking of glycoproteins or cells. They interact with immunoglobulins through sugar residues.

A. Plant Lectins

Jacalin, a 65-kDa protein isolated from seeds of a tropical plant, jackfruit, has a strong affinity for galactose β1→3 linked to *N*-acetylgalactosamine (Kabir, 1998). There are four aromatic residues in the sugar-combining site of jacalin. The lectin binds to serum or secretory human IgA_1, but not to IgA_2 of both allotypic variants or to other Ig classes or IgA of other mammals (with the exception of chimpanzee and rabbit secretory IgA). Jacalin interacts with human IgD, which contains galactose β1→3 linked to *N*-acetylgalactosamine in the hinge region. The jacalin specificity for IgA_1 is due to the presence of five nonsialylated galactose-containing *O*-linked oligosaccharides located in the IgA_1 hinge. The IgA_2 molecules have no glycans in this region due to a deletion of 13 residues. Jacalin is used effectively for the purification of human IgA_1 and its separation from IgA_2 (Haun *et al.*, 1989). A small fraction of rabbit IgG is also bound by jacalin. The interaction is probably mediated through *O*-linked glycans present on the H chain (Kabir *et al.*, 1995). A technique was developed to measure IgA_1 concentration in serum and saliva using jacalin. Jacalin is also a useful tool in studies of IgA nephropathy, which is characterized by the deposition of IgA_1 in renal tissue and by elevated IgA_1 concentration in blood.

The galactose-specific lectin from castor beans, *Ricinus communis*, reacts specifically with accessible terminal galactose residues of the Fc and Fab portions of IgG molecules. The affinity of the lectin is higher after the aggregation of IgG, and increases with the mass of IgG aggregates. The lentil, *Lens culinaris* lectin, specific for α-mannose residues, binds to IgM molecules and can be used for purification of monoclonal IgM antibodies from ascites fluid. Several lectins were isolated from Malaysian champedak seeds. The first of them is a 52-kDa galactose-binding *lectin-C* that reacted with human IgA_1 and colostral IgA, but not with IgA_2 and other immunoglobulins (Hashim *et al.*, 1991). The second lectin, a 64-kDa mannose-binding *lectin-M*, interacts strongly with a number of human serum proteins and IgA, as well as with γ- and μ-chains, but not with intact IgG molecules (Hashim *et al.*, 2001).

B. Animal Lectins

1. Galectin-3 *and* Galectin-1

A 31-kDa IgE-binding protein, *galectin-3* (Gal-3), is expressed on the surface, as well as in the cytoplasm and nucleus, of different kinds of cells, including mast cells, macrophages, neutrophils, and eosinophils. It also exists in the extracellular medium. Gal-3 is a highly conserved multifunctional protein with β-galactoside specificity belonging to the galectin (S-type) animal lectin family (Liu, 1990, 1993; Rabinovich *et al.*, 2002). The receptors for Gal-3 on IgE are *N*-linked oligosaccharides, and the interaction depends on sialylation of their terminal residue (Robertson and Liu, 1991). Gal-3 could act as an amplifier of the inflammatory cascade because it recognizes not only IgE, but also the Fc_ε receptor on mast cells. The activation of these cells by Gal-3 is achieved by cross-linking either the Fc_ε receptors or receptor-bound IgE molecules or both.

Another member of the galectin family, *galectin-1* (Gal-1), is a multifunctional homodimer protein with β-galactoside binding activity (Rabinovich *et al.*, 2002). Gal-1 is expressed on the surface of various cells, including cells of the immune system, and reacts with a number of cell surface receptors, initiating signal-transduction events. It was found that the reaction of human pre-B cells with stromal cells is accounted for by the ability of the pre-B cell receptor to interact with Gal-1, as well as with some other stromal cell receptors (Gauthier *et al.*, 2002). The interactions result in pre-B cell triggering. NH_2-terminal part of the invariant λ-like peptide, a part of the surrogate L chains of the human pre-B cell receptor, is responsible for the interaction with Gal-1 ($Ka \sim 10^6 M^1$). In contrast, binding of a murine pre-B cell receptor to stromal cells depends on interactions of λ-like peptide (λ_5) of the receptor with stroma cell-associated heparan sulfate (Bradl *et al.*, 2003).

2. Mannose-Binding Protein A *and* Mannose *Macrophage* Receptor

A 650-kDa serum lectin, *mannose-binding protein* A (MBP-A), is a hexamer of trimeric units forming a "bouquet"-type structure, which structurally resembles C1q. Its carbohydrate recognition domains bind not only mannose, but also *N*-acetylglucosamine and some other sugar residues (Holmskov *et al.*, 1994). MBP-A is a member of the collectin family and plays an important role in innate immunity, as it mediates the activation of the classical complement pathway in an antibody independent way (Epstein *et al.*, 1996). MBP-A binds to IgG molecules, the N-terminal sugar residue of which is *N*-acetylglucosamine, but not galactose. The MBP-A interaction with IgG results in complement activation. As the levels of agalacto-IgG are markedly increased in patients with rheumatoid arthritis, the interaction can induce chronic inflammatory reactions of the synovial membranes of injured joints (Malhotra *et al.*,

1995). IgM molecules have a high content of oligomannose, and MBP-A could be used for the isolation and detection of this immunoglobulin (Koppel and Solomon, 2001; Nevens *et al.*, 1992). *Mannose receptor*, a C-type lectin expressed on macrophages, dendritic cells, and epithelial cells, binds to agalacto-IgG in a manner similar to MBP-A (Dong *et al.*, 1999).

3. Galactosyltransferase

An enzyme involved in the biosynthesis of oligosaccharides, *β-1,4-galactosyltransferase (GT)*, either in soluble or membrane-bound form, binds to molecules of main Ig classes and acts as a lectin (Tomana *et al.*, 1993a). The interactions with immunoglobulins can be explained by incomplete galactosylation of their oligosaccharides and the ability of GT to bind galactose residues. The most effective binding is to polymeric IgA_2 molecules and less effective to IgG. Isolated Ig peptide chains interact more efficiently with GT than intact Ig molecules, which could be explained by the higher accessibility of terminal residues of oligosaccharide moieties. The more effective interactions of GT with IgA, IgM, and their H chains (as compared with IgG) could be explained by the presence of several carbohydrate chains on α-, μ-, and J-chains, which in higher proportion lack terminal galactose or sialic acid residues and therefore are substrates for galactosylation. The functional significance of GT–Ig complexes is not so clear, but they could probably prevent the interactions of immunoglobulins with cell receptors. The membrane-bound GT found on the surface of various types of cells, including human lymphocytes, is also able to bind immunoglobulins and Ig peptide chains and acts as a lectin-like receptor (Tomana *et al.*, 1993b).

VII. Proteins from Pathogens Reacting with Immunoglobulins

A. Bacterial Immunoglobulin-Binding Proteins

Proteins with the ability to bind Ig molecules in a nonimmune fashion are expressed on the surface of many microorganisms. They are involved in the process of infection and are able to weaken the immune response. Bacterial Ig-binding proteins have been widely applied for isolation and quantitation of immunoglobulins and their fragments.

1. Staphylococcal Protein A

a. Structure and Fc-Binding Site. *Protein A* (SpA) is a component of the *Staphylococcus aureus* cell wall, consisting of an extracellular part, which reacts with the Fc_γ region, and a cell wall-binding part (Forsgren *et al.*, 1983). The extracellular part contains a tandem of five highly homologous monovalent Fc-binding domains designated E, D, A, B, and C. Each domain is composed

of about 60 residues (6.6 kDa). Domains A–D have similar affinities for Fc_γ but the affinity of the E domain is lower (Moks *et al.*, 1986).

The B fragment (or its recombinant counterpart, Z) is composed of a bundle of three α-helices, all of them being retained in the B–Fc_γ complex, but only two (helices I and II) making contact with Fc_γ (Kato *et al.*, 1993). The three-dimensional structure of the B–Fc_γ complex has been resolved by X-ray diffraction analysis (Deisenhofer, 1981), and the contact residues between B and Fc were confirmed by an NMR study (Kato *et al.*, 1993), as well as by mutation analysis (Cedergren *et al.*, 1993). The residues of the Z fragment involved in contact with Fc are located in helix I (Phe-5, Glu-9, Asn-11, Phe-13, Tyr-14, and Leu-17) and helix II (Asn-28, Phe-30, Ile-31, Glu-32, and Lys-35). From these residues, Leu-17, Asn-28, Ile-31, and Lys-35 are all clustered at the $C_\gamma2$–$C_\gamma3$ interface, establishing hydrophobic and hydrogen bond contacts (Tashiro and Montelione, 1995).

b. Amino Acid Residues of the Fc_γ Region Involved in SpA Binding. By X-ray diffraction analysis, it was shown that the previously-mentioned residues of the B fragment may establish contact with some residues of human Fc_γ, which are located in three separate loops of the $C_\gamma2$–$C_\gamma3$ interface (252–254, 308–312, and 433–436) (Deisenhofer, 1981). The participation of some of these residues in binding the B fragment was confirmed by NMR analysis, indicating the key role of Ile-253, Ser-254, His-310, and Gln-311 in $C_\gamma2$ and His-433, His-435, and His-436 in $C_\gamma3$ (Kato *et al.*, 1993). These findings were confirmed by site-directed mutagenesis of mouse Fc_γ showing that the mutations of Ile-253, His-310, His-435, and, to a lesser extent, His-433, Asn-434, and His-436 diminish the reactivity with immobilized SpA (Kim *et al.*, 1994a) (Table VI). The mutation of His-435 (in human IgG_1) by Arg (as in a human IgG_3 allotype) decreased SpA binding, shortened the IgG half-life in mice (Kim *et al.*, 1999), and reduced *in situ* transmission through the placenta (Firan *et al.*, 2001).

The residues of the three loops of the $C_\gamma2$–$C_\gamma3$ cleft show species variation (with the exception of Ile-253, His-310, Gln-311, and Asn-434) that may explain the variable affinity of SpA for IgG belonging to different species and isotypes (Forsgren *et al.*, 1983). The reaction of SpA and SpG with IgG was extensively exploited for the measurement and purification of IgG antibodies and various antigens (Boyle, 1990), for the isolation of IC (Nezlin, 2000), as well as for the detection, isolation, and purification of a variety of cell populations (Ghetie and Sjöquist, 1984a, 1984b).

c. The Stoichiometry of the IgG–SpA Interaction. The reaction of the monovalent B fragment of SpA with human Fc and rabbit and mouse Fc_γ and IgG yields soluble complexes B–Fc_γ or B–IgG with a molar ratio of 2:1,

indicating that both $C_\gamma2$–$C_\gamma3$ clefts are equally available for interactions with the B fragment (Deisenhofer, 1981; Dima *et al.*, 1984).

The stoichiometry of the reaction between IgG and intact SpA is difficult to estimate, because IgGs of many species form insoluble or weak complexes that cannot be properly characterized. The only exception is rabbit IgG, which forms stable soluble complexes with SpA and some of its fragments. In a wide range of molar ratios (from 1:4 to 4:1), only two main types of soluble rabbit IgG–SpA complexes were identified (Dima *et al.*, 1984; Langone *et al.*, 1978; Mihaescu *et al.*, 1979; Mota *et al.*, 1978). One with a molecular mass of 190 kDa and a molecular formula of IgG1–SpA1 forms in excess of SpA, and the other is obtained in excess of IgG with a molecular mass of 680 kDa and a molecular formula of IgG4–SpA2. Since the IgG1–SpA2 complex was not identified, it was concluded that the reaction of SpA with one side of Fc_γ exerts a steric constraint on the other site or masks it (Hanson and Schumacher, 1984). The apparent monovalency of IgG in the reaction with SpA is reminiscent to the behavior of human IgG, which was able to interact with two Fab fragments of RF (Corper *et al.*, 1997), but only with one intact RF molecule (Nardella *et al.*, 1981). Evidently, the reaction of intact RF with one of the available $C_\gamma2$–$C_\gamma3$ clefts makes the other cleft sterically inaccessible.

d. The Binding of SpA to the Fab Region of IgG. In addition to the Fc_γ binding site, SpA has another distinct site with the ability to interact with the Fab region of IgG and some other Ig classes (Romagnani *et al.*, 1982). Through this interaction, a significant percent of human IgM, IgA, IgE, and IgG binds

TABLE VI
AMINO ACID RESIDUES OF MURINE Fc FRAGMENT INVOLVED IN THE BINDING OF IMMOBILIZED SpA[a]

Amino acid residue[b]	Binding to SpA[c]
Wild type	100
Ile-253	23
His-310	12
His-433	38
Asn-434	62
His-435	10
His-436	79

[a]Kim *et al.* (1994) and Medesan *et al.* (1997).

[b]All residues were mutated to Ala.

[c]Radiolabeled wild-type mouse Fc (from IgG_1) and its mutants were reacted with SpA-Sepharose, and the amount of the bound Fc was measured. The amount of the bound wild-type Fc was considered as 100%.

to SpA (Inganas, 1981; Inganas *et al.*, 1980). The Fab-binding site seems to be located on all of the five domains (Ljungberg *et al.*, 1993; Roben *et al.*, 1995).

Human and mouse immunoglobulins that interact with SpA in the Fab region are encoded by gene segments belonging to the V_H3 or J606 and S107 V_H families (Potter *et al.*, 1996; Roben *et al.*, 1995; Sasso *et al.*, 1991; Seppälä *et al.*, 1990). The pattern of SpA binding to human immunoglobulins indicates not only that residues in FR1, CDR2, and FR3 are involved in the interaction, but also that all these three regions are required for binding to occur. The interaction with SpA does not block the antigen-combining site, leading to the conclusion that the contact occurs outside this site and the SpA molecule could be considered as a B cell superantigen (Silverman, 1997).

The crystal structure of the complex between fragment D and the Fab fragment of a human IgM antibody indicated that α-helices II and III of fragment D interact with the V_H3 region through framework residues without any involvement of the CDRs (Graille *et al.*, 2000). The contact residues are highly conserved in both the V region of the antibody and in the D domain of SpA. The interacting surfaces are mainly hydrophilic, involving polar side chains and salt linkages, as also indicated by an NMR study of the reaction of SpA fragment E with a Fv fragment derived from a human antibody (Meininger *et al.*, 2000).

e. SpA Mimetics. Several peptidyl-SpA mimetics with molecular sizes smaller than the B(Z) domain were obtained (Braisted and Wells, 1996; Nilson *et al.*, 1987). From a combinatorial phage display library, a decapeptide was selected that resembles residues of SpA responsible for interaction with Fc (FCRLVSSIRY). Since it was eluted from an immobilized Fc fragment with SpA, it is quite possible that some of these decapeptides react with the same sites on Fc (C_H2–C_H3 cleft) as SpA does (Krook *et al.*, 1998). Another SpA mimetic (*PAM*) consisting of a tetrameric tripeptide (Tyr-Thr-Arg linked to polylysine) was able to inhibit the binding of SpA to IgG and to react with mammalian IgGs and chicken IgY. It was used effectively for the isolation of immunoglobulins from serum and the purification of monoclonal antibodies from ascites (Fassina, 2000; Fassina *et al.*, 1996). Nonpeptidyl ligands that mimic SpA were also synthetized (Kabir, 2002). Using a computer modeling, a series of nonpeptidyl biomimetic molecules around residues Phe-132/Tyr-133 involved in the reaction with Fc were designed (Li *et al.*, 1998). One of them (*ApA*) was used for the purification of IgG.

The SpA mimetics could also be valuable for the development of a new family of candidate drugs for the control of IgG overproduction by accelerating the autoantibodies, catabolism (Marino *et al.*, 2000). Similar to SpA or its B fragment, these drugs may block the interaction of IgG with FcRn (Dima *et al.*, 1984; Raghavan *et al.*, 1994) and as a consequence may accelerate

the catabolism of IgG (including autoantibodies) and its removal from the circulation.

f. Therapeutic Implications. Immobilized SpA (e.g., Prosorba) was used for the extracorporeal selective removal of plasma proteins to achieve immune modulation and a therapeutic effect. This procedure has a beneficial clinical effect in various diseases such as cancer (Messerschmidt *et al.*, 1989), idiopathic thrombocytopenia (Guthrie and Oral, 1989; Muroi *et al.*, 1989), rheumatoid arthritis (Felson *et al.*, 1999), SLE (Braun *et al.*, 2000), and various renal diseases (Belson *et al.*, 2001; Esnault *et al.*, 1999). It also causes the modulation of the immune response in patients with autoimmune diseases (Braun and Riesler, 1999; Snyder *et al.*, 1989).

2. Streptococcal Protein G

a. Structure and Fc_γ-Binding Site. Protein G (SpG) originates from two main streptococcal strains, C and G (Boyle, 1990). SpG from strain G (65 kDa) consists of several repetitive domains. Three of them placed at the C-terminal half of the molecule, with very similar if not identical structures and designated C1, C2, and C3 or B1, B2, and B3, are involved in the binding to Fc_γ (Sjobring *et al.*, 1991). The two or three other domains located in the N-terminal half of the molecule have been found to bind human serum albumin (Åckerström *et al.*, 1987).

Each of the B/C domains of SpG consists of approximately 60 residues and presents a high degree of homology, if not identity (Sjobring *et al.*, 1991; Tashiro and Montelione, 1995). The structure of the three recombinant B/C domains reveals the presence of one single α-helix positioned diagonally across four β sheets (β-1 to β-4) (Achari *et al.*, 1992; Gallagher *et al.*, 1994; Lian *et al.*, 1992; Sauer-Eriksson *et al.*, 1995). The residues involved in the binding to Fc_γ are localized in two section of the B/C domain, namely, in the central part of the α-helix and in the N-terminal end of the β-3 sheet. The α-helix contacts contain a tandem of two pairs of residues (Lys-28/Glu-32 and Glu-27/Lys-31) whose side chains are exposed on the opposite side of the α-helix. The N-terminal end of the β-3 sheet contains Glu-42 and Trp-43 in close spatial proximity to Asp-35, and Asp-40 situated at the end of the α-helix and in the loop connecting the α-helix with the β-3 sheet, respectively. These findings resulted from NMR and crystallographic studies and are also supported by the fact that a short fragment containing residues 34–44 is able to inhibit binding SpG to Fc_γ (Frick *et al.*, 1992).

b. Amino Acid Residues of the Fc Region Involved in the Binding of SpG. The residues of Fc_γ that interact with the B/C residues are localized in all three hydrophobic loops of the $C_\gamma2$–$C_\gamma3$ cleft, and some of them are also involved in

TABLE VII
INTERACTION OF AMINO ACID RESIDUES OF Fc REGION WITH SpG AND SpA[a]

Fc residues	SpG (C2 domain residues)	SpA (B domain residues)
Loop 1 (C_H2 domain)		
Leu-251 (MC)[b]	Lys-31 (SC)	Gln-10 (SC)
Met-252 (H)	Lys-28 (H)	Phe-5 (H)
Ile-253 (H)	Trp-43 (H)	Phe-13 (H)
Ser-254 (SC)	Glu-27 (SC)	Gln-9 (SC)
Loop 2 (C_H2 domain)		
Glu-311 (SC)	Glu-42 (SC)	Asn-18 (SC)
Leu-314 (H)	—	Leu-17 (H)
Loop 3 (C_H3 domain)		
Met-428 (H)	Lys-28 (H)	—
His-433 (SC)	Asn-35 (SC)	—
Asn-434 (SC)	Asn-35 (SC)	Asn-11 (SC)
	Val-39 (SC)	
	Trp-43 (MC)	
His-435 (H)		Tyr-14 (H)
		Leu-17 (H)
His-436 (MC)	Lys-28 (H)	—
	Asn-35 (SC)	
Gln-438 (SC)	Gln-32 (SC)	—

[a]Adapted from Sauer-Eriksson *et al.* (1995).
[b]MC, polar interaction with main-chain atoms; SC, polar or charged interaction; H, hydrophobic interaction.

binding SpA. A comparison of the Fc_γ residues involved in binding SpG and SpA is presented in Table VII. In addition to these residues, there are two residues (Gln-380 and Glu-382) in the $C_\gamma3$ domain outside the $C_\gamma2$–$C_\gamma3$ cleft that establish a bridge with Lys-28 of the C2 domain of SpG (Sauer-Eriksson *et al.*, 1995). These two $C_\gamma3$ residues are not involved in binding SpA. His-310, which plays a key role in binding FcRn (Table III) and SpA (Table VI), seems not involved in binding SpG and RF (Corper *et al.*, 1997; Deisenhofer, 1981; Medesan *et al.*, 1997). Four of the $C_\gamma2$–$C_\gamma3$ interface residues (Ile-253, Ser-254, Glu-311, and Asn-434) are of particular importance because their side chains interact with the side chains of both SpA and SpG (Table VII). Since there is no structural homology between SpG and SpA, it is not surprising that these two proteins use particular modes of interaction with Fc_γ. Thus SpG makes contact with His-433, Glu-380, Gln-382, and Glu-438 (the last three residues being outside the $C_\gamma2$–$C_\gamma3$ cleft), whereas SpA interacts with the side chains of Leu-314 and His-435. The lack of interaction of position 435 with SpG but not with SpA explains why the latter cannot interact with an allotype of human IgG_3 with Arg-435. In fact, the introduction of Arg in position 435 can

be accommodated into the SpG C2–Fc_γ complex, but not in the SpA B–Fc_γ complex, where His-435 is in close proximity to several hydrophobic residues of the B fragment (Deisenhofer, 1981). All these differences in the reaction of Fc_γ with SpG and SpA are reflected in the remarkable differences in their affinity and pH dependence for IgG. Thus the affinity of SpG for many IgGs is 10-fold higher than that of SpA (1–10 n*M*) (Boyle, 1990). The optimal binding of Fc_γ to SpG takes place at around pH 5.0, while SpA requires a neutral or, for some classes and subclasses of IgG, an alkaline pH, both complexes dissociating at lower pH than 5.0. Interestingly, some IgGs bound to SpG-Sepharose at pH 5.0 can be eluted by raising the pH in the alkaline region (Åckerström and Björk, 1986), a behavior reminiscent of the ability of Fc_γ to bind FcRn at acid pH and release it at a slightly alkaline pH (Ghetie and Ward, 2000).

c. Interaction of SpG with the Fab Region. Different portions of the B/C domains are involved in binding the Fc_γ and Fab_γ regions (Derrick and Wigley, 1994; Lian *et al.*, 1994). The affinity for Fab_γ is, however, 10 times lower (Björck and Kronval, 1981). The SpG–Fab_γ interaction involves exclusively the β-2 strand of the B/C domain. This strand interacts with the last, surface-exposed β strand of the $C_\gamma1$ domain. The $C_\gamma1$ residues that are in contact with the B/C domain are highly conserved in human and mouse IgG (Pro-126, Val-128, Tyr-129, Ser-209, Ser-210, Thr-211, and Lys-215). These residues establish main chain/main chain hydrogen bonds and van der Waals contacts with residues 11–17 of the β-2 strand of the B/C domain (Derrick and Wigley, 1994). The antiparallel pairing of these two β sheets from Fab_γ and the B/C domain is a novel protein–protein recognition system different from that of the key/lock type of the SpG–Fc_γ interaction (Kuehn *et al.*, 1993).

The highly conserved nature of $C_\gamma1$ residues explains the reaction of SpG with Fab of all Ig isotypes of human, mouse, and probably other species. This is in contrast with SpA, which recognizes only particular sequences of some human and mouse IgG belonging to restricted V_H families (Tashiro and Montelione, 1995).

3. Streptococcal Protein H

Protein H (SpH) is a polyreactive molecule expressed on the surface of some strains of *Streptococcus pyogenes*. It belongs to the large family of fibrous surface M proteins, which have structural similarity to a number of host proteins such as tropomyosin, myosin, and laminin. SpH interacts by its N-terminal domains with the Fc region of human IgG with a high affinity ($K_a = 1.6 \times 10^9\ M^{-1}$). The Fc-binding site is located at the $C_\gamma2$–$C_\gamma3$ interface, nearly in the same region as the binding sites for SpA and SpG (Frick *et al.*, 1994, 1995). However, the Fc_γ-binding sites for these proteins have different structures. The SpH–Fc_γ interaction is temperature dependent with high

affinity at 4 °C and 22 °C, but no reaction is registered near 37 °C when SpH is in an unfolded inactive form (Nilson *et al.*, 1995). At low temperatures, SpH exists as a dimer, but at 37°C it can be found only as monomer. Most likely, SpH has to be in a dimeric form for effective binding to IgG.

4. Peptostreptococcal Protein L

Protein L (PpL) (76–106 kDa), expressed by some strains of *Peptostreptococcus magnus*, binds exclusively to the V regions of κ L chains of human and other mammalian immunoglobulins with high affinity. The binding activity is located at four or five small homologous extracellular domains in the N-terminal end of PpL. The three-dimensional structure of these domains is in general very similar to that of the SpG domains despite the fact that SpG and PpL have no significant sequence homology. Both types of domains are composed of α-helix on top of two antiparallel β-stranded sheets (Wikström *et al.*, 1994). The interaction between human L chains and PpL ($K_a = 10^9$ M^{-1}) involves only V regions of three κ subgroups, $V_{\kappa I}$, $V_{\kappa III}$, and $V_{\kappa IV}$ (i.e., about a half of the V_κ repertoire), but not of $V_{\kappa II}$ or V_λ (Nilson *et al.*, 1992). The PpL–κ chain complex has a structure similar to the SpG–C_γ1 complex and involves β sheet interactions (Wikström *et al.*, 1995).

An X-ray crystallographic study was performed on the complex between a single domain of PpL (61 residues) and Fab fragments isolated from an IgM rheumatoid factor with $V_{\kappa I}$ chains (Graille *et al.*, 2001). The complex contains two Fabs and one PpL domain located between them. Contact areas include similar V_L framework areas, which are located distantly from V_L hypervariable loops and are remote from H chains. The interaction does not block antigen-binding activity because contact areas locate away from the antigen-combining site. However, both Fab–PpL interactions are characterized by significantly different affinities despite the fact that total buried solvent-accessible areas are nearly similar (1300 and 1400 Å^2). The first Fab–PpL interaction is dominated and encompasses 13 V_L residues, 10 of which are located in FR1. Twelve residues of the PpL domain participate in these contacts. The second V_L contact area is built from 15 residues, and 10 of them are similar to those that participate in the first interaction. Fourteen residues construct the second contact area of the PpL domain. The first interface is characterized by six hydrogen bonds. The second interaction is also mediated by six hydrogen bonds and, in addition, by two salt bridges. No conformational changes were registered in the backbone of Fab and the PpL domain upon binding. The first type of contact between human $V_{\kappa I}$ and PpL is conserved and similar to that of mouse V_κ and PpL (Graille *et al.*, 2002). According to these data, the Fab–PpL complex could be assigned to the major group of protein–protein complexes, which are characterized by "standard-size" interfaces with a total buried area in the recognition site equal to 1600 (400) Å^2. Such interactions are sufficient

to form stable specific complexes without large conformational changes (Lo Conte *et al.*, 1999).

PpL and bacterial cells bearing this protein on their surface can activate mast cells, which are effector cells synthesizing and releasing vasoactive and proinflammatory molecules. After the addition of PpL or bacterial cells, mast cells release presynthesized active mediators and also begin to synthesize leukotriene C_4 *de novo* (Genovese *et al.*, 2000, 2003). Monoclonal IgE with κ chains completely blocks PpL activity, whereas IgE_λ has no such effect. The activation of mast cells by PpL could be caused by cross-linking IgE_κ molecules bound to the Fc_ε receptors, which are anchored to the surface of mast cells. Bacterial cells that have SpA or PpL on their surface could be important factors in the pathogenesis of inflammatory processes, particularly in heart and cardiovascular diseases.

5. *IgA- and IgD-Binding Bacterial Protein*

A major human pathogen, *Streptococcus pyogenes*, expresses IgA-binding proteins, which are members of the M protein family. A 37-kDa protein, *Sir22*, interacts with IgA and IgG molecules with similar affinity (Stenberg *et al.*, 1994). A 50-residue peptide Sap was derived from this protein with specific IgA-binding activity. Sap reacts with both human IgA subclasses, as well as with secretory IgA, but does not bind mouse IgA. The immobilized Sap peptide can be used for purification of IgA, as well as for detection of IgA antibodies in complexes with antigen (Sandin *et al.*, 2002).

A 40-kDa protein, *Arp*, was isolated from group A *Streptococcus* (Åckerström *et al.*, 1994) and a 124-kDa β *protein* from group B *Streptococcus* (Hedén *et al.*, 1991). Protein Arp is homologous to protein Sir and has higher affinity for serum IgA than for secretory IgA, as the secretory component interferes with Arp binding. The oligomerization of IgA has no influence on the affinity of the interaction with Arp, and the formation of the Arp–IgA complex is independent of the J chain. The Arp protein has only one binding site and cannot cross-link IgA molecules. The β protein binds serum IgA of both subclasses, but poorly interacts with secretory IgA.

The previously-mentioned IgA-binding proteins interact with the closely related binding sites at the $C_\alpha2$–$C_\alpha3$ interface, which overlap that used by the human $Fc_\alpha RI$ cell receptor. Therefore the IgA-binding proteins can block the interactions of IgA molecules with $Fc_\alpha RI$ and in this way interfere with IgA effector functions, thereby contributing to bacterial virulence.

Diplococcus *Moraxella catarrhalis* cells that frequently colonize the respiratory tract express a 200-kDa IgD-binding protein, *MID* (Forsgren *et al.*, 2001). MID protein is bound only by IgD, but not by molecules of other Ig classes. It is able to induce a proliferative response in peripheral B cells.

B. Viral Immunoglobulin-Binding Proteins

1. Herpes Simplex Virus Proteins

Virus particles of herpes simplex virus (HSV-1) bind to the Fc region of human IgG molecules. Two viral glycoproteins, *gE* and *gI*, together form an Fc receptor on the virion envelope. The cells infected by this virus express the same two proteins on their surfaces and are able to bind IgG molecules. Soluble extracellular domains of gE and gI proteins (42 and 36 kDa, correspondingly) assemble in a stable heterodimer with a 1:1 stoichiometry. This complex interacts with IgG also in a 1:1 ratio with relatively high affinity (Chapman *et al.*, 1999). Protein gE alone binds only to IgG aggregates, but not to IgG monomers, whereas soluble gI has no binding activity. Rodent IgGs do not react with HSV-1. A part of protein gE (residues 323–359) participating in Fc binding has sequence similarity with Ig chains and shares homology with human Fc receptors (Dubin *et al.*, 1994). A mutation at gE position 339 causes loss of IgG binding, as the binding site was destroyed (Nagashunmugam *et al.*, 1998).

Human IgG_1, IgG_2, and IgG_4 bind to the viral Fc receptor, but IgG_3 binding is dependent on the IgG_3 phenotype. Only IgG_3 molecules from Oriental populations with an Fc phenotype different from that of white populations have HSV-1 Fc_γ-binding activity (Johansson *et al.*, 1994). The Fc_γ-binding site for HSV-1 is located at the $C_\gamma2$–$C_\gamma3$ region (Johansson *et al.*, 1988). For interaction of the gE–gI complex with Fc_γ residue, His-435 is critical, as IgG_3 allotypes with Arg-435 are unable to bind HSV-1 proteins (Chapman *et al.*, 1999). His-435 located at the $C_\gamma2$–$C_\gamma3$ interface is also a contact residue for interactions with SpA and some RFs. Nonreactive IgG_3 variants can be converted into HSV-binding molecules after a single residue change Arg-435→His-435, and the His-435→Arg-435 change results in loss of binding activity.

Several other residues important for IgG–gE–gI interactions were found in experiments on interactions of various human IgG_1 allotypes with the cell membrane gE–gI complex (Armour *et al.*, 2002). Molecules G1m(3), common in white populations, have Arg in position 214 of the $C_\gamma1$ domain, and molecules G1m(null) have Thr-214. The latter molecules have good binding activity, but the G1m(3) molecules possess only weak affinity, which indicates the importance of the residue in position 214 for IgG binding. As IgG_4 molecules with high binding activity also have Arg-214, the context of residues in position 214 seems very important. Molecules G1m(17) and Gm(1,17), which varied in binding affinity, have different residues at positions 356 and 358 of the $C_\gamma3$ domain. Most probably these residues also contribute to binding specificity. Therefore residues located in the $C_\gamma1$ and $C_\gamma3$ domains influence binding

to gE–gI, in addition to the $C_\gamma2$–$C_\gamma3$ interface residues. The IgG-binding hierarchy for this interaction is $IgG_4 > IgG_1 \geq IgG_2$ (Atherton *et al.*, 2000).

a. Functional Implications. Specific anti-HSV antibodies are relatively ineffective against HSV infection, and the ability of the virus to react with IgG with relatively high affinity explains this phenomenon. The IgG molecules bind to virion particles in the circulation and inhibit the neutralizing activity of anti-HSV antibodies. The expression of gE–gI virus proteins on the surface of infected cells protects them from destruction by effector mechanisms that require the presence of the free Fc region. Anti-HSV antibodies readily destroy cells infected by mutant HSV virions lacking the IgG-binding site, whereas the same antibodies were ineffective against cells infected with wild-type virus (Nagashunmugam *et al.*, 1998). The presence of an Fc-binding site protects cells infected by HSV from antibody-dependent cell-mediated cytotoxicity (Dubin *et al.*, 1991). The mechanism of the protection could be explained by antibody bipolar bridging (i.e., by binding antiviral antibodies to cell surface antigens by the antigen-combining site and simultaneous binding the Fc region by the gE–gI complex). Such a "head and tail" interaction is possible due to the pronounced flexibility of IgG molecules (Nezlin, 1990). As a result, the Fc region of antiviral antibodies is blocked, which prevents the Fc-dependent immune attack. Indeed, antibodies bound to noninfected cells have free Fc, as they can react with SpA. In contrast, antibodies bound to the HSV-infected cells have the blocked Fc region and are unable to react with SpA (Van Vliet *et al.*, 1992). The antibody bipolar bridging also blocks binding of C1q to Fc of antiviral IgG antibodies on infected cells and protects from complement-mediated cytolysis.

The protection from the host immune attack by expression of Fc receptors on infected cells is a common strategy among some other viruses. Cells infected by varicella-zoster virus (Litwin *et al.*, 1992), pseudorabies virus (Favorell *et al.*, 1997), and human cytomegalovirus (Antonsson and Johansson, 2001) also express viral Fc_γ-binding proteins on the surface. Studies of Fc_γ viral receptors could be important for selecting the strategy for the preparation of virus vaccines.

2. *HIV-1 Protein* gp120

The major envelope protein, *gp120*, of HIV-1 virus is bound by Ig molecules, the V_H domains of which are coded preferentially by the V_H3 gene family, the largest family of the V regions genes (Berberian *et al.*, 1993). Ig molecules with other V_H segments do not interact with gp120. The isolated gp120 reacts with a monoclonal IgM, which possesses the V_H3 region with a high affinity ($K_d = 8.6 \times 10^9\ M^{-1}$), whereas the binding activity of IgG with the V_H3 region for gp120 is significantly lower. However, not all V_H3 Ig molecules are able to react with

gp120, as only molecules with V_H3–23, V_H3–30, V_H3–15, and V_H3–73 gene segments, comprising 25% of all V_H3, have gp120-binding activity (Karray *et al.*, 1998). These V_H3 segments have germline sequences, whereas nonreactive V_H3 encompass between 2 and 15 amino acid substitutions. Apparently the ability to bind gp120 is very sensitive to somatic hypermutations of V_H3.

SpA also reacts with V_H3 segments, but binding sites for SpA and gp120 are not identical. It was found that about 16 nonsequential residues of FR1 and FR3 and CDR1 and CDR2 loops correlate with gp120 binding. These residues locate in the solvent-exposed regions mainly outside the antigen-combining site on the face of the V_H region opposite to the contact area with L chains. Only three of 16 residues are common for SpA and gp120-binding sites (Karray *et al.*, 1998).

The ability of the gp120 protein to react with V_H3 immunoglobulins could lead to serious pathological consequences. In AIDS patients there is a clonal deficit of V_H3-expressing nonimmune B lymphocytes, which is preceded by a stimulation and expansion of these B cells on earlier steps of the disease. Such progressive cell depletion could be accounted for by the fact that the gp120 protein can target the membrane anchored V_H3 immunoglobulins and activate B lymphocytes that bear these surface Ig molecules as cell receptors. The V_H3 Ig family comprises nearly half of the expressed human antibody repertoire, and the elimination of V_H3 B cells could cause significant lymphocyte deficiency and, as a consequence, diminished antibody response in HIV-infected patients.

VIII. The Promiscuity of the $C_\gamma2$–$C_\gamma3$ Interface

The promiscuous ability of the $C_\gamma2$–$C_\gamma3$ interface to bind at least six proteins (SpA, SpG, SpH, RF, HSV-1 proteins, and FcRn) and some synthetic peptides (Braisted and Wells, 1996; DeLano *et al.*, 2000), all of them lacking any structural similarity, is mainly determined by its physical and chemical features.

The junction between the $C_\gamma2$ and $C_\gamma3$ domains creates a large cavity in the Fc_γ region consisting of three loops, one on the face of $C_\gamma3$ (residues 433–436) and two on the opposite side of the $C_\gamma2$ face, one proximal to the $C_\gamma3$ loop (residues 252–254) and the second distal (residues 309–311) (Fig. 6). The surface area of this junction cavity ($\sim$2000 Å^2) can be expanded and diminished by the independent and free movement of two $C_\gamma2$ domains, which do not interact with each other (Edmundson *et al.*, 1995). The $C_\gamma2$ motion pivots around a helical loop (Pro-257 to Pro-270) that forms the principal contact interface between the $C_\gamma2$ and $C_\gamma3$ domains (Harris *et al.*, 1997). This segmental flexibility of the $C_\gamma2$–$C_\gamma3$ cleft was proven by comparing the angle between the $C_\gamma2$ and $C_\gamma3$ domains with or without the hinge region (e.g., IgG Mcg) (Guddat *et al.*, 1993). IgG Mcg, with a lower angle between the two domains (67° vs. 90°), has an enlarged $C_\gamma2$–$C_\gamma3$ cavity, as shown by the

increased distance between Met-252 and His-435 from 7.9 to 13.6 Å. If the disulfide bonds of the hinge region are cleaved or missing, the Fab arms can move freely along with their attached $C_\gamma2$ domains, the junction with the $C_\gamma3$ domain behaving like an *alternative* hinge (Seegan *et al.*, 1979). This intrinsic physical property of the $C_\gamma2$–$C_\gamma3$ cleft makes it adaptable for interaction with a variety of proteins with different sizes and shapes, offering them a surface area of approximately one third of the whole surface area of the cavity (700–800 $Å^2$).

The chemical accessibility of the $C_\gamma2$–$C_\gamma3$ cleft by so many unrelated ligands is determined by the unique combination of the amino acid residues, with distinct characteristics present in all three binding loops. The majority of these 10 residues is well conserved across species and is able to establish a variety of noncovalent interactions, some common but others distinct for each ligand. Thus Ile-253 makes hydrophobic interactions with all tested ligands such as SpA (Phe-13), SpG (Trp-43), RF (Tyr-98), FcRn (Trp-133), and Val-10 in a small synthetic peptide (DeLano *et al.*, 2000). In contrast, His-310 is important in the binding of FcRn (Glu-117) and possibly SpA, probably by salt bridges, but has no reported role in binding RF or SpG. However, the adjacent residue (Glu-311) does not participate in the binding of FcRn and RF, but is essential for the interaction of SpA (Asn-18) and SpG (Glu-42). In the $C_\gamma3$ loop, His-435 is necessary for binding FcRn (Glu-132) and SpA (Tyr-14) but not SpG, while the neighbor residue (Asn-434) has no involvement in binding FcRn, but is essential for the polar or charged interaction with SpA (Asn-11), SpG (Asn-35), and RF.

These few examples suggest that the interaction of multiple ligands with the residues of the $C_\gamma2$–$C_\gamma3$ binding loops is elective, "choosing" only the residues of the ligands that allow the appropriate noncovalent pairing. The fact that the reaction of the multiple ligands with IgGs has a characteristic pH dependency and binding affinity clearly indicates that the $C_\gamma2$–$C_\gamma3$ cleft has not only physical but also chemical flexibility. The flexibility and accessibility of this region are also supported by experiments showing that from a vast library of peptides selected to bind *any* region of the Fc_γ fragment, the dominating peptides were those reacting with the $C_\gamma2$–$C_\gamma3$ interface (DeLano *et al.*, 2000). Therefore it will not be surprising to find in the future new natural proteins and synthetic peptides with affinity for this region of IgG.

IX. Concluding Remarks

Many Ig interactions with various ligands occur in the circulation, when both components are in soluble form, whereas others take place on the cell surface, as one of the components is localized on the cell membrane (Table VIII). Both types of interactions have functional consequences essential for the development

TABLE VIII
IMMUNOGLOBULIN INTERACTION SITES

Ligands	Localization of Ig sites
Cell receptors	
FcγRI, II, III	Low hinge, $C_\gamma2$
Fc_εRI	$C_\varepsilon3$ and $C_\varepsilon2$–$C_\varepsilon3$ linkers
Fc_αRI	$C_\alpha2$–$C_\alpha3$ cleft
PolyIgR	$C_\alpha3$
FcRn	$C_\gamma2$–$C_\gamma3$ cleft
Complement components	
C3b	$C_\gamma1$, $C_\gamma2$–$C_\gamma3$ cleft
C1q	Low hinge and $C_\gamma2$; $C_\mu3$
Anaphylatoxins	Fab_γ
Bacterial proteins	
SpA	$C_\gamma2$–$C_\gamma3$ cleft, V_H3
SpG	$C_\gamma2$–$C_\gamma3$ cleft, $C_\gamma1$
SpH	$C_\gamma2$–$C_\gamma3$ cleft
PpL	V_κ
Arp, Sir22	$C_\alpha2$–$C_\alpha3$ cleft
Viral proteins	
HSV-1	$C_\gamma2$–$C_\gamma3$ cleft
HIV-1	V_H3
Lectins	Ig glycans
Prolactin	Fd_γ
Fibronectin	Fc
Rheumatoid factor	$C_\gamma2$–$C_\gamma3$ cleft
CD4	V_H
Histidine-rich glycoprotein	Fab
Clusterin	Fab and Fc
Peptides	Fc_γ
Protein Fv	V_H

of the immune response, as well as for the progress of various disorders. The initiation of the complement cascade follows the reaction of the complement component C1q with IC, and the covalent binding of C3 and C4 is important for the elimination of antigens from the circulation. The formation of the complexes can close some functionally important sites. An example of such reactions is shielding virion particles with Fc receptors by Ig molecules, which inhibits inactivation of viruses by neutralizing antibodies. Histidine-rich glycoprotein can inhibit the formation of insoluble IC and also affects the binding of IgG and IC by cells. Biologically active substances (for example, anaphylatoxins) could be eliminated from the circulation after the binding to immunoglobulins.

The interactions with cell-bound ligands are just as important. Various Fc receptors are expressed on the surface of many types of cells, and binding Ig molecules to these receptors could activate or inhibit cell activity or stimulate

the transportation of Ig molecules through cell membranes. A complex of an Ig molecule with a ligand can react simultaneously with two corresponding types of cell receptors—with Fc receptors by the Fc moiety and with specific receptors for the Ig-bound ligand. Such "double" interactions can amplify signaling and change the cell response (the effect of coligation). A special case of the receptor coligation is the antibody bipolar bridging, when virus-neutralizing antibodies react with antigens expressed on cells infected with some viruses. Due to the pronounced flexibility, antibody molecules bind by the antigen-combining sites to cell-surface viral antigens and interact simultaneously by their Fc regions, with Fc viral receptors expressed on the cell surface. Such "head and tail" antibody binding results in blocking the antibody Fc region and preventing the host Fc-dependent immune attack. Ig interactions with cell-bound ligands could have important consequences for the development of some disorders. For example, fibronectin associated with matrix participates in removing IC from the circulation and deposing them in tissues, which could cause serious pathological complications.

Studies of Ig–ligand interactions also have a methodological aspect. Ligands with specificity for immunoglobulins, such as SpA and SpG, are effectively used for detection and isolation of Ig molecules. Lectins could be helpful for structural studies of oligosaccharides linked to Ig molecules. IVIG preparations, which contain mainly IgG, are widely used for prophylaxis and treatment of various pathologies. Therefore it is important to know whether these preparations also contain complexes of IgG with some biologically active substances, which could be harmful for the body.

Ig peptide chains have a modular structure and are built from several compact folds or domains homologous to those of proteins, which are members of the large Ig superfamily (Nezlin, 1998). A very stable structure of Ig fold has been conserved in evolution and can be found in protein molecules of a wide variety of species, including invertebrates and ancient vertebrates (Barclay *et al.*, 1997). Molecules of the Ig superfamily are able to bind ligands of various structures and dimensions from complex proteins to small molecules. Therefore the Ig fold can be considered a scaffold on which arrays of various binding sites are displayed either on β strands or at the loops connected to the strands or on both.

Amino acid residues of nearly all Ig domains participate in the construction of binding sites for interactions with various ligands. Some ligands can bind to only one Ig domain, whereas others interact with binding sites localized on different Ig domains. A large number of ligands react with the C_H2–C_H3 interface. The promiscuity of this location could be at least partly explained by the flexible structure of the Fc region and, as a consequence, by the ability of Fc to adapt more easily to various spatial configurations important for the interactions with different ligands.

Studies on Ig interactions with various ligands are continuing, and we can anticipate that new findings would be valuable both for further understanding of the immune response, as well as for clinical immunology. On the basis of X-ray crystallography data, the modeling of inhibitors of harmful Ig interactions could be performed, which provides the possibility of creating new, effective drugs. We can expect that new types of Ig interactions would be found. In particular, it seems to be important to identify ligands forming complexes with receptors of B cell precursors, which could give new insight on B cell development. Information on the evolutionary aspects of Ig interactions could help to explain the development of the interplay between host and parasites and provide new ideas about the evolution of the immune system.

References

Achari, A., Hale, S. P., Howard, A. J., Clore, M. G., Gronenborn, A. M., Hardman, K. D., and Whitlow, M. (1992). 1.67 Å X-ray structure of the B2 immunoglobulin-binding domain of streptococcal protein G and comparison to the NMR structure of B1 domain. *Biochemistry* **31,** 10449–10457.

Åckerström, B., and Björck, L. (1986). A physicochemical study of protein G, a molecule with unique immunoglobulin-binding properties. *J. Biol. Chem.* **261,** 10240–10247.

Åckerström, B., Nielsen, E., and Björck, L. (1987). Definition of IgG- and albumin-binding regions of streptococcal protein G. *J. Biol. Chem.* **262,** 13388–13391.

Åckerström, B., Lindquist, A., Maelen, C. V., Grubb, A., Lindahl, G., and Vaerman, J.-P. (1994). Interaction between streptococcal protein Arp and different molecular forms of human immunoglobulin A. *Mol. Immunol.* **31,** 393–400.

Andrieux, C., Pires, R., Moreau, M.-C., and Bouvet, J.-P. (1998). Release of the soluble co-receptor (protein Fv) of secretory immunoglobulins after colonization of axenic rats by the human gut microflora. *Scand. J. Immunol.* **48,** 192–195.

Antón, L. C., Ruiz, S., Barrio, E., Marques, G., Sánchez, A., and Vivanco, F. (1994). C3 binds with similar efficiency to Fab and Fc regions of IgG immune aggregates. *Eur. J. Immunol.* **24,** 599–604.

Antonsson, A., and Johansson, P. J. (2001). Binding of human and animal immunoglobulins to the IgG receptor induced by human cytomegalovirus. *J. Gen. Virol.* **82,** 1137–1145.

Armour, K. L., Atherton, A., Williamson, L. M., and Clark, M. R. (2002). The contrasting IgG-binding interactions of human and herpes simplex virus Fc receptors. *Biochem. Soc. Transact.* **30,** 495–499.

Artandi, S. E., Canfield, S. M., Tao, M.-H., Calame, K. L., Morrison, S. L., and Bonagura, V. R. (1991). Molecular analysis of IgM rheumatoid factor binding chimeric IgG. *J. Immunol.* **146,** 603–610.

Arya, S., Chen, F., Spycher, S., Isenman, D. E., Shulman, M. J., and Painter, R. H. (1994). Mapping of amino acid residues in the Cμ3 domain of mouse IgM important in macromolecular assembly and complement-dependent cytolysis. *J. Immunol.* **152,** 1206–1212.

Atherton, A., Armour, K. L., Bell, S., Minson, A. C., and Clark, M. R. (2000). The herpes simplex virus type 1 receptor discriminates between IgG1 allotypes. *Eur. J. Immunol.* **30,** 2540–2547.

Baird, B., Zheng, Y., and Holowka, D. (1993). Structural mapping of IgE-FcεRI, an immunoreceptor complex. *Acc. Chem. Res.* **26,** 428–434.

Bakos, M. A., Kurosky, A., and Goldblum, R. M. (1991). Characterization of a critical binding site of human polymeric Ig on secretory component. *J. Immunol.* **147,** 3419–3426.

Bakos, M. A., Widen, S. G., and Goldblum, R. M. (1994). Expression and purification of biologically active domain I of the human polymeric immunoglobulin receptor. *Mol. Immunol.* **31,** 165–171.

Barclay, A. N., Brown, M. H., Law, S. K. A., McKnight, A. J., Tomlinson, M. G., and van der Merwe, P. A. (1997). "The Leucocyte Antigen Facts Book," 2nd ed. Academic Press, London.

Basta, M., Van Goor, F., Luccioli, S., Billings, E. M., Vortmeyer, A. O., Baranyi, L., Szebeni, J., Alving, C. R., Carroll, M. C., Berkower, I., Stojilkovic, S. S., and Metcalf, D. D. (2003). $F(ab)'_2$-mediated neutralization of C3a and C5a anaphylatoxins: A novel effector function of immunoglobulins. *Nat. Med.* **9,** 431–438.

Belson, A., Yorgin, P. D., Al-Uzri, A.-Y., Salvatierra, O., Higgins, J., and Alexander, S. R. (2001). Long-term plasmaphoresis and protein A column treatment of recurrent FSGS. *Pediatr. Nephrol.* **16,** 985–989.

Berberian, L., Goodglick, L., Kipps, T. J., and Braun, J. (1993). Immunoglobulin V_H3 gene products: Natural ligands for HIV gp120. *Science* **261,** 1588–1591.

Björck, L., and Kronval, G. (1981). Purification and some properties of streptococcal protein G. A novel IgG-binding reagent. *J. Immunol.* **133,** 969–974.

Bonagura, V. R., Artandi, S. E., Agostino, N., Tao, M.-H., and Morrison, S. L. (1992). Mapping rheumatoid factor binding sites using genetically engineered chimeric IgG antibody. *DNA Cell Biol.* **11,** 245–252.

Bonagura, V. R., Artandi, S. E., Davidson, A., Randen, I., Agostino, N., Thompson, K., Natvig, J. B., and Morrison, S. L. (1993). Mapping studies reveal unique epitopes on IgG recognized by rheumatoid arthritis derived monoclonal rheumatoid factors. *J. Immunol.* **151,** 3840–3852.

Borvak, J., Richardson, J., Medesan, C., Antohe, F., Radu, C., Simionescu, M., Ghetie, V., and Ward, E. S. (1998). Functional expression of the MHC class I-related receptor, FcRn in endothelial cells of mice. *Int. Immunol.* **10,** 1289–1298.

Bouvet, J.-P., Pirès, R., and Quan, C. P. (1996). Protein Fv (Fv fragment binding protein): A mucosal human superantigen reacting with normal immunoglobulins. *In* "Human B Cell Superantigens" (M. Zouali, Ed.), pp. 179–188. R. C. Landes Co., Austin, TX.

Boyle, M. D. P. (1990). Bacterial Immunoglobulin-binding Proteins. Academic Press, San Diego.

Bradl, H., Wittmann, J., Milius, D., Vettermann, C., and Jäck, H.-M. (2003). Interaction of murine precursor B cell receptor with stroma cells is controlled by the unique tail of λ5 and stroma cell-associated heparan sulfate. *J. Immunol.* **171,** 2338–2348.

Brady, R. L., and Barclay, A. N. (1996). The structure of CD4. *In* "The CD4 Molecule" (D. R. Littman, Ed.), pp. 1–18. Springer-Verlag, Berlin.

Braisted, A. C., and Wells, J. A. (1996). Minimizing a binding domain from protein A. *Proc. Natl. Acad. Sci. USA* **93,** 5688–5692.

Braun, N., and Riesler, T. (1999). Immunoadsorption as a tool for the immunomodulation of humoral and cellular immune system in autoimmune diseases. *Ther. Apheresis* **3,** 240–245.

Braun, N., Erley, C., Klein, R., Koffler, I., Saal, J., and Riesler, T. (2000). Immunoadsorption onto protein A induced remission in severe systemic lupus erithematosus. *Nephrol. Dial. Transplant.* **15,** 1367–1372.

Bray, B. A., Osman, M., and Turino, G. M. (1994). Evidence that fibronectin-immunoglobulin complexes occur normally in plasma. *Proc. Soc. Exp. Biol. Med.* **207,** 324–331.

Brüggemann, M., Teale, C., Clark, M., Bindon, C., and Waldmann, H. (1989). A matched set of rat/mouse chimeric antibodies. Identification and biological properties of rat H chain constant regions μ, γ1, γ2a, γ2b, γ2c, ε, and α. *J. Immunol.* **142,** 3145–3150.

Burmeister, W. P., Gastinel, L. N., Simister, N. E., Blum, M. L., and Bjorkman, P. J. (1994a). Crystal structure at 2.2 Å resolution of the MHC-related neonatal Fc receptor. *Nature* **372,** 336–343.

Burmeister, W. P., Huber, A. H., and Bjorkman, P. J. (1994b). Crystal structure of the complex of rat neonatal Fc receptor with Fc. *Nature* **372,** 379–383.

Canfield, S. M., and Morrison, S. L. (1991). The binding affinity of human IgG for its high affinity Fc receptor is determined by multiple amino acids in the C_H2 domain and is modulated by the hinge region. *J. Exp. Med.* **173,** 1483–1491.

Cedergren, L., Andersson, R., Jansson, B., Uhlen, M., and Nilsson, B. (1993). Mutational analysis of the interaction between staphylococcal protein A and human IgG. *Protein Eng.* **6,** 441–448.

Chapman, T. L., You, I., Joseph, I. M., Bjorkman, P. J., Morrison, S. L., and Raghavan, M. (1999). Characterization of the interaction between the herpes simplex virus type I Fc receptor and immunoglobulin G. *J. Biol. Chem.* **274,** 6911–6919.

Chen, P. P., and Carson, D. A. (1994). New insight on the physiological and pathological rheumatoid factors in humans. *In* "Autoimmunity: Physiology and Disease" (A. Cutinho and M. Kazatchkine, Eds.), pp. 247–268. Wiley, New York.

Christianson, G. J., Brooks, W., Vekasi, S., Manolfi, E. A., Wiles, J., Roopenian, S. L., Rothlein, R., and Roopenian, D. C. (1997). β2-microglobulin-deficient mice are protected from hypergammaglobulinemia and have defective antibody response because of the increased catabolism. *J. Immunol.* **159,** 4781–4792.

Cianga, P., Medesan, C., Richardson, J. A., Ghetie, V., and Ward, E. S. (1999). Identification and function of neonatal Fc receptor in mammary gland of lactating mice. *Eur. J. Immunol.* **29,** 2515–2523.

Coloma, M. J., Clift, A., Wims, L., and Morrison, S. L. (2000). The role of carbohydrate in the assembly and function of polymeric IgG. *Mol. Immunol.* **37,** 1081–1090.

Corper, A. L., Sohi, M. K., Bonagura, V. R., Steinitz, M., Jefferis, R., Feinstein, A., Beale, D., Taussig, M. J., and Sutton, B. J. (1997). Structure of human IgM rheumatoid factor Fab bound to its autoantigen IgGFc reveals a novel topology of antibody-antigen interaction. *Nat. Struct. Biol.* **4,** 374–381.

Corthesy, B. (2002). Recombinant immunoglobulin A: Powerful tools for fundamental and applied research. *Trends Biotechnol.* **20,** 65–71.

Coyne, R. S., Siebrecht, M., Peitsch, M. C., and Casanova, J. E. (1994). Mutational analysis of polymeric immunoglobulin receptor/ligand interactions. Evidence for the involvement of multiple complementary determining region (CDR)-like loops. *J. Biol. Chem.* **269,** 31620–31625.

Crottet, P., and Corthesy, B. (1998). Secretory component delays the conversion of secretory IgA into antigen-binding competent $F(ab')_2$: A positive implication for mucosal defense. *J. Immunol.* **161,** 879–888.

Crottet, P., and Corthesy, B. (1999). Mapping the interaction between immune IgA and murine secretory component carrying epitope binding to IgA. *J. Biol. Chem.* **274,** 31456–31462.

Dall'Acqua, W. F., Woods, R. M., Ward, E. S., Palaszynski, S. R., Patel, N. K., Brewah, Y. A., Wu, H., Kiener, P. A., and Langermann, S. (2002). Increasing affinity of a human IgG1 for the neonatal Fc receptor: Biological consequences. *J. Immunol.* **169,** 5171–5180.

Deisenhofer, J. (1981). Crystallographic refinement and atomic models of human Fc fragment and its complex with fragment B of protein A from *Staphylococcus aureus* at 2.9 and 2.8 Å resolution. *Biochemistry* **20,** 2361–2370.

DeLano, W. L., Ultsch, M. H., deVos, A. M., and Wells, J. A. (2000). Convergent solution to binding at a protein-protein interface. *Science* **287,** 1279–1283.

Delespesse, G., Sarfati, M., Wu, C. Y., Fournier, S., and Letellier, M. (1992). The low-affinity receptor for IgE. *Immunol. Rev.* **125,** 77–97.

Derrick, J. P., and Wigley, D. B. (1994). The third IgG-binding domain from streptococcal protein G. An analysis by X-ray crystallography of the structure alone and in complex with Fab. *J. Mol. Biol.* **243,** 906–918.

Dima, S., Medesan, C., Mota, G., Moraru, I., Sjöquist, J., and Ghetie, V. (1984). Effect of protein A and its fragment B on the catabolism and Fc receptor sites of IgG. *Eur. J. Immunol.* **13,** 605–614.

Dong, X., Storkus, W. J., and Salter, R. D. (1999). Binding and uptake of agalactosyl IgG by mannose receptor on macrophages and dendritic cells. *J. Immunol.* **163,** 5427–5434.

Dubin, G., Socolof, E., Frank, I., and Friedman, H. M. (1991). Herpes simplex virus type 1 Fc receptor protects infected cells from antibody-dependent cellular cytotoxicity. *J. Virol.* **65,** 7046–7050.

Dubin, G., Basu, S., Mallory, D. L., Basu, M., Tal-Singer, R., and Friedman, H. M. (1994). Characterization of domains of herpes simplex virus type 1 glycoprotein E involved in Fc binding activity for IgG aggregates. *J. Virol.* **68,** 2478–2486.

Dudich, E. I., and Dudich, I. V. (1983). Polarization fluorescence, spin label and ultracentrifugal studies of specific interaction of low molecular weight proteins with the Fc fragment of human immunoglobulin G. *Mol. Immunol.* **20,** 1267–1272.

Dudich, E. I., Nezlin, R., and Franěk, F. (1978). Fluorescence polarization analysis of various immunoglobulins. Dependence of rotational relaxation time on protein concentration and on ability to precipitate with antigen. *FEBS Lett.* **89,** 89–92.

Duncan, A. R., and Winter, G. (1988). The binding site for C1q on IgG. *Nature* **332,** 738–740.

Edmundson, A. B., Guddat, L. W., Rosauer, R. A., Andersen, K. W., Shan, L., and Fan, Z-C. (1995). Three-dimensional aspects of IgG structure and function. *In* "The Antibodies" (M. Zanetti and J. D. Capra, Eds.), Vol. I, pp. 41–100. Harwood Academic Publ., Luxembourg.

Epstein, J., Eichbaum, Q., Sheriff, S., and Esekovitz, R. A. B. (1996). The collectins in innate immunity. *Curr. Opin. Immunol.* **8,** 29–35.

Esnault, V. L. M., Besnier, D., Testa, A., Coville, P., Simon, P., Subra, J.-P., and Andrain, M. A. P. (1999). Effect of protein A immunoadsorption in nephrotic syndrome of various etiologies. *J. Am. Soc. Nephrol.* **10,** 2014–2017.

Fallgreen-Gebauer, E., Gebauer, W., Bastian, A., Kratzin, H. D., Eiffert, H., Zimmermann, B., Kavas, M., and Hilschmann, N. (1993). The covalent linkage of secretory component to IgA. Structure of sIgA. *Biol. Chem. Hoppe Seyler* **375,** 1023–1028.

Fassina, G. (2000). Protein A mimetic (PAM) affinity purification. *In* "Methods in Molecular Biology" (P. Bailon, G. K. Ehrlich, W.-J. Fung, and W. Berthold, Eds.), Vol. 147, pp. 57–68. Humana Press, Totowa, NJ.

Fassina, G., Verdoliva, A., Odlerna, M. R., and Cassini, G. (1996). Protein A mimetic peptide ligand for affinity purification of antibodies. *J. Mol. Recog.* **9,** 564–569.

Favorell, H. W., Nauwynck, H. J., van Oostveldt, P., Mettenleiter, T. C., and Pensaert, M. B. (1997). Antibody-induced and cytoskeleton-mediated redistribution and shedding of viral glycoproteins, expressed on pseudorabies virus-infected cells. *J. Virol.* **71,** 8554–8561.

Felson, D. T., Lavalley, M. P., Baldassare, A. R., Block, J. A., Caldwell, J. R., Cannon, G. W., Deal, C., Evans, S., Fleischmann, R., Gendreau, R. M., Harvis, E. R., Matteson, E. L., Roth, S. H., Schumacher, R., Weisman, M. H., and Furst, D. E. (1999). The Prosorba column for treatment of refractory rheumatoid arthritis. *Arthritis Rheum.* **42,** 2153–2159.

Firan, M., Bawdon, R., Radu, C., Ober, R. J., Eaken, D., Antohe, F., Ghetie, V., and Ward, E. S. (2001). The MHC class I related receptor FcRn plays an essential role in the maternofetal transfer of gamma-globulin in humans. *Int. Immunol.* **13,** 993–1002.

Forsgren, A., Ghetie, V., Lindmark, R., and Sjöquist, J. (1983). Protein A and its exploitation. *In* "Staphylococcal and Streptococcal Infections" (C. S. F. Eastmon and C. Adlam, Eds.), Vol. I, pp. 429–480. Academic Press, New York.

Forsgren, A., Brant, M., Möllenkvist, A., Muyombwe, A., Janson, H., Woin, N., and Riesbeck, K. (2001). Isolation and characterization of a novel IgD-binding protein from *Moraxella catarrhalis*. *J. Immunol.* **167,** 2112–2120.

Frick, I.-M., Wikstrom, M., Forsen, S., Drakenberg, T., Gomi, H., Sjobring, V., and Björck, L. (1992). Convergent evolution among immunoglobulin-binding bacterial proteins. *Proc. Natl. Acad. Sci. USA* **89,** 8532–8536.

Frick, I.-M., Åkesson, P., Cooney, J., Sjöbring, U., Schmidt, K.-H., Gomi, H., Hattori, S., Tagawa, C., Kishimoto, F., and Björck, L. (1994). Protein H—a surface protein of *Streptococcus pyogenes* with separate binding sites for IgG and albumin. *Mol. Microbiol.* **12,** 143–151.

Frick, I.-M., Crossin, K. L., Edelman, G. M., and Björck, L. (1995). Protein H a bacterial surface protein with affinity for both immunoglobulin and fibronectin type III domains. *EMBO J.* **14,** 1674–1679.

Frutiger, S., Hughes, G. J., Hanly, W. C., Kingzette, M., and Jaton, J. C. (1986). The amino-terminal domain of rabbit secretory component is responsible for the noncovalent binding to immunoglobulin A dimers. *J. Biol. Chem.* **261,** 16673–16681.

Gallagher, T., Alexander, P., Bryan, P., and Gilliland, G. L. (1994). Two crystal structure of the B1 immunoglobulin-binding domain of streptococcal protein G and comparison with NMR. *Biochemistry* **33,** 4721–4729.

Garman, S. C., Kinet, J.-P., and Jardetzky, T. S. (1998). Crystal structure of the human high-affinity IgE receptor. *Cell* **95,** 951–961.

Garman, S. C., Wurzburg, B. A., Tarchevskaya, S. S., Kinet, J.-P., and Jardetzky, T. S. (2000). Structure of the Fc fragment of human IgE bound to its high-affinity receptor FcεRIα. *Nature* **406,** 259–266.

Garman, S. C., Sechl, S., Kinet, J.-P., and Jardetzky, T. S. (2001). The analysis of the human affinity IgE receptor FcεRIα from multiple crystal forms. *J. Mol. Biol.* **311,** 1049–1062.

Gauthier, L., Rossi, B., Roux, F., Termine, E., and Schiff, C. (2002). Galectin-1 is a stromal cell ligand of the pre-B cell receptor (BCR) implicated in synapse formation between pre-B and stromal cells in pre-BCR triggering. *Proc. Natl. Acad. Sci. USA* **99,** 13014–13019.

Genovese, A., Bouvet, J.-P., Florio, G., Lamparter-Schummert, B., Björck, L., and Marone, G. (2000). Bacterial immunoglobulin superantigen proteins A and L activate human heart mast cells by interacting with immunoglobulin E. *Immun. Infect.* **68,** 5517–5524.

Genovese, A., Borgia, G., Björck, L., Petraroli, A., de Paulis, A., Piazza, M., and Marone, G. (2003). Immunoglobulin superantigen protein L induces IL-4 and IL-13 secretion from human FcεRI$^+$ cells through interaction with the κ light chains of IgE. *J. Immunol.* **170,** 1854–1861.

Ghetie, V., and Sjöquist, J. (1984a). Use of protein A in the detection and quantitation of immunoglobulin G on the surface of lymphocytes. *Methods Enzymol.* **108,** 405–413.

Ghetie, V., and Sjöquist, J. (1984b). Separation of cells by affinity chromatography on protein A gels. *Methods Enzymol.* **108,** 132–138.

Ghetie, V., and Ward, E. S. (2000). Multiple roles for the major histocompatibility complex class I-related receptor. *Annu. Rev. Immunol.* **18,** 739–766.

Ghetie, V., Hubbard, J. G., Kim, J.-K., Tsen, M.-F., Lee, Y., and Ward, E. S. (1996). Abnormally short serum half-lives of IgG in beta 2-microglobulin deficient mice. *Eur. J. Immunol.* **26,** 690–696.

Ghetie, V., Popov, S., Borvak, J., Radu, C., Matesoi, D., Medesan, C., Ober, R., and Ward, E. S. (1997). Serum persistence of an IgG fragment by random mutagenesis. *Nat. Biotechnol.* **15,** 637–640.

Ghetie, V., Ward, E. S., and Vitetta, E. S. (2004). The pharmacokinetics of antibody and immunotoxins in mice and humans. *In* "Pharmacokinetics and Pharamacodynamics of Anti-Cancer Agents" (W. D. Figg and H. McLeod, Eds.). Humana Press, Totawa, NJ (in press).

Gorgani, N. N., Parish, C. R., Easterbrook-Smith, S. B., and Altin, J. G. (1997). Histidine-rich glycoprotein binds to human IgG and C1q and inhibits the formation of insoluble immune complexes. *Biochemistry* **36,** 6653–6662.

Gorgani, N. N., Parish, C. R., and Altin, J. G. (1999a). Differential binding of histidine-rich glycoprotein (HRG) to human IgG subclasses and IgG molecules containing kappa and lambda light chains. *J. Biol. Chem.* **274,** 29633–29640.

Gorgani, N. N., Altin, J. G., and Parish, C. R. (1999b). Histidine-rich glycoprotein prevents the formation of insoluble immune complexes by rheumatoid factor. *Immunology* **98,** 456–463.

Gorgani, N. N., Altin, J. G., and Parish, C. R. (1999c). Histidine-rich glycoprotein regulates the binding of monomeric IgG and immune complexes to monocytes. *Int. Immunol.* **11,** 1275–1282.

Gould, H. J., Sutton, B. J., Beavil, A. J., Beavil, R. J., McCloskey, N., Coker, H. A., Fear, D., and Smurthwaite, L. (2003). The biology of IgE and the basis of allergic disease. *Annu. Rev. Immunol.* **21,** 579–628.

Graille, M., Stura, E. A., Corper, A. L., Sutton, B. J., Taussig, M. J., Charbonnier, J.-B., and Silverman, G. J. (2000). Crystal structure of a *Staphylococcus aureus* protein A domain complexed with the Fab fragment of a human IgG antibody. Structural basis for recognition of B-cell receptors and superantigen activity. *Proc. Natl. Acad. Sci. USA* **97,** 5399–5404.

Graille, M., Stura, E. A., Housden, N. G., Beckingham, J. A., Bottomley, S. P., Beale, D., Taussig, M. J., Sutton, B. J., Gore, M. G., and Charbonnier, J.-B. (2001). Complex between *Peptostreptococcus magnus* protein L and a human antibody reveals structural convergence in the interaction modes of Fab binding proteins. *Structure* **9,** 679–687.

Graille, M., Harrison, S., Crump, M. P., Findlow, S. C., Housden, N. G., Muller, B. H., Batail-Poirot, N., Sibaï, G., Sutton, B. J., Taussig, M. J., Jolivet-Reynaud, C., Gore, M. G., and Stura, E. A. (2002). Evidence for plasticity and structural mimicry at the immunoglobulin light chain-protein L interface. *J. Biol. Chem.* **277,** 47500–47506.

Guddat, L. W., Herron, J. N., and Edmundson, A. B. (1993). Three-dimensional structure of human immunoglobulin with hinge deletion. *Proc. Natl. Acad. Sci. USA* **90,** 4271–4275.

Guthrie, T. H., and Oral, A. (1989). Immune trombocytopenia purpura: A pilot study of staphylococcal protein A immunomodulation in refractory patients. *Semin. Hematol.* **26,** 3–9.

Hanson, D. C., and Schumaker, V. N. (1984). A model for the formation and interconversion of protein A–IgG soluble complexes. *J. Immunol.* **132,** 1397–1409.

Harris, L. J., Larson, S. B., Hasel, K. W., and McPherson, A. (1997). Refined structure of an intact IgG2a monoclonal antibody. *Biochemistry* **36,** 1581–1597.

Harrison, P. T., and Allen, J. M. (1998). High affinity IgG binding to FcγRI (CD64) is modulated by two distinct IgSF domains and the transmembrane domain of the receptor. *Protein Eng.* **11,** 225–232.

Hashim, O. H., Ng, C. L., Gedeh, G. S., and Jaafar, M. I. N. (1991). IgA binding lectins isolated from distinct *Artocarpus* species demonstrate differential specificity. *Mol. Immunol.* **28,** 393–398.

Hashim, O. H., Ahmad, F., and Shuib, A. S. (2001). The application of *Artocarpus integer* seed lectin-M in the detection and isolation of selective human serum acute-phase proteins and immunoglobulins. *Immunol. Commun.* **30,** 131–141.

Hashimoto, T., Takishita, M., Kosaka, M., Sano, T., and Matsumoto, T. (2001). Superantigens and autoantigens may be involved in the pathogens of gastric mucosa-associated lymphoid tissue lymphoma. *Int. J. Hematol.* **74,** 197–204.

Haun, M., Incledon, B., Alles, P., and Wasi, S. (1989). A rapid procedure for the purification of IgA_1 and IgA_2 subclasses from normal human serum using protein G and jackfruit lectin (jacalin) affinity chromatography. *Immunol. Lett.* **22,** 273–280.

Hedén, L.-O., Frithz, E., and Lindahl, G. (1991). Molecular characterization of an IgA receptor from group B streptococci: Sequence of the gene, identification of a proline-rich region with unique structure and isolation of N-terminal fragments with IgA-binding capacity. *Eur. J. Immunol.* **21,** 1481–1490.

Herr, A. B., Ballister, E. R., and Bjorkman, P. J. (2003). Insights into IgA-mediated immune responses from the crystal structures of human FcαRI and its complex with IgA1-Fc. *Nature* **423,** 614–620.

Hexham, J. M., White, K. D., Carayannopoulos, L. N., Mandecki, W., Brisette, R., Yang, Y.-S., and Capra, J. D. (1999). A human immunoglobulin (Ig)A $C_\alpha 3$ domain motif directs polymeric Ig receptor-mediated secretion. *J. Exp. Med.* **189,** 747–751.

Hogarth, P. M. (2002). Fc receptors are major mediators of antibody-based inflammation in autoimmunity. *Curr. Opin. Immunol.* **14,** 798–802.

Holmskov, U., Malhotra, R., Sim, R. B., and Jensenius, J. C. (1994). Collectins: Collagenous C-type lectins of the innate immune defense system. *Immunol. Today* **15,** 67–74.

Hugly, T. E. (1984). Structure and function of the anaphylatoxins. *Springer Semin. Immunopathol.* **7,** 193–219.

Hugly, T. E., and Müller-Eberhard, H. J. (1978). Anaphylatoxins: C3a and C5a. *Adv. Immunol.* **26,** 1–53.

Hulett, M. D., and Hogarth, P. M. (1994). Molecular basis of Fc receptor function. *Adv. Immunol.* **57,** 1–127.

Hulett, M. D., and Hogarth, P. M. (1998). The second and third extracellular domains of FcγRI (CD64) confer the unique high affinity binding of IgG2a. *Mol. Immunol.* **35,** 989–996.

Hulett, M. D., Witort, E., Brinkworth, R. I., McKenzie, I. F., and Hogarth, P. M. (1994). Identification of the IgG binding site of the human low affinity receptor for IgG Fc gamma RII. Enhancement and ablation of binding by site-directed mutagenesis. *J. Biol. Chem.* **270,** 21188–21194.

Idusogie, E. E., Presta, L. G., Gazzano-Santoro, H., Totpal, K., Wong, P. Y., Ultsch, M., Meng, Y. G., and Mulkerrin, M. G. (2000). Mapping of the C1q binding site on Rutixan, a chimeric antibody with human IgG1 Fc. *J. Immunol.* **164,** 4178–4184.

Inganas, M. (1981). Comparison of mechanism of interaction between protein A from *Staphylococcus aureus* and human monoclonal IgG, IgA and IgM in relation with classical Fc and alternative $F(ab')_2$–protein A interaction. *Scand J. Immunol.* **13,** 343–352.

Inganas, M., Johansson, S. G. O., and Bennich, H. H. (1980). Interaction of human polyclonal IgE and IgG from different species with protein A from *Staphylococcus aureus*: Demonstration of protein A-reactive sites located in the $F(ab')_2$ fragment of human IgG. *Scand. J. Immunol.* **12,** 23–31.

Israel, E. J., Patel, V. K., Taylor, S. F., Marshak-Rothstein, A., and Simister, N. E. (1995). Requirement for β2-microglobulin-associated Fc receptor for acquisition of maternal IgG by fetal and neonatal mice. *J. Immunol.* **154,** 6246–6251.

Israel, E. J., Wilsker, D. F., Hayes, K. C., Schonfeld, D., and Simister, N. E. (1996). Increased clearance of IgG in mice that lack beta 2-microglobulin: possible protective role of FcRn. *Immunology* **89,** 573–578.

Jefferis, R., and Lund, J. (2002). Interaction sites on human IgG-Fc for FcγR: Current models. *Immunol. Lett.* **82,** 57–65.

Johansson, P. J. H., Nardella, F. A., Sjöquist, J., Schryder, A. K., and Christensen, P. (1988). Herpes simplex type 1-induced Fc receptor binds to the Cγ2–Cγ3 interface region of IgG in the area that binds staphylococcal protein A. *Immunology* **66,** 8–18.

Johansson, P. J. H., Otam, T., Tsuchiya, N., Malone, C. C., and Williams, R. C. (1994). Studies of protein A and herpes simplex virus-1 induced Fcγ-binding specificities. Different binding patterns for IgG3 from Caucasian and Oriental subjects. *Immunology* **83,** 631–638.

Jones, S. E., and Jomary, C. (2002). Clusterin. *Intern. J. Biochem. Cell. Biol.* **34,** 427–431.

Junghans, R. P., and Anderson, C. L. (1996). The protection receptor of IgG catabolism is the beta-2-microglobulin containing neonatal intestinal transport receptor. *Proc. Natl. Acad. Sci. USA* **93,** 5512–5516.

Kabir, S. (1998). Jacalin: A jackfruit (*Artocarpus heterophylus*) seed-derived lectin of versatile applications in immunobiological research. *Immunol. Methods* **212,** 193–211.

Kabir, S. (2002). Immunoglobulin purification by affinity chromatography using protein A mimetic ligands prepared by combinatorial chemical synthesis. *Immunol. Invest.* **31,** 263–278.

Kabir, S., Ahmed, I. S. A., and Daar, A. S. (1995). The binding of jacalin with rabbit immunoglobulin G. *Immunol. Invest.* **24,** 725–735.

Kacskovics, I., Wu, Z., Simister, N. E., Frenyo, L. V., and Hammarstrom, L. (2000). Cloning and characterization of bovine MHC class I-like Fc receptor. *J. Immunol.* **164,** 1889–1897.

Karray, S., Joumpan, L., Maroun, R. C., Isenberg, D., Silverman, G. J., and Zouali, M. (1998). Structural basis of the gp120 superantigen-binding site on human immunoglobulins. *J. Immunol.* **161,** 6681–6688.

Kato, K., Gouda, H., Takaha, W., Yoshiro, A., Matsunaga, C., and Arata, Y. (1993). ^{13}C NMR study of the mode of interaction in solution of the B fragment of staphylococcal protein A and the Fc fragments of mouse immunoglobulin G. *FEBS Lett.* **328,** 49–54.

Kato, K., Sautes-Fridman, C., Yamada, W., Kobayashi, K., Uchiyama, S., Kim, H., Enokizono, J., Galinha, A., Kobayashi, Y., Fridman, W. H., Arata, Y., and Shimada, I. (2000a). Structural basis of the interaction between IgG and Fcγ receptors. *J. Mol. Biol.* **295,** 213–224.

Kato, K., Fridman, W. H., Arata, Y., and Sautes-Fridman, C. (2000b). A conformational change in the Fc precludes the binding of two Fcγ receptor molecules to one IgG. *Immunol. Today* **21,** 310–313.

Kilmon, M. A., Ghirlando, R., Strub, M.-P., Beavil, R. L., Gould, H. J., and Conrad, D. H. (2001). Regulation of IgE production requires oligomerization of CD23. *J. Immunol.* **167,** 3139–3145.

Kim, J.-K., Tsen, M.-F., Ghetie, V., and Ward, E. S. (1994a). Identifying amino acid residues that influence plasma clearance of murine IgG1 fragments by site-directed mutagenesis. *Eur. J. Immunol.* **24,** 542–548.

Kim, J.-K., Tsen, M.-F., Ghetie, V., and Ward, E. S. (1994b). Localization of the site of the murine IgG1 molecule that is involved in binding to the murine intestinal Fc receptor. *Eur. J. Immunol.* **24,** 2429–2434.

Kim, J.-K., Tsen, M.-F., Ghetie, V., and Ward, E. S. (1994c). Catabolism of the murine IgG1 molecule: Evidence that both CH2-CH3 domain interfaces are required for persistence of IgG1 in the circulation of mice. *Scand. J. Immunol.* **40,** 457–465.

Kim, J.-K., Tsen, M.-F., Ghetie, V., and Ward, E. S. (1995). Evidence that the hinge region plays a role in maintaining serum levels of the murine IgG1 molecule. *Mol. Immunol.* **32,** 467–475.

Kim, J.-K., Firan, M., Radu, C. G., Kim, C.-H., Ghetie, V., and Ward, E. S. (1999). Mapping the site of human IgG for binding of the MHC class I-related receptor FcRn. *Eur. J. Immunol.* **29,** 2819–2825.

Kishore, U., and Reid, K. B. M. (1999). Modular organization of proteins containing C1q-like globular domain. *Immunopharmacology* **42,** 15–21.

Koppel, R., and Solomon, B. (2001). IgM detection via selective recognition by mannose-binding protein. *J. Biochem. Biophys. Methods* **49,** 641–647.

Krook, M., Mosbach, K., and Ramström, O. (1998). Novel peptides binding to the Fc-portion of immunoglobulins obtained from a combinatorial phage display peptide library. *J. Immunol. Methods* **221,** 151–157.

Kuehn, M. J., Ogg, D. J., Kihlberg, J., Slonim, L. N., Flemmer, K., Bergfors, T., and Hultgren, S. J. (1993). Structural basis of pilus recognition by the PapD chaperone. *Science* **262,** 1234–1241.

Lakins, J. N., Poon, S., Easterbrook-Smith, S. B., Carver, J. A., Tenniswood, M. P. R., and Wilson, M. R. (2002). Evidence that clusterin has discrete chaperone and ligand binding sites. *Biochemistry* **41,** 282–291.

Langone, J. J., Boyle, M. D. P., and Borsos, T. (1978). Studies on the interaction between protein A and immunoglobulin G. II. Composition and activity of complexes formed between protein A and IgG. *J. Immunol.* **121,** 333–341.

Law, S. K. A., and Dodds, A. W. (1997). The internal thioester and the covalent binding properties of the complement proteins C3 and C4. *Protein Sci.* **6,** 263–274.

Leadbetter, E. A., Rifkin, I. R., Hohlbaum, A. M., Beaudette, B. C., Shlomchik, M. J., and Marshak-Rothstein, A. (2002). Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature* **416,** 603–607.

Lenert, P., Kroon, D., Spiegelberg, H., Golub, E. S., and Zanetti, M. (1990). Human CD4 binds immunoglobulins. *Science* **248,** 1639–1643.

Lenert, P., Lenert, G., and Zanetti, M. (1995). Human recombinant CD4 and CD4-derived synthetic peptides agglutinate immunoglobulin-coated latex particles. Evidence that residues 25–28 and 35–38 of human CD4 form two separate immunoglobulin binding sites. *Mol. Immunol.* **32,** 1399–1404.

Levinson, A. I. (1989). Nature and stimulus for rheumatoid factor production. *In* "Autoantibodies to Immunoglobulins" (F. Shakib, Ed.), pp. 130–150. Karger, Basel, Switzerland.

Li, R., Dowd, V., Stewart, D. J., Burton, S. J., and Lowe, C. R. (1998). Design, synthesis, and application of protein A mimetic. *Nat. Biotechnol.* **16,** 190–195.

Lian, L.-Y., Derrick, J. P., Sutcliffe, M. J., Yang, J. C., and Roberts, G. C. K. (1992). Determination of the solution structure of domain II and III of protein G from *Streptococcus* by 1H nuclear magnetic resonance. *J. Mol. Biol.* **228,** 1219–1234.

Lian, L.-Y., Barsukov, I. L., Derrick, J. P., and Roberts, G. C. K. (1994). Mapping the interactions between streptococcal protein G and the Fab fragment of IgG in solution. *Nat. Struct. Biol.* **1,** 355–357.

Litwin, V., Jackson, W., and Grose, C. (1992). Receptor properties of two varicella–zoster virus glycoproteins, gpI and gpIV, homologous to herpes simplex virus gE and gI. *J. Virol.* **66,** 3643–3651.

Liu, F.-T. (1990). Molecular biology of IgE-binding protein, IgE-binding factors and IgE receptors. *Crit. Rev. Immunol.* **10,** 289–306.

Liu, F.-T. (1993). S-type mammalian lectins in allergic inflammation. *Immunol. Today* **14,** 486–490.

Liu, Z., Roopenian, D. C., Zhou, X., Christianson, G. J., Diaz, L. A., Sedmark, D. D., and Anderson, C. L. (1997). β2-microglobulin-deficient mice are resistant to bullous pemphigoid. *J. Exp. Med.* **186,** 777–783.

Ljungberg, U. K., Jansson, B., Niss, U., Nilsson, R., Sandberg, B. E., and Nilsson, B. (1993). The interaction between different domains of staphylococcal protein A and human polyclonal IgG, IgA, IgM and $F(ab')_2$: Separation of affinity from specificity. *Mol. Immunol.* **30,** 1279–1285.

Lo Conte, L., Chothia, C., and Janin, J. (1999). The atomic structure of protein-protein recognition sites. *J. Mol. Biol.* **285,** 2177–2196.

Malhotra, R., Wormald, M. R., Rudd, P. M., Fischer, P. B., Dwek, R. A., and Sim, R. B. (1995). Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement via the mannose-binding protein. *Nat. Med.* **1,** 237–243.

Marino, M., Ruvo, M., Defalco, S., and Fassina, G. (2000). Prevention of systemic lupus erithematosus in MRL/lpr mice by administration of an immunoglobulin-binding peptide. *Nat. Biotechnol.* **18,** 735–739.

Martin, W. L., and Bjorkman, P. J. (1999). Characterization of the 2:1 complex between the class I MHC-related Fc receptor and its Fc ligand in solution. *Biochemistry* **38,** 12639–12647.

Martin, W. L., West, A. P., Gau, L., and Bjorkman, P. J. (2001). Crystal structure at 2.8 Å of an FcRn/heterodimeric Fc complex: Mechanism of pH-dependent binding. *Mol. Cell* **7,** 867–877.

Masson, P. L. (1993). Elimination of infectious agents and increase of IgG catabolism as possible modes of action of IVIG. *J. Autoimmun.* **6,** 683–689.

Matsumoto, H., Ho, S., Miyazaki, T., and Ohta, T. (1983). Structural studies of a human gamma-3 myeloma protein (JIV) bearing the allotypic marker Gm(st). *J. Immunol.* **131,** 1865–1870.

Maxwell, K. F., Powell, M. S., Hulett, M. D., Barton, P. A., McKenzie, F. C., Garrett, T. P. J., and Hogarth, P. M. (1999). Crystal structure of the human leukocyte Fc receptor, FcRIIa. *Nat. Struct. Biol.* **6,** 437–442.

Medesan, C., Radu, C., Kim, J.-K., Ghetie, V., and Ward, E. S. (1996). Localization of the site of IgG molecule that regulates maternofetal transmission in mice. *Eur. J. Immunol.* **26,** 2533–2536.

Medesan, C., Matesoi, D., Radu, C., Ghetie, V., and Ward, E. S. (1997). Delineation of the amino acid residues involved in the transcytosis and catabolism of mouse IgG1. *J. Immunol.* **158,** 2211–2217.

Medesan, C., Cianga, P., Mummert, M., Stanescu, D., Ghetie, V., and Ward, E. S. (1998). Comparative studies of rat IgG to further delineate the Fc:FcRn interaction site. *Eur. J. Immunol.* **28,** 2092–2100.

Meininger, D. P., Rance, M., Starovasnik, M. A., Fairbrother, W. J., and Skelton, N. J. (2000). Characterization of the binding interface between E-domain of staphylococcal protein A and an antibody Fv-fragment. *Biochemistry* **39,** 26–36.

Messerschmidt, G. L., Henry, H. W., Snyder, H. W., Bertram, J., Mittelman, A., Ainsworth, S., Fiore, J., Viola, M. V., Louise, J., Ambinder, E., MacKintosh, F. R., Higby, D. J., O'Brien, P., Kiprov, D., Hamburger, M., Balint, J. P., Fisher, L. D., Perkins, W., Pinsky, C. M., and Jones, F. R. (1989). Protein A immunotherapy in the treatment of cancer: An update. *Semin. Hematol.* **26,** 19–24.

Metzger, H. (2002). Molecular versatility of antibodies. *Immunol. Rev.* **185,** 186–205.

Mihaescu, S., Sulica, A., Sjoquist, J., and Ghetie, V. (1979). Affinity of rabbit IgG antibody complexed with protein A of *S. aureus*. *Rev. Roum. Biochim.* **16,** 57–60.

Miletic, V. D., and Frank, M. M. (1995). Complement-immunoglobulin interactions. *Curr. Opin. Immunol.* **7,** 41–47.

Moks, J., Abrahmsen, L., Nilsson, B., Hellman, U., Sjoquist, J., and Uhlen, M. (1986). Staphylococcal protein A consists of five IgG-binding domains. *Eur. J. Biochem.* **156,** 637–643.

Monteiro, R. C., and van de Winkel, J. C. J. (2003). IgA Fc receptors. *Annu. Rev. Immunol.* **21,** 177–204.

Morgan, A., Jones, N. D., Nesbitt, A. M., Chaplin, L., Bodmer, M. W., and Emtage, J. S. (1995). The N-terminal end of the C_H2 domain of chimeric human IgG1 anti-HLA-DR is necessary for C1q, FcγRI and FcγRIII binding. *Immunology* **86,** 319–324.

Mota, G., Ghetie, V., and Sjöquist, J. (1978). Characterization of the soluble complex formed by reacting rabbit IgG with protein A of *Staphylococcus aureus*. *Immunochemistry* **15,** 639–642.

Muñoz, E., Vidarte, L., Casado, M. T., Pastor, C., and Vivanco, F. (1998a). The C_H1 domain of IgG is not essential for C3 covalent binding: Importance of the other constant domains as targets for C3. *Int. Immunol.* **10,** 97–106.

Muñoz, E., Vidarte, L., Pastor, C., Casado, M., and Vivanco, F. (1998b). A small domain (6.5 kDa) of bacterial protein G inhibits covalent binding to the Fc region of IgG immune complexes. *Eur. J. Immunol.* **28,** 2591–2597.

Muroi, K., Sasaki, R., and Miura, Y. (1989). The effect of immunoadsorption therapy by a protein A column on patients with trombocytopenia. *Semin. Hematol.* **25,** 10–14.

Nagashunmugam, T., Lubinski, J., Wang, L., Goldstein, L. T., Weeks, B. S., Sundaresan, P., Kang, E. H., Dubin, G., and Friedman, H. M. (1998). In vivo immune evasion mediated by the herpes simplex type 1 IgG Fc receptor. *J. Virol.* **72,** 5351–5359.

Nardella, F. A., Teller, D. C., and Mannik, M. (1981). Studies on the antigenic determinants of self-association of IgG rheumatoid factor. *J. Exp. Med.* **154,** 112–125.

Nevens, J. R., Mallia, A. K., Wendt, M. W., and Smith, P. K. (1992). Affinity chromatographic purification of immunoglobulin M antibodies utilizing immobilized mannan-binding protein. *J. Chromatogr.* **597,** 247–256.

Newkirk, M. (1996). Fc glycosylation and rheumatoid factors. *In* "Abnormalities of IgG Glycosylation and Immunological Disorders" (D. A. Isenberg and T. W. Rademacher, Eds.), pp. 119–130. Wiley, London.

Nezlin, R. (1990). Internal movements in immunoglobulin molecules. *Adv. Immunol.* **40,** 1–40.

Nezlin, R. (1993). Detection of the C3a complement component in commercial gamma globulins by dot blotting. *J. Immunol. Methods* **163,** 269–272.

Nezlin, R. (1998). "The Immunoglobulins. Structure and Function." Academic Press, San Diego.

Nezlin, R. (2000). A quantitative approach to the determination of antigen in immune complexes. *J. Immunol. Methods* **237,** 1–17.

Nezlin, R., and Freywald, A. (1992). Complexes of IgG molecules and C3a and C4a complement components in human serum. *Eur. J. Immunol.* **22,** 1955–1957.

Nezlin, R., Freywald, A., and Oppermann, M. (1993). Proteins separated from human IgG molecules. *Mol. Immunol.* **30,** 935–940.

Nilson, B., Moks, T., Jansson, B., Abrahamsen, L., Elmblad, A., Holmgren, E., Henrichson, C., Jones, T. A., and Uhlen, M. (1987). A synthetic IgG-binding domain based on staphylococcal protein A. *Protein Eng.* **1,** 107–113.

Nilson, B. H. K., Solomon, A., Björck, L., and Åkerström, B. (1992). Protein L from *Peptostreptococcus magnus* binds to the κ light chain variable domain. *J. Biol. Chem.* **267,** 2234–2239.

Nilson, B. H. K., Frick, I.-M., Åkesson, P., Forsén, S., Björck, L., Åkerström, B., and Wikström, M. (1995). Structure and stability of protein H and the M1 protein from *Streptococcus pyogenes*. Implications for other surface proteins of gram-positive bacteria. *Biochemistry* **34,** 13688–13698.

Nissim, A., Schwarzbaum, S., Siraganian, R., and Eshhar, Z. (1993). Fine specificity of the IgE interaction with the low and high affinity Fc receptor. *J. Immunol.* **150,** 1365–1374.

Norderhaug, I. N., Johansen, F.-E., Schjerven, H., and Brandzaeg, P. (1999). Regulation of the formation and external transport of secretory immunoglobulins. *Crit. Rev. Immunol.* **19,** 481–508.

Ober, R. J., Radu, C., Ghetie, V., and Ward, E. S. (2001). Differences in promiscuity for antibody-FcRn interaction across species: Implications for therapeutic antibodies. *Int. Immunol.* **13,** 1551–1559.

Ohno, T., Kubagawa, H., Sanders, S. K., and Cooper, M. D. (1990). Biochemical nature of an Fcμ receptor on human B-lineage cells. *J. Exp. Med.* **172,** 1165–1175.

Oppliger, I. R., Nardella, F. A., Stone, G. C., and Mannik, M. (1987). Human rheumatoid factors bear the internal image of the Fc binding region of Staphylococcal protein A. *J. Exp. Med.* **166,** 702–710.

Patella, V., Giuliano, A., Bouvet, J.-P., and Marone, G. (1998). Endogenous superallergen protein Fv induces IL-4 secretion from human FcεRI cells through interaction with the VH3 region of IgE. *J. Immunol.* **161,** 5647–5655.

Perkins, S. J., Nealis, A. S., Sutton, B. J., and Feinstein, A. (1991). Solution structure of human and mouse IgM by synchrotron X-ray scattering and molecular graphics modeling. A possible mechanism for complement binding. *J. Mol. Biol.* **221,** 1345–1366.

Phalipon, A., Cardona, A., Kraehenbuhl, J.-P., Edelman, L., Sansonette, P. J., and Corthesy, B. (2002). Secretory component: A new role in secretory IgA-mediated immune exclusion in vivo. *Immunity* **17,** 107–115.

Pleass, R. J., Areschoug, T., Lindahl, G., and Woof, J. M. (1999). Streptococcal IgA-binding proteins bind in the Cα2-Cα3 interdomain region and inhibit binding of IgA to human CD89. *J. Biol. Chem.* **276,** 8197–8204.

Pleass, R. J., Dunlop, J. J., Anderson, C. M., and Woof, J. M. (2001). Identification of residues in the CH2/CH3 domain interface of IgA essential for interaction with the human Fcα receptor (FcαR) CD89. *J. Biol. Chem.* **274,** 23508–23514.

Popov, S., Hubbard, J. G., Kim, J.-K., Ober, B., Ghetie, V., and Ward, E. S. (1996). The stoichiometry and affinity of the interaction of murine Fc fragments with the MHC class I-related receptor, FcRn. *Mol. Immunol.* **33,** 521–530.

Potter, K. N., Li, Y., and Capra, J. D. (1996). Staphylococcal protein A simultaneously interact with framework region 1, complementarity-determining region 2 and framework region 3 on human VH3-encoded Igs. *J. Immunol.* **157,** 2982–2988.

Praetor, A., Jones, R. M., Wong, W. L., and Hunziker, W. (2002). Membrane-anchored human FcRn can oligomerize in the absence of IgG. *J. Mol. Biol.* **321,** 277–284.

Rabinovich, G. A., Baum, L. G., Tinari, N., Paganelli, R., Natoli, C., Liu, F.-T., and Iacobelli, S. (2002). Galectins and their ligands: Amplifiers, silencers or tuners of the inflammatory response? *Trends Immunol.* **23,** 313–320.

Radaev, S., and Sun, P. D. (2001). Recognition of immunoglobulins by Fcγ receptors. *Mol. Immunol.* **38,** 1073–1083.

Radaev, S., Motyka, S., Fridman, W. H., Sautes-Fridman, C., and Sun, P. D. (2001). The structure of a human type III Fcγ receptor in complex with Fc. *J. Biol. Chem.* **276,** 16469–16477.

Raghavan, M., and Bjorkman, P. J. (1996). Fc receptors and their interactions with immunoglobulins. *Annu. Rev. Cell Dev. Biol.* **12,** 181–220.

Raghavan, M., Chen, M. Y., Gastinel, L. N., and Bjorkman, P. J. (1994). Investigation of the interaction between class I MHC-related Fc receptor and its immunoglobulin G ligand. *Immunity* **1,** 303–315.

Ravetch, J. V., and Bolland, S. (2001). IgG Fc receptors. *Annu. Rev. Immunol.* **19,** 275–290.

Reid, K. B. M. (1996). The complement system. *In* "Molecular Immunology" (B. D. Hames and D. M. Glover, Eds.), pp. 326–381. IRL Press, Oxford, UK.

Roben, P. W., Salem, A. N., and Silverman, G. J. (1995). VH3 family antibodies bind domain D of staphylococcal protein A. *J. Immunol.* **154,** 6437–6445.

Robertson, M. W., and Liu, F.-T. (1991). Heterogeneous IgE glycoforms characterized by differential recognition of an endogenous lectin (IgE-binding protein). *J. Immunol.* **147,** 3024–3030.

Romagnani, S., Giudizi, M. G., delPrete, E., Maggi, R., Biagiotti, F., Almerigogna, F., and Ricci, M. (1982). Demonstration on protein A of two district immunoglobulin-binding sites and their role in the mitogenic activity of *Staphylococcus aureus* Cowan-1 on human B cells. *J. Immunol.* **129,** 596–602.

Roopenian, D. C., Christanson, G. J., Sproule, T. J., Brown, A. C., Akilesh, S., Jung, N., Petkova, S., Avanessian, L., Choi, E. Y., Shaffer, D. J., Eden, P. A., and Anderson, C. L. (2003). The MHC class I-like IgG receptor controls perinatal IgG transport, IgG homeostasis, and fate of IgG-Fc-coupled drugs. *J. Immunol.* **170,** 3528–3533.

Rostagno, A., Frangione, B., and Gold, L. I. (1991). Biochemical studies of the interaction of fibronectin with Ig. *J. Immunol.* **146,** 2687–2693.

Rostagno, A., Williams, M., Frangione, B., and Gold, L. I. (1996). Biochemical analysis of the interaction of fibronectin with IgG and localization of the respective binding sites. *Mol. Immunol.* **33,** 561–572.

Rostagno, A. A., Gallo, G., and Gold, L. I. (2002). Binding of polymeric IgG to fibronectin in extracellular matrices: An in vitro paradigm for immune-complex deposition. *Mol. Immunol.* **38,** 1101–1111.

Ruffet, E., Pirès, R., Pillot, J., and Bouvet, J.-P. (1994). Activation of the classical pathway of complement by non-immune complexes of immunoglobulins with human protein Fv (Fv fragment-binding protein). *Scand. J. Immunol.* **40,** 359–362.

Sanchez, L. M., Penny, D. M., and Bjorkman, P. J. (1999). Stoichiometry of the interaction between the MHC-related Fc receptor and its Fc ligand. *Biochemistry* **38,** 9471–9476.

Sandin, C., Linse, S., Areschoug, T., Woof, J. M., Reinholdt, J., and Lindahl, G. (2002). Isolation and detection of human IgA using a streptococcal IgA-binding peptide. *J. Immunol.* **169,** 1357–1364.

Sasso, E. H., Silverman, G. J., and Mannik, M. (1991). Human IgA and IgG $F(ab')_2$ that bind to staphylococcal protein A belong to the V_H3 subgroup. *J. Immunol.* **147,** 1877–1883.

Sauer-Eriksson, A. E., Keywegt, G. J., Uhlen, M., and Jones, T. A. (1995). Crystal structure of the C2 fragment of streptococcal protein G in complex with the Fc domain of human IgG. *Structure* **3,** 265–278.

Schuck, R., Radu, C., and Ward, E. S. (1999). Sedimentation equilibrium analysis of recombinant mouse FcRn with murine IgG1. *Mol. Immunol.* **36,** 1117–1125.

Seegan, G. W., Smith, C. A., and Schumacher, V. N. (1979). Changes in the quaternary structure of IgG upon reduction of the interheavy-chain disulfide bonds. *Proc. Natl. Acad. Sci. USA* **76,** 907–911.

Sensel, M. G., Kane, L. M., and Morrison, S. L. (1997). Amino acid differences in the N-terminus of C_H2 influence the relative abilities of IgG2 and IgG3 to activate complement. *Mol. Immunol.* **34,** 1019–1029.

Seppälä, I., Kaartinen, M., Ibrahim, S., and Mäkelä, O. (1990). Mouse Ig coded by V_H families S107 or J666 bind to protein A. *J. Immunol.* **145,** 2989–2993.

Sharon, N., and Lis, H. (2003). "Lectins." Kluwer Academic Publishers, Dordrecht, The Netherlands.

Shibuya, A., Sakamoto, N., Shimizu, Y., Shibuya, K., Osawa, M., Hiroyama, T., Eyre, H. J., Sutherland, G. R., Endo, Y., Fujita, Y., Miyabayashi, T., Sakano, S., Tsuji, T., Nakayama, E., Phillips, J. H., Lanier, L. L., and Nakauchi, H. (2000). Fcα/μ receptor mediates endocytosis of IgM coated microbes. *Nat. Med.* **1,** 441–446.

Shields, R. L., Namenuk, A. K., Hong, K., Meng, G., Rae, J., Briggs, J., Xie, D., Lai, J., Stadlen, A., Li, B., Fox, J. A., and Presta, L. G. (2001). High resolution mapping of the binding site of human IgG1 for FcγRI, FcγRII, FcγRIII and FcRn and design of the IgG1 variants with improved binding to FcγR. *J. Biol. Chem.* **276,** 6591–6604.

Shohet, J. M., Pemberton, P., and Carroll, M. C. (1993). Identification of a major binding site for complement C3 on the IgG_1 heavy chain. *J. Biol. Chem.* **268,** 5866–5871.

Silverman, G. J. (1997). B-cell superantigens. *Immunol. Today* **18,** 379–386.

Silverman, G. J., Pirès, R., and Bouvet, J.-P. (1996). An endogenous sialoprotein and a bacterial B cell superantigen compete in their VH family-specific binding interactions with human Igs. *J. Immunol.* **157,** 4496–4502.

Simister, N. E. (1998). Multiple roles of FcRn. *In* "The Immunoglobulin Receptors and Their Physiological and Pathological Roles in Immunology" (J. G. J. Van de Winkel and P. M. Hogarth, Eds.), pp. 63–71. Kluwer Academic Publishers, London.

Simister, N. E., and Mostov, K. E. (1989). An Fc receptor structurally related to MHC class I antigen. *Nature* **337,** 184–187.

Sjobring, U., Björck, L., and Kastern, W. (1991). Streptococcal protein G. Gene structure and protein-binding properties. *J. Biol. Chem.* **266,** 399–405.

Snyder, H. W., Balint, J. P., and Jones, F. R. (1989). Modulation of immunity in patients with autoimmune diseases and cancer treated by extracorporeal immunoadsorption with Prosorba^R columns. *Semin. Hematol.* **26,** 31–41.

Sondermann, P., Huber, R., and Jacob, U. (1999). Crystal structure of the soluble form of the human Fcγ-receptor IIb: A new member of the immunoglobulin superfamily at 1.7 Å resolution. *EMBO J.* **18,** 1095–1103.

Sondermann, P., Huber, R., Oosthuizen, V., and Jacob, U. (2000). The 3.2-Å crystal structure of the human IgG1 Fc fragment–FcγRIII complex. *Nature* **406,** 267–273.

Stenberg, L., O'Toole, P. W., Mestecky, J., and Lindahl, G. (1994). Molecular characterization of protein Sir, a streptococcal cell surface protein that binds both immunoglobulin A and immunoglobulin G. *J. Biol. Chem.* **269,** 13458–13464.

Sutton, B., Corper, A., Bonagura, V. R., and Taussig, M. (2000). The structure and origin of rheumatoid factor. *Immunol. Today* **21,** 177–180.

Takai, T. (2002). Roles of the Fc receptors in autoimmunity. *Nat. Rev. Immunol.* **2,** 580–592.

Tamm, A., and Schmidt, R. E. (1997). IgG binding sites on human Fc gamma receptors. *Int. Rev. Immunol.* **16,** 57–85.

Tao, M.-H., Smith, R. I. F., and Morrison, S. L. (1993). Structural features of human immunoglobulin G that determine isotype-specific differences in complement activation. *J. Exp. Med.* **178,** 661–667.

Tashiro, M., and Montelione, G. T. (1995). Structures of bacterial immunoglobulin-binding domains and their complexes with immunoglobulin. *Curr. Opin. Struct. Biol.* **5,** 471–481.

Thommesen, J. E., Michaelsen, T. E., Loset, G. Å., Sandlie, I., and Brekke, O. H. (2000). Lysine 322 in the human IgG3 C_H2 domain is crucial for antibody dependent complement activation. *Mol. Immunol.* **37,** 995–1004.

Tomana, M., Zikan, J., Kulhavy, R., Bennett, J. C., and Mestecky, J. (1993a). Interactions of galactosyltransferase with serum and secretory immunoglobulins and their component chains. *Mol. Immunol.* **30,** 277–286.

Tomana, M., Zikan, J., Moldoveanu, Z., Kulhavy, R., Bennett, J. C., and Mestecky, J. (1993b). Interactions of cell-surface galactosyltransferase with immunoglobulins. *Mol. Immunol.* **30,** 265–275.

Tsuchiya, N., Williams, R. C., and Hatt-Flechter, L. M. (1990). Rheumatoid factors may be the internal image of Fc-binding protein of herpes simplex virus type I. *J. Immunol.* **144,** 4742–4748.

Turner, H., and Kinet, J.-P. (1999). Signalling through the high-affinity IgE receptor FcεRI. *Nature* **402,** 24–30.

Vaerman, J. P., Langendries, A., Giffroy, D. A., Brandzaeg, P., and Kobayashi, K. (1998). Lack of SC/pIgR-mediated epithelial transport of a human polymeric IgA devoid of J chain: In vitro and in vivo studies. *Immunology* **95,** 90–96.

Van Vliet, K. E., De Graaf-Miltenburg, L. A. M., Verhoef, J., and Van Strijp, J. A. G. (1992). Direct evidence for antibody bipolar bridging on herpes simplex virus-infected cells. *Immunology* **77,** 109–115.

Vaughn, D. E., Milburn, C. M., Penny, D. M., Martin, W. L., Johnson, J. L., and Bjorkman, P. J. (1997). Identification of critical IgG binding epitopes on the neonatal Fc receptor. *J. Mol. Biol.* **274,** 597–607.

Vivanco, F., Muñoz, E., Vidarte, L., and Pastor, C. (1999). The covalent interaction of C3 with IgG immune complexes. *Mol. Immunol.* **36,** 843–852.

Walker, A. M., Montgomery, D. W., Saraiya, S., Ho, T. W. C., Garewal, H. S., Wilson, J., and Lorand, L. (1995). Prolactin-immunoglobulin G complexes from human serum act as costimulatory ligands causing proliferation of malignant B lymphocytes. *Proc. Natl. Acad. Sci. USA* **92,** 3278–3282.

Wan, T., Beavil, R. L., Fabiane, S. M., Beavil, A. J., Sohi, M. K., Keown, M., Young, R. J., Henry, A. J., Owens, R. J., Gould, H. J., and Sutton, B. J. (2002). The crystal structure of IgE Fc reveals an asymmetrical bent conformation. *Nature* **3,** 681–686.

Ward, E. S., Zhou, J., Ghetie, V., and Ober, R. J. (2003). Evidence to support the cellular mechanism involved in serum IgG homeostasis in humans. *Int. Immunol.* **15,** 1–9.

West, A. P., and Bjorkman, P. J. (2000). Crystal structure and immunoglobulin G binding properties of the human major histocompatibility complex related Fc receptor. *Biochemistry* **39,** 9698–9708.

Wikström, M., Drakenberg, T., Forsén, S., Sjöbring, U., and Björck, L. (1994). Three-dimensional solution structure of an immunoglobulin light chain-binding domain of protein L. Comparison with the IgG-binding domains of protein G. *Biochemistry* **33,** 14011–14017.

Wikström, M., Sjöbring, U., Drakenberg, T., Forsén, S., and Björck, L. (1995). Mapping of the immunoglobulin light chain-binding site of protein L. *J. Mol. Biol.* **250,** 128–133.

Wilson, M. R., and Easterbrook-Smith, S. B. (1992). Clusterin binds by a multivalent mechanism to the Fc and Fab regions of IgG. *Biochim. Biophys. Acta* **1159,** 319–326.

Wilson, M. R., Roeth, P. J., and Easterbrook-Smith, S. B. (1991). Clusterin enhances the formation of insoluble immune complexes. *Biochem. Biophys. Res. Commun.* **177,** 985–990.

Wines, B. D., Sardjono, C. T., Trist, H. M., Lay, C.-S., and Hogarth, P. M. (2001). The interaction of FcαRI with IgA and its implications for ligand binding by immunoreceptors of the leukocyte receptor cluster. *J. Immunol.* **166,** 1781–1789.

Wright, A., and Morrison, S. L. (1994). Effect of altered C_H2-associated carbohydrate structure on the functional properties and in vivo fate of chimeric mouse-human immunoglobulin G1. *J. Exp. Med.* **180,** 1087–1096.

Wright, J. F., Shulman, M. J., Isenman, D. E., and Painter, R. H. (1990). C1 binding by mouse IgM. The effect of abnormal glycosylation at position 402 resulting from a serine to asparagine exchange at residue 406 of the μ chain. *J. Biol. Chem.* **265,** 10506–10513.

Wurzburg, B. A., and Jardetzky, T. S. (2001). Structural insights into the interactions between human IgE and its high affinity receptor FcεRI. *Mol. Immunol.* **38,** 1063–1072.

Youngblood, K., Fruchter, L., Ding, G., Lopez, J., Bonagura, V., and Davidson, A. (1994). Rheumatoid factors from the peripheral blood of two patients with rheumatoid arthritis are genetically heterogeneous and somatically mutated. *J. Clin. Invest.* **93,** 852–861.

Yu, Z., and Lennon, V. A. (1999). Mechanism of intravenous immune globulin therapy in antibody-mediated diseases. *N. Engl. J. Med.* **340,** 227–228.

Zhang, Y., Boesen, C. C., Radaev, S., Brooks, A. G., Fridman, W. H., Sautes-Fridman, C., and Sun, P. D. (2000). Crystal structure of the extracellular domain of a human FcγRIII. *Immunity* **13,** 387–395.

Zuckier, L. S., Chang, C. J., Scharff, M. D., and Morrison, S. L. (1998). Chimeric human-mouse IgG antibodies with shuffled constant region exons demonstrate that multiple domains contribute to in vivo half-life. *Cancer Res.* **58,** 3905–3908.